METABOLIC INVESTIGATIONS OF THE PIPERIDINE-TYPE PHENOTHIAZINE ANTIPSYCHOTIC AGENTS WITH EMPHASIS ON THE LACTAM METABOLITES

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy
in the
College of Pharmacy
University of Saskatchewan
Saskatoon

By
Ge Lin, M. Sc.

June, 1992

In presenting this thesis in partial fulfilment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of the University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Dean of the College of Pharmacy
University of Saskatchewan
Saskatoon, Saskatchewan, Canada
S7N 0W0

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Drs. E.M. Hawes and K.K. Midha for their personal interest, guidance and encouragement at all stages of this research and thesis work. I would also like to thank Drs. R.T. Coutts, R.S. Abrams, J.R. Dimmock, G. McKay and K.W. Hindmarsh for their helpful suggestions and advice.

Thanks are also due to Mr. N. Pidskalny (College of Pharmacy) for running mass spectra, Dr. K. Brown (Department of Chemistry) for measuring nmr spectra, Mr. K. Thoms (Department of Chemistry) for performing elemental analyses, and Dr. R.J. Baldessarini and Ms. N.S. Kula (Harvard Medical School, Belmont, MA 02178-9106, USA) for pharmacological activity assays.

Financial supports from the Medical Research Council of Canada (Program Grant PG-34), and in the form of graduate scholarship from the College of Graduate Studies and Research, University of Saskatchewan, are gratefully acknowledged.

Abstract

Thioridazine, mesoridazine and sulforidazine are piperidine-type phenothiazine antipsychotic agents, which only differ from one another in the oxidative state of the sulfur atom at the 2-position of the phenothiazine ring. These drugs are extensively metabolized in humans and animals. There has been no systematic investigation of the metabolites in any species although there is substantial documentation that the pathways include S-oxidations of both the phenothiazine ring and side chain sulfur atoms, aromatic hydroxylation of the phenothiazine ring followed by conjugation and N-demethylation of the piperidine ring N-substituent. There are few reports on the metabolism of the piperidine ring itself; thioridazine N-oxide is a minor metabolite of thioridazine in rat and tentative identification has been made of the lactams of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide in the urine of patients. For other drugs with saturated nitrogen-containing heterocyclic ring systems, including piperidine, it is well established in various species that the metabolites include the lactams, ring opened products and N-oxides. Therefore, studies were conducted to investigate systematically the metabolic profiles in urine after oral administration of thioridazine, mesoridazine and sulforidazine in rat, dog and human, which included the

definitive identification and quantification of those metabolites resultant from metabolism of the piperidine ring such as lactam and N-oxide derivatives.

Synthetic procedures were developed for the lactam analogues of thioridazine, mesoridazine and sulforidazine, their corresponding lactam ring sulfoxides, the lactam of sulforidazine ring sulfone and the N-oxide of sulforidazine. In addition, sulforidazine was synthesized and an investigational new drug certificate was obtained that enabled its administration to human.

An extraction procedure was developed which took into consideration the thermolability of S-oxides and N-oxides and the photoinduced racemization of ring sulfoxide derivatives. The analytes in organic extracts of urine were separated by HPLC and analyzed by MS in the cases of rat and dog administered sulforidazine, while an on-line HPLC-MS technique with a plasmaspray interface was used to analyze qualitatively the urinary extracts in all other cases. Except for the phenolic metabolites and mesoridazine N-oxide, the identity of each compound was confirmed by direct comparison of its chromatographic behaviour and MS data with those of an authentic synthetic sample. The lactams of mesoridazine, mesoridazine ring sulfoxide, sulforidazine and sulforidazine ring sulfoxide were unequivocally identified for the first time. At least one lactam metabolite was

detected in urinary extracts of each species for each drug. Mesoridazine N-oxide was found in human and sulforidazine N-oxide was identified in both human and dog. The other metabolites which were identified resulted from the S-oxidations of both the phenothiazine ring and side chain sulfur atoms, aromatic hydroxylation followed by conjugation and N-demethylation. Such metabolites that were detected in any species for the first time were N-desmethylmesoridazine ring sulfoxide and N-desmethylsulforidazine ring sulfoxide.

The unchanged drugs, their lactam derivatives and various other metabolites were quantified by HPLC methods with UV detection. Some of the ring sulfoxide metabolites were successfully separated into their diastereomeric pairs and the mean diastereomeric ratios (concentrations of the fast eluting diastereomer to the slow eluting diastereomer) were determined. In contradiction to previous reports in some of the cases examined there was stereoselectivity in ring S-oxidation. For example, the mean diastereomeric ratios for thioridazine ring sulfoxide in rat, dog and human administered thioridazine were 1.39±0.14, 1.70±0.06 and 1.86±0.12, respectively. The renal excretion of lactam metabolites was more pronounced in human and rat than dog. For example, the percentage of the drug accounted for that was excreted in the form of lactam metabolites for these drugs in human, rat and dog was in the range 11-39, 7-18 and

0.1-0.2%, respectively. In the case of N-oxide metabolites only trace amounts were present in the urine of human and dog.

Interspecies comparison, both qualitatively and quantitatively, of the overall urinary metabolic profiles, including lactam metabolites, showed that rat more closely resembled human than dog. Therefore, rat would be a suitable animal for further study of the importance of the C-oxidation of the piperidine ring of this class of drug.

Table of Contents

List of Figures

List of Tables

List of Schemes

List of Abbreviations

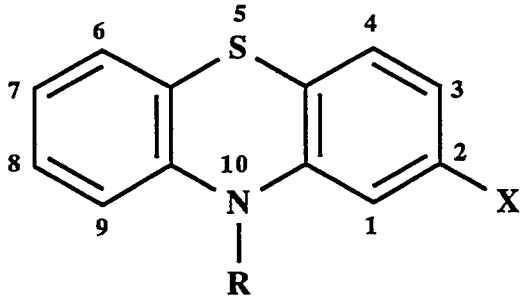
AMP	Adenosine monophosphate
AUFS	Absorbance units full scale
bp	Boiling point
BuOH	n-Butanol
CI	Chemical ionization
DMF	Dimethylformamide
EI	Electron impact
Et_2NH	Diethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
FAB	Fast atom bombardment
GLC	Gas liquid chromatography
HPLC	High performance liquid chromatography
IR	Infrared
mp	Melting point
MS	Mass spectrometer, mass spectrometry or mass spectrum, where appropriate
^{1}H NMR	Hydrogen-1 nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
RIA	Radioimmunoassay
RRA	Radioreceptor assay
RTIC	Reconstructed total ion chromatogram
TLC	Thin layer chromatography
UV	Ultraviolet

1. Introduction

1.1. Phenothiazine antipsychotic agents

There are several classes of organic compounds which are used as antipsychotic drugs, and these include phenothiazines, thioxanthenes, butyrophenones, dibenzoxazepines, dibenzodiazepines and indolines (Hollister 1986, Meltzer 1986). Among these classes, phenothiazine derivatives are the most widely used antipsychotic agents. This class was the first group of drugs in which a pronounced antipsychotic effect was demonstrated and they have been used clinically for about 40 years. The prototype antipsychotic agent, chlorpromazine (Figure 1) which belongs to this class was synthesized in the late 1940s and was first used in the treatment of psychotic patients in the early 1950s (Baldessarini 1990). Its impact on the treatment of psychiatric patients was remarkable; for example, it enabled many institutionalized patients to become outpatients.

Phenothiazine derivatives are generally divided into three groups according to the type of their N-10 substituted side chain. These three groups are referred to as aliphatic-, piperazine- and piperidine-type phenothiazine antipsychotic agents since the N-10 substituent is an aliphatic tertiary aminopropyl, piperazinylpropyl and either a 2-piperidinylethyl or a 1-piperidinylpropyl group,



Class	R	Example of drug
Aliphatic-type	$CH_2CH_2CH_2$-$N(CH_3)_2$	Chlorpromazine
Piperazine-type	$CH_2CH_2CH_2$ – N N – Z	Trifluoperazine
Piperidine-type	CH_2CH_2 – N, CH_3	Thioridazine
	$CH_2CH_2CH_2$ - N – Z	Pipothiazine

Figure 1. Classification of phenothiazine antipsychotic agents with respect to the N-10 substituted side chain.

respectively (Figure 1). Chlorpromazine, is a representative drug of the aliphatic-type group of phenothiazines. The piperazine-type phenothiazines (e.g. trifluoperazine and fluphenazine) are generally the most potent of the three groups. Thioridazine, mesoridazine and sulforidazine are examples of the piperidine-type phenothiazines.

The structure-activity relationships of tricyclic antipsychotic agents have been thoroughly studied. For example, the central ring angle formed between the two benzene rings and the central atoms (N-S in the case of phenothiazines) must approach 135° for retention of antipsychotic effects. Furthermore, the antipsychotic potency is influenced by the nature and position of an aromatic ring substituent within the heterocyclic nucleus. For example, substitution of an electron-withdrawing group (e.g. CF_3, Cl, SCH_3, $SOCH_3$, SO_2CH_3) in the 2-position of the phenothiazine nucleus significantly increases antipsychotic potency (Figure 2). In fact, virtually all the marketed phenothiazine drugs have such a 2-substituent.

In general, maximum antipsychotic potency of the phenothiazine class is achieved when the basic amino group of the side chain is separated from the nitrogen atom (N-10) of the central ring by a saturated three carbon bridge bearing no substituent (Figure 2). Shortening or

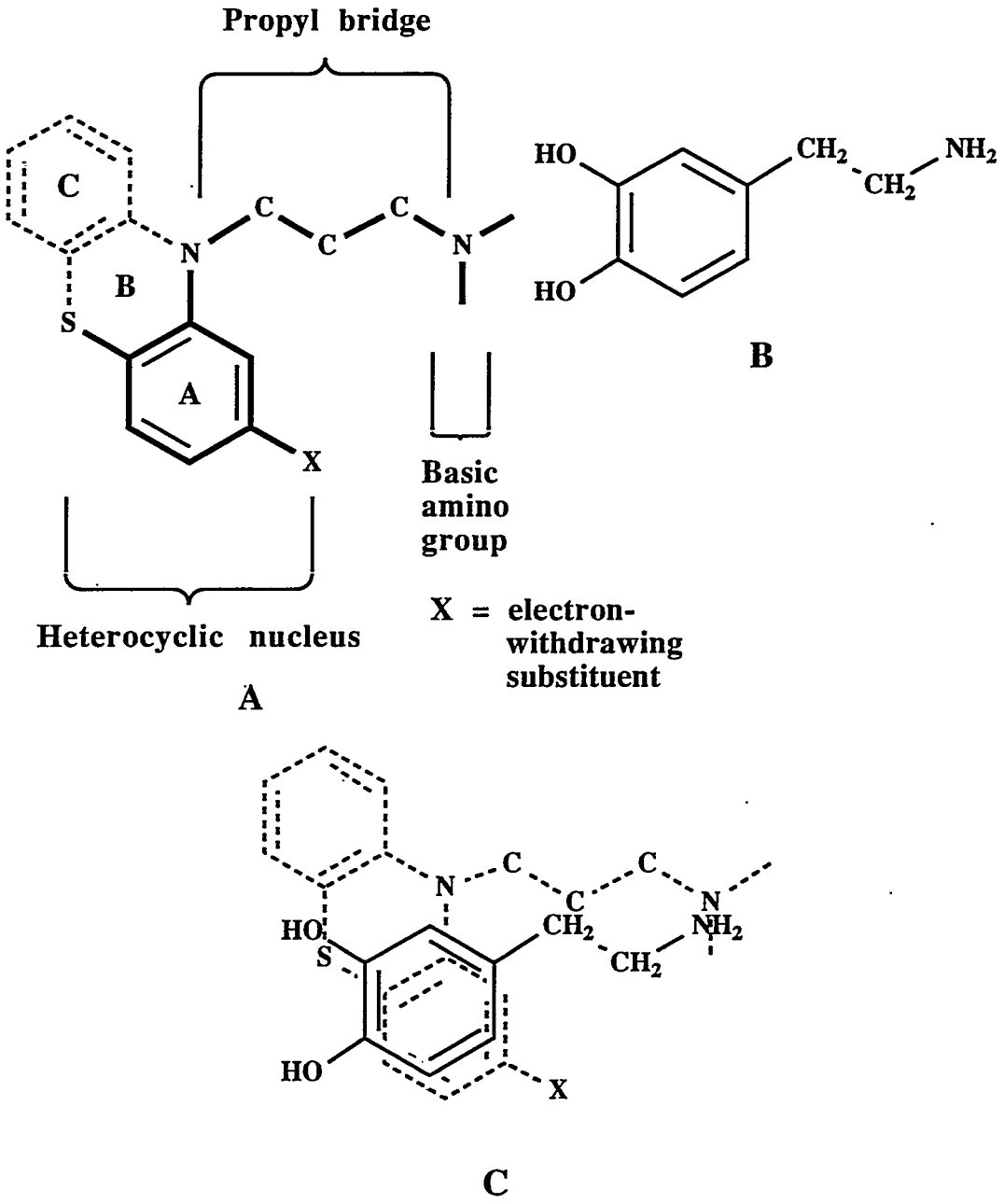


Figure 2. The three segments of an essential structural fragment (solid lines) of phenothiazine antipsychotic agents for maximal activity (A), the structure of dopamine (B) and the superimposition of phenothiazine antipsychotic agents and dopamine (C).

lengthening of this three carbon bridge generally reduces activity. Finally both secondary and primary amino analogues are less potent than the marketed compounds which possess a tertiary amino group (Vida and Tenthorey 1986).

Various investigations of the structural relationships between the phenothiazine class and dopamine have been performed. The conformations of the two structures overlap at certain sites (Figure 2) and indeed phenothiazine antipsychotic agents bind to the same receptors as dopamine (Horn and Snyder 1971, McDowell 1974, 1975, Dahl et al. 1982, Lipkowitz et al. 1986). In fact, the mechanism of action of phenothiazines and other antipsychotic classes in the therapy of schizophrenic disorders is believed to involve the blockade of dopaminergic receptors in the mesolimbic area of the brain. On the other hand blockade of receptors in the nigrostriatal dopaminergic pathway is thought to produce unwanted extrapyramidal side effects.

There are multiple types of dopamine receptors. Two subtypes have been definitively shown to exist and these have been widely studied. These are the D_1 receptor, which activates the enzyme adenylyl cyclase and increases intracellular levels of cyclic AMP, and the D_2 receptor, which exerts an inhibitory influence on adenylyl cyclase (Kebabian et al. 1972, Miller et al. 1974, Clement-Cormier

et al. 1974, Kebabian and Calne 1979). Recently three further subtypes, namely D_3, D_4 and D_5 receptors have been identified through molecular cloning techniques (Reynolds 1992, Sibley and Monsma 1992). Both structurally and functionally the D_3 and D_4 receptors show resemblance to the D_2 receptor (Sokoloff et al. 1990, Van Tol 1991), while the D_5 receptor shows such resemblance to the D_1 receptor (Sunahara et al. 1991). In general, antipsychotic drugs block both D_1 and D_2 receptors with more potent affinity to the D_2 than to the D_1 site. Indeed it is generally hypothesised that blockade of D_2 receptors plays a major role in producing both the antipsychotic and extrapyramidal effects of antipsychotic agents including phenothiazines. The ongoing work regarding the multiplicity and function of dopamine receptors may clarify the mechanism of action of antipsychotic drugs (Seeman et al. 1976, Creese et al. 1976, Reynolds 1992, Sibley and Monsma 1992).

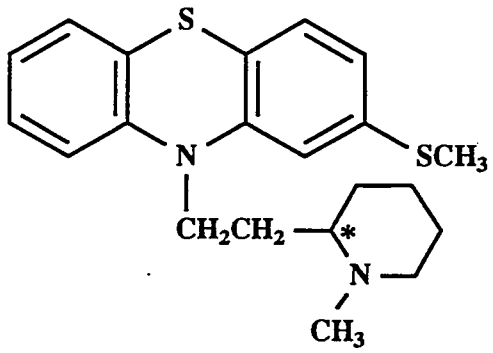
In addition to the blockade of dopaminergic receptors, phenothiazine derivatives also act in varying degrees on other receptors and exhibit a number of unwanted side effects. Thus α-adrenoreceptor blockade produces effects, such as orthostatic hypotension, while central histamine H_1-receptor blockade contributes to the commonly encountered sedative side effects of these drugs. Moreover antimuscarinic receptor blockade causes side effects, such

as dry mouth, blurred vision, constipation and urinary retention (Baldessarini 1990, Hollister 1986).

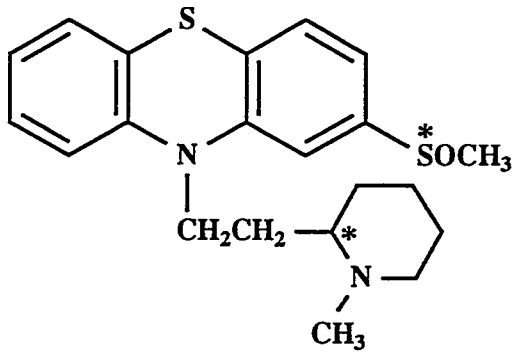
In general there is a relationship between potency in producing certain side effects and the nature of the N-10 side chain. For example, with respect to hypotensive, antimuscarinic and sedative side effects, the potencies to produce these effects decrease in the order of aliphatic-type, piperidine-type and piperazine-type, whereas for extrapyramidal side effects the corresponding order is piperazine-type, aliphatic-type and piperidine-type (Baldessarini 1990). With other side effects no relationship exists between structure and potency. For example, the serious disorder known as tardive dyskinesia is a major problem of all phenothiazines and its incidence increases with factors which include exposure to total dose used rather than the structural characteristics of the drug.

1.2. Thioridazine, mesoridazine and sulforidazine

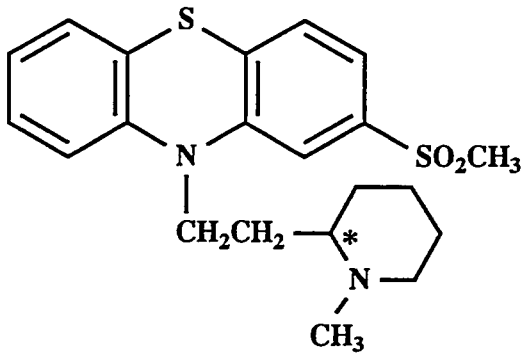
Thioridazine (**1**), mesoridazine (**2**) and sulforidazine (**3**) are piperidine-type phenothiazine antipsychotic agents. These three drugs differ from each other only in the oxidative state of the sulfur atom in the phenothiazine ring 2-substituent (Figure 3). Thioridazine has been used for the treatment of schizophrenia and a variety of related disorders since 1959. Mesoridazine and sulforidazine are



Thioridazine (1)



Mesoridazine (2)



Sulforidazine (3)

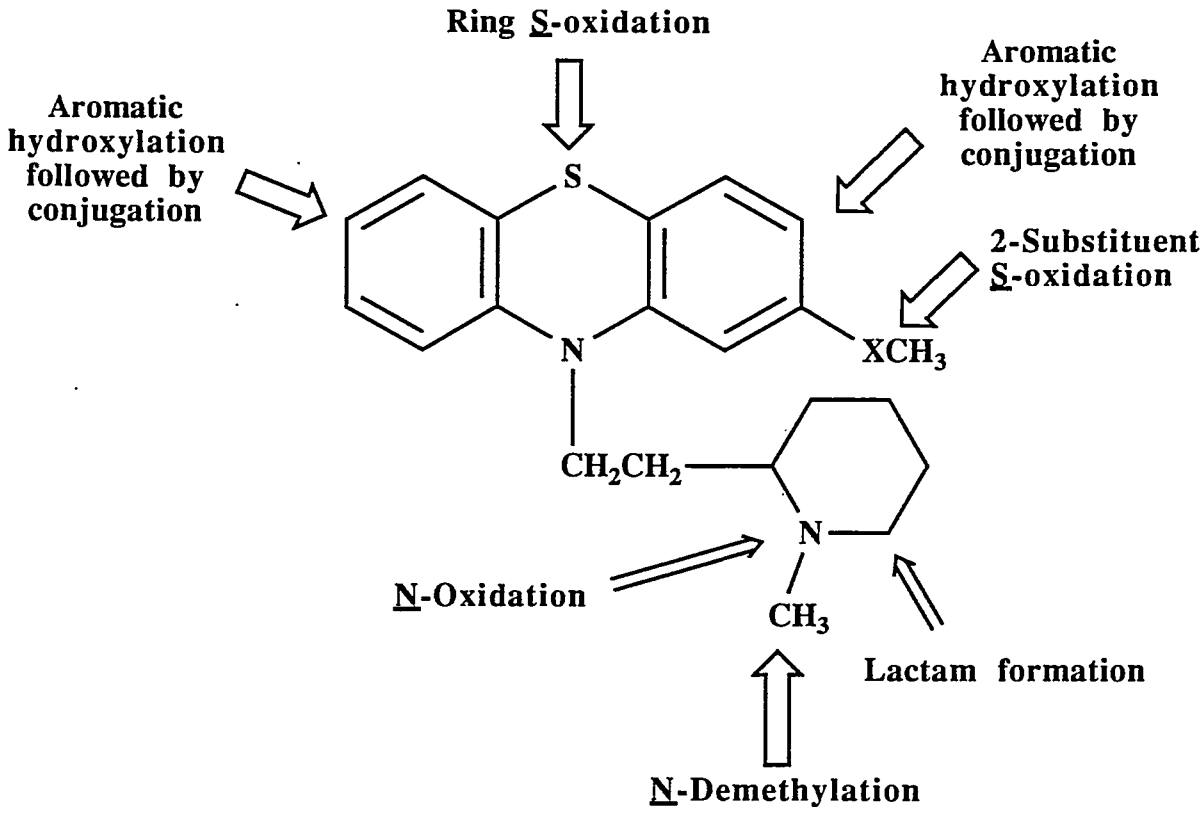
Figure 3. Chemical structures of thioridazine (1), mesoridazine (2) and sulforidazine (3).
* Chiral centres.

therapeutically active metabolites of thioridazine, and in certain screens for activity are respectively four and five times more potent than thioridazine (Axelsson 1977, Papadopoulos et al. 1980, Kilts et al. 1984, Niedzwicki et al. 1984). All three drugs are considered to be atypical antipsychotic agents because of a lower incidence of extrapyramidal side effects than the typical antipsychotic drugs, which include all non-piperidine type phenothiazines (Hollister 1986). Both thioridazine and sulforidazine possess a single chiral centre at the 2-position of the piperidine ring, while mesoridazine has an additional chiral centre at the 2-substituent sulfoxide moiety of the phenothiazine ring (Figure 3). Therefore, in the cases of thioridazine and sulforidazine they each consist of a mixture of two enantiomers while mesoridazine consists of a mixture of two diastereomeric pairs of enantiomers. Thioridazine, mesoridazine and sulforidazine are marketed under trade names, such as Mellaril® (Sandoz, Canada), Serentil® (Sandoz, Canada) and Inofal® (Sandoz, FRG), respectively. However, sulforidazine is not available for human administration in either Canada or the USA (Davis 1985).

1.2.1. Metabolism of thioridazine, mesoridazine and sulforidazine

The previous reports to the metabolites of thioridazine have been limited to human (Mårtensson et al. 1975, Papadopoulos et al. 1985, Jørgensen 1986, Papadopoulos and Crammer 1986, Ganes 1988) and rat (Zehnder et al. 1962, Watkins et al. 1986). Although there has been no systematic investigation of the metabolism of mesoridazine in any species, a number of publications refer to its metabolites in medicated patients (Axelsson 1977, Kinon et al. 1979, Green et al. 1980, Sakalis et al. 1980, Papadopoulos et al. 1985, Papadopoulos and Crammer 1986, Ganes 1988). In the case of sulforidazine, there have been few reports on the metabolites of this drug in any species (Axelsson 1977, Papadopoulos et al. 1985, Papadopoulos and Crammer 1986). The established routes of metabolism of these three drugs in human and animals involve S-oxidations of the phenothiazine ring and the ring 2-substituent (except sulforidazine) sulfur atoms, aromatic hydroxylation of the phenothiazine ring followed by conjugation and N-demethylation of the piperidine ring N-substituent (Figure 4).

There is little known about the metabolism of the piperidine ring of these drugs. In one document thioridazine N-oxide was identified as a minor metabolite of thioridazine in rat (Watkis et al. 1986). In addition, two lactam metabolites, namely the lactam of mesoridazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide,



Thioridazine (1): X = S

Mesoridazine (2): X = SO

Sulforidazine (3): X = SO_2

Figure 4. Metabolic pathways of thioridazine, mesoridazine and sulforidazine.

Closed arrows indicate the established metabolic routes, opened arrows indicate the metabolic routes which need further investigation.

have been tentatively identified as present in the extracts of the urine of patients receiving chronic oral treatment with these drugs (Papadopoulos and Crammer 1986). However, for other drugs with saturated nitrogen-containing heterocyclic ring systems, including piperidine, it is well established that the metabolites include lactams, ring opened products and N-oxides (Damani and Crooks 1982, Hawes et al. 1991, Oelschläger and Al Shaik 1985). A general discussion of the metabolism of saturated nitrogen-containing heterocyclic rings is given later (Section 1.3.).

1.2.1.1. S-Oxidation of the phenothiazine ring 2-substituent sulfur atom

According to Traficante et al. (1979), the ring 2-substituent sulfur atom in thioridazine is the preferred site of S-oxidation. The 2-sulfide group of thioridazine (Figure 3, **1**) is oxidized to form thioridazine-2-sulfoxide, which is named mesoridazine (Figure 3, **2**). In turn the 2-sulfoxide group of mesoridazine is further oxidized to thioridazine-2-sulfone, which is named sulforidazine (Figure 3, **3**) (Zehnder 1962, Gruenke et al. 1975, Mårtensson et al. 1975, Axelsson 1977, Axelsson and Mårtensson 1977, Sakalis et al. 1980, Widerl et al. 1982, Papadopoulos et al. 1985, McKay et al. 1985, Jørgensen 1986, Svendsen and Bird 1986). Mesoridazine is one of the major metabolites of

thioridazine, while sulforidazine is produced in a far lesser amount (Traficante et al. 1979). For example, after a single oral dose of thioridazine to healthy male volunteers (n = 5) the mean plasma concentrations of thioridazine, mesoridazine and sulforidazine were determined to be 149±75, 328±110 and 52±27 ng/ml, respectively (Chakraborty 1987, Chakraborty et al. 1987a, 1988). However, in the case of the determination of the concentrations of thioridazine and its active metabolites in post mortem brain, the concentrations of thioridazine were far higher than mesoridazine concentrations, whereas sulforidazine was hardly detectable (Svendsen et al. 1988).

Regarding the further metabolism of the side chain sulfoxide and sulfone groups, after oral administration of mesoridazine to man, both mesoridazine and sulforidazine were found in the serum together with trace amounts of thioridazine (Ganes 1988). When sulforidazine was given, only the parent drug and trace amounts of its N-demethylated metabolite were found (Axelsson 1977). These observations suggest that the formation of the side chain sulfoxide from the sulfide is a reversible process wherein the sulfoxide is the preferred metabolite and formation of the side chain sulfone from the sulfoxide appears to be an unidirectional process (Mitchell 1989).

1.2.1.2. S-Oxidation of the phenothiazine ring sulfur atom

Ring S-oxidation is a major route of metabolism of all phenothiazine antipsychotic agents. Thus in chronically medicated patients thioridazine ring sulfoxide, mesoridazine ring sulfoxide and sulforidazine ring sulfoxide have been found to be major metabolites of thioridazine, mesoridazine and sulforidazine, respectively. Also the ring sulfoxides of mesoridazine and sulforidazine were found as metabolites of thioridazine, and sulforidazine ring sulfoxide was also detected as a metabolite of mesoridazine (Mårtensson 1975, Axelsson 1977, Axelsson and Mårtensson 1977, 1983, Vanderheeren and Muusze 1977, Papadopoulos et al. 1980, 1985, Papadopoulos and Crammer 1986).

Oxidation of the ring sulfur atom to the corresponding ring sulfoxide creates a chiral centre so that ring sulfoxide metabolites with one further chiral centre will exist as two diastereomeric pairs of enantiomers (Juenge et al. 1983). It is well documented that the ring sulfoxide metabolites of phenothiazine drugs can be chromatographically separated into their diastereomeric pairs (Poklis et al. 1982, Wells et al. 1983, Hale and Poklis 1985, Papadopoulos et al 1985, Papadopoulos and Crammer 1986, Watkis et al. 1986, Ganes and Midha 1987). In fact, the ring sulfoxides of thioridazine (Brookes et al. 1978) and sulforidazine (Papadopoulos and Crammer 1986), but

not mesoridazine have been successfully separated. The measurement in urine and/or serum of roughly equal amounts of the diastereomers of sulforidazine ring sulfoxide in patients treated with thioridazine (Hale et al. 1985, Papadopoulos and Crammer 1986) and in rat dosed with thioridazine (Hale and Poklis 1984a, 1986a, Hale et al. 1985, Watkins et al. 1986, Ganes 1988) suggested that there was lack of stereoselectivity in the ring S-oxidation of piperidine-type phenothiazine antipsychotic agents. However, it was noted in one publication that in all plasma samples from healthy volunteers administered a single dose of sulforidazine the fast eluting diastereomer of sulforidazine ring sulfoxide was present in slightly greater concentration than the other diastereomer (Ganes and Midha 1987). Furthermore, after the work in this thesis was completed a report indicated that there was stereoselectivity in the ring S-oxidation of thioridazine in that the mean concentration of the fast eluting diastereomer of thioridazine ring sulfoxide was greater than that of the slow eluting diastereomer, about 1.37 fold in plasma or 1.54 fold in urine of eleven patients medicated with thioridazine (Eap et al. 1991).

The literature also contains conflicting reports as to whether ring sulfoxide compounds are further metabolized to form ring sulfone metabolites. Only two such metabolites,

namely thioridazine ring sulfone and sulforidazine ring sulfone, have been identified as metabolites of thioridazine and sulforidazine, respectively, in human and rat (Zehnder et al. 1962, Mårtensson et al. 1975, Sakalis et al. 1980). Regarding other phenothiazines, chlorpromazine ring sulfone has been identified in the urine of patients chronically treated with chlorpromazine. However, this metabolite only accounted for a very small fraction of the total dose (Breyer-Pfaff et al. 1978). This general lack of further oxidation of phenothiazine ring sulfoxides to the corresponding sulfone is presumably due in part to the relatively large number of alternative metabolic pathways that these drugs can undergo and also the polarity of sulfoxide metabolites in that such polar metabolites are rapidly excreted.

Racemic thioridazine ring sulfoxide was reported to be psychopharmacologically inactive (Muusze and Huber 1973, Axelsson 1977, Bylund 1981, Kilts et al. 1984). Conformational studies of phenothiazine derivatives suggested that the sulfur atom of the phenothiazine nucleus binds with the dopamine receptor (Figure 2), and that oxidation of this sulfur atom would interfere with this binding, therefore, giving explanation as to why ring sulfoxide metabolites of phenothiazines are inactive (see Section 1.1.). However, cardiovascular side effects have

been reported to be associated with high serum or plasma concentrations of racemic thioridazine ring sulfoxides (Gottschalk et al. 1978, 1979, Axelsson and Mårtensson 1980, Heath et al. 1985). Each separated diastereomer of this metabolite was potent in producing cardiovascular side effects in the isolated perfused rat heart (Hale and Poklis 1984b, 1986b). Also investigations have shown that whereas patients who responded well to thioridazine therapy had relatively high levels of thioridazine itself or its active metabolites, those who responded poorly tended to have a preponderance of ring sulfoxide metabolites. For example, when patients were treated with thioridazine, three non-responders had plasma levels of thioridazine ring sulfoxide, mesoridazine and sulforidazine of 291±157, 96±90 and 48±29 ng/ml, respectively, whereas in three responders these levels were 212±73, 237±62 and 68±52 ng/ml, respectively (Sakalis et al. 1977).

1.2.1.3. Aromatic hydroxylation of the phenothiazine ring

Aromatic hydroxylation of the phenothiazine ring plays a significant role in the metabolism of piperidine-type phenothiazine antipsychotic agents (Brookes et al. 1978, Papadopoulos et al. 1985). Thioridazine, mesoridazine and sulforidazine were all metabolized by this route to form the corresponding 7-hydroxylated metabolites in both human and

rat (Zehnder et al. 1962, Mårtensson et al. 1975, Papadopoulos et al. 1985, Jørgensen 1986, Svendsen and Bird 1986, Watkins et al. 1986), and also 3-hydroxythioridazine, 3-hydroxy-N-desmethylthioridazine, 7-hydroxy-N-desmethylthioridazine and 7-hydroxy-N-desmethylsulforidazine have been identified as metabolites of thioridazine in human (Papadopoulos et al. 1985, Papadopoulos and Crammer 1986). In general with phenothiazine antipsychotic agents hydroxylation occurs predominantly in the 3- and 7-positions of the phenothiazine ring (Papadopoulos et al. 1985, Jørgensen 1986). However, when the 2-position is substituted with a bulky and/or strong electron-withdrawing group, such as the methylsulfonyl group in sulforidazine, 3-hydroxylation is unlikely to occur. In fact, only 7-hydroxysulforidazine was found as the phenolic metabolite of sulforidazine in human urine, whereas with the 2-methylthio substituted drug thioridazine both 3- and 7-hydroxy metabolites were identified (Papadopoulos et al. 1985).

Hydroxylated metabolites are either excreted as their free phenolic form or further biotransformed to the corresponding phenolic conjugate(s). According to recent studies (Papadopoulos et al. 1985), after oral administration of thioridazine to patients, both free and conjugated (determined indirectly by means of ß-glucuronidase treatment) phenols were present in urine to

the extent of 1 and 8% of the dose administered, respectively, whereas about 6% of the dose was excreted in faeces as free phenols. Also in some cases different phenolic metabolites were found in the urine and faeces of these patients. Thus 7-hydroxymesoridazine was the major phenolic metabolite of thioridazine in urine, but it could not be detected in faeces, where 7-hydroxythioridazine dominated. These observations suggest that hydrolysis of conjugates and extensive reduction of the 2-sulfinyl group may occur in the gastrointestinal tract prior to excretion in the faeces. However, this reduction did not occur with the 2-sulfonyl group since only phenolic sulforidazine derivatives were found in both urine and faeces in the case of patients administered sulforidazine (Papadopoulos et al. 1985).

The contribution of phenolic metabolites to the therapeutic effects of piperidine-type phenothiazine antipsychotic agents is unknown. However, the 7-hydroxy metabolite of chlorpromazine has been shown to have pharmacological activity almost equal to that of the parent drug in a number of tests in human and animals (Buckley et al. 1974, Meltzer et al. 1977, Kleinman et al. 1980). In addition, X-ray crystallography studies of chlorpromazine have indicated that the essential structural features for antipsychotic activity are retained in 7-hydroxy-

chlorpromazine in that the 7-substituent is in the so-called C ring of the phenothiazine nucleus, a ring which is likely not involved in dopamine-receptor interaction (Figure 2) (Horn and Snyder 1971, Dahl et al. 1982).

1.2.1.4. N-Demethylation of the piperidine ring N-substituent

N-Dealkylation of the side chain N-substituent(s) is another established route of metabolism of phenothiazine antipsychotic drugs. In the cases of the piperidine-type phenothiazines, N-demethylation has been reported for thioridazine, mesoridazine and sulforidazine in both man and rat. The reported N-demethylated metabolites of these drugs include N-desmethylthioridazine, N-desmethylmesoridazine, N-desmethylsulforidazine, N-desmethylthioridazine ring sulfoxide, 3-hydroxy-N-desmethylthioridazine, 7-hydroxy-N-desmethylthioridazine and 7-hydroxy-N-desmethyl-sulforidazine (Zehnder et al. 1962, Mårtensson et al. 1975, Papadopoulos et al. 1985, Papadopoulos and Crammer 1986, Jørgensen 1986). However, according to one report this N-dealkylation route occurs to a much larger extent in rat compared to man (Lewis et al. 1984).

The contribution of N-desmethyl metabolites to the antipsychotic effects of these drugs is not clear. Where studied, the N-demethylated metabolites were present only in

relatively low concentration in the plasma or serum of patients medicated with these drugs (Mårtensson et al. 1975, Axelsson and Mårtensson 1977, Svendsen et al. 1988). Although N-desmethylthioridazine was inactive when tested by a dopamine receptor binding assay, it was active with respect to binding to α-adrenergic and muscarinic cholinergic receptors (Dahl 1982).

1.3. The metabolism of saturated nitrogen-containing heterocyclic ring systems

In general the metabolism of drugs containing saturated nitrogen-containing heterocyclic ring systems has been widely studied. The documented phase I metabolic pathways of the ring itself include N-oxidation and C-oxidation (Damani and Crooks, 1982, Oelschläger and Al Shaik 1985, Hawes et al. 1991). According to the literature in general for such ring systems N-oxidation appears to play a less important role than oxidative reactions at carbon atoms. The sole products of N-oxidation of tertiary amines are N-oxides, which are sometimes metabolically reconverted to the parent compounds. This reversible metabolic phenomenon has been demonstrated for several drugs in human (Jenner et al. 1973, Powis et al. 1979, Al-Waiz et al. 1987, Jaworski et al. 1990), rat (Heimans et al. 1971, Dajani et al. 1985) and dog (McMahon and Sullivan 1977, Jaworski et al. 1990), and

conversion of N-oxides to the parent drugs is more extensive in rat than dog or human (Jaworski et al. 1988, 1990, Midha et al. 1991). In general, amine oxides possess a higher dipole moment than the corresponding tertiary amines, and are thus usually more water soluble and are rapidly excreted. In the case of piperidine-type phenothiazines the only report to a N-oxide metabolite involves thioridazine N-oxide as a minor metabolite of thioridazine in rat (Watkis et al. 1986).

Metabolic pathways which involve the carbon atom of saturated nitrogen-containing heterocyclic ring systems are complex. For example, oxidation can occur at each carbon atom and also metabolic ring opening can occur leading to metabolites which are usually subject to further metabolism. However, almost invariably the metabolism of these ring systems involves in part oxidation of the methylene group vicinal to the nitrogen atom to produce various metabolites that include the 2-one (-NR-CO-) analogues. Such cyclic amides are called lactams. These metabolic conversions of saturated nitrogen-containing heterocyclic ring systems to lactam and related metabolites is reviewed in the next section.

1.3.1. Lactam and related metabolites

It was previously mentioned that the only report on

metabolites resulting from C-oxidation of the piperidine ring of piperidine-type phenothiazine antipsychotic agents was the tentative identification (based on electron impact mass spectral evidence) of the lactam of mesoridazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide in the urine of patients treated with these drugs (Papadopoulos and Crammer 1986). In general lactam formation is well established as a metabolic pathway for other drugs with saturated nitrogen-containing heterocyclic systems (Damani and Crooks 1982, Oelschläger and Al Shaik 1985). For example, lactam metabolites have been identified for numerous drugs containing various saturated heterocyclic systems including pyrrolidine [e.g. nicotine (Gorrod and Jenner 1975)], piperidine [e.g. cyproheptadine (Porter et al. 1975)], hexahydroazepine [e.g. meptazinol (Franklin et al. 1977)], piperazine [e.g. tiospirone (Mayol et al, 1991)] and morpholine [e.g. indeloxazine (Kamimura et al. 1986)] (Table 1). Some of the drugs with a piperidine ring which are known to produce lactam metabolite(s) in vivo in man and/or animals are listed in Table 2. In general, large interspecies variations in lactam formation have been observed. The present literature search indicated that lactam metabolites were more pronounced in human and rat than dog. In fact, for all the studies examined in dog, lactam metabolites were present either in trace amounts

[e.g. diphenidol (Kaiser et al. 1972) and quinpirole (Whitaker and Lindstrom 1987)] or were undetected [e.g. cisapride (Meuldermans et al. 1988a) and roxatidine (Honma et al. 1987, Iwamura et al. 1987)].

The mechanism of lactam formation has been investigated, especially in the cases of nicotine (Murphy 1973, Peterson et al. 1987) and phencyclidine (Ward et al. 1982a, 1982b, Hallström et al. 1983, Holsztynska and Domino 1986). In Figure 5 the generally recognized mechanism of formation of lactam metabolites is shown; phencyclidine is used as an example. Cytochrome P-450 mediated oxidation of the 2-position of the piperidine ring leads to the formation of an unstable carbinolamine intermediate, that is readily dehydrated to generate an electrophilic iminium ion. Both carbinolamine and iminium ion intermediates are further metabolized by cytoplasmic aldehyde dehydrogenase to produce the lactam metabolite (Murphy 1973, Holsztynska and Domino 1986, Masumoto et al. 1991). Also these two intermediates exist in equilibrium by reversible dehydration and hydration, and are both chemically reactive.

The existence of iminium ions during the formation of lactam metabolites has been indicated by in vitro trapping experiments. Thus on incubation of an appropriate substrate with liver microsomes in the presence of cyanide salts (i.e. NaCN or KCN), stable α-aminonitrile adducts have been

Table 1. Saturated heterocyclic ring systems which are known to produce lactam metabolites *in vivo*.

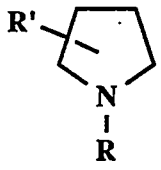
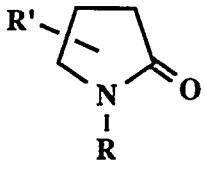
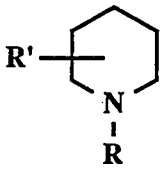
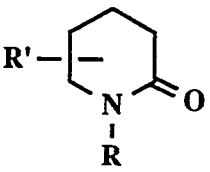
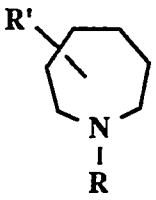
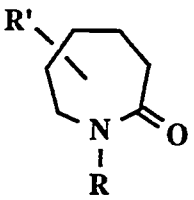
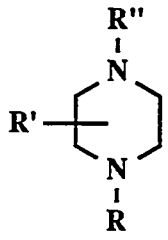
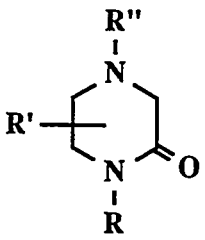
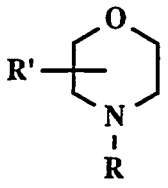
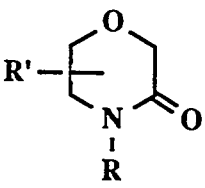
Saturated heterocyclic ring Name	Structure	Lactam metabolite	Documented example
Pyrrolidine			Nicotine
Piperidine			Cyproheptadine
Hexahydro-azepine			Meptazinol
Piperazine			Tiospirone
Morpholine			Indeloxazine

Table 2. Drugs containing a piperidine ring which are known to produce lactam metabolite(s) in vivo. The arrow shows the position at which a carbonyl group is added.

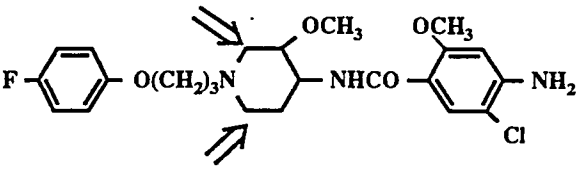
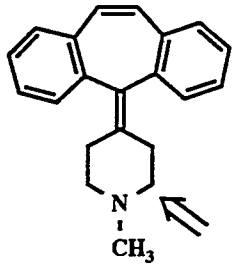
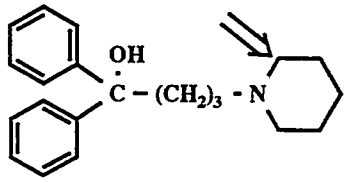
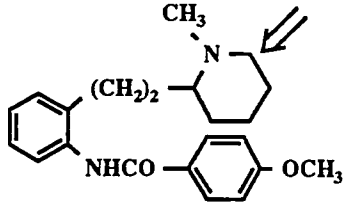
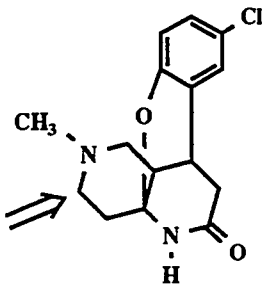
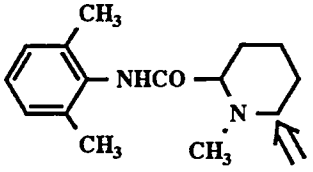
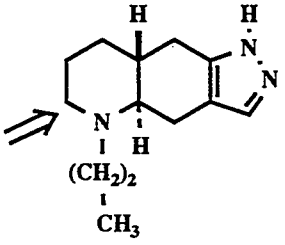
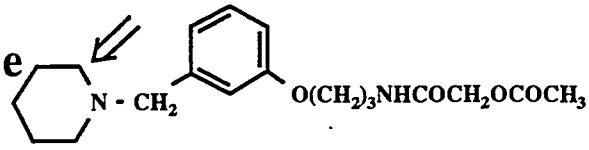
Drug	Structure	Species (references)
Cisapride		Rat, Man (Meuldermans et al. 1988a, 1988b)
Cyproheptadine		Man (Porter et al. 1975)
Diphenidol		Dog, Man (Kaiser et al. 1972)
Encainide		Man (Jajoo 1990)

Table 2. Continued.

Drug	Structure	Species (references)
Lortalamine		Man (Elsom et al. 1985)
Mepivacaine		Man (Meffin et al. 1973)
Quinpirole		Rat, Mouse, Dog, Monkey (Whitaker and Lindstrom 1987)
Roxatidine		Rat (Honma et al. 1987, Iwamura et al. 1987)

isolated (Figure 5) (Murphy 1973, Ward *et al*. 1982a, Masumoto *et al*. 1991). Also when the *in vitro* metabolism of nicotine was performed under an atmosphere of $^{18}O_2$, the isolated cotinine (nicotine lactam) was devoid of the stable isotope labelled oxygen (Murphy 1973). This observation is compatible with the proposed mechanism (Figure 5) in that although an ^{18}O-labelled carbinolamine would be formed initially, the label would be lost in subsequent steps. Furthermore, recently the iminium ions of some substrates, such as phencyclidine (Hoag *et al*. 1987) and *N*-benzylpiperidine (Masumoto *et al*. 1991), were directly assayed in incubation preparations. Also in each case the lactam metabolite was isolated after incubation of the iminium ion with liver microsomes. Therefore, in these cases the iminium ion was directly proven to be a precursor of the lactam metabolite.

Iminium ions are electrophilic and consequently unstable and chemically reactive in character. This suggests that *in vivo* covalent binding between iminium ions and nucleophilic macromolecules may be associated with the toxicities of drugs which metabolically produce iminium ions. Various studies have been carried out to investigate the extent of covalent binding of drugs metabolized *via* iminium intermediates. For example, incubation of isotope labelled nicotine with microsomal preparations from rabbit

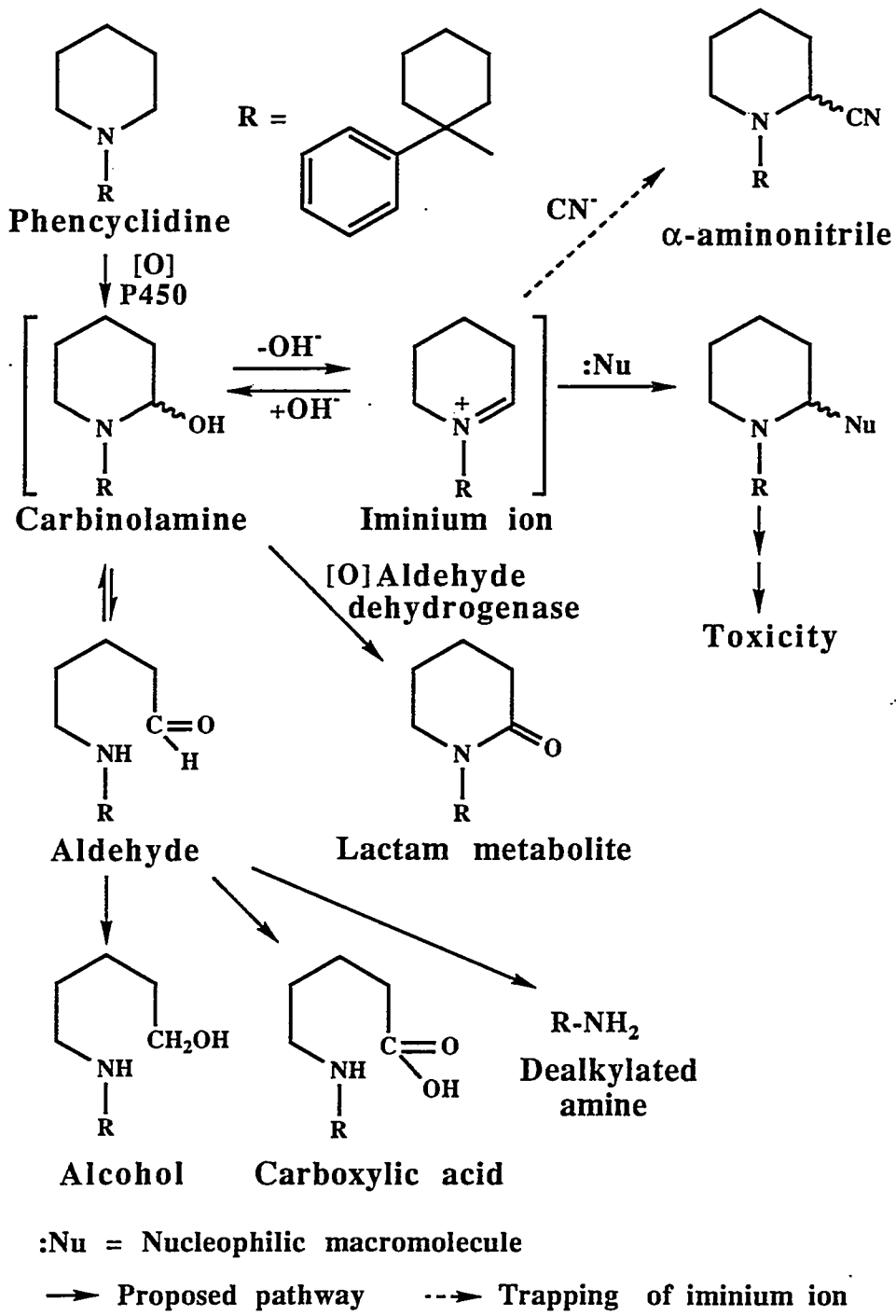


Figure 5. The mechanism of formation of the lactam and ring opened metabolites of phencyclidine.

liver and lung, and human liver tissues (Shigenaga et al. 1988) showed that the covalent binding of labelled compound to liver macromolecules was metabolism-dependent. Thus addition of either cytochrome P-450 enzyme inhibitors, such as SKF 525A, or sodium cyanide, which forms stable adducts with the cytochrome P-450-generated iminium ion, inhibited the covalent binding. Similar studies have been performed with phencyclidine (Ward et al. 1982b, Hoag et al. 1987). For example, incubation of isotope labelled phencyclidine iminium ion with liver microsomes resulted in concentration-dependent, irreversible covalent binding of radioactive material to macromolecules.

The carbinolamine intermediate is also in equilibrium with its ring opened aldehyde form (Figure 5) and the latter form is more stable than the former one. Since it is difficult to directly isolate and analyze such unstable carbinolamines, they are usually identified through derivatization, such as formation of the trimethylsilyl derivative, after treatment with an appropriate derivatizing reagent. However, in the case of phencyclidine the carbinolamine has been directly isolated in the urine of mouse dosed with this drug (Gole et al. 1988). On the other hand, the aldehyde is further metabolized to generate various highly polar ring opened metabolites such as carboxylic acids and alcohols, either where the amino

functional group has been retained or where it has been deaminated (McKennis et al. 1958, Hallström et al. 1983, Masumoto et al. 1989, 1991). Quantitatively, these ring opened metabolites are important in the overall metabolism of a number of drugs. For example, four ring opened metabolites of the piperidine ring of phencyclidine, namely the aldehyde, the alcohol, the carboxylic acid and the dealkylated amine (N-phenylcyclohexylamine) (Figure 5) have been identified upon in vitro incubation with rabbit liver preparations. Among them the alcohol and the carboxylic acid were present as major metabolites (Hallström et al. 1983). Furthermore, the ring opened metabolites may contribute to the therapeutic effects of the parent drugs. For instance the psychomotor stimulant drug prolintane, which contains a pyrrolidine ring, is metabolized to a ring opened carboxylic acid metabolite that has depressive properties on the psychomotor system (Yoshihara and Yoshimura 1974).

1.4. Enzyme systems involved in the phase I metabolism of phenothiazine antipsychotic drugs

A large proportion of the enzymatic studies of the metabolism of phenothiazine antipsychotic agents has focused on sulfoxidations. In general, the oxidation of sulfur can be catalyzed by a variety of enzyme systems of which the two

major ones are the microsomal cytochrome P-450 and microsomal flavin-containing monooxygenase families of enzymes (Ziegler 1980, 1982, Ziegler et al. 1980, Watanabe 1982, Oae et al. 1985). The major determinant of which of these two enzyme systems is involved in the oxidation of a particular substrate is the electromolecular environment in which the sulfur atom occurs. In simple terms, the microsomal cytochrome P-450 system will preferentially oxidize those sulfur atoms which have reduced nucleophilicity resulting from delocalization of their electronic shield via conjugated bond systems, while the flavin-containing monooxygenases will tend to oxidize those sulfur atoms which are more nucleophilic, such as those in aliphatic or alicyclic environments (Mitchell and Waring 1986, Taylor and Ziegler 1987).

Regarding phenothiazine derivatives the cyclic sulfur atom in the aromatic nucleus of chlorpromazine which is of relatively low nucleophilicity has been shown to be oxidized to the ring sulfoxide by the cytochrome P-450 system (Coccia and Westerfeld 1967, Gorrod et al. 1974, Traficante et al. 1979). Also the sulfur atom of the 2-sulfinyl substituent of thioridazine has been demonstrated to be oxidized to the corresponding sulfoxide by the same cytochrome P-450 isozyme (P450IID6) which catalyzes the 4-hydroxylation of debrisoquine. This isozyme of cytochrome P-450 in not

involved in catalyzing the ring oxidation of thioridazine, although the isozyme(s) involved remain to be determined in this case (Meyer et al. 1990, von Bahr et al. 1991). Information regarding further oxidation of phenothiazine sulfoxides to sulfones is scarce but tends to suggest that more than one enzyme system is involved.

The N-oxidation of tertiary amines to form N-oxides is catalyzed by the cytochrome P-450 and/or flavin-containing monooxygenase enzyme systems. In general those nitrogen atoms of tertiary amines of relative low nucleophilicity are oxidized via cytochrome P-450 oxidation, while flavin-containing monooxygenases catalyze the oxidation of those nitrogen atoms of higher nucleophilicity (Ziegler 1980, 1984, 1988, 1991). Therefore, the tertiary amines in the N-10 side chain of phenothiazine derivatives are good substrates for flavin-containing monooxygenases (Bickel 1971). In fact, the N-oxidation of various phenothiazine antipsychotic agents was reported to be catalyzed by porcine liver flavin-containing monooxygenases (Ziegler 1991).

There is little information available about the enzyme systems involved in the reduction of the sulfoxide groups of phenothiazines. In fact, as compared to the sulfoxide oxidases, far less is known about the nature and catalytic mechanisms of sulfoxide reductases. However, two cytosolic enzyme systems are known to be involved in the reduction of

the sulfoxide functional group. These two enzyme systems are thioredoxin/thioredoxin reductase (Anders et al. 1980, 1981, Kitamura et al. 1981) and aldehyde oxidase (Tatsumi 1982, Tatsumi et al. 1982, Kitamura and Tatsumi 1983, Tatsumi and Kitamura 1983, Yoshihara and Tatsumi 1985). The thioredoxin system is also present in coliform bacteria and the gut microflora, and thus it may be important in the reduction of sulfoxides following their enterohepatic circulation (Mannervik 1982, Renwick et al. 1982, Strong et al. 1984, Yoshihara 1985).

1.5. Analytical techniques utilized in the metabolic studies of thioridazine, mesoridazine and sulforidazine

Several of the techniques used for the qualitative and quantitative analysis of piperidine-type phenothiazine antipsychotic agents and their metabolites rely on the fact that the phenothiazine nucleus is a conjugated system with a characteristic ultraviolet (UV) absorption spectrum which can differ markedly between the parent compounds and certain metabolites, such as the sulfoxides. Moreover coloured quasi-stable ions or complexes are formed in strong acid solutions or with metal ions. These characteristics provide the opportunity to detect and quantify these compounds spectrophotometrically. In addition, quantification has been performed electrochemically by means of reduction-

oxidation reactions (Fairbrother 1979).

The first techniques employed for the quantification of thioridazine and its metabolites involved colorimetry (Neve 1961) and inverse isotope dilution analysis (Zehnder et al. 1962). Several quantitative methods for the determination of plasma or serum concentrations of these drugs and some of their major metabolites were developed afterwards, and included fluorometric and gas liquid chromatographic (GLC) techniques. The fluorometric method (Pacha 1969) was not specific because it measured both thioridazine and its non-phenolic metabolites (Ng and Crammer 1977). A number of GLC procedures using flame ionization detection have been developed for the quantification of thioridazine, mesoridazine, sulforidazine and thioridazine ring sulfoxide in biological fluids (Curry and Mould 1969, Dinovo et al. 1974, 1976, Ng and Crammer 1977, Debruyne et al. 1980, Papadopoulos et al. 1980, Shvartsburd et al. 1984). GLC techniques using electron capture detection and nitrogen phosphorus detection were also reported to be sensitive enough to quantify serum and cerebrospinal fluid concentrations of thioridazine and its major metabolites in psychiatric patients (Nyberg et al. 1978, 1981). However, because of the thermolability of certain metabolites such as ring sulfoxide and N-oxide metabolites, the use of GLC in the determination of these drugs and their metabolites is

not favoured (Hall et al. 1982).

Several methods based on high performance liquid chromatography (HPLC) have been developed for the quantitation of thioridazine, mesoridazine and sulforidazine and some of their major metabolites, such as the ring sulfoxides in plasma or serum. The majority of the reported methods used normal phase systems (Muusze and Huber 1974, Shinner et al. 1981, Kilts et al. 1982, Wells et al. 1983, Hale and Poklis 1984a, 1985, Ganes 1988), while reversed phase systems have been utilized also such as the use of a nitrile column (McKay et al. 1985) and a C18 column (Svendsen and Bird 1986). Various types of detection systems have been used including UV (Muusze and Huber 1973, Shinner et al. 1981, Kilts et al. 1982, Hale and Poklis 1984a, 1985, McKay et al. 1985), fluorescence (Muusze and Huber 1974, Wells et al. 1985) and electrochemical (Svendsen and Bird 1986).

Biological methods such as radioreceptor assay (RRA) and radioimmunoassay (RIA) have been employed in the determination of thioridazine, mesoridazine and sulforidazine in plasma or serum. The RRA procedure is non-specific in that all active molecules bind to the dopamine receptors of the rat caudate tissue employed (Creese and Snyder 1977). On the other hand, the RIAs of thioridazine (Chakraborty et al. 1987b), mesoridazine (Chakraborty et al.

1987a) and sulforidazine (Chakraborty et al. 1988) have been validated against respective HPLC procedures.

1.6. High performance liquid chromatography-mass spectrometry interfaces

The importance of coupling HPLC and MS is well recognized today since the combination of these two techniques provides a powerful means of compound separation followed by detection and quantification, thereby allowing very useful and sensitive, qualitative and quantitative procedures to be developed (Games 1983, Vestal 1984, Karger and Vouros 1985). However, there were major problems in developing HPLC-MS interfaces and the first one was developed only two decades ago. Experimentally, the major problem experienced in the development of HPLC-MS interfaces was the incompatibility between the operating pressures of the MS analyzer (about 10^{-6} Torr) and the LC system (at $\geq$760 Torr). The pioneer work demonstrating the potential for such an interface was accomplished in the late 1960s (Tal'roze et al. 1968) and a few years later the first commercial interface, a moving belt, was introduced (Scott et al. 1974, McFadden et al. 1976). To date a number of interfaces have been developed, but no single methodology has yet emerged as the universal choice. Although the development of HPLC-MS interfaces is still undergoing rapid

evolution there are three interfaces that are commercially available. These three types involve mechanical transport of solute to the MS after solvent removal (i.e. transport or moving belt interface), bulk solute introduction with or without splitting (i.e. direct liquid introduction) and direct ionization methods involving the entire solvent stream (i.e. thermospray and plasmaspray). In addition, other important HPLC-MS interfaces have been developed during the past few years, such as electrospray (Yamashita and Fenn 1984, Whitehouse, et al. 1985, Edmons et al. 1989, Smith et al. 1990), continuous flow fast atom bombardment (Caprioli 1989) and particle beam ionization (Yergey et al. 1989a).

In mechanical transport interfaces, such as the moving belt, the HPLC eluent is physically transported from the end of the column to the ion source while the mobile phase is simultaneously being removed prior to entry into the ion source (Scott et al. 1974, McFadden et al. 1976, Games and Martinez 1989). This interface has several features which are advantageous. Since the mobile phase has been evaporated by the time the analyte has reached the ion source, this interface is compatible with a variety of ionization modes including electron impact (EI), chemical ionization (CI) and fast atom bombardment (FAB). Furthermore, non-volatile buffers can be employed in the

HPLC mobile phase since such buffers are readily removed from the belt by means of a washing mechanism after it has exited the source. The disadvantages of the moving belt interface include the limitation of the range of analytes to be analyzed to thermally stable compounds and the potentially high background from the belt and carryover from previous analyses.

The direct liquid introduction interface is the simplest approach to HPLC-MS. In commercial interfaces of this type, a solvent jet is formed by passing the HPLC eluent through a laser-drilled pinhole (usually 2-5 μm in diameter) in a replaceable diaphragm. This jet then passes though a desolvation chamber where the droplets are vaporized, and the vapor enters the MS source. Due to the limited amount of solvent which can be tolerated by the vacuum system of the MS, direct liquid introduction interfaces for conventional HPLC columns incorporate a splitter, usually a simple needle valve and a water flow-line located downstream from the orifice. This allows a certain percentage of the solute to form the jet and yet provides the back-pressure necessary for jet formation (Baldwin and McLafferty 1973, Parker et al. 1985, Tomer and Parker 1989). Unfortunately, one consequence of the use of the splitter is that only a portion of the analyte enters the source. The subsequent development of microbore and

minibore HPLC systems enabled improvements in the ratio of analyte entering the source or even the use of the interface without the split valve (Bruins 1985, Karger and Vouros 1985). Direct liquid introduction interfaces can be used with a conventional MS modified by the addition of a desolvation chamber. The resulting high source pressures yield CI spectra, both positive and negative ion, with the mobile phase forming the reagent gas. Ionization occurs with proton transfer in the positive ion mode and proton abstraction and electron capture in the negative ion mode (Parker et al. 1985). Since the solvent vaporizes within the MS, certain solvent restrictions are encountered, and only volatile solvents and volatile buffers can be used, such as ammonium acetate and ammonium formate.

In the case of thermospray, the flow from the column containing an electrolytic salt (usually ammonium acetate) is directed into a stainless steel tube which acts as a vaporizer when heated. The temperature is controlled to allow the solvent stream to emerge as a fine spray at the exit end of the vaporizer. As the liquid emerges from the vaporizer tip, the aerosol droplets evaporate rapidly with a small fraction of the solution ions retaining their charge in the vapour state. Sample ions are then formed by interaction of the solute with the electrolyte ions, either in solution to form dissolved ions, or as the finely divided

aerosol droplets evaporate. A typical thermospray spectrum exhibits sample pseudo-molecular ions, $[M+H]^+$ and $[M+NH_4]^+$ (using ammonium acetate buffer) of high intensity (Blakley et al. 1978, 1980, Blakley and Vestal 1983, Arpino 1985, Bursey et al. 1985, Covey et al. 1985, Alexander and Kebarle 1986, Tomer and Parker 1989, Yergey et al. 1989b).

The plasmaspray interface is an adaptation of the thermospray interface. It uses the same type of thermospray interface consisting of a directly heated stainless steel capillary inlet system but with the addition of a discharge electrode to vaporize the mobile phase. The ionization is achieved by initiating a glow discharge inside the ion source, where the probe tip acts as the cathode and the ion source block as the anode. Components separated by the HPLC enter the MS in a heated solvent spray. Positive ions formed in the plasma from the solvent molecules are accelerated back toward the HPLC probe inlet by the potential difference applied between the source block (anode) and the probe tip (cathode) where they ionize incoming components. Plasmaspray MS usually exhibits an intense protonated molecular ion (MH^+) with a variety of fragment ions. The number and intensity of the latter ions are largely dependent upon the probe temperature and the structure of the molecule. This interface has some advantages over the thermospray interface. For example,

whereas the thermospray interface can be used only with buffered reversed phase systems the plasmaspray interface operates equally well with buffered or non-buffered systems and thereby can be used with normal or reversed phase systems. Moreover, since the ionization occurs over a wider range of probe tip temperatures, the probe temperatures can be used to control the degree of fragmentation and consequently more structural information can be obtained (Griffiths et al. 1988, Mills et al. 1988, Tomer and Parker 1989, Shindo et al. 1990).

2. Rationale and objectives

Previous metabolic studies of thioridazine, mesoridazine and sulforidazine have established that there are several metabolic pathways which are commonly encountered with these three drugs in human and animals. These well documented routes include S-oxidations of the phenothiazine ring and the ring 2-substituent (except sulforidazine) sulfur atoms , aromatic hydroxylation of the phenothiazine ring followed by conjugation and N-demethylation of the piperidine ring N-substituent (see also Section 1.2.1.).

Until recently there was little known about the metabolism of the piperidine ring of these drugs. Thioridazine N-oxide was found to be a minor metabolite of thioridazine in rat (Watkis et al. 1986) and the lactam derivatives of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide have been tentatively identified on the basis of the electron impact MS of the isolated metabolites from the extracts of the urine of patients treated with these drugs (Papadopoulos and Crammer 1986). However, for other drugs with saturated nitrogen-containing heterocyclic ring systems, including piperidine, it is well established that the metabolites include lactams, ring opened products and N-oxides, and also chemically reactive intermediates, such as carbinolamines and iminium ions, that are involved in the

formation of lactam metabolites (see Section 1.3.). There has been no systematic study of the metabolism of any one of the piperidine-type phenothiazine antipsychotic agents. Therefore, in the present study the overall objective was to systematically investigate the urinary metabolic profiles of thioridazine, mesoridazine and sulforidazine in three species including human. Particular emphasis was paid to include identification of those metabolites that result from metabolism of the piperidine ring such as the lactams and N-oxide derivatives. The animal species chosen for these studies were dog and rat. A literature search indicated that in general for drugs with an appropriate cyclic tertiary amine group lactam metabolites are more pronounced in rat than dog (Section 1.3.1.) whereas N-oxide metabolites are present in greater amounts in dog than rat. This latter interspecies difference is due to predominant reduction of such metabolites back to the parent compounds in rat (see Section 1.3.).

The urinary metabolic profiles of thioridazine, mesoridazine and sulforidazine obtained in three different species would enable interspecies comparisons to be made for these three structurally related drugs. These studies would enable investigation of whether dog or rat more closely resembled human in terms of its metabolic profiles including metabolic pathways such as lactam formation and N-oxidation.

Consequently such studies would provide the basis for the rational selection of a suitable animal for further investigation of the metabolism of piperidine-type phenothiazine antipsychotic drugs.

It was planned to synthesize certain lactam and N-oxygenated derivatives of these drugs in order to utilize them as authentic reference standards. Moreover sulforidazine was synthesized in order that an investigational new drug certificate could be obtained for its in vivo studies in human.

It was necessary to develop procedures that enabled the isolation, purification and identification of the unchanged drugs and their intact metabolites from the urine of man and animals. In the development of these procedures the instability of S-oxide and N-oxide metabolites and photo-induced racemization of ring S-oxide metabolites were considered. It is well recognized that phenolic metabolites do not chromatograph easily as distinct peaks during analysis because of their polar nature, therefore it was planned to derivatize these metabolites with appropriate reagents following their isolation. Moreover no attempt was made to directly identify intact phase II metabolites. They were identified as phase I metabolites following enzymic treatment of their extracts with ß-glucuronidase and sulfatase.

In order to determine the relative importance of the metabolism resulting from oxidation of the piperidine ring suitable quantitative analytical procedures for these metabolites in the urine of each of the three species were developed. It is well established that the diastereomeric isomers of sulfoxide metabolites can be separated by HPLC procedures. Therefore, it was planned to develop HPLC procedures for the quantification of lactams, unchanged drug and selected metabolites and where appropriate it was planned to quantify each diastereomer of each ring sulfoxide metabolite in order to determine stereoselectivity in the ring sulfoxidation of this class of drug.

3. Experimental

3.1. Materials and Methods

3.1.1. Chemicals

Ruthenium (IV) oxide hydrate was obtained from Fluka Chemical Corp., Hauppauge, NY, USA. 2-Methylthio-10H-phenothiazine, 2-methylsulfinyl-10H-phenothiazine, and the reference standards of thioridazine, mesoridazine and sulforidazine were gifts from Sandoz Inc., East Hanover, NJ, USA and Sandoz Pharmaceuticals, Dorval, Quebec. β-Glucuronidase (Type H-2, also containing sulfatase activity, 100,100 units/ml of β-glucuronidase activity and 6300 units/ml of sulfatase activity) was obtained from Sigma Chemical Company, St. Louis, MO, USA. N-Methyl-N-(tert.-butyldimethylsilyl)-N-trifluoroacetamide (MTBSTFA) and bis(trimethylsilyl)acetamide (BSA) were from Pierce, Rockford, IL, USA. All other chemicals were of commercial analytical grade and were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI, USA or BDH Chemicals Canada Ltd., Edmonton, Alberta.

2-Methylsulfonyl-10H-phenothiazine, mesoridazine, thioridazine ring sulfoxide, mesoridazine ring sulfoxide, sulforidazine ring sulfoxide (Mohammad et al. 1989), thioridazine (Mohammad et al. 1988) and prochlorperazine ring sulfoxide were synthesized in our laboratories. The purity and structural identity of synthesized compounds were

confirmed by TLC, mass spectrometry, NMR, IR and elemental analyses.

Solvents used for synthetic reactions, extraction and for the preparation of the HPLC mobile phase were HPLC grade (BDH Chemicals Canada Ltd.). Ether refers to diethyl ether. In the cases of extractions of both reaction mixtures and biological samples all combined organic extracts were dried over anhydrous sodium sulfate.

3.1.2. Instrumentation

Melting points (mp) were determined on a Gallenkamp melting point apparatus and are reported uncorrected. Observed boiling points (bp) are also uncorrected. The removal of solvent from reaction mixtures and from extracts was carried out on either a Buchi Rotavapor-R connected to a water aspirator or a SpeedVac concentrator (model RH 60-17 100, Savant Instruments Inc., Farmingdale, NY, USA). TLC was performed on aluminum sheets precoated (0.2 mm) with Kieselgel 60 F_{254} (E. Merck, purchased from Terochem Laboratories Ltd., Edmonton, Alberta) and spots were examined either with or without an UV lamp (254 or 365 nm), or 0.5% ninhydrin in butan-1-ol spray followed by heat treatment. Flash column chromatography was performed with Merck silica gel 60 (40-63 μm). Lyophilization was carried out on a Labconco Freeze Dryer-18 (Fisher Scientific and

Co., Edmonton, Alberta). The IR spectra were obtained on either Beckman Acculab 4 or Bio FTS-40 infrared spectrophotometers. The ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker AM-300 spectrometer. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. The abbreviations used to describe the multiplicity of peaks are as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer.

Direct probe mass spectrometry The electron impact (EI) mass spectra were obtained in the positive and/or negative ion modes using a direct insertion probe technique on a VG Micromass 7070HE instrument coupled to a DEC PDP 11-250J data system operating at an ionizing potential of 70 eV, source temperature of 180°C and emission current of 200 μA. The fast atom bombardment (FAB) mass spectra were also recorded on the same instrument in the positive ion mode where a matrix of either glycerol or 3-nitrobenzyl alcohol was employed, and operated at a resolution of 1,500 under the following operating conditions: argon atom beam, gun emission 1.2 mA, 7 kV, source pressure 3 x 10^{-5} mbar and ambient source temperature. The mass spectral data were reported in both text and graphic output. Only those peaks with relative intensities

greater than 10% of the base peak were reported in the text output unless the ion was considered diagnostic.

Plasmaspray mass spectrometry without column The plasmaspray mass spectra were obtained on a VG 70 SQ hybrid mass spectrometer in positive ion mode. The discharge voltage was adjusted to obtain optimal spectra. The temperatures of ion source and probe tip were 270°C and 250°C, respectively. Two solvent systems flowing to the ionization source were utilized. Solvent system A consisted of 50.0% methanol, 47.5% double distilled water, 2.5% 0.1 M ammonium acetate and 0.1% dimethylamine and was used for reference standards. Solvent system B consisted of 70.0% acetonitrile, 20.0% methanol and 10% 0.1 M ammonium acetate and was used for both standards and biological samples. The flow rates of the two solvent systems were 1.2 ml/min and 0.9 ml/min, respectively.

GLC-mass spectrometry The GLC-mass spectrometric analysis was performed on a VG analytical 70 SQ hybrid mass spectrometer equipped with a DEC PDP 11-250J data system and interfaced by a direct inlet system to a HP 5890 gas chromatograph. The gas chromatograph was fitted with a DB-5 capillary column (30 m x 0.32 mm int. diam. x 0.25 μm film thickness). The GLC-mass spectrometer system was used in the elucidation of the structures of two metabolites of sulforidazine in rat, namely the lactam of sulforidazine and

the diastereomers of N-desmethylsulforidazine ring sulfoxide. Identical operating conditions were used for the identification of these metabolites unless noted in parentheses. The injection technique was splitless with a purge time of 0.5 min and an injection port temperature of 310°C. The carrier gas was helium with a head pressure of 5 psi which gave a total column flow rate of 2 ml/min. The oven temperature was held at 275°C for 1 min, increased to 310°C (320°C) at a rate of 5°C/min and held for 15 min. The interface temperature was held constant at 310°C. The mass spectrometer was operated under EI at 70 eV in the positive ion mode with an accelerating voltage of 6 kV. The emission current and the source temperature were held constant at 100 μA and 275°C (220°C), respectively.

Conventional HPLC analysis A Perkin-Elmer liquid chromatograph (Series 10, Perkin-Elmer Corporation, Norwalk, CT, USA) with a 100 μl sample loop (model 7125 injector) was connected to a Perkin-Elmer fixed wavelength (254 nm) UV spectrophotometer (model LC-15B). The data were recorded using a Shimadzu integrator (model C-R3A, Shimadzu Corporation, Kyoto, Japan). The chromatographic column (150 x 4.6 mm int. diam.) was packed with 3 μm Spherisorb cyano packing (packed in-house using a Shandon column packer).

Two different mobile phase systems were utilized for the qualitative and quantitative studies of the metabolism

of sulforidazine. Mobile phase A, used for the qualitative study, consisted of 70.0% 2,2,4-trimethylpentane, 15.0% dichloromethane, 15.0% methanol and 0.1% diethylamine and mobile phase B, used for the quantitative study, consisted of 75.0% 2,2,4-trimethylpentane, 15.0% dichloromethane, 10.0% methanol and 0.1% diethylamine. The mobile phase systems were degassed before use by filtration (HVLP type membrane filter, Millipore Canada Ltd., Mississauga, Ontario). The chromatographic column was operated at ambient temperature with a flow rate of 0.8 ml/min. The detector was operated at a sensitivity of 0.128 absorbance units full scale (AUFS).

In addition, in the cases of further purification of the diastereomers of N-desmethylsulforidazine ring sulfoxide isolated from urine of rat dosed with sulforidazine, and analysis of these metabolites and their N-acetyl derivatives, mobile phase C was employed. This mobile phase system only differed from mobile phase B in that the dichloromethane was saturated with 1% ammonium acetate.

The mobile phase systems used in the other studies are now described. Two mobile phase systems were utilized in the study of the metabolism of mesoridazine. Mobile phase D consisted of 82.0% 2,2,4-trimethylpentane, 10.0% dichloromethane, 8.0% methanol and 0.01% diethylamine with a flow rate of 1.3 ml/min. Mobile phase E consisted of

92.0% acetonitrile, 8.0% 0.05 M ammonium acetate and 0.05% diethylamine with a flow rate of 1.0 ml/min. The AUFS values were 0.08 for human, and 0.128 for rat and dog urine samples, respectively.

In the case of the study of the metabolism of thioridazine the mobile phase systems E and F were utilized with a flow rate of 1.1 ml/min. The mobile phase system F only differed from mobile phase D in that 0.1% diethylamine was utilized. In these studies the AUFS value was 0.08 for all urine samples.

HPLC-mass spectrometry HPLC-mass spectrometry was carried out on a VG analytical 70 SQ hybrid mass spectrometer (connected to a DEC PDP 11-250J data system) equipped with a plasmaspray interface with a discharge of 320 V. All plasmaspray mass spectra were obtained in positive ion mode. In the case of the study of sulforidazine in rat, the column (150 x 4.6 mm int. diam.) was packed with spherisorb® 3 μm cyano packing. The mobile phase consisted of 97.0% acetonitrile, 2.0% methanol, 1.0% 0.1 M ammonium acetate and 0.025% diethylamine, and was filtered and degassed before use (HVLP type membrane filter). The column was maintained at ambient temperature with a flow rate of 1.0 ml/min. After elution from the chromatographic column the mobile phase was mixed in a short glass bead column with methanol-water (1:1 v/v) delivered at

a rate of 0.4 ml/min. The ion source temperature of the mass spectrometer was 270°C, and the plasmaspray probe tip ranged from 130°C to 140°C.

In all other cases, the column (250 x 4.6 mm int. diam.) was a Serva HPLC silica column (Si 100 polyol, 5 μm, Terochem Laboratories Ltd.). The mobile phase consisted of 83.0% acetonitrile and 17.0% 0.047 M ammonium acetate, and was filtered and degassed before use (HVLP type membrane filter). The column was maintained at ambient temperature with a flow rate of 1.0 ml/min. The ion source temperature of the mass spectrometer was 250°C and the plasmaspray probe tip ranged from 200°C to 300°C.

3.2. Chemical synthesis

3.2.1. Synthesis of 2-(2-chloroethyl)piperidine hydrochloride (**5**) (Scheme 1)

Thionyl chloride (20 ml, excess) was added dropwise to a stirred and cooled (0°C) solution of 2-(2-hydroxyethyl)-piperidine (**4**) (20.0 g, 0.155 mole) in anhydrous chloroform (150 ml). After warming gradually to room temperature, the reaction mixture was heated under reflux for 2 h. The solvent and excess thionyl chloride were removed under reduced pressure. The brown solid residue was washed with acetone and then dissolved in methanol. Addition of acetone gave white needles which were filtered and crystallized from

acetone-methanol to yield the hydrochloride salt **5** (27.0 g, 93%) as colourless needles, mp 158-159°C [lit. (Carmosin and Carson 1987) mp 160-162°C]; TLC R_f 0.42 (n-BuOH-AcOH-H_2O, 4:2:1); IR (potassium bromide): υ 3480 (N-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.58 (2H, br, N^+H_2 deuterium oxide exchangeable), 3.87 (1H, m, CHCl), 3.70 (1H, m, CHCl), 3.49 (1H, m, C_6-H), 3.26 (1H, br, C_2-H), 2.98 (1H, m, C_6-H), 2.55 (1H, m, C<u>H</u>CH_2Cl), 2.22 (1H, m, C<u>H</u>CH_2Cl), 2.06 (5H, m, C_3-H_2, C_4-H, C_5-H_2), 1.54 (1H, m, C_4-H); MS (EI): m/z (relative intensity %) 149/147 (0.9/2.2, $M^{+\cdot}$), 84 (100); MS (FAB): m/z (relative intensity %) 150/148 (30/90, MH^+), 144 (11), 114 (100), 84 (26); Anal. Calcd. for $C_7H_{15}Cl_2N$: C, 45.90; H, 8.20; N, 7.65; Found: C, 45.73; H, 8.51; N, 7.38.

3.2.2. Synthesis of 1-acetyl-2-(2-chloroethyl)piperidine (**6**) (Scheme 1)

To a solution of **5** (10.0 g, 54.8 mmoles) in pyridine (80 ml) was added acetic anhydride (60 ml). The solution was stirred at room temperature for 1 h and then poured into water and extracted with ethyl acetate. The combined organic extracts were washed with 1 N hydrochloric acid and then water. The extract was dried and evaporated under reduced pressure. The resultant yellow oil was distilled *in vacuo* to obtain the N-acetylpiperidine **6** (9.7 g, 93%) as a colourless liquid, bp 127°C (1.5 mm Hg); TLC R_f 0.24

(toluene-EtOH, 4:1); IR (neat): υ 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.92 (1H, m, C_6-H), 4.54 (1H, m, C_2-H), 4.20 (1H, m, C_6-H), 3.52 (2H, m, CH_2Cl), 3.15 (1H, m, C<u>H</u>CH_2Cl), 2.53 (1H, m, C<u>H</u>CH_2Cl), 2.17 (3H, s, CH_3), 1.75-1.60 (6H, m, C_3-H_2, C_4-H_2, C_5-H_2); MS (EI): <u>m/z</u> (relative intensity %) 191/189 (3.4/7.0, $M^{+\cdot}$), 154 (19), 126 (45), 84 (100); MS (FAB): <u>m/z</u> (relative intensity %) 192/190 (31/100, MH^+), 154 (13), 146 (11), 126 (15), 84 (27); Anal. Calcd. for $C_9H_{16}ClNO$: C, 57.14; H, 8.47; N, 7.41; Found: C, 57.38; H, 8.78; N, 7.31.

3.2.3. <u>Synthesis of 1-acetyl-6-(2-chloroethyl)-2-piperidinone (7) (Scheme 1)</u>

Ruthenium tetroxide oxidation was employed to produce compound **7** by using a literature procedure (Yoshifuji <u>et al</u>. 1985, Mohammad <u>et al</u>. 1988). A solution of **6** (10.0 g, 52.9 mmoles) in ethyl acetate (160 ml) was added dropwise to a stirred mixture of a catalytic amount of ruthenium (IV) oxide hydrate (350 mg) in aqueous 10% sodium metaperiodate (500 ml). After the addition was completed, the stirring was continued at room temperature for 4 h. The two layers were separated and the aqueous solution was extracted with ethyl acetate. The combined organic extracts were treated with isopropyl alcohol (20 ml) for 2 h to destroy excess ruthenium oxidant. The black precipitate was filtered, and the filtrate was washed with water, dried and evaporated <u>in</u>

vacuo. Distillation of the residual oil afforded **7** (8.4 g, 78%) as a colourless oil, bp 125-126°C (1.0 mm Hg); TLC R_f 0.57 (toluene-EtOH, 4:1); IR (neat): υ 1700 (d, C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (1H, m, C_6-H), 3.57 (2H, m, CH_2Cl), 2.60 (2H, m, C_3-H_2), 2.44 (3H, s, CH_3), 2.15-1.62 (6H, m, C_4-H_2, C_5-H_2, CH_2CH_2Cl); MS (EI): m/z (relative intensity %) 205/203 (0.4/1.0, $M^{+\cdot}$), 168 (22), 163/161 (7/20), 140 (13), 126 (13), 112 (15), 99 (100), 71 (11); MS (FAB): m/z (relative intensity %) 206/204 (15/48, MH^+), 168 (22), 162 (100), 98 (55); Anal. Calcd. for $C_9H_{14}ClNO_2$: C, 53.20; H, 6.90; N, 6.90; Found: C, 52.90; H, 7.06; N, 6.83.

3.2.4. Synthesis of 6-(2-chloroethyl)-2-piperidinone (**8**) (Scheme 1)

A mixture of **7** (3.5 g, 17.2 mmoles) and 70.0 g of basic aluminum oxide in 400 ml of n-hexane-ethyl acetate (1:2) was stirred at room temperature overnight. The reaction mixture was filtered and the residual aluminum oxide was washed with dichloromethane. The combined filtrates were evaporated under reduced pressure. Crystallization from ether with a few drops of dichloromethane gave **8** (1.8 g, 66%) as colourless needles, mp 125.5-126°C; TLC R_f 0.37 (toluene-EtOH, 4:1); IR (potassium bromide): υ 3220 (N-H), 1655 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.76 (1H, br, NH), 3.68 (2H, m, CH_2Cl), 3.49 (1H, m, C_6-H), 2.35 (2H, m, C_3-H_2), 2.05-1.68

(4H, m, C_5-H_2, $C\underline{H}_2CH_2Cl$), 1.45 (1H, m, C_4-H), 1.22 (1H, m, C_4-H); MS (EI): (relative intensity %) 163/161 (33/100, $M^{+\cdot}$), 128 (10), 105 (8); MS (FAB): (relative intensity %) 164/162 (34/100, MH^+), 128 (20); Anal. Calcd. for $C_7H_{12}ClNO$: C, 52.17; H, 7.45; N, 8.70; Found: C, 52.23; H, 7.58; N, 8.43.

3.2.5. Synthesis of 6-(2-chloroethyl)-1-methyl-2-piperidinone (**9**) (Scheme 1)

To a mixture of **8** (1.2 g, 7.5 mmoles) and methyl iodide (5 ml, excess) in dimethylformamide (60 ml) was added sodium hydride (0.5 g, 80% dispersion in mineral oil, freshly washed with toluene). The reaction mixture was stirred at room temperature overnight, and then filtered, poured into water and extracted with ether. The combined extracts were dried, decolorized by charcoal and evaporated *in vacuo*. The residual oil was distilled to produce synthon **9** (1.1 g, 87%) as a colourless liquid, bp 127°C (1.4 mm Hg); TLC R_f 0.70 (*n*-BuOH-AcOH-H_2O: 4:2:1); IR (neat): υ 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.59 (3H, m, C_6-H, CH_2Cl), 2.95 (3H, s, CH_3), 2.36 (2H, m, C_3-H_2), 2.18 (1H, m, $C\underline{H}CH_2Cl$), 2.04-1.74 (5H, m, C_4-H_2, C_5-H_2, $C\underline{H}CH_2Cl$); MS (EI): *m/z* (relative intensity %) 177/175 (1.8/4.4, $M^{+\cdot}$), 112 (100), 84 (16); MS (FAB): *m/z* (relative intensity %) 178/176 (33/100, MH^+), 140 (12), 112 (35); Anal. Calcd. for $C_8H_{14}ClNO$: C, 54.86; H, 8.00; N, 8.00; Found: H, 55.04; H, 7.75; N, 7.75.

3.2.6. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylthio-10H-phenothiazine (lactam of thioridazine) (**13**) (Scheme 1)

To a solution of 2-methylthio-10H-phenothiazine (**10**) (0.49 g, 2.0 mmoles) and the chloro compound **9** (0.39 g, 2.2 mmoles) in dry toluene (10 ml) was added a suspension of sodium hydride (0.36 g, 80% dispersion in mineral oil, freshly washed with toluene) in dry toluene (10 ml). The resultant mixture was heated under reflux for 22 h under nitrogen in the absence of direct intense light. After cooling, the mixture was poured into water (200 ml) containing ammonium chloride (2.0 g) and extracted with toluene. The combined extracts were successively washed with 1 N hydrochloric acid and water, and then dried. Evaporation of toluene under vacuum provided a crude product which was purified by silica gel flash chromatography using 3% methanol in toluene as eluent to give compound **13** (0.36 g, 78%) as a thick yellow oil; TLC R_f 0.42 (toluene-EtOH, 2:1); IR (dichloromethane): υ 1645 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.20-6.79 (7H, m, aromatic H_7), 3.96 (1H, m, phenothiazinyl CH), 3.86 (1H, m, phenothiazinyl CH), 3.49 (1H, m, piperidinone C_6-H), 2.71 (3H, s, NCH_3), 2.47 (3H, s, SCH_3), 2.30 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 1.94-1.72 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); ^{13}C NMR ($CDCl_3$): δ 169.7 (s, CO), 145.8 (s, C-10a), 144.6 (s, C-

9a), 137.7 (s, C-2), 127.7 (d, C-4), 126.9 (d, C-6), 126.7 (d, C-8), 126.4 (s, C-5a), 122.9 (d, C-9), 122.7 (s, C-4a), 120.9 (d, C-7), 117.7 (d, C-1), 116.3 (d, C-3), 57.4 (d, piperidinone C-6), 44.1 (t, piperidinone C-3), 33.6 (q, NCH_3), 32.4 (t, phenothiazinyl CH_2), 28.7 (t, piperidinonyl CH_2), 26.4 (t, piperidinone C-5), 17.3 (t, piperidinone C-4), 15.9 (q, SCH_3); MS (EI): m/z (relative intensity %) 384 (31, $M^{+\cdot}$), 258 (9), 244 (20), 139 (70), 112 (34), 96 (20), 83(100); MS (FAB): m/z (relative intensity %) 385 (42, MH^+), 258 (24), 245 (20), 244 (17), 112 (100); Anal. Calcd. for $C_{21}H_{24}N_2OS_2$: C, 65.63; H, 6.25; N, 7.29; Found: C, 65.85; H, 6.46; N, 7.43.

3.2.7. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylsulfinyl-10H-phenothiazine (lactam of mesoridazine) (**14**) (Scheme 1)

This compound was prepared from 2-methylsulfinyl-10H-phenothiazine (**11**) and the chloro compound **9** by the method described for **13**. Compound **14** (77%) was obtained as a thick yellow oil; TLC R_f 0.37 (toluene-EtOH, 1:2); IR (dichloromethane): υ 1645 (C=O), 1060 (S=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.30-6.92 (7H, m, aromatic H_7), 4.03 (1H, m, phenothiazinyl CH), 3.93 (1H, m, phenothiazinyl CH), 3.49 (1H, m, piperidinone C_6-H), 2.73 (3H, s, NCH_3), 2.70 (3H, s, $SOCH_3$), 2.30 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2),

1.92-1.74 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); ^{13}C NMR ($CDCl_3$): δ 169.9 (s, CO), 146.6 (s, C-10a), 144.0 (s, C-9a), 138.4 (s, C-2), 128.7 (s, C-5a), 128.2 (d, C-4), 127.9 (d, C-6), 127.6 (d, C-8), 124.9 (d, C-9), 124.8 (s, C-4a), 123.6 (d, C-7), 117.5 (d, C-1), 116.1 (d, C-3), 57.3 (d, piperidinone C-6), 44.1 (t, piperidinone C-3), 33.6 (q, NCH_3), 32.2 (t, phenothiazinyl CH_2), 28.7 (t, piperidinonyl CH_2), 26.4 (t, piperidinone C-5), 21.8 (q, $SOCH_3$), 17.3 (t, piperidinone C-4); MS (EI): m/z (relative intensity %) 400 (100, $M^{+\cdot}$), 386 (19), 385 (44), 384 (44), 260 (25), 259 (19), 258 (22), 246 (16), 245 (27), 244 (33), 140 (20), 112 (94); MS (FAB): m/z (relative intensity %) 401 (42, MH^+), 384 (14), 258 (13), 245 (12), 244 (12), 112 (100); Anal. Calcd. for $C_{21}H_{24}N_2O_2S_2$: C, 63.00; H, 6.00; N, 7.00; Found: C, 62.86; H, 6.28; N, 6.86.

3.2.8. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylsulfonyl-10H-phenothiazine (lactam of sulforidazine) (**15**) (Scheme 1)

This compound was prepared from 2-methylsulfonyl-10H-phenothiazine (**12**) and the chloro compound **9** by the procedure described for **13**. Compound **15** was obtained (72%) as a thick yellow oil; TLC R_f 0.53 (toluene-EtOH, 1:2); IR (dichloromethane): υ 1645 (C=O), 1325 (SO_2), 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35-6.92 (7H, m, aromatic H_7), 3.99

(1H, m, phenothiazinyl CH), 3.91 (1H, m, phenothiazinyl CH), 3.49 (1H, m, piperidinone C_6-H), 3.04 (3H, s, SO_2CH_3), 2.75 (3H, s, NCH_3), 2.30 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 1.91-1.71 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); ^{13}C NMR ($CDCl_3$): δ 170.0 (s, CO), 146.6 (s, C-10a), 144.0 (s, C-9a), 139.7 (s, C-2), 134.0 (s, C-5a), 129.7 (d, C-4), 128.8 (d, C-6), 128.6 (d, C-8), 124.7 (s, C-4a), 124.5 (d, C-9), 122.5 (d, C-7), 116.9 (d, C-1), 114.5 (d, C-3), 57.3 (d, piperidinone C-6), 44.1 (t, piperidinone C-3), 33.6 (q, NCH_3), 32.2 (t, phenothiazinyl CH_2), 32.1 (q, SO_2CH_3), 28.7 (t, piperidinonyl CH_2), 26.4 (t, piperidinone C-5), 17.3 (t, piperidinone C-4); MS (EI): m/z (relative intensity %) 416 (85, $M^{+\cdot}$), 291 (10), 290 (25), 277 (26), 276 (19), 211 (11), 198 (12), 197 (16), 196 (10), 140 (27), 139 (11), 113 (13), 112 (100), 98 (14); MS (FAB): m/z (relative intensity %) 417 (59, MH^+), 290 (20), 277 (17), 137 (57), 112 (100); Anal. Calcd. for $C_{21}H_{24}N_2O_3S_2$: C, 60.58; H, 5.77; N, 6.73; Found: C, 60.87; H, 5.69; N, 6.79.

3.2.9. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylthio-10H-phenothiazine-5-sulfoxide (lactam of thioridazine ring sulfoxide) (**16**) (Scheme 3)

A solution of 10% aqueous nitrous acid (30 ml) was added dropwise to a stirred solution of compound **13** (300 mg, 0.78 mmole) in acetone (30 ml). The reaction mixture was

stirred at room temperature for a further 3 h. After neutralization with aqueous ammonia, the mixture was extracted with dichloromethane. The combined extracts were dried and the solvent was removed on a rotavapor. Crystallization from hexane with a few drops of dichloromethane gave ring sulfoxide **16** (280 mg, 90%) as red crystals, mp 99-100°C; TLC R_f 0.24 (toluene-EtOH, 1:2); IR (potassium bromide): υ 1643 (C=O), 1040 (S=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.94-7.08 (7H, m, aromatic H_7), 4.26 (2H, m, phenothiazinyl CH_2), 3.51 (1H, m, piperidinone C_6-H), 2.85 (3H, s, NCH_3), 2.57 (3H, s, SCH_3), 2.38 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 2.06-1.77 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); MS (EI): m/z (relative intensity %) 400 (32, $M^{+\cdot}$), 385 (14), 384 (39), 382 (20), 259 (27), 258 (100), 245 (21), 244 (39), 226 (21), 211 (13), 185 (16), 180 (10), 138 (24), 112 (54), 84 (12); MS (FAB): m/z (relative intensity %) 401 (100, MH^+), 384 (22), 258 (41), 244 (32), 112 (83); Anal. Calcd. for $C_{21}H_{24}N_2O_2S_2$: C, 63.00; H, 6.00; N, 7.00; Found: C, 62.72; H, 5.78; N, 6.80.

3.2.10. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylsulfinyl-10H-phenothiazine-5-sulfoxide (lactam of mesoridazine ring sulfoxide) (**17**) (Scheme 3)

This compound was prepared from compound **14** by the method described for **16** except that the reaction mixture was

stirred for 8 h. The compound **17** was obtained (82%) as a red solid, mp 90-92°C; TLC R_f 0.37 (toluene-EtOH, 1:2); IR (potassium bromide): υ 1645 (C=O), 1065 (S=O), 1043 (S=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.08-7.21 (7H, m, aromatic H_7), 4.37 (2H, m, phenothiazinyl CH_2), 3.65 (1H, m, piperidinone C_6-H), 2.86 (3H, s, NCH_3), 2.81 (3H, s, $SOCH_3$), 2.41 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 2.09-1.80 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); MS (EI): m/z (relative intensity %) 416 (20, $M^{+\cdot}$), 401 (14), 400 (37), 399 (11), 398 (11), 385 (13), 384 (15), 276 (19), 275 (27), 274 (100), 261(13), 260 (19), 259 (33), 258 (29), 246 (12), 245 (21), 244 (21), 228 (11), 212 (10), 211 (11), 197 (12), 138 (21), 112 (64), 84 (14); MS (FAB): m/z (relative intensity %) 417 (81, MH^+), 401 (37), 274 (28), 245 (34), 112 (100); Anal. Calcd. for $C_{21}H_{24}N_2O_3S_2$: C, 60.58; H, 5.77; N, 6.73; Found: C, 59.40[1], H, 5.61; N, 7.11.

3.2.11. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylsulfonyl-10H-phenothiazine-5-sulfoxide (lactam of sulforidazine ring sulfoxide) (**18**) (Scheme 3)

Compound **15** (139 mg, 0.33 mmole) was dissolved in a mixture of methanol and acetone (6:1, 35 ml). Hydrogen peroxide (30%, 5 ml) was added to the mixture dropwise. The

---

[1]Acceptable analysis (within 0.5% of calculated values) for carbon could not be obtained for this compound.

reaction solution was stirred at room temperature for 36 h and then poured into water and extracted with dichloromethane. The combined extracts were dried and evaporated. The residue was crystallized from hexane with a few drops of dichloromethane to give compound **18** (116 mg, 82%) as yellowish crystals, mp 207-208°C; TLC R_f 0.45 (toluene-EtOH, 1:2); IR (potassium bromide): υ 1640 (C=O), 1325 (SO_2), 1165 (SO_2), 1040 (S=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.15-7.35 (7H, m, aromatic H_7), 4.36 (2H, m, phenothiazinyl CH_2), 3.49 (1H, m, piperidinone C_6-H), 3.11 (3H, s, SO_2CH_3), 2.87 (3H, s, NCH_3), 2.40 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 2.16-1.82 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); MS (EI): m/z (relative intensity %) 432 (10, $M^{+\cdot}$), 417 (18), 416 (66), 292 (13), 291 (25), 290 (100), 277 (44), 276 (21), 258 (12), 211 (16), 198 (26), 197 (22), 196 (13), 179 (10), 140 (22), 139 (11), 138 (11), 113 (12), 112(98), 98 (58); MS (FAB): m/z (relative intensity %) 433 (100, MH^+), 416 (9), 290 (30), 276 (12), 112 (87); Anal. Calcd. for $C_{21}H_{24}N_2O_4S_2$: C, 58.33; H, 5.56; N, 6.48; Found: C, 57.84; H, 5.46; N, 6.25.

3.2.12. Synthesis of 10-[2-(1-methylpiperidin-2-on-6-yl)ethyl]-2-methylsulfonyl-10H-phenothiazine-5-sulfone (lactam of sulforidazine ring sulfone) (**19**) (Scheme 3)

This compound was prepared from compound **15** by the

method described for **18** except that the reaction mixture was heated under reflux for 10 h. The ring sulfone **19** was obtained (80%) as an orange powder, mp 259-260°C; TLC R_f 0.35 (toluene-EtOH, 1:2); IR (potassium bromide): υ 1640 (C=O), 1320 (br, SO_2), 1165 (br, SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.39-7.48 (7H, m, aromatic H_7), 4.28 (2H, m, phenothiazinyl CH_2), 3.49 (1H, m, piperidinone C_6-H), 3.12 (3H, s, SO_2CH_3), 2.80 (3H, s, NCH_3), 2.48 (4H, m, piperidinonyl CH_2, piperidinone C_3-H_2), 1.95-1.75 (4H, m, piperidinone C_4-H_2, piperidinone C_5-H_2); MS (EI): m/z (relative intensity %) 448 (0.5, $M^{+\cdot}$), 416 (13), 401 (18), 400 (53), 385 (22), 384 (34), 277 (17), 276 (12), 275 (14), 274 (73), 261 (12), 260 (19), 259 (27), 258 (33), 246 (13), 245 (25), 244 (29), 226 (10), 211 (12), 198 (22), 197 (15), 185 (11), 179 (10), 140 (15), 138 (15), 113 (13), 112 (100), 100 (10), 98 (39); MS (FAB): m/z (relative intensity %) 449 (75, MH^+), 310 (20), 279 (16), 149 (60), 112 (100); Anal. Calcd. for $C_{21}H_{24}N_2O_5S_2$: C, 56.25; H, 5.36; N, 6.25; Found: C, 55.97; H, 5.80; N, 6.34.

3.2.13. Synthesis of 1-methyl-2-(2-chloroethyl)piperidine (**20**) (Scheme 4)

To a stirred mixture of 36% formaldehyde (14 ml, excess) and 98% formic acid (36 ml) was added slowly compound **5** (10.0 g, 54.6 mmoles). The solution was heated under reflux for 18 h. The reaction mixture was cooled to

room temperature, neutralized with aqueous ammonia and then extracted with ethyl acetate. The combined extracts were dried and evaporated on a rotavapor. The residue was purified by silica gel flash chromatography with 25% ethanol in dichloromethane as eluent. Distillation of the residual oil afforded the piperidine **20** (7.0 g, 80%) as a colourless oil, bp 63°C (1.5 mm Hg) [mp of hydrochloride salt of this compound reported in the literature (Norton et al. 1946) mp 132-133°C]; TLC R_f 0.55 (EtOH-CH_2Cl_2, 1:1, NH_4OH saturated); IR (neat): υ 1378 (CH_3) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.55 (2H, m, CH_2Cl), 2.80 (1H, m, C_2-H), 2.24 (3H, s, NCH_3), 2.10-1.31 (10H, $C\underline{H}_2CH_2Cl$, C_3-H_2, C_4-H_2, C_5-H_2, C_6-H_2); MS (EI): m/z (relative intensity %) 163/161 (1.0/3.1, $M^{+\cdot}$), 99 (11), 98 (100), 70 (23); MS (FAB): m/z (relative intensity %) 164/162 (29/100, MH^+), 148 (21), 144 (21), 136 (22), 98 (33), 91 (16), 90 (11), 89 (20).

3.2.14. Synthesis of sulforidazine (3) (Scheme 4)

To a solution of 2-methylsulfonyl-10H-phenothiazine (**12**) (6.0 g, 24.4 mmoles) and the chloro compound (**20**) (4.8 g, 29.8 mmoles) in dry toluene (350 ml) was added a suspension of sodium hydride (1.0 g, 80% dispersion in mineral oil, freshly washed with toluene) in dry toluene (10 ml). The resultant mixture was refluxed for 20 h under a nitrogen atmosphere in the absence of direct light. After

cooling, the mixture was poured into water (400 ml) containing ammonium chloride (4.0 g) and extracted with toluene. The combined extracts were washed with water and then dried. Evaporation of toluene in vacuum provided a crude product which was purified by silica gel flash chromatography using 3% methanol in toluene as eluent. Crystallization from ethyl acetate gave sulforidazine (**3**) (5.1 g, 52%) as pale yellow rhombic crystals, mp 121-122°C [lit. (Renz et al. 1964, 1966, 1968) mp 121-123°C]; TLC R_f 0.30 (toluene-EtOH, 1:5, NH_4OH saturated); IR (potassium bromide): υ 1320 (SO_2), 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.47 (1H, dd, J = 1.73, 8.09 Hz, aromatic C_3-H), 7.41 (1H, d, J = 1.78 Hz, aromatic C_1-H), 7.33 (1H, d, J = 8.00 Hz, aromatic C_4-H), 7.23 (1H, dt, J = 1.43, 7.56 Hz, aromatic C_8-H), 7.13 (1H, dd, J = 1.44, 6.92 Hz, aromatic C_6-H), 7.07 (1H, dt, J = 1.43, 7.56 Hz, aromatic C_7-H), 6.97 (1H, dd, J = 1.40, 6.98 Hz, aromatic C_9-H), 3.98 (2H, m, phenothiazinyl CH_2), 3.11 (3H, s, SO_2CH_3), 2.79 (1H, m, piperidine C_2-H), 2.16 (2H, m, piperidine C_6-H_2), 2.11 (3H, s, NCH_3), 1.75-1.31 (8H, m, piperidinyl CH_2, piperidine C_3-H_2, piperidine C_4-H_2, piperidine C_5-H_2); ^{13}C NMR ($CDCl_3$): δ 147.5 (s, C-10a), 145.0 (s, C-9a), 141.2 (s, C-2), 134.1 (s, C-5a), 128.6 (d, C-4), 128.0 (d, C-6), 127.6 (d, C-8), 125.0 (s, C-4a), 124.5 (d, C-9), 123.5 (d, C-7), 118.5 (d, C-1), 114.5 (d, C-3), 63.4 (d, piperidine C-2), 57.2 (t, piperidine C-6), 45.2 (t,

phenothiazinyl CH_2), 45.0 (q, NCH_3), 44.1 (q, SO_2CH_3), 30.3 (t, piperidinyl CH_2), 30.1 (t, piperidine C-3), 26.5 (t, piperidine C-5), 25.5 (t, piperidine C-4); MS (EI): m/z (relative intensity %) 402 (12, $M^{+\cdot}$), 290 (5), 126 (7), 99 (12), 98 (100); MS (FAB): m/z (relative intensity %) 403 (25, MH^+), 290 (5), 126 (9), 124 (6), 99 (7), 98 (100).

3.2.15. Synthesis of sulforidazine N-oxide (**21**) (Scheme 5)

To a solution of sulforidazine (**3**) (0.5 g, 1.2 mmoles) in tetrahydrofuran (10 ml) (purified as according to Furniss et al. 1978) at -70°C was added 3-chloroperoxybenzoic acid (0.24 g, 1.4 mmoles) (purified as according to Fieser and Fieser 1967). After stirring the reaction mixture for 20 min it was treated with diethylamine (0.25 ml) for 10 min to destroy the excess 3-chloroperoxybenzoic acid. The reaction mixture was slowly brought to room temperature after which the solvent was removed on a rotavapor. The crude product was purified by silica gel flash chromatography using ethanol-dichloromethane-ammonium hydroxide (15:100:1) as eluent. The appropriate fraction was collected and the solvent was removed on a rotavapor. The residue was crystallized from acetone to give compound **21** (400 mg, 77%) as white crystals, mp 159-160°C; TLC R_f 0.42 (EtOH-dichloromethane-ammonium hydroxide, 3:6:0.1); IR (potassium bromide): υ 1300 (SO_2), 1160 (SO_2) cm^{-1}; ^1H NMR (CD_3OD): δ

7.47 (1H, dd, J = 1.7, 8.1 Hz, aromatic C_3-H), 7.42 (1H, d, J = 1.7 Hz, aromatic C_1-H), 7.28 (1H, d, J = 7.9 Hz, aromatic C_4-H), 7.24 (1H, dt, J = 1.5, 7.7 Hz, aromatic C_8-H), 7.12 (1H, dd, J = 1.5, 7.7 Hz, aromatic C_6-H), 7.01 (1H, dt, J = 1.5, 7.7 Hz, aromatic C_7-H), 6.96 (1H, dd, J = 1.5, 7.7 Hz, aromatic C_9-H), 4.05 (2H, m, phenothiazinyl CH_2), 3.21 (3H, m, piperidine C_2-H, piperidine C_6-H_2), 3.15 (3H, s, SO_2CH_3), 2.89 (3H, s, $NOCH_3$), 1.83-1.29 (8H, m, piperidinyl CH_2, piperidine C_3-H_2, piperidine C_4-H_2, piperidine C_5-H_2); ^{13}C NMR (DMSO-d_6): δ 145.9 (s, C-10a), 143.5 (s, C-9a), 140.5 (s, C-2), 131.0 (s, C-5a), 129.0 (d, C-4), 128.5 (d, C-6), 127.5 (d, C-8), 123.8 (d, C-9), 123.5 (s, C-4a), 121.0 (d, C-7), 116.0 (d, C-1), 113.9 (d, C-3), 71.5 (d, piperidine C-2), 68.0 (t, piperidine C-6), 58.0 (q, $NOCH_3$), 44.5 (t, phenothiazinyl CH_2), 43.7 (q, SO_2CH_3), 27.5 (t, piperidinyl CH_2), 27.5 (t, piperidine C-3), 23.2 (t, piperidine C-5), 21.3 (t, piperidine C-4); MS (EI^+): m/z (relative intensity %) 418 (5, $M^{+\cdot}$), 416 (12), 402 (21), 388 (11), 290 (31), 278 (19), 277 (60), 276 (44), 211 (14), 198 (26), 197 (35), 196 (21), 140 (17), 126 (43), 99 (13), 98 (100); MS (EI^-): m/z (relative intensity %) 418 (40 $M^{-\cdot}$), 417 (17), 404 (33), 403 (84), 402 (17), 401 (44), 389 (17), 388 (12), 387 (36), 293 (13), 292 (45), 278 (21), 277 (24), 276 (100), 262 (12); MS (FAB): m/z (relative intensity %) 419 (32, MH^+), 290 (10), 176 (20), 142 (19), 120 (11), 98 (100); Anal. Calcd. for

$C_{21}H_{26}N_2O_3S_2$: C, 60.13; H, 6.13; N, 6.68; Found: C, 60.28; H, 6.22; N, 6.69.

3.2.16. Synthesis of sulforidazine N,S-dioxide (**22**) (Scheme 5)

A solution of 30% hydrogen peroxide (excess) was added dropwise to a solution of **3** (7.0 g, 17.4 mmoles) in ethanol (140 ml) at 0°C. The reaction mixture was stirred at room temperature for 20 h and then treated with small portions of manganese dioxide power until the absence of hydrogen peroxide was indicated with potassium iodide starch paper. The excess manganese dioxide was filtered and the solvent from the filtrate was removed on a rotavapor. The residue was crystallized from methanol to give the N,S-dioxide **22** (1.5 g, 20%) as white crystals, mp 178-181°C; TLC R_f 0.36 (methanol saturated with aqueous ammonia); IR (potassium bromide): υ 1320 (SO_2), 1165 (SO_2), 1040 (SO) cm^{-1}; ^1H NMR (CD_3OD): δ 8.26 (1H, d, J = 8.1 Hz, aromatic C_4-H), 8.14 (1H, d, J = 1.4 Hz, aromatic C_1-H), 8.05 (1H, dd, J = 0.8, 7.6 Hz, aromatic C_6-H), 7.95 (1H, bd, J = 8.6 Hz, aromatic C_9-H), 7.85 (1H, dt, J = 0.8, 7.5 Hz, aromatic C_8-H), 7.81 (1H, dd, J = 1.3, 8.2 Hz, aromatic C_3-H), 7.43 (1H, dt, J = 0.8, 7.5 Hz, aromatic C_7-H), 4.59 (2H, m, phenothiazinyl CH_2), 3.57 (1H, m, piperidine C_2-H), 3.26 (3H, s, SO_2CH_3), 3.16 (2H, m, piperidine C_6-H_2), 2.96 (3H, s, $NOCH_3$), 1.91-1.69 (8H, m,

piperidinyl CH_2, piperidine C_3-H_2, piperidine C_4-H_2, piperidine C_5-H_2); ^{13}C NMR (DMSO-d_6): δ 144.9 (s, C-10a), 138.0 (s, C-9a), 137.1 (s, C-2), 133.9 (d, C-4), 132.8 (d, C-6), 131.8 (d, C-8), 127.5 (s, C-5a), 123.8 (s, C-4a), 123.0 (d, C-9), 119.1 (d, C-7), 117.3 (d, C-1), 115.2 (d, C-3), 74.6 (d, piperidine C-2), 71.1 (t, piperidine C-6), 59.2 (q, $NOCH_3$), 45.9 (t, phenothiazinyl CH_2), 43.7 (q, SO_2CH_3), 28.5 (t, piperidinyl CH_2), 25.6 (t, piperidine C-3), 23.2 (t, piperidine C-5), 22.8 (t, piperidine C-4); MS (EI^+): m/z (relative intensity %) 402 (16), 401 (41), 387 (14), 291 (14), 290 (52), 277 (16), 198 (11), 197 (14), 196 (11), 179 (13), 178 (31), 166 (16), 165 (17), 124 (12), 114 (23), 110 (25), 100 (19), 99 (16), 98 (100), 97 (12), 84 (42); MS (EI^-): m/z (relative intensity %) 434 (15, $M^{-\cdot}$), 420 (23), 419 (39), 418 (91), 406 (12), 405 (34), 404 (77), 403 (100), 390 (16), 389 (60), 293 (13), 292 (27), 278 (18); MS (FAB): m/z (relative intensity %) 435 (75, MH^+), 419 (12), 290 (5), 276 (13), 220 (13), 142 (16), 136 (34), 107 (21), 105 (11); Anal. Calcd. for $C_{21}H_{26}N_2O_4S_2(H_2O)$: C, 55.73; H, 6.24; N, 6.19; Found: C, 55.88; H, 6.10; N, 6.27.

3.2.17. Synthesis of N-acetyl-N-desmethylsulforidazine (**28**) (Scheme 6)

Sulforidazine (**3**) was treated with 2,2,2-trichloroethyl chloroformate to give N-desmethylsulforidazine (**25**)

(Montzka et al. 1974). Subsequently a mixture of the product **25** (0.89 g, 2.20 mmoles), acetic anhydride (130 ml excess) and ethyl acetate (100 ml) was stirred at 65°C for 1 h. The reaction mixture was poured into water, treated with aqueous ammonia and then extracted with ethyl acetate. The combined organic extracts were dried and the solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography eluting with 1% of ethanol in toluene. The collected fractions were dried and crystallized from 1% hexane in ethyl acetate. Compound **28** was obtained (0.81 mg, 82%) as red crystals, mp 128-129°C; R_f 0.71 (toluene-EtOH, 1:2, NH_4OH saturated); IR (potassium bromide): υ 1625 (CO), 1313 (SO_2), 1155 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.44-6.87 (7H, m, aromatic H_7), 3.95 (2H, m, phenothiazinyl CH_2), 3.15 (1H, m, piperidine C_2-H), 3.05 (3H, s, SO_2CH_3), 2.19 (2H, m, piperidine C_6-H_2), 2.04 (3H, $COCH_3$), 1.78-1.33 (8H, piperidinyl CH_2, piperidine C_3-H_2, piperidine C_4-H_2, piperidine C_3-H_2); ^{13}C NMR ($CDCl_3$): δ 169.8 (s, CO), 145.6 (s, C-10a), 144.2 (s, C-9a), 139.5 (s, C-2), 133.6 (s, C-5a), 129.0 (d, C-4), 128.9 (d, C-6), 128.7 (d, C-8), 125.3 (d, C-9), 123.4 (s, C-4a), 121.5 (d, C-7), 118.0 (d, C-1), 113.9 (d, C-3), 51.5 (d, piperidine C-2), 45.1 (q, SO_2CH_3), 44.8 (t, piperidine C-6), 42.5 (t, phenothiazinyl CH_2), 29.8 (t, piperidinyl CH_2), 28.0 (t, piperidine C-3), 27.0 (t, piperidine C-5), 22.5 (q, $CO\underline{C}H_3$), 19.8 (t,

piperidine C-4); MS (EI): m/z (relative intensity %) 430 (32, $M^{+\cdot}$), 277 (6), 290 (6), 197 (10), 155 (12), 154 (100), 126 (8), 84 (44).

3.2.18. Synthesis of N-acetyl-N-desmethylthioridazine ring sulfoxide (**29**) (Scheme 6)

A published two step procedure for the N-demethylation of tertiary amines that utilizes 2,2,2-trichloroethyl chloroformate as reagent was adapted to convert thioridazine (**1**) to N-desmethylthioridazine (**23**) (Montzka et al. 1974). The product **23** obtained was subsequently reacted with acetic anhydride (excess) at 65°C for 1 h to produce N-acetyl-N-desmethylthioridazine (**26**). The latter was then treated with 10% nitrous acid (excess) at room temperature for 1 h to give N-acetyl-N-desmethylthioridazine ring sulfoxide (**29**). The product was obtained in only sufficient quantity to allow characterization by HPLC-mass spectrometry.

3.2.19. Synthesis of N-acetyl-N-desmethylmesoridazine ring sulfoxide (**30**) (Scheme 6)

N-Desmethylmesoridazine (**24**) was prepared by modification of a literature procedure (Renz et al. 1964). Thus reaction of 2-methylsulfinyl-10H-phenothiazine (**11**) with 2-(2-chloroethyl)piperidine (**5**) using sodium hydride as a base catalyst provided compound **24**. Subsequent N-

acetylation and then ring sulfoxidation of compound **24** (by the same reaction conditions as described for the preparation of product **29** from compound **23** in Section 3.2.18.) gave N-acetyl-N-desmethylmesoridazine ring sulfoxide (**30**). Compound **30** was obtained in only sufficient quantity to allow characterization by plasmaspray HPLC-mass spectrometry.

3.2.20. Synthesis of N-acetyl-N-desmethylsulforidazine ring sulfoxide (**31**) (Scheme 6)

N-Acetyl-N-desmethylsulforidazine (**28**) (200 mg, 0.46 mmole) was dissolved in a mixture of methanol and acetone solution (6:1, 35 ml), and 30% hydrogen peroxide (20 ml, excess) was added to the mixture. The reaction solution was stirred at room temperature for 1 h, and then poured into water and extracted with dichloromethane. The combined organic extracts were dried and evaporated. The residue was crystallized from ethyl acetate to yield compound **31** (150 mg, 73%) as white crystals, mp 184-186°C; R_f 0.49 (toluene-EtOH, 1:2, NH_4OH saturated); IR (potassium bromide): υ 1625 (CO), 1320 (SO_2), 1160 (SO_2), 1040 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.24-7.39 (7H, m, aromatic H_7), 4.55 (2H, m, phenothiazinyl CH_2), 3.87 (1H, m, piperidine C_2-H), 3.25 (3H, s, SO_2CH_3), 2.42 (2H, m, piperidine C_6-H_2), 2.22 (3H, s, $COCH_3$), 1.81-1.64 (8H, m, piperidinyl CH_2, piperidine C_3-H_2,

piperidine C_4-H_2, piperidine C_5-H_2); ^{13}C NMR ($CDCl_3$): δ 172.1 (s, CO), 145.8 (s, C-10a), 139.1 (s, C-9a), 138.4 (s, C-2), 136.1 (d, C-4), 134.4 (d, C-6), 133.1 (d, C-8), 129.1 (s, C-5a), 127.5 (s, C-4a), 123.2 (d, C-9), 120.1 (d, C-7), 117.0 (d, C-1), 115.2 (d, C-3), 49.0 (d, piperidine C-2), 46.6 (t, piperidine C-6), 44.0 (q, SO_2CH_3), 43.5 (t, phenothiazinyl CH_2), 30.0 (t, piperidinyl CH_2), 28.0 (t, piperidine C-3), 27.0 (t, piperidine C-5), 22.9 (q, $CO\underline{C}H_3$), 19.8 (t, piperidine C-4); MS (EI^+): m/z (relative intensity %) 430 (28), 290 (8), 277 (9), 154 (100), 126 (9), 84 (40); MS (EI^-): m/z (relative intensity %) 446 (3, $M^{-\cdot}$), 431 (7), 416 (33), 415 (100); MS (FAB): m/z (relative intensity %) 447 (2, MH^+), 431 (25), 430 (31), 290 (9), 277 (3), 126 (18), 84 (100).

3.2.21. Synthesis of N-desmethylsulforidazine ring sulfoxide (**34**) (Scheme 6)

The N-acetyl compound **31** (20 mg, 0.04 mmole) was treated with 2 N HCl under reflux for 12 h. The reaction mixture was washed with dichloromethane, treated with aqueous ammonia and then extracted with dichloromethane. The organic extracts were dried and evaporated under reduced pressure. The resultant complex mixture was separated by preparative HPLC to yield N-desmethylsulforidazine ring sulfoxide (**34**) (3 mg, 19%) as a white solid; R_f 0.14

(toluene-EtOH, 1:2, NH_4OH saturated); MS (EI^+): m/z (relative intensity %) 404 (3, $M^{+\cdot}$), 388 (22), 387 (25), 386 (18), 290 (49), 277 (18), 276 (8), 258 (7), 198 (19), 197 (12), 112 (16), 110 (42), 98 (23), 84 (100); MS (EI^-) m/z (relative intensity %) 404 (100, $M^{-\cdot}$), 390 (10), 389 (42).

3.3. Metabolic studies

3.3.1. Protocols

3.3.1.1. Drug administrations in rat

Female Lewis rats (n = 15, body weight 200-250 g) were dosed by gastric intubation with 20 mg/kg of sulforidazine (as an aqueous solution of hydrochloride salt prepared *in situ*).

Female Lewis rats (n = 5, body weight 200-260 g) were dosed by gastric intubation with 28.1 mg/kg of mesoridazine besylate (the equivalent of 20 mg/kg mesoridazine) in aqueous solution.

Female Lewis rats (n = 5, body weight 220-250 g) were dosed by gastric intubation with 21.9 mg/kg of thioridazine hydrochloride (the equivalent of 20 mg/kg thioridazine) in aqueous solution.

The animals were placed in individual metabolic cages immediately after drug administration. Food was withheld for 24 h after administration but access to water was allowed at all times. Urine was collected for 24 h after

drug administration. The urine samples were frozen (-20°C) until analyzed.

3.3.1.2. Drug administrations in dog

The drugs were separately administered in extemporaneously prepared capsules to female beagle dogs (n = 3, body weight 10-15 kg) that were fasted overnight. Sulforidazine (37.5 mg) was given over 30 h in three divided doses. Mesoridazine (70.3 mg of besylate salt, the equivalent of 50 mg mesoridazine) and thioridazine (109.7 mg of hydrochloride salt, the equivalent of 100 mg thioridazine) were separately administered over 30 h in four divided doses.

The animals were placed in individual metabolic cages immediately after drug administration and were allowed free access to food and water at all times. Urine was collected for 48 h in the case of sulforidazine and for 72 h in the cases of thioridazine and mesoridazine following the first dose of the drug. The urine samples were frozen (-20°C) until analyzed.

3.3.1.3. Drug administrations in human

In the case of sulforidazine an investigational new drug certificate (Health Protection Branch, Ottawa, Ontario: IND control number 3HP902252) was obtained to enable its

oral administration to healthy subjects. The protocols (Appendix A) were approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation. Three healthy adult males (26-49 years old, body weight 68-83 kg) after an overnight fast ingested a single oral dose of 25 mg of sulforidazine under medical supervision. Similarly on separate occasions three healthy male volunteers (26-31 years old, body weight 62-83 kg) after an overnight fast were given 35.2 mg of mesoridazine besylate (the equivalent of 25 mg mesoridazine) and 54.9 mg of thioridazine hydrochloride (the equivalent of 50 mg thioridazine) under medical supervision. Two of the three volunteers who received mesoridazine and thioridazine also received sulforidazine. There was at least a three week washout period between doses.

For each of the drugs urine was collected for 48 h after drug administration. The urine samples were frozen (-20°C) until analyzed.

3.3.2. Qualitative studies

3.3.2.1. Characterization of underivatized metabolites of sulforidazine

The urine sample from each rat (n = 5) and a one-tenth portion of the urine sample from each dog (n = 3) and human (n = 3) was individually lyophilized, extracted with

methanol and the extract evaporated to dryness. The residues were taken up in 0.3 M phosphate buffer (pH 7.2, 100 ml) and then extracted with dichloromethane (3 x 60 ml). The extracts were washed in turn with phosphate buffer (3 x 30 ml) and distilled water (3 x 30 ml), dried and evaporated to dryness. The residues were dissolved in acetonitrile and injected into the HPLC system.

In the case of extracts of rat and dog urine individual metabolites were separated using mobile phase A and the portions of mobile phase corresponding to the appropriate peaks of the HPLC chromatograms were collected. The collected fractions were further purified by re-injecting them into the HPLC system and recollecting the appropriate portions of mobile phase. These collected portions of mobile phase were evaporated to dryness and analyzed by EI mass spectrometry as direct insertion probe samples.

Additionally with respect to the lactam of sulforidazine and the diastereomers of N-desmethyl-sulforidazine ring sulfoxide in the extracts of rat urine, the appropriate purified fractions collected from the HPLC system were also examined by GLC-mass spectrometry. The collected HPLC fractions were evaporated to dryness and the dried residues extracted with methanol prior to GLC-mass spectrometry.

With respect to both dog and human urine samples,

aliquots of the acetonitrile solution were directly analyzed by HPLC-mass spectrometry with a plasmaspray interface (Section 3.1.2.).

In all cases blank urine samples, both with and without added authentic reference standards, were treated as above before analysis by the appropriate procedure.

3.3.2.2. Characterization of underivatized metabolites of mesoridazine

One-tenth portions of urine samples from each subject of each species were individually lyophilized and extracted by the same method described in Section 3.3.2.1. An aliquot of the acetonitrile solution of each extract from each of the three species was directly analyzed by HPLC-mass spectrometry with a plasmaspray interface. In addition, the aliquots of acetonitrile extracts of rat urine were also analyzed using the conventional HPLC system with mobile phase D. The individually collected fractions of the appropriate mobile phase were dried and analyzed by direct probe plasmaspray mass spectrometry (Section 3.1.2.).

In all cases blank urine samples, both with and without added authentic standards, were treated and analyzed by the same procedure.

3.3.2.3. Characterization of underivatized metabolites of

thioridazine

One-tenth portions of the urine samples from each individual rat, dog and human administered thioridazine were individually lyophilized and extracted by the same procedure described in Section 3.3.2.1. An aliquot of the acetonitrile solution of each extract from each subject of each species was directly analyzed by plasmaspray HPLC-mass spectrometry (Section 3.1.2.). Blank urine samples, both with and without added authentic standards, were treated and analyzed in the same manner.

3.3.2.4. Characterization of derivatized metabolites of sulforidazine

In the case of the diastereomers of N-desmethyl-sulforidazine ring sulfoxide found in rat urine extracts, the dried residues obtained after HPLC separation of the organic extracts from rat urine (Section 3.3.2.1.) were derivatized with either a silylating reagent (BSA or MTBSTFA) or the acylating reagent, acetic anhydride. Thus silylation was performed with either 100 μl of BSA and 200 μl of ethyl acetate at 65°C for 1 h or 50 μl of MTBSTFA and 100 μl of acetonitrile at 65°C for 10 min. Acetylation was carried out with 50 μl of acetic anhydride and 100 μl of ethyl acetate at 65°C for 1 h. After each of the above derivatization reactions the samples were dried under

nitrogen and redissolved in methanol prior to HPLC using mobile phase C and GLC-mass spectrometric analysis.

The presence of phenols in rat urine sample was investigated by means of HPLC-mass spectrometric analysis of the silylated (MTBSTFA) extracts. The procedure which was followed allowed distinction of whether each phenol was unconjugated or conjugated. A brief description of the procedure with or without enzymic treatment prior to extraction is given below. The bulked urine sample from another five rats administered sulforidazine was lyophilized. The residue was dissolved in water (50 ml) and a volume of saturated Na_2CO_3 solution was added to adjust the pH to the 9.0-9.5 range. The resultant solution was washed with ether (4 x 25 ml) and then adjusted to pH 7.0-7.2 with 1.0 N HCl. The solution was extracted with dichloromethane (4 x 25 ml) and the resultant organic extract was dried under nitrogen. The residue was redissolved in acetonitrile (0.8 ml) and half of this organic solution was treated with a solution of MTBSTFA (30 μl) in acetonitrile (0.4 ml). The reaction mixture was incubated at 65°C for 10 min.

To the aqueous layer obtained after dichloromethane extraction was added a solution of 2 M sodium acetate buffer (pH 5.2, 10 ml) and the resultant solution was incubated with 0.8 ml of β-glucuronidase at 37°C for 12 h. Phosphate buffer (0.3 M, pH 7.2) was added to the above solution and

extracted with dichloromethane (4 x 15 ml). This organic extract was subsequently treated with the silylating reagent as above. The resultant mixtures obtained after derivatization with MTBSTFA (both where prior treatment had or had not included enzymic incubation) were then dried under nitrogen. Each residue was dissolved in acetonitrile and a portion (about one-tenth) was injected into the HPLC-mass spectrometry system.

In the case of the extracts obtained from each individual dog and human, the procedure that was adapted for the identification of both unconjugated and conjugated phenols was simpler than that described above. In this procedure the residues obtained after lyophilization were immediately take up in 0.3 M phosphate buffer (pH 7.2) and then extracted with dichloromethane. Also the aqueous solution obtained after organic extraction was enzymically hydrolyzed and then extracted with dichloromethane. The portions of the above extracts from dog and human both with and without prior enzymic hydrolysis were silylated with MTBSTFA, and then investigated by means of plasmaspray HPLC-mass spectrometric analysis.

3.3.2.5. Characterization of derivatized metabolites of mesoridazine

The presence of N-desmethylmesoridazine ring sulfoxide

in the urine of all three species administered mesoridazine was confirmed *via* conversion of this metabolite to its *N*-acetyl derivative. The derivatizing procedure was carried out as follows. Portions of the acetonitrile solutions obtained from each species (Section 3.3.2.2.) were separated by HPLC using mobile phase D, and the individual portions of mobile phase corresponding to the appropriate peak of the HPLC chromatograms were collected. The combined fractions were further purified by re-injecting them into the HPLC system and recollecting the appropriate portions of mobile phase. The dry residue obtained after evaporation of the mobile phase was treated with 50 μl of ethyl acetate and 25 μl of acetic anhydride at 65°C for 1 h. The resultant sample was dried under nitrogen and dissolved in acetonitrile prior to HPLC-mass spectrometric analysis.

The presence of phenolic metabolites in each urine sample with and without β-glucuronidase treatment was investigated by means of HPLC-mass spectrometric analysis of extracts derivatized with the silylating reagent MTBSTFA. The procedure was the same as that for the analysis of phenolic metabolites in the extracts of urine samples from dog and human dosed with sulforidazine as previously described (Section 3.2.2.4.).

3.3.2.6. Characterization of derivatized metabolites of

thioridazine

In the case of N-desmethylthioridazine ring sulfoxide found in both rat and dog urine, its presence was confirmed via conversion to its N-acetyl derivative. Portions of the acetonitrile extracts from each rat and dog urine (Section 3.3.2.3.) were separated by the HPLC system using mobile phase E. The collected fractions corresponding to the appropriate peak of the respective metabolite from the HPLC chromatograms were further purified, acetylated and analyzed in the same manner as described for the characterization of N-desmethylmesoridazine ring sulfoxide in Section 3.3.2.5.

The presence of phenolic metabolites in each urine sample from each species was investigated by means of HPLC-mass spectrometric analysis of extracts from each individual species (Section 3.3.2.3.) treated with the silylating reagent MTBSTFA. The procedure which allowed distinction of whether each phenol was unconjugated or conjugated was the same as that described for the study of phenolic metabolites in the extracts of urine obtained from dog and human dosed with sulforidazine (Section 3.3.2.4.).

3.3.3. Quantitative studies

The amounts of the unchanged drug and some of its metabolites present in urine of each of the three species administered each of the three substrates were determined by

a HPLC technique with UV detection utilizing different mobile phase systems. All manipulations in each assay were performed in subdued light in order to avoid decomposition, light-induced racemization and photolysis (Watkins et al. 1986, Eap et al. 1991).

3.3.3.1. Quantification of sulforidazine and some of its metabolites

The amounts of sulforidazine, sulforidazine ring sulfoxide, the lactam of sulforidazine and the lactam of sulforidazine ring sulfoxide present in each individual urine sample obtained from each species dosed with sulforidazine were determined by modification of the procedure used for the characterization of underivatized metabolites of sulforidazine (Section 3.3.2.1.). Note that in the case of rat the urine samples examined in this quantitative study were obtained from different rats (n = 5) than those examined in either of the qualitative studies (Sections 3.3.2.1. and 3.3.2.4.).

A stock solution in acetonitrile (1 mg/ml in each case) was prepared for each of the authentic standards of analytes and the internal standard (the lactam of mesoridazine). Dilutions were made with blank urine in order to give solutions with concentrations of 0.5, 1, 2, 4 and 6 μg/ml of sulforidazine, 0.5, 1, 5, 10 and 12 μg/ml of each

diastereomer of sulforidazine ring sulfoxide, 50, 100, 150, 200 and 250 ng/ml of the lactam of sulforidazine and 0.5, 1, 3, 6 and 10 μg/ml of the lactam of sulforidazine ring sulfoxide. Each standard sample was prepared in triplicate.

The internal standard (1 μg) was added to each urine sample (1 ml for rat and dog, and 10 ml for human urine[2], spiked or unknown) and each sample was lyophilized. The residue was extracted with methanol (3 x 3 ml) by shaking for 15 min using an Ika-Vibrax shaker (Terochem laboratories Ltd.). The combined methanolic extracts were evaporated to dryness at <45°C in a SpeedVac concentrator. The residue was dissolved in 0.3 M phosphate buffer (pH 7.2, 2 ml) and extracted with dichloromethane (3 x 3 ml). The combined organic extracts were washed with phosphate buffer (2 x 2 ml) and distilled water (2 x 2 ml), dried and evaporated to dryness. The residue was reconstituted with acetonitrile (100 μl) and 10 μl aliquots injected into the HPLC system that employed mobile phase B. Standard curves were constructed by plotting peak height ratios versus concentration of the analyte.

For the determination of recoveries blank urine samples spiked at three different concentrations over the standard curve range of each of the analytes were run through the

---

[2]10 ml of each human urine sample was utilized because the concentrations of analytes present in human urine were generally far lower than in rat and dog urine.

above described procedure except that the internal standard was added after extraction. The peak height ratios obtained for the extracted samples were compared with those obtained for solutions of the analytes in acetonitrile. Also quality control samples were prepared in duplicate to contain each analyte at the high, intermediate and low concentrations of each standard curve. They were analyzed as unknowns (analyst blind) at the same time as samples for standard curve and test samples.

3.3.3.2. Quantification of mesoridazine and some of its metabolites

Mesoridazine and some of its metabolites, namely mesoridazine ring sulfoxide, sulforidazine, sulforidazine ring sulfoxide, the lactam of mesoridazine, the lactam of mesoridazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide were quantified in each urine sample. Two different HPLC-UV assays were employed that utilized two different mobile phase systems. Mobile phase D was used for the quantification of the three lactam metabolites while mobile phase E was used for the quantification of the other analytes. Moreover the use of mobile phase D allowed the quantification of each of the diastereomers of the lactam of mesoridazine ring sulfoxide while with the use of mobile phase E sulforidazine ring sulfoxide diastereomers were

quantified.

A stock solution in acetonitrile (1.0 mg/ml in each case) was prepared for each of the authentic standards of mesoridazine, its metabolites and the internal standard (prochlorperazine ring sulfoxide). The stock solutions were stored in the dark at 4°C. The concentrations of analytes in human urine were generally far lower than in dog or rat urine, therefore, two series of standard curve samples were prepared for each analyte. Appropriate dilutions of each stock solution of analyte were made with blank human urine to give two series of solutions each at five different concentrations over the standard curve ranges, which are listed below in the order of the concentrations for human urine sample analysis first followed by the concentrations for dog and rat urine sample analysis: mesoridazine, 10, 50, 100, 300 and 600 ng/ml and 1, 5, 10, 15 and 20 μg/ml; mesoridazine ring sulfoxide, 20, 50, 100, 200 and 300 ng/ml and 1, 5, 10, 15 and 20 μg/ml; sulforidazine, 5, 10, 20, 50 and 100 ng/ml and 0.5, 1, 2, 4 and 5 μg/ml; each diastereomer of sulforidazine ring sulfoxide, 10, 50, 100, 300 and 500 ng/ml and 1, 5, 10, 15 and 20 μg/ml; the lactam of sulforidazine ring sulfoxide, 5, 10, 40, 70 and 100 ng/ml and 0.1, 0.5, 1.0, 2.0 and 2.5 μg/ml; and each diastereomer of the lactam of mesoridazine ring sulfoxide, 5, 10, 40, 70 and 100 ng/ml and 0.1, 0.5, 1.0, 1.5 and 2.0 μg/ml. In

addition, the standard solutions of the lactam of mesoridazine were prepared with concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 μg/ml for rat urine analysis only. Each standard sample was prepared in triplicate.

In the case of human 10 ml urine samples (spiked or unknown) were taken and 1 μg/ml of the internal standard (1 ml of 10 μg/ml solution) added. For dog and rat 1 ml urine samples (spiked or unknown) were taken and 5 μg/ml of the internal standard (50 μl of 100 μg/ml solution) added. The procedure used for the analysis of each urine sample from the three species was the same as described in Section 3.3.3.1. Standard curves were constructed by plotting peak height ratios versus concentration of the analyte. All standards and unknowns were analyzed concurrently.

3.3.3.3. Quantification of thioridazine and some of its metabolites.

Thioridazine and six of its metabolites were quantified by HPLC-UV methods. Two different mobile phase systems were employed in the assays. Mobile phase E was used for the quantification of mesoridazine, mesoridazine ring sulfoxide and each of the diastereomers of sulforidazine ring sulfoxide, while mobile phase F was used for the quantification of thioridazine, the lactam of sulforidazine ring sulfoxide, and each of the diastereomers of both

thioridazine ring sulfoxide and the lactam of mesoridazine ring sulfoxide.

A stock solution in acetonitrile (1.0 mg/ml in each case) was prepared for each of the authentic standards of analytes and internal standards (prochlorperazine ring sulfoxide for mobile phase E and the lactam of mesoridazine for mobile phase F). Appropriate dilutions of the stock solution of each analyte were made with blank human urine to give a series of solutions at five different concentrations for each of the standard curve ranges. These concentrations were 50, 100, 500, 800 and 1000 ng/ml for thioridazine and mesoridazine, 20, 50, 100, 400 and 800 ng/ml for mesoridazine, each diastereomer of thioridazine ring sulfoxide and each diastereomer of sulforidazine ring sulfoxide, 50, 100, 200, 300 and 500 ng/ml for each diastereomer of the lactam of mesoridazine ring sulfoxide and 20, 50, 100, 300 and 500 ng/ml for the lactam of sulforidazine ring sulfoxide. Each standard sample was prepared in triplicate.

The concentrations of analytes in dog and rat urine were generally far greater than in human urine, therefore, the urine samples (1 ml) obtained from the former two species were diluted to 10 ml with blank human urine before analysis. Each 10 ml urine sample (spiked or unknown) was taken and 100 ng/ml of the internal standard (10 μl of 100

μg/ml solution) added. Each sample was lyophilized and the residue was extracted and analyzed by the same procedure as described in Section 3.3.3.1. Standard curves were constructed by plotting peak height ratios versus concentration of the analyte.

For the determination of extraction efficiencies blank urine samples spiked at three different concentrations over the standard curve range of each analyte were run through the above described procedure except that the internal standard was added after extraction. The peak height ratios obtained for the extracted samples were compared with those for solutions of the analytes in acetonitrile. Also quality control samples were prepared in duplicate to contain each analyte at the low, intermediate and high concentrations of each standard curve range. They were analyzed as unknowns (analyst blind) at the same time as samples for standard curve and test samples.

4. Results and discussion

4.1. Chemical synthesis

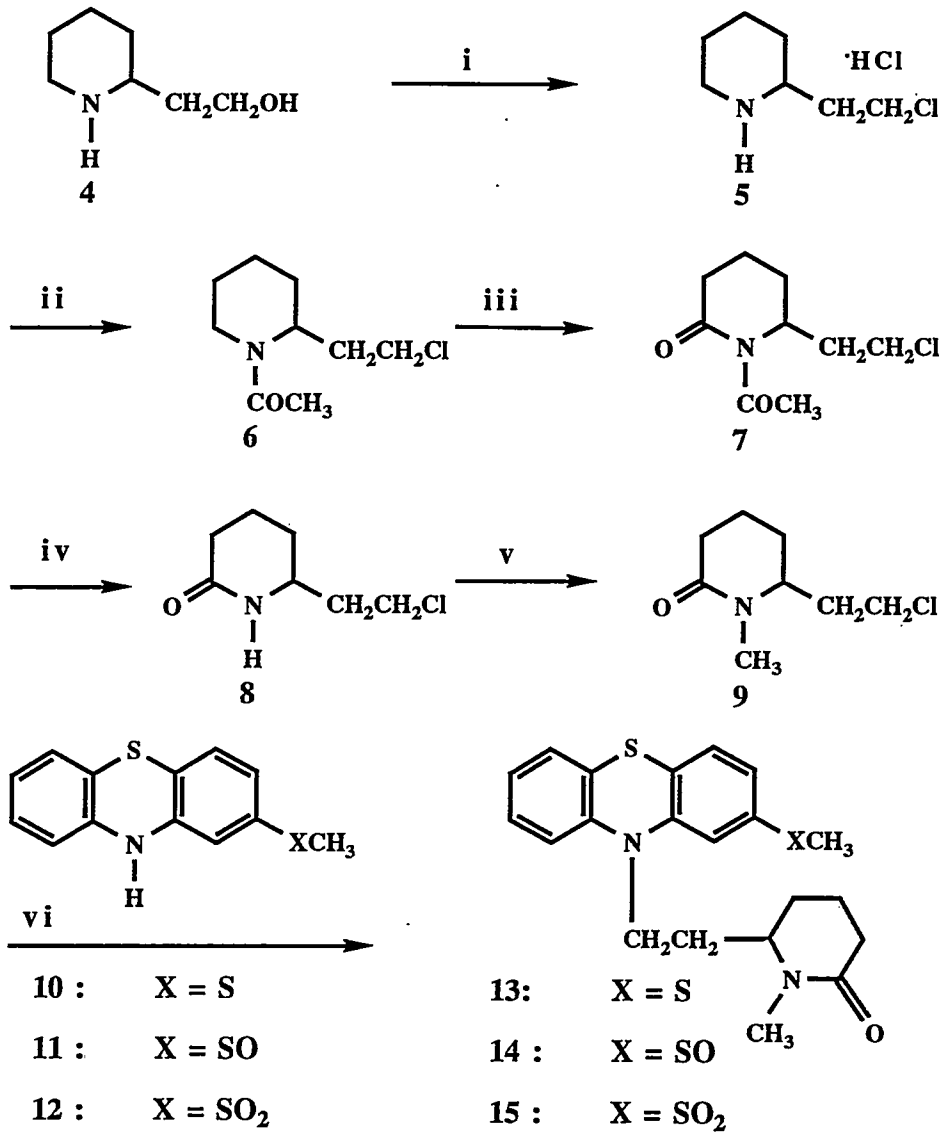
4.1.1. Synthesis of lactam metabolites

The approach taken to the synthesis of the lactam metabolites of piperidine-type phenothiazine antipsychotic agents **13-15** was to react the synthon 6-(2-chloroethyl)-1-methyl-2-piperidinone (**9**) with the appropriate 2-substituted phenothiazine **10-12**. There are previous reports to synthon **9** (Wakabayashi et al.1978) or the suitable intermediate 6-(2-hydroxyethyl)-1-methyl-2-piperidinone (Kametani et al. 1968, Wakabayashi et al. 1977). These reported synthetic approaches involved in the key step either cyclization of an amino acid derivative (Wakabayashi et al. 1977) or reduction of a 2-pyridinone (Kametani et al. 1968). However, both these approaches required multiple steps (nine and eight, respectively) to the synthon from commercially available starting materials.

Ruthenium tetroxide oxidation of acylated cyclic amines to give lactams and/or imides (Sheehan and Tulis 1974) has recently found use in the conversion of 2-substituted piperidines to the analogous piperidinones (Yoshifuji et al. 1985, Mohammad et al. 1988). Thus a synthetic approach to synthon **9** was developed where in the key step the lactam functional group was introduced in the piperidine ring by oxidation using ruthenium tetroxide. The relatively cheap

commercially available compound 2-(2-hydroxyethyl)piperidine (**4**) was used as starting material. The initial attempt to obtain the synthon **9** from **4** involved in turn acylation of both NH and OH groups, oxidation to provide the piperidinone derivative, N- and O-deacylation, N-methylation and chlorination. Unfortunately in the case of the penultimate reaction the N,O-dimethylated compound was obtained as the major product (Mohammad, unpublished results). Hence for the present work reported in this thesis the synthetic route to **9** was modified as shown in Scheme 1. In the first step the alcohol **4** was converted to the chloro compound **5**. Subsequent reaction with acetic anhydride in pyridine produced the N-acetyl derivative **6**, which although stable at low temperature (0-4°C) slowly decomposed at room temperature to the acyl ester of **4**. Treatment of compound **6** with a catalytic amount of ruthenium dioxide and an excess of sodium metaperiodate in a two-phase system of ethyl acetate-water (Yoshifuji et al. 1985) provided the piperidinone **7**. Subsequent deacylation and methylation gave the synthon **9**. Finally N-10 alkylation of the appropriate phenothiazines **10-12** utilizing sodium hydride as base afforded racemic mixtures of the desired lactam compounds **13-15**. The overall yields in the synthesis of compounds **13-15** from **4** in six steps were in the range 28-30%.

The structures of all the desired products were



Reagents:

i : $SOCl_2$, $CHCl_3$

ii : Ac_2O, C_5H_5N

iii : $RuO_2.xH_2O$, $NaIO_4$, EtOAc-H_2O

iv : Al_2O_3, n-Hexane-EtOAc

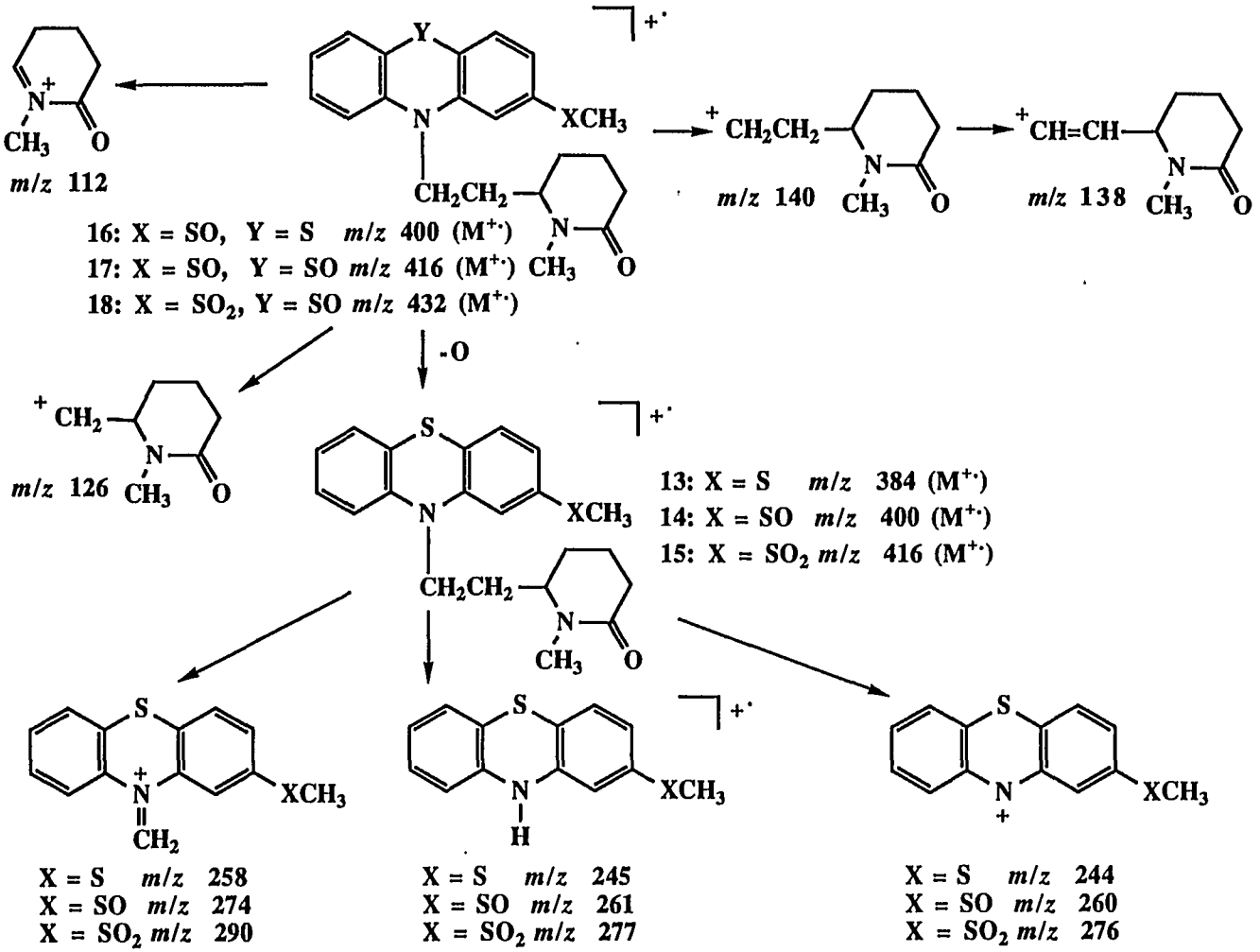
v : CH_3I, NaH, DMF

vi : NaH, Toluene

Scheme 1. Synthesis of the lactam metabolites of thioridazine, mesoridazine and sulforidazine.

substantiated by spectroscopic analysis. The IR spectra of all lactam products showed an amide absorption band (1645 cm^{-1}). The positive ion EI MS of each lactam metabolite exhibited a molecular ion ($M^{+\cdot}$), a high intensity ion at m/z 112 corresponding to the piperidinone moiety and other diagnostic fragmentation ions as shown in Scheme 2. For example, in each case ions were present which could be interpreted as due to the phenothiazine ring without the entire N-10 side chain, the tricyclic ring with only a hydrogen at N-10 and the tricyclic ring with a N-10 methylene group: at m/z 244, 245 and 258 for the lactam of thioridazine, at m/z 260, 261 and 274 for the lactam of mesoridazine, and at m/z 276, 277 and 290 for the lactam of sulforidazine, respectively. In addition all lactam compounds gave rise to ions at m/z 140, 138 and 126 with differing intensities which could be interpreted as arising because of the fragmentations of the N-10 side chain. In the FAB MS of all lactam analogues, each showed a pseudo-molecular ion (MH^+) and the diagnostic piperidinone ion at m/z 112. Moreover, high resolution ^1H NMR and ^{13}C NMR spectra, and elemental analysis of all synthesized lactams were consistent with the desired structures.

The ring sulfoxide analogues **16-18** of the lactams of thioridazine, mesoridazine and sulforidazine were obtained by use of a suitable oxidizing agent as shown in Scheme 3.



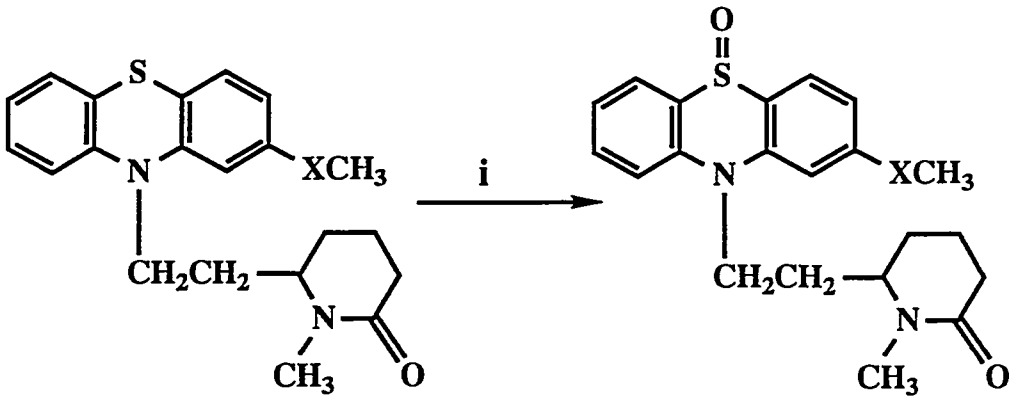
Scheme 2. Typical fragmentations in the positive ion electron impact mass spectra of the lactam analogues of thioridazine, mesoridazine and sulforidazine.

Nitrous acid is often favoured for such oxidations (Juenge et al. 1983) but, whereas it was convenient for the synthesis of compounds **16** and **17**, it did not react with sulforidazine. Therefore, a stronger oxidizing agent, hydrogen peroxide, was used for such conversion of sulforidazine where at room temperature the sulfoxide **18** was obtained while the sulfone **19** was obtained after reflux.

The purity and structural identity of these products were also confirmed by spectroscopic and elemental analyses. The IR spectra of all ring sulfoxide lactams exhibited both amide and sulfoxide absorption bands at 1640-1645 cm^{-1} and 1040-1043 cm^{-1}, respectively, while for the lactam sulfone **19** in addition to the above absorption bands it also showed sulfone absorption bands at 1165 and 1320 cm^{-1}. The positive ion EI and FAB MS of ring S-oxidized lactams were similar to those of lactam analogues as shown in Scheme 2, except that the positive ion EI MS of the former lactams generally gave a weak molecular ion. In the ^{1}H NMR and ^{13}C NMR spectra of ring S-oxygenated lactams, the signals for all aromatic protons and aromatic carbon atoms, respectively, were shifted downfield compared to those of the lactam analogues.

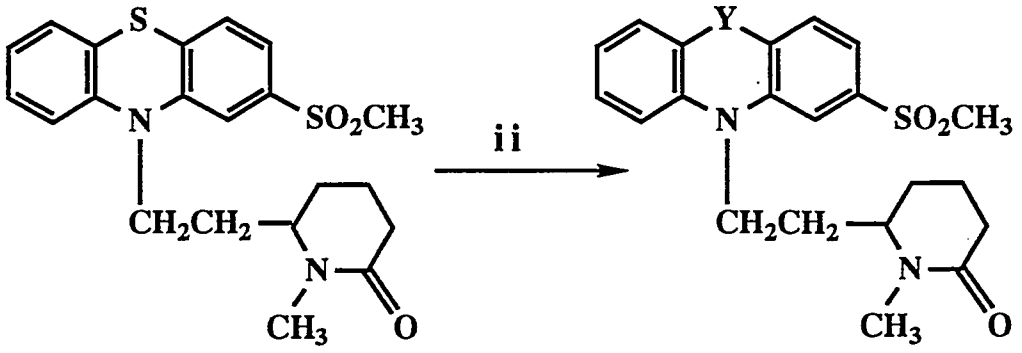
4.1.2. Synthesis of sulforidazine

Since sulforidazine is not marketed in Canada, it was necessary to synthesize sulforidazine for human



13: X = S
14: X = SO

16: X = S
17: X = SO



15

18: Y = SO
19: Y = SO_2

Reagents i: HNO_2, CH_3COCH_3-H_2O

ii: H_2O_2, CH_3COCH_3-CH_3OH

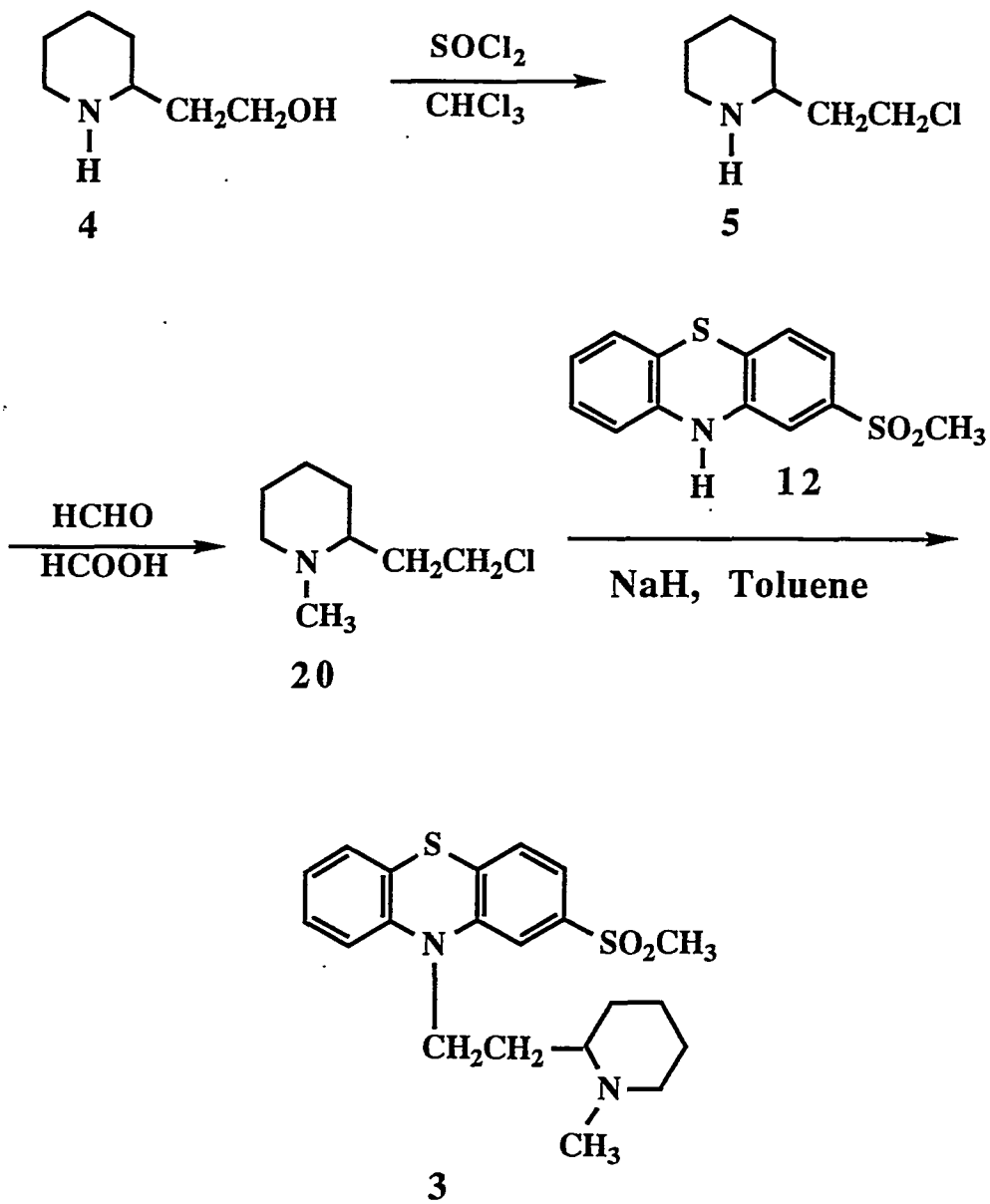
Scheme 3. Synthesis of the lactam ring sulfoxides of thioridazine, mesoridazine and sulforidazine, and the lactam ring sulfone of sulforidazine.

administration and to obtain an investigational new drug certificate in order to enable studies in human experimentation.

The previous reports to the synthesis of sulforidazine are patents (Renz et al. 1964, 1966, 1968), and none of these reports involves the widely used method to the synthesis of phenothiazine antipsychotic agents where in one step the appropriate amine and haloalkyl compound are reacted in the presence of a base catalyst. Such an approach was recently adopted to the synthesis of sulforidazine in these laboratories (Mohammad et al. 1988), and was modified in the present work as shown in Scheme 4. Commercially available 2-(2-hydroxyethyl)piperidine (**4**) was converted to the chloro compound **5**, which was then methylated to compound **20**. Finally N-10 alkylation of 2-methylsulfonylphenothiazine (**12**) in the presence of sodium hydride as a base catalyst afforded sulforidazine. The product was identical to an authentic reference sample (gift from Sandoz Inc., USA and Sandoz Pharmaceuticals, Canada) in terms of UV, IR, MS, ^{1}H NMR and ^{13}C NMR spectra, chromatographic behaviours, melting point and elemental analyses data.

4.1.3. Synthesis of N-oxide and N,S-dioxide of sulforidazine

The synthesis of the N-oxide of any phenothiazine



Scheme 4. Synthesis of sulforidazine.

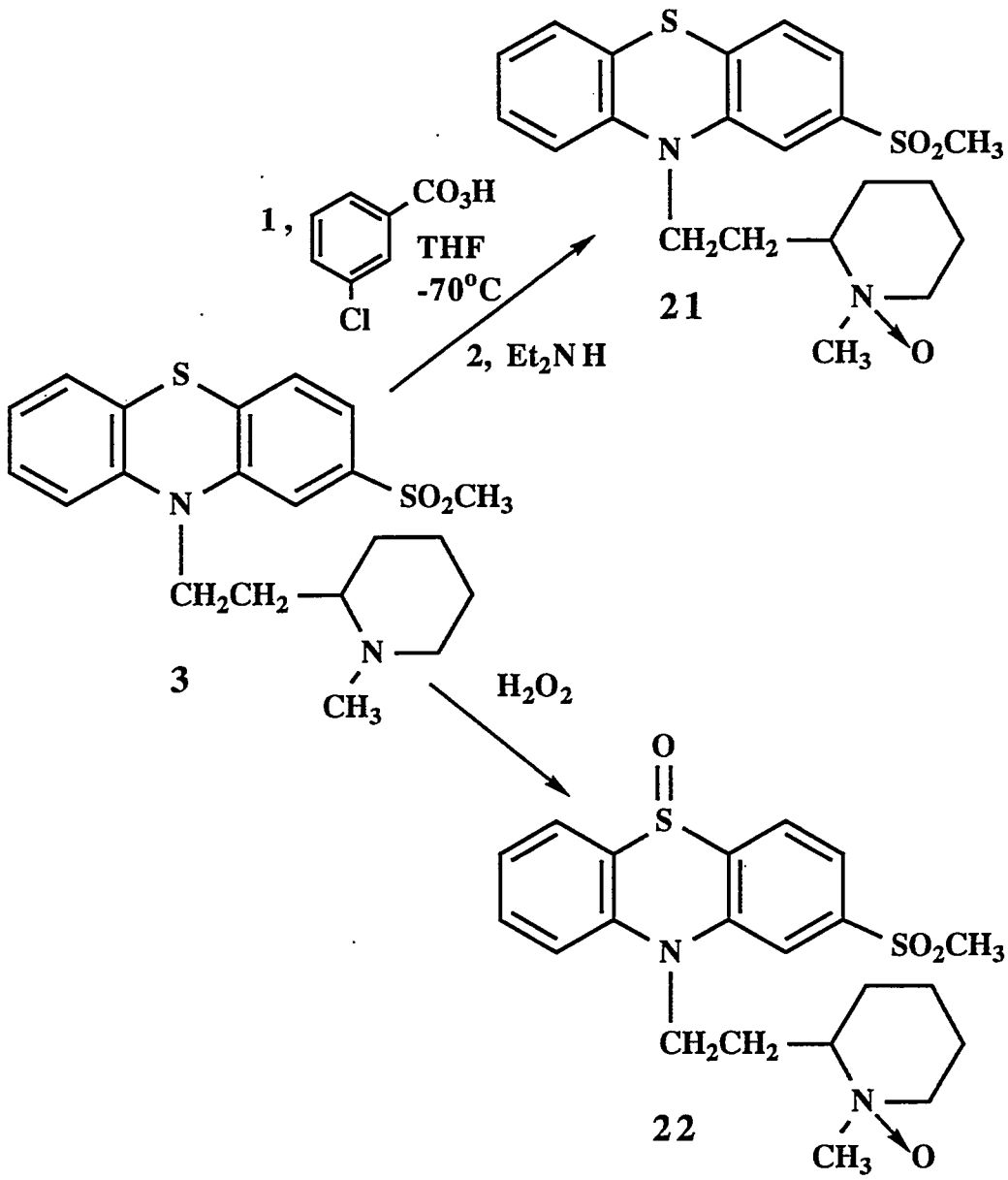
antipsychotic agent is generally believed to be difficult due to the presence of not only one or more oxidizable nitrogen atoms but also one or more oxidizable sulfur atoms. For example, in the cases of thioridazine and mesoridazine, in addition to the ring sulfur atom, these drugs have an incompletely oxidized sulfur atom at the 2-position of the phenothiazine ring. In the present work, it was intended to synthesize the N-oxide metabolite of sulforidazine, a compound which contains a fully oxidized sulfur atom at the 2-substituent.

Various oxidizing reagents have been employed in the synthesis of the N-oxide metabolites of phenothiazine antipsychotic agents (Sumita and Nishino 1960, Beckett et al. 1974, Craig et al. 1978, Watkins et al. 1986). However, it must be emphasized that all of them are also known to convert the sulfide group to the sulfoxide group (Madeschaire 1986). In the present study the original published general procedure (Craig and Purushothaman 1970) to the synthesis of tertiary amine N-oxides by use of 3-chloroperoxybenzoic acid as an oxidizing agent and chlorinated hydrocarbon as solvent was modified to obtain sulforidazine N-oxide (**21**) in a relatively high yield (77%). The oxidation was successfully performed in tetrahydrofuran at low temperature (-70°C) with the use of 1.2 equivalents of 3-chloroperoxybenzoic acid as oxidant (Scheme 5). The

solvent was selected since in earlier work when the N-oxidation of the phenothiazine antipsychotic agent chlorpromazine was investigated lower yields of desired product were obtained with all other solvents examined, including the chlorinated hydrocarbon solvents commonly employed in such types of oxidation reactions (Jaworski et al. 1992).

In order to synthesize directly the N,S-dioxide derivative of sulforidazine, a different oxidizing reagent, hydrogen peroxide, was utilized. This is a stronger oxidizing reagent than 3-chloroperoxybenzoic acid and has been employed in the synthesis of both N- and S-oxygenated derivatives of phenothiazines (Anderson et al. 1987). In the present work, although it was found to produce a complex mixture of oxidized products, sulforidazine N,S-dioxide (**22**) could be isolated in satisfactory yield (20%) (Scheme 5).

The structural assignments of N-oxide products were substantiated by spectroscopic data. The IR spectrum of **22** showed an intense absorption band (1040 cm^{-1}) that could be assigned to the sulfoxide group. Although, the positive ion EI MS exhibited either a molecular ion of very low intensity (in the case of **21**) or no molecular ion (in the case of **22**), the positive ion FAB MS and negative ion EI MS showed the presence of quasi-molecular ions (MH^+) and molecular ions ($M^{\cdot-}$), respectively. In the high resolution ^1H NMR and ^{13}C NMR

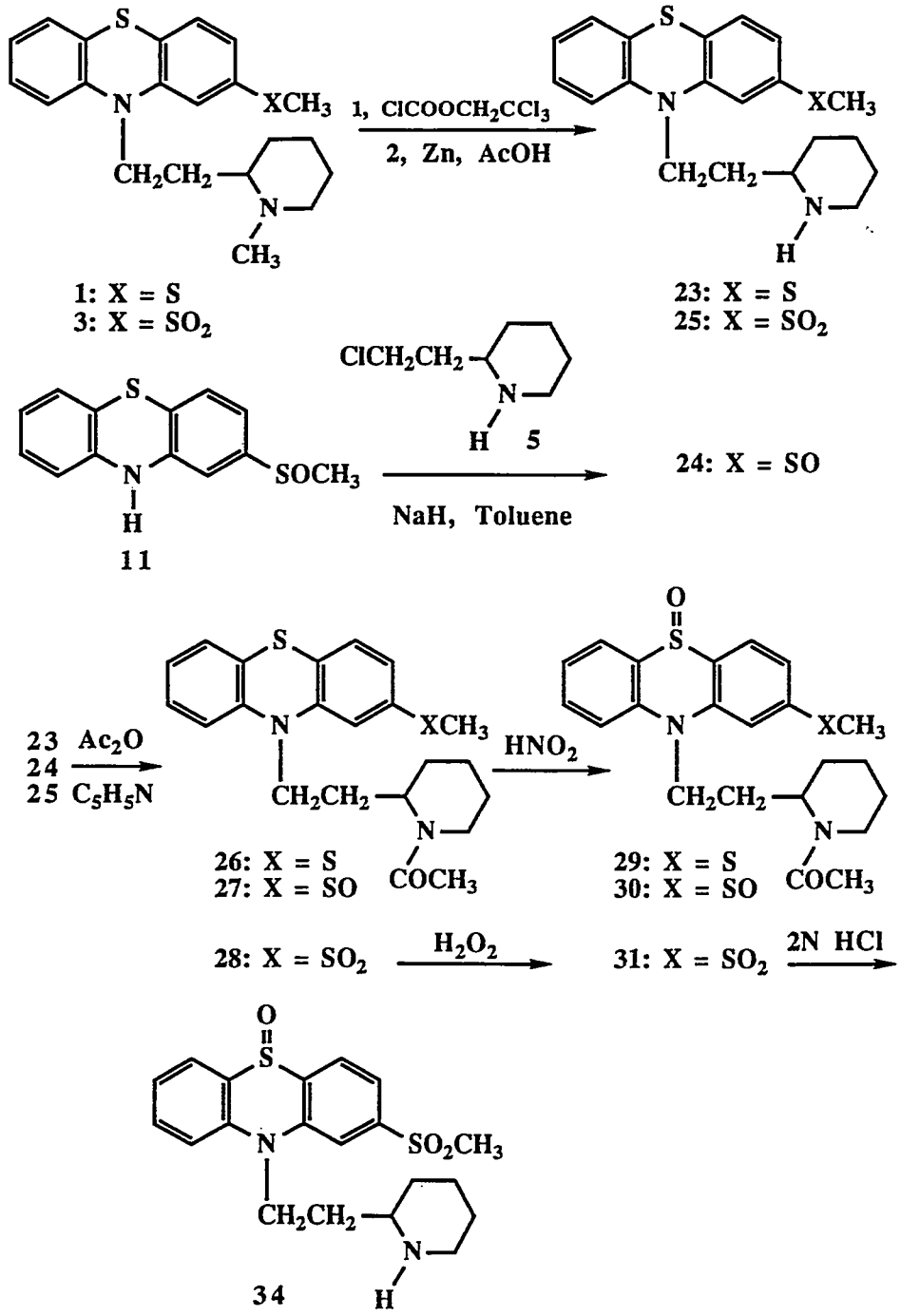


Scheme 5. Synthesis of sulforidazine N-oxide and sulforidazine N,S-dioxide.

spectra, in comparison to the spectra of sulforidazine, the protons vicinal to the site of N-oxidation and carbons attached to the oxidized nitrogen, respectively, were greatly shifted downfield for both **21** and **22** whereas such was the case with all aromatic protons and aromatic carbons in the ring S-oxidized product **22**. The assignments made for the aromatic protons of sulforidazine N,S-dioxide were confirmed by spin decoupling experiments. For example, irradiation of the signal assigned to aromatic C_6-H at δ 8.05 resulted in the collapse of the signals assigned to aromatic C_7-H at δ 7.43 (changed from dt to dd) and aromatic C_8-H at δ 7.85 (changed from dt to t), while irradiation of the signal assigned to aromatic C_7-H at δ 7.43 resulted in change of the signals assigned to aromatic C_6-H (δ 8.05, from dd to d), aromatic C_8-H (δ 7.85, from dt to dd) and aromatic C_9-H (δ 7.95, from bd to d).

4.1.4. Synthesis of N-demethylated metabolites

Attempts were made to synthesize the N-desmethyl ring sulfoxide metabolites of thioridazine, mesoridazine and sulforidazine. A published two-step procedure for the N-demethylation of tertiary amines that utilizes 2,2,2,-trichloroethyl chloroformate as reagent was adapted to convert the parent drugs thioridazine (**1**) and sulforidazine (**3**) to the corresponding N-desmethyl derivatives **23** and **25**



Scheme 6. Synthesis of the N-acetyl-N-desmethyl ring sulfoxide analogues of thioridazine, mesoridazine and sulforidazine, and N-desmethylsulforidazine ring sulfoxide.

(Montzka et al. 1974). However, this approach could not be used to directly prepare N-desmethylmesoridazine (**24**) from mesoridazine (**2**), since under these reaction conditions the sulfoxide group is readily converted to the corresponding sulfide (Oae 1977). Therefore, N-desmethylmesoridazine was synthesized by a modified literature approach (Renz et al. 1963) that involved the reaction of 2-methylsulfinyl-phenothiazine (**11**) with the chloro compound **5** using sodium hydride as a catalyst. Subsequent treatment of the N-desmethyl compounds **23-25** with acetic anhydride gave the respective N-acetyl derivatives **26-28**, which were in turn oxidized with either nitrous acid or hydrogen peroxide to produce the appropriate N-acetyl-N-desmethyl ring sulfoxides **29-31** (Scheme 6). However, the N-acetyl-N-desmethyl ring sulfoxide analogues of thioridazine and mesoridazine were obtained in insufficient quantities for further reaction. Thus only the N-acetyl-N-desmethyl ring sulfoxide of sulforidazine was deacetylated with 2 N HCl under reflux to produce the N-desmethyl ring sulfoxide metabolite of sulforidazine (**34**) (Scheme 6).

The synthetic samples of N-desmethylsulforidazine ring sulfoxide **34** and the N-acetyl-N-desmethyl ring sulfoxide analogues **29-31** of all three drugs were all used as reference standards in the metabolic studies described in this thesis.

4.2. Metabolic studies

4.2.1. Extraction of metabolites from urine samples and subsequent derivatization

The extraction procedures used in the present studies are summarized in Scheme 7. In initial investigations attempts were made to develop procedures where analytes were selectively extracted. Thus blank urine spiked with authentic standards was adjusted to various pH values. These solutions were extracted with organic solvents of varying polarity (e.g. hexane, ether, ethyl acetate and dichloromethane) and the extracts examined by TLC. However, none of these extractions was selective for the analytes examined. Therefore, a simple extraction procedure was employed for metabolic studies of all three species. In this procedure, urine samples were initially lyophilized, the methanolic soluble extracts taken up in 0.3 M phosphate buffer (pH 7.2) and then extracted with dichloromethane. The compounds in these extracts were subsequently separated by HPLC and/or analyzed by HPLC-MS.

In the initial metabolic study of sulforidazine in rat a more elaborate extraction scheme was used to isolate the phenolic metabolites (Scheme 7). However, later work showed that this more elaborate extraction scheme was unnecessary and consequently in all subsequent studies only the simplified method that used dichloromethane for extraction

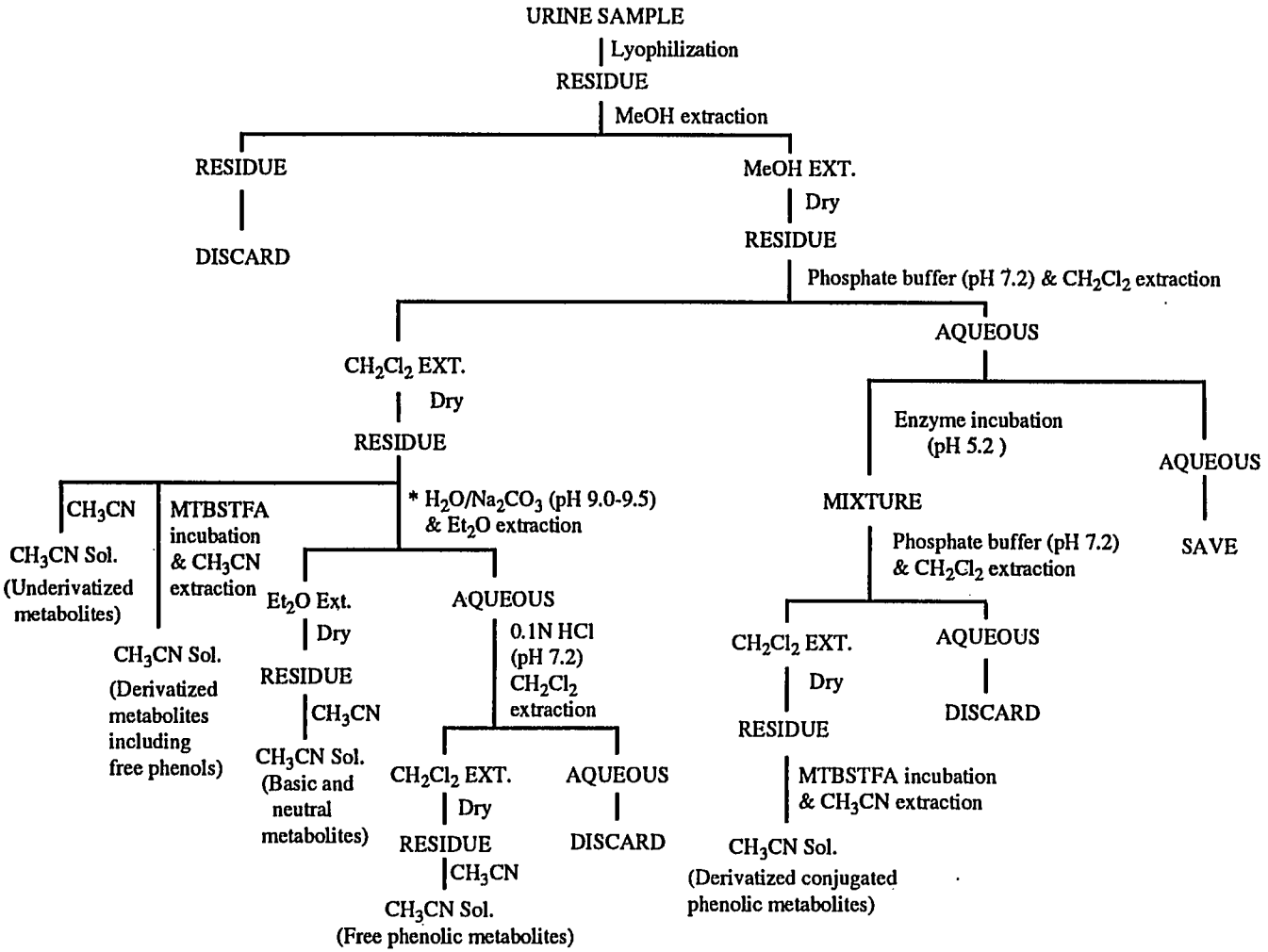
at pH 7.2 was employed (Scheme 7). Extraction of metabolites with the pH of the medium close to pH 7.0 avoided decomposition of metabolites such as N-oxides (Hubbard et al. 1985, Midha et al. 1991).

In order to investigate the presence of phenolic metabolites, extracts were derivatized with a silylating reagent (MTBSTFA). Also since no attempt was made in the present work to identify polar phase II metabolites, the presence of conjugated forms of phenolic metabolites was investigated by extraction of the corresponding free form of phenols after ß-glucuronidase (also containing sulfatase activity) hydrolysis. Subsequently the silylated free forms of phenolic metabolites in the MTBSTFA derivatized extracts with or without prior enzymic treatment were analyzed by plasmaspray HPLC-mass spectrometry.

In the present work, the extraction recoveries of quantified analytes were determined to be relatively high (80-92%). In all cases, the entire extraction process was performed in subdued light in order to avoid decomposition, light induced racemization and photolysis (Watkins et al. 1986, Eap et al. 1991).

4.2.2. Urinary metabolites of sulforidazine

4.2.2.1. Characterization of metabolites of sulforidazine in rat



Scheme 7. Extraction procedures used in studies of the metabolism of thioridazine, mesoridazine and sulforidazine in rat, dog and human.

*In the case of the study of the phenolic metabolites of sulforidazine in rat urine only.

When the available authentic standards were subjected to conventional HPLC analysis using mobile phase A, each chromatographed as a cluster of peaks as shown in Figure 6(B). The HPLC chromatogram (Figure 6(C)) of a urine extract from a rat dosed with sulforidazine contained six clusters of peaks which were not present in the chromatogram (Figure 6(A)) of an extract from control rat urine. In the case of the first four peaks their order of elution and the retention times were identical with those obtained for authentic sulforidazine (**3**), the lactam of sulforidazine (**15**), sulforidazine ring sulfoxide (**35**) and the lactam of sulforidazine ring sulfoxide (**18**) added to control rat urine extract (Figure 6(B)). Also for the first, third and fourth of these peaks the concentrations were relatively high such that the identities of the compounds eluting as these peaks were readily confirmed since the appropriate fraction collected from the HPLC gave, after sample preparation, virtually the same MS (positive and negative ion EI modes) as a sample of reference standard prepared similarly (Table 3).

In the case of the lactam of sulforidazine (**15**), its concentration in the urine extract was low such that its identity could not be confirmed by use of direct probe MS analysis of the appropriate fraction collected from HPLC. However, analysis of this same fraction by the more

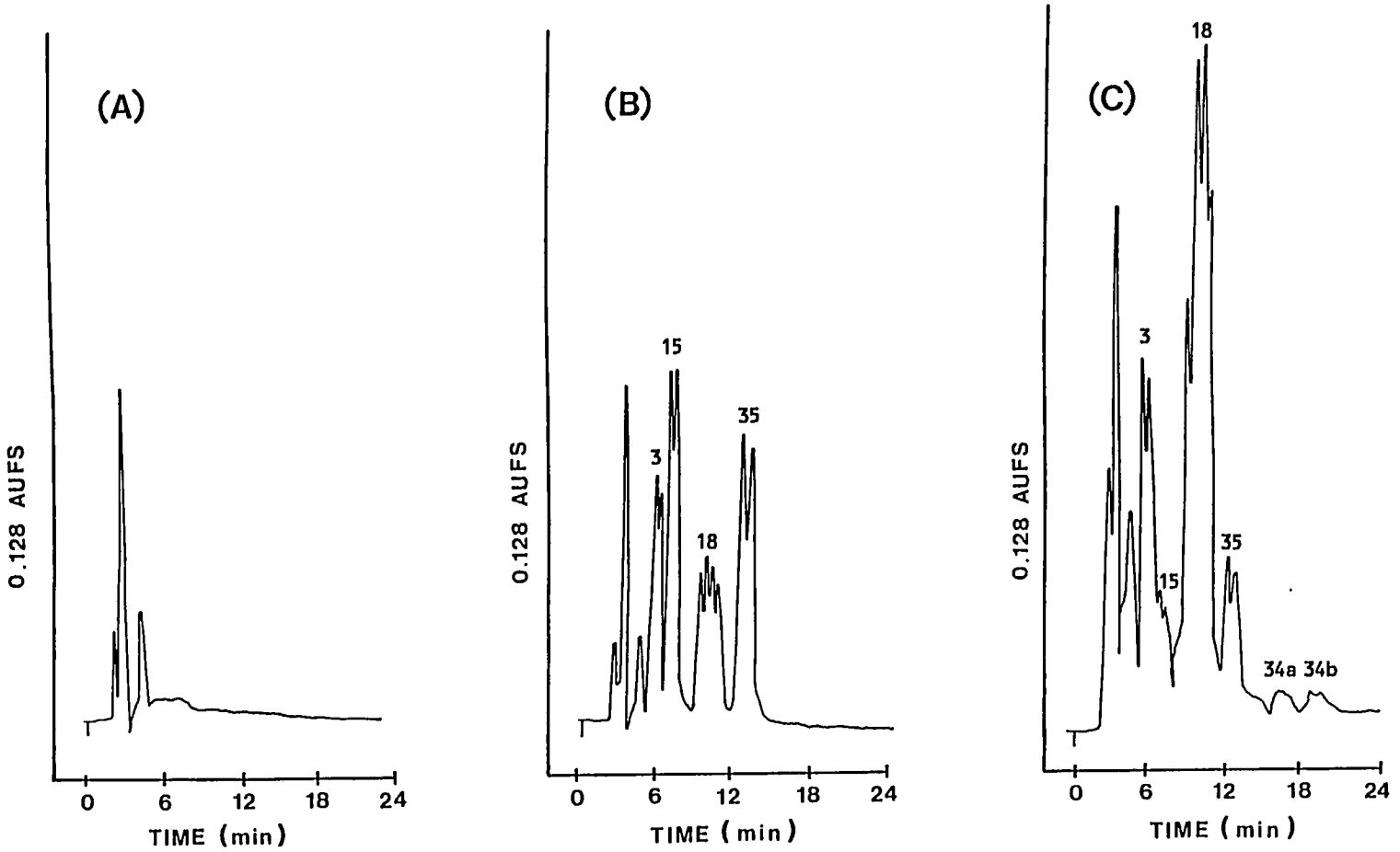


Figure 6. HPLC chromatograms of extracts of (A) blank urine, (B) blank urine with added authentic standards and (C) urine of a rat dosed with sulforidazine.

Mobile phase A, 70% 2,2,4-trimethylpentane, 15% dichloromethane, 15% methanol, 0.1% diethylamine. **3**, Sulforidazine; **15**, lactam of sulforidazine; **18**, lactam of sulforidazine ring sulfoxide; **34a** and **34b**, diastereomers of N-desmethylsulforidazine ring sulfoxide; **35**, sulforidazine ring sulfoxide.

sensitive GLC-MS technique enabled its confirmation. The retention time and positive ion EI MS were identical to those of a similarly prepared extract of blank urine spiked with authentic sulforidazine lactam (Figure 7, Table 3). It should be noted that sulforidazine was also identified in this collected fraction during GLC-MS analysis since sulforidazine and its lactam eluted close to one another (Figure 6) and the parent drug was present in a far greater amount.

Regarding the last two peaks (**34a** and **34b**) of the HPLC chromatogram of the urinary extract of a dosed rat (Figure 6(C), retention times of 16.3 min and 19.1 min, respectively) the concentrations of the analytes eluted were high enough to be individually collected and examined by MS analysis in both positive and negative ion EI modes. Since the utilization of mobile phase A for the HPLC separation of the two compounds and their subsequent MS analysis indicated that an impurity co-eluted with the second metabolite their further purification was carried out using a different mobile phase system (mobile phase C). The two appropriate peaks were collected individually from the HPLC using mobile phase C, and then analyzed by direct probe MS in both positive and negative ion modes. The MS of both metabolites were virtually identical to one another as well as with that of the unresolved synthetic standard of the diastereomers of

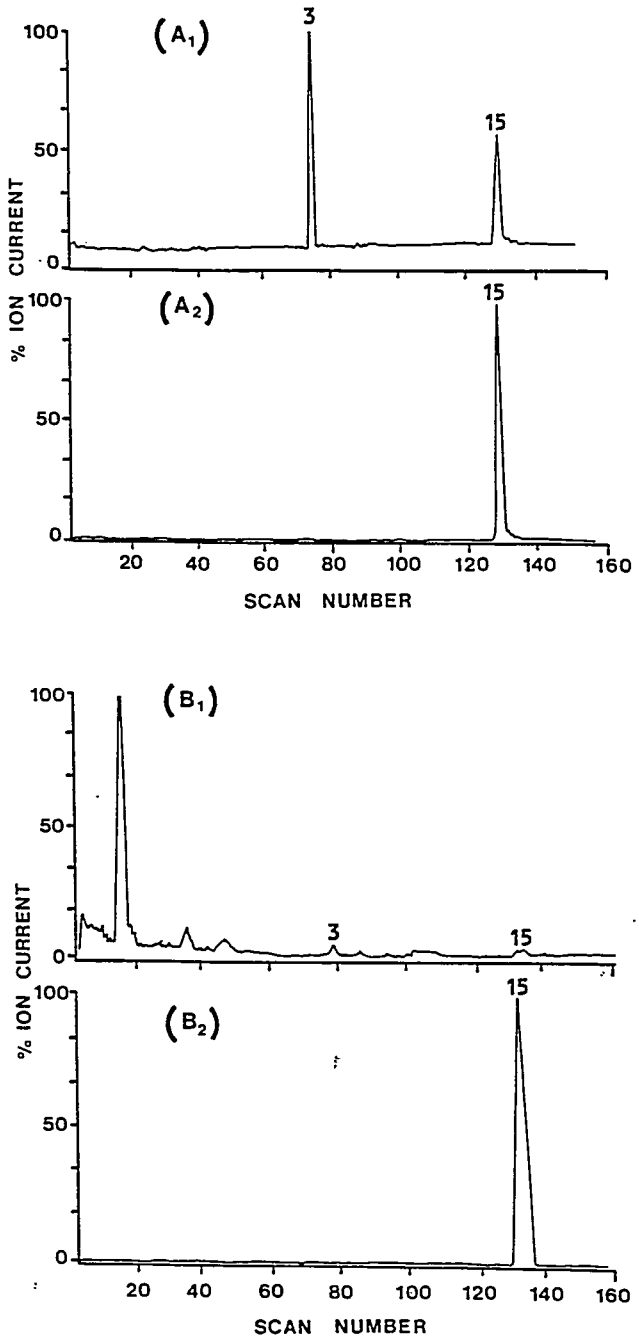


Figure 7. Ion chromatograms of GLC-mass spectrometric analysis of collected HPLC fractions obtained from extracts of (A) blank urine with added sulforidazine (**3**) and the lactam of sulforidazine (**15**) and (B) urine from a rat dosed with sulforidazine.

A_1 and B_1, total ion chromatograms; A_2 and B_2, reconstructed ion chromatograms for m/z 416 (molecular ion of **15**).

Table 3. HPLC retention times and mass spectral data of sulforidazine and its metabolites and authentic standards isolated from various extracts of rat urine[a].

Compound number	Identity	HPLC R_t value (min)[b]	Mass spectral data[c]	
			EI^+	EI^-
3	Sulforidazine	5.5	402(14/18, $M^{+\cdot}$) 290(4/5) 277(3/5) 126(19/11) 114(42/5) 98(100/100)	402(1/2, $M^{-\cdot}$) 401(6/8) 387(100/100)
15	Lactam of sulforidazine[d]	6.6	416(66/85, $M^{+\cdot}$) 290(22/25) 277(17/25) 211(11/11) 198(7/12) 197(16/16) 140(23/27) 112(100/100)	
35	Sulforidazine ring sulfoxide	9.4	418(1/2, $M^{+\cdot}$) 403(8/9) 402(18/19) 401(36/38) 290(34/34) 277(8/9) 126(10/6) 98(100/100)	418(100/100, $M^{-\cdot}$) 403(46/49) 292(5/6)
18	Lactam of sulforidazine ring sulfoxide	12.4	432(6/3, $M^{+\cdot}$) 417(13/7) 416(49/28) 290(62/36) 277(36/25) 276(17/13) 258(8/5) 198(24/17) 139(27/59) 126(17/22) 112(100/100) 84(22/21)	432(100/100, $M^{-\cdot}$) 417(70/57) 401(26/23) 292(37/31)

[a] Mobile phase A: 70% 2,2,4-trimethylpentane, 15% dichloromethane, 15% methanol, 0.1% diethylamine. Samples were run in the electron impact mode, positive ion (EI^+) and except in one case negative ion (EI^-).

[b] Refers to the mid-point of each cluster of peaks.

[c] Results show prominent fragments (m/z) with the corresponding relative abundance (%) of authentic compound/metabolite in parentheses. The relative abundances of ions were determined by comparison to the same base peak in both authentic standard and unknown.

[d] Mass spectral data obtained from GLC-mass spectral analysis of fractions collected from HPLC.

N-desmethylsulforidazine ring sulfoxide (**34**) (Table 4).

Further confirmation of the identities of the two diastereomeric metabolites was obtained by treatment of each fraction collected from the HPLC (mobile phase C) with derivatizing reagents. BSA and MTBSTFA, which readily silylate phenols and alcohols failed to react with each metabolite as determined by both HPLC and GLC-MS of the reaction mixtures. This gave further indication that the metabolites were oxygenated in such a manner that there was no phenolic or alcoholic functional group formed. On the other hand, when these metabolites were separately treated with acetic anhydride, a derivative of each metabolite was obtained and its HPLC retention time and EI MS (positive and negative ion modes) were identical to those of synthetic N-acetyl-N-desmethylsulforidazine ring sulfoxide (**31**) (Table 4).

Attempts were made to determine whether or not phenols were present in the combined urine sample from the rats (n = 5) dosed with sulforidazine. An extraction procedure for phenolic metabolites was developed and applied to samples both with and without prior enzymic deconjugation. Plasmaspray HPLC-MS analysis which included selected ion monitoring was carried out on these extracts. No unconjugated phenolic metabolite could be identified in any of the underivatized extracts. However, when the same

Table 4. HPLC retention times and mass spectral data of N-desmethylsulforidazine ring sulfoxide diastereomers and their N-acetyl derivatives, and unresolved authentic standards isolated from extracts of rat urine.

Source (compound number)	Identity	HPLC R_t value (min)[a]	Mass spectral data[b]	
			EI^+	EI^-
Metabolite (34a)	Fast eluting N-desmethyl-sulforidazine ring sulfoxide diastereomer	21.8	404(2, $M^{+\cdot}$) 388(25) 387(29) 386(19) 290(59) 277(22) 276(11) 258(9) 211(12) 198(11) 197(14) 112(21) 110(47) 98(18) 84(100)	404(100, $M^{-\cdot}$) 390(15) 389(69) 373(9) 292(7)
Metabolite (34b)	Slow eluting N-desmethyl-sulforidazine ring sulfoxide diastereomer	25.1	404(4, $M^{+\cdot}$) 388(22) 387(22) 386(16) 290(47) 277(19) 276(8) 258(8) 211(10) 198(10) 197(13) 112(19) 110(42) 98(16) 84(100)	404(100, $M^{-\cdot}$) 390(13) 389(57) 373(1) 292(7)
Synthetic Standard (34)	Unresolved N-desmethyl-sulforidazine ring sulfoxide diastereomers	21.8 25.1	404(3, $M^{+\cdot}$) 388(22) 387(25) 386(18) 290(49) 277(18) 276(8) 258(7) 211(9) 198(19) 197(12) 112(16) 110(42) 98(23) 84(100)	404(100, $M^{-\cdot}$) 390(10) 389(42) 373(3) 292(7)
Derivative of 34a metabolite (31a)	Fast eluting N-acetyl-N-desmethyl-sulforidazine ring sulfoxide diastereomer	10.5	430(21) 290(8) 277(7) 154(100) 126(15) 112(3) 84(34)	446(2, $M^{-\cdot}$) 431(7) 416(29) 415(100)
Derivative of 34b metabolite (31b)	Slow eluting N-acetyl-N-desmethyl-sulforidazine ring sulfoxide diastereomer	10.6	430(24) 290(6) 277(6) 154(100) 126(7) 112(3) 84(34)	446(1, $M^{-\cdot}$) 431(4) 416(29) 415(100)
Synthetic Standard (31)	Unresolved N-acetyl-N-desmethyl-sulforidazine ring sulfoxide diastereomers	10.5 10.6	430(28) 290(8) 277(9) 154(100) 126(9) 112(3) 84(40)	446(3, $M^{-\cdot}$) 431(7) 416(33) 415(100)

[a] Mobile phase C: 75% 2,2,4-trimethylpentane, 15% dichloromethane (saturated with 1% ammonium acetate), 10% methanol, 0.1% diethylamine.
[b] Samples were run in the electron impact mode, both positive ion (EI^+) and negative ion (EI^-).Results show prominent fragments (m/z) with the corresponding relative abundances (%) in parentheses.

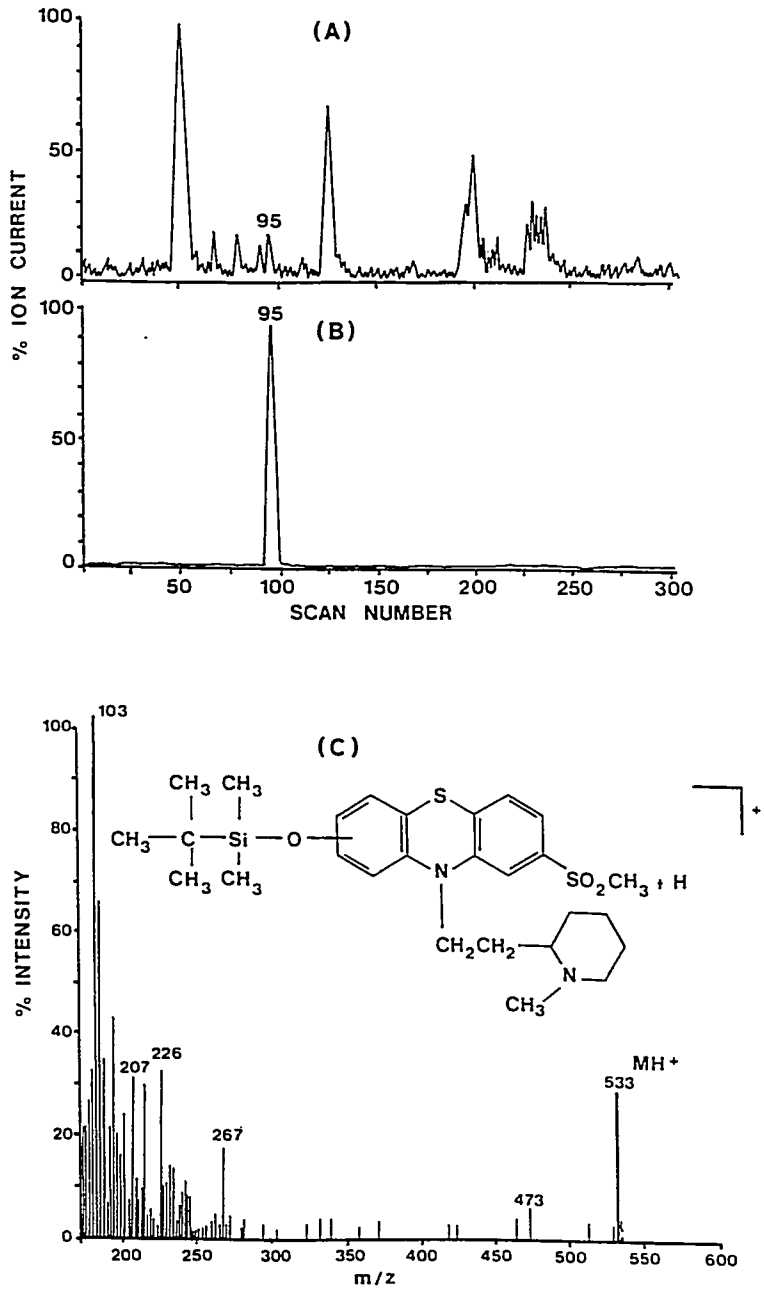


Figure 8. Positive ion HPLC-mass spectrometric analysis with a plasmaspray interface of a MTBSTFA-treated extract of urine from rats dosed with sulforidazine. (A) Total ion chromatogram in the range m/z 325-450; (B) reconstructed ion chromatogram for m/z 533 (protonated molecular ion of tert.-butyldimethylsilyl derivative of **36**); (C) positive ion mass spectrum for scan 95 of (A). **36**, unconjugated hydroxylated sulforidazine.

extracts, both with and without prior enzymic hydrolysis were treated with MTBSTFA prior to HPLC-MS analysis, the resultant chromatograms in each case showed one intense peak which was not present in chromatograms of the underivatized extracts. The positive ion plasmaspray MS of this peak gave a protonated molecular ion at m/z 533 that corresponded to the tert.-butyldimethylsilyl derivative of a phenolic metabolite of sulforidazine. These chromatograms and MS for the extract without prior enzymic treatment are shown in Figures 8(A-C). However, the position of the aromatic hydroxyl substituent could not be assigned as no appropriate authentic synthetic standards were available.

4.2.2.2. Characterization of metabolites of sulforidazine in dog

Sulforidazine and its examined metabolites gave a cluster of multiple peaks for each analyte in HPLC chromatograms using mobile phase A. However, it was subsequently found that by altering the proportions of mobile phase A this problem could be avoided. Therefore, in subsequent work mobile phase B was used instead of mobile phase A. This improvement can be demonstrated by comparison between the HPLC chromatograms for extracts of rat dosed with sulforidazine using mobile phase A (Figure 6(C)) and mobile phase B (Figure 9(C)). The HPLC chromatogram (Figure

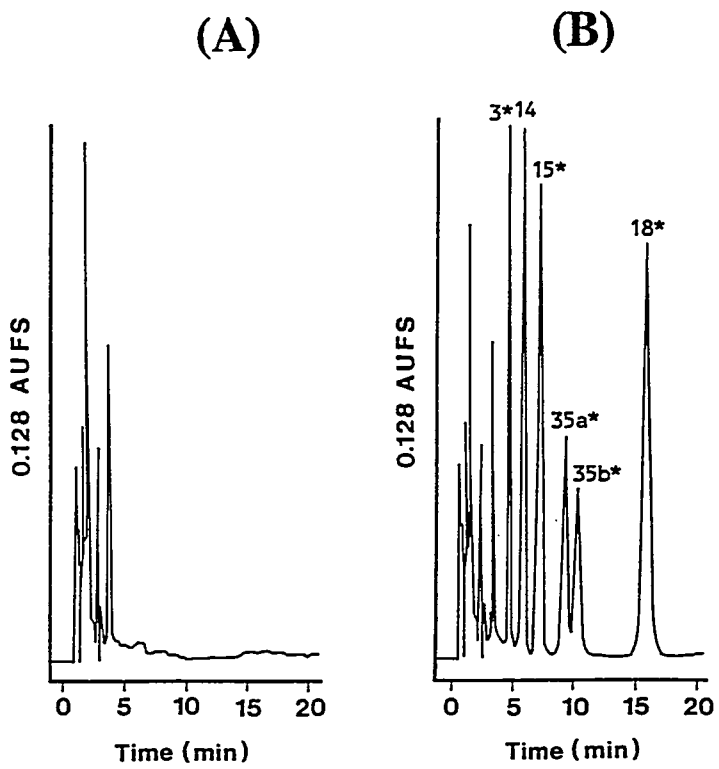


Figure 9. HPLC chromatograms of extracts of (A) blank human urine, (B) blank human urine with added authentic standards, (C) urine from a rat dosed with sulforidazine, (D) urine from a dog dosed with sulforidazine and (E) urine from a man dosed with sulforidazine.

Mobile phase B: 75% 2,2,4-trimethylpentane, 15% dichloromethane, 10% methanol, 0.1% diethylamine. Quantified compounds are indicated by an asterisk. **3**, Sulforidazine; **15**, lactam of sulforidazine; **18**, lactam of sulforidazine ring sulfoxide; **35a**, fast eluting diastereomer of sulforidazine ring sulfoxide; **35b**, slow eluting diastereomer of sulforidazine ring sulfoxide; **34a** and **34b**, diastereomers of N-desmethylsulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **14**, lactam of mesoridazine (internal standard).

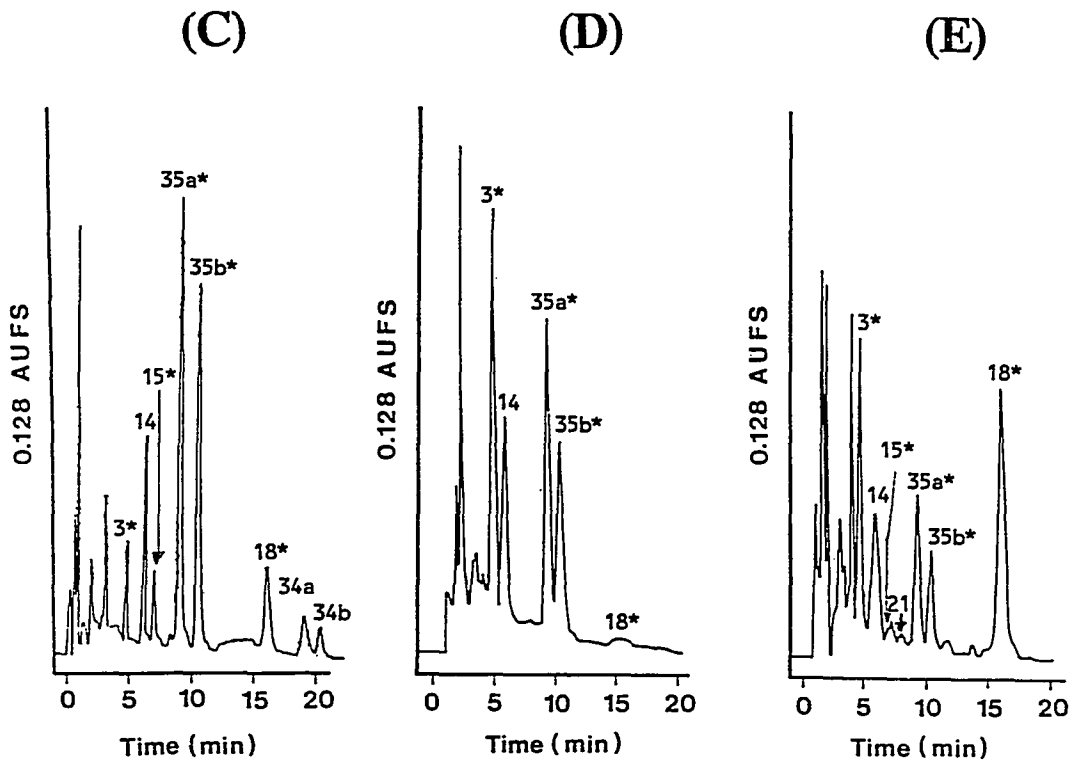


Figure 9. Continued.

9(D)) of a urine extract from a dog dosed with sulforidazine using mobile phase B gave four peaks which were not present in the chromatogram of an extract from blank urine. The order of elution and the retention times of these four peaks were identical with those obtained for authentic sulforidazine (**3**), sulforidazine ring sulfoxide diastereomers (**35a, 35b**) and the lactam of sulforidazine ring sulfoxide (**18**) (Figure 9(B)). The identity of each of these compounds was confirmed in that the appropriate fraction collected from the HPLC gave virtually the same MS as the reference standard.

The extraction procedure used for the identification of both unconjugated and conjugated phenols in rats dosed with sulforidazine (Section 3.3.2.4.) was simplified for all other studies of the metabolism of piperidine-type phenothiazine antipsychotic agents in three species. Thus in the simplified procedure crude extracts, both with and without prior enzymic hydrolysis were silylated with MTBSTFA before HPLC-MS analysis. In the case of the extract obtained after enzymic treatment the HPLC analysis showed one intense peak which was not present in the chromatograms of the underivatized extracts (data not shown). The positive ion plasmaspray MS of this peak gave a highest mass ion at m/z 533 that corresponded to the protonated molecular ion of the tert.-butyldimethylsilylated phenolic metabolite

of sulforidazine. The position(s) of the aromatic hydroxyl group could not be assigned as no appropriate authentic synthetic standards were available.

4.2.2.3. Characterization of metabolites of sulforidazine in human

There were six prominent peaks observed in the HPLC chromatogram of a urine extract from a volunteer dosed with sulforidazine (Figure 9(E)) which were not present in the chromatogram of the extract from control human urine (Figure 9(A)). The six peaks coincided in order of elution and retention times as the peaks due to authentic sulforidazine (**3**), the lactam of sulforidazine (**15**), sulforidazine N-oxide (**21**), two diastereomers of sulforidazine ring sulfoxide (**35a**, **35b**) and the lactam of sulforidazine ring sulfoxide (**18**) (Figure 9(B)).

The confirmation for the presence of these compounds in urinary extracts was accomplished by plasmaspray HPLC-MS analysis. The total ion chromatogram and the reconstructed ion chromatograms which utilized a specific m/z value corresponding to the appropriate protonated molecular ion for each suspected metabolite were shown in Figure 10(B). The plasmaspray MS for each of the metabolites was thus obtained (Figure 11(B)). The identities of these compounds were confirmed by direct comparison of their order of

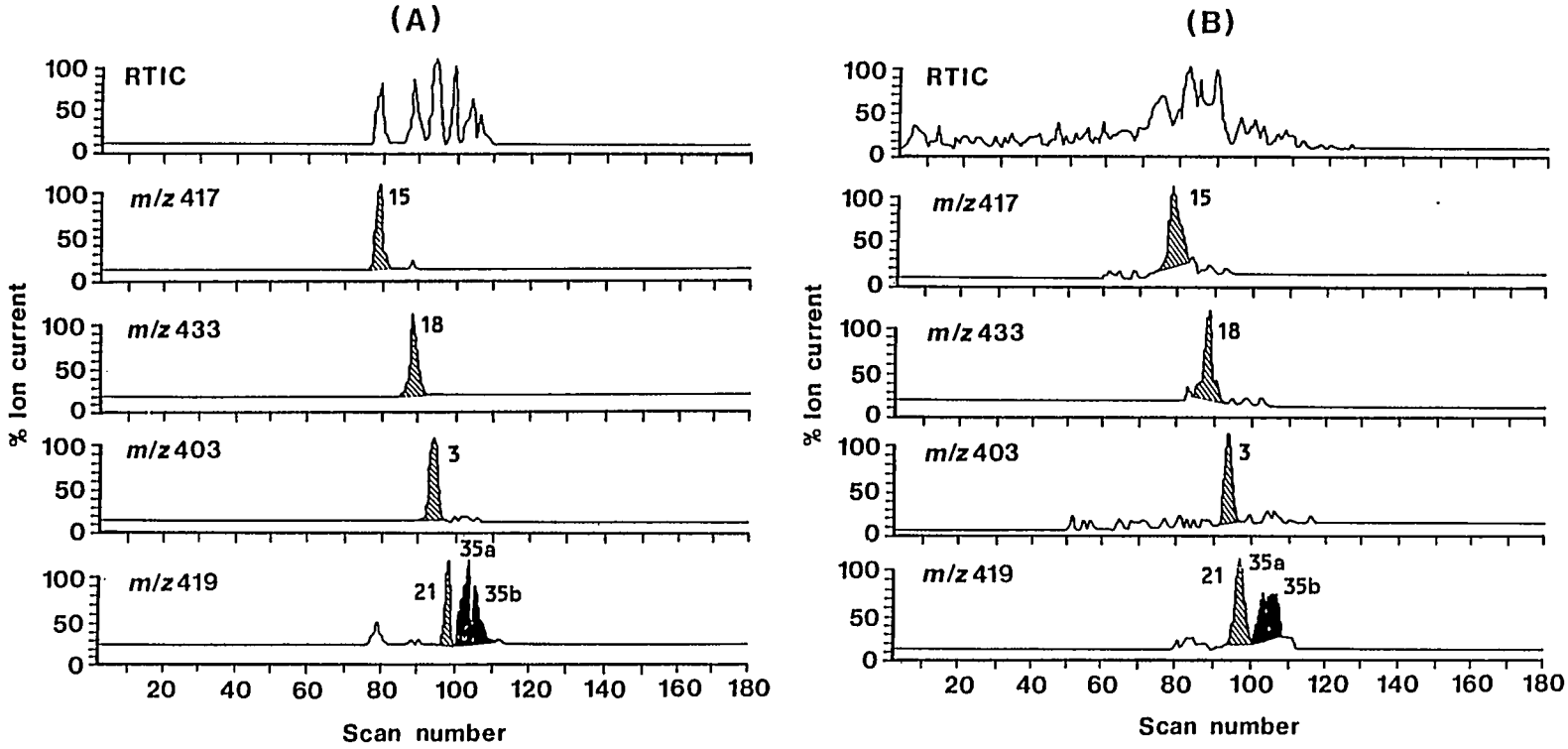


Figure 10. Reconstructed total ion chromatogram and reconstructed ion chromatograms of plasmaspray HPLC-mass spectrometric analysis of extracts of (A) blank urine with added reference standards and (B) urine of a man dosed with sulforidazine.

RTIC, reconstructed total ion current. **3**, Sulforidazine; **18**, the lactam of sulforidazine ring sulfoxide; **15**, the lactam of sulforidazine; **35a** and **35b**, diastereomers of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide.

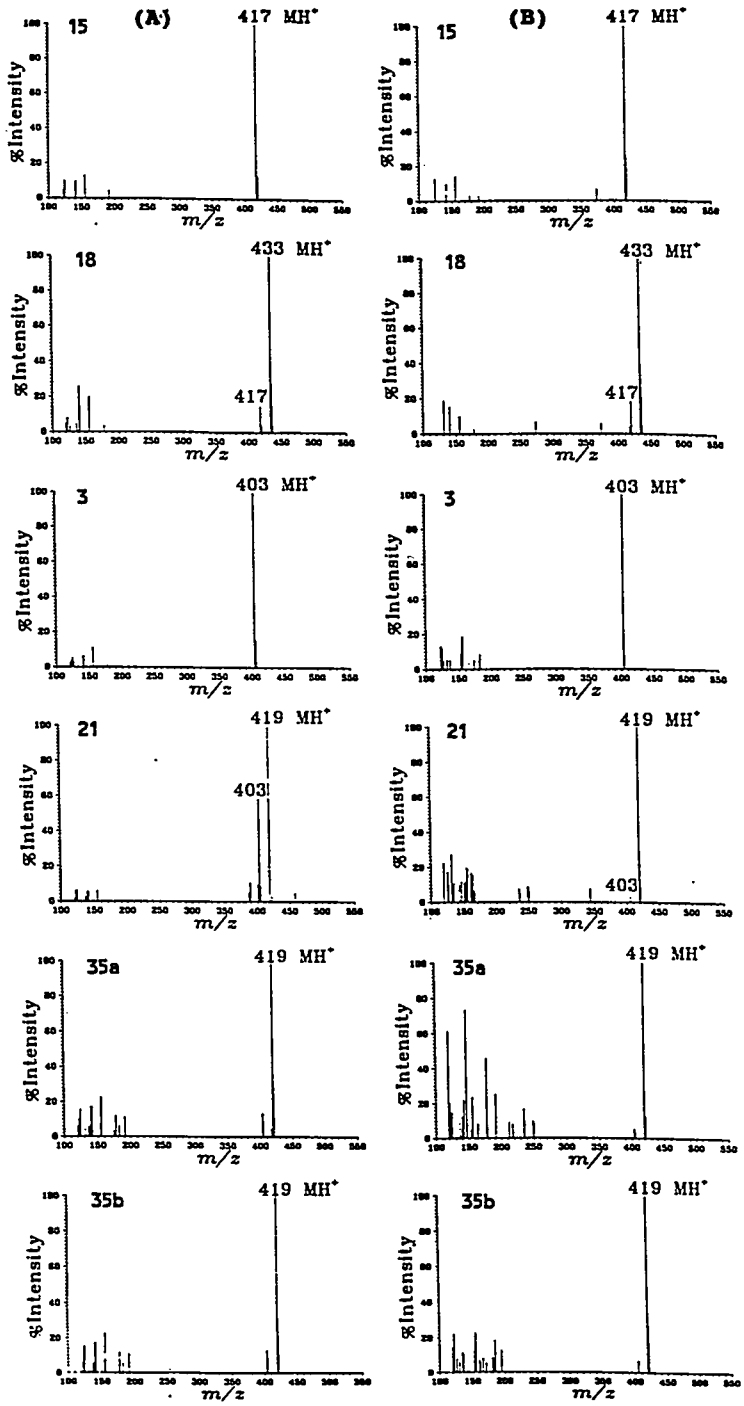


Figure 11. Plasmaspray mass spectra of each individual compound corresponding to the appropriate reconstructed ion chromatogram in Figure 10.

(A) Authentic standards and (B) isolated metabolites. The identities of the compounds are indicated in Figure 10.

elution, retention time and MS data (Figures 10(B) and 11(B)) with those obtained for authentic standards (Figures 10(A) and 11(A)). It should be noted that the order of elution of the peaks was different between the HPLC-MS and conventional HPLC systems. Moreover, the peaks due to the diastereomers of sulforidazine ring sulfoxide were more clearly resolved in the conventional HPLC system. Finally HPLC-MS analysis of derivatized extracts (protonated molecular ion at m/z 533: tert.-butyldimethylsilyl derivative) indicated that both unconjugated (**36**) and conjugated (**37**) phenolic derivatives of sulforidazine were present in the urine of human volunteers.

4.2.2.4. Quantification of metabolites of sulforidazine in three species

To determine the relative importance of the lactam metabolites of sulforidazine present in urine of the three species, their quantification as well as that of sulforidazine and sulforidazine ring sulfoxide was performed using an HPLC-UV method. Mobile phase B was employed in which sulforidazine ring sulfoxide was resolved into two diastereomers. The regression lines for each analyte over the stated standard curve ranges are summarized in Table 5. The mean recoveries of each analyte at three different concentrations over these standard curve ranges were

Table 5. Calibration curve data for the quantification of sulforidazine and some of its metabolites in rat, dog and human urine by HPLC-UV procedures[a].

Analyte (Compound number)	Extraction efficiency (%)[b]	Standard curve (r^2)	Concentration range (µg/ml)[c]	Mean % CV[d]
Sulforidazine (3)	88.1±9.6	$y = -0.066x^2+1.376x+0.371$ (0.98)	0.5-6	4.5
Fast eluting diastereomer of sulforidazine ring sulfoxide (35a)	86.9±10.4	$y = 0.02x^2+0.228x+0.158$ (0.98)	0.5-12	5.9
Slow eluting diastereomer of sulforidazine ring sulfoxide (35b)	87.4±14.8	$y = 0.021x^2+0.245x+0.085$ (0.98)	0.5-12	6.0
Lactam of sulforidazine (15)	91.6±4.3	$y = 0.03x-0.026$ (0.94)	0.05-0.25	8.7
Lactam of sulforidazine ring sulfoxide (18)	85.4±10.2	$y = -0.014x^2+0.449x+0.103$ (0.99)	0.5-10	3.6

[a] Mobile phase B: 75% 2,2,4,-trimethylpentane, 15% dichloromethane, 10% methanol, 0.1% diethylamine.

[b] The overall mean±S.D. extraction efficiency of the mean values (n = 3) obtained at each point of three different concentrations over the standard curve range.

[c] Five concentrations over each standard curve range were prepared in triplicate.

[d] The overall mean coefficient of variation of the mean values (n = 5) obtained at each concentration point of the standard curve range.

determined to be 88.1±9.6, 86.9±10.4, 87.4±14.8, 91.6±4.3 and 85.4±10.2% for sulforidazine, the fast eluting diastereomer of sulforidazine ring sulfoxide, the slow eluting diastereomer of sulforidazine ring sulfoxide, the lactam of sulforidazine and the lactam of sulforidazine ring sulfoxide, respectively. The quality control samples of each analyte gave values in each case such that four or more of the six samples were within ±15% of the nominal values. This criterion allowed the laboratory to accept the results of quantification.

The mean (rats, n = 5; dogs and healthy volunteers, n = 3) urinary excretions of sulforidazine, the lactam of sulforidazine ring sulfoxide and the total of the two diastereomers of sulforidazine ring sulfoxide given in the order of rat, dog and human in each case were 2.3±0.4, 7.2±1.9 and 5.9±0.7%, 3.2±2.6, <0.1 and 7.5±2.8%, and 12.1±1.6, 13.3±4.4 and 13.2±4.6%, respectively. The corresponding excretion of the lactam of sulforidazine in rat and human urine was 0.1±0.1% in each case, while visual inspection of the chromatograms indicated that sulforidazine N-oxide was also a minor metabolite in human urine (Table 6). Finally, the mean total excretions of the measured analytes in rat, dog and human were 17.7±5.4, 20.6±6.2 and 26.8±8.1%, respectively.

In the case of sulforidazine ring sulfoxide the mean

Table 6. The mean renal excretion of sulforidazine and its metabolites in rats (n =5), dogs (n = 3) and healthy male volunteers (n = 3) administered oral doses of sulforidazine.

Compound number	Identity	% Dose excretion for[a]		
		Rat	Dog	Human
3	Sulforidazine	2.3±0.4	7.2±1.9	5.9±0.7
15	Lactam of sulforidazine	0.1±0.1	nd	0.1±0.1
18	Lactam of sulforidazine ring sulfoxide	3.2±2.6	<0.1	7.5±2.8
35	Sulforidazine ring sulfoxide	12.1±1.6	13.3±4.4	13.2±4.6
21	Sulforidazine N-oxide	nd	nd	+
34	N-Desmethyl-sulforidazine ring sulfoxide	+	nd	nd
36	Unconjugated hydroxylated sulforidazine	+	nd	+
37	Conjugated hydroxylated sulforidazine	+	+	+

[a] nd, Not detected; +, detected but not measured. The data are expressed as means±S.D. Dose of sulforidazine was 20 mg/kg, 37.5 mg over 30 h and 25 mg in the case of rat, dog and human, respectively. Urine was collected over 0-24 h for rat and over 0-48 h for human and dog.

diastereomeric ratios of the concentrations of the fast eluting diastereomer to that of the slow eluting diastereomer were 1.24±0.05, 1.37±0.04 and 1.42±0.04 for rat (n = 5), dog (n =3) and human (n = 3), respectively.

4.2.2.5. Discussion of the results

There have been few reports on the metabolism of sulforidazine in any species. Apart from unchanged drug, N-desmethylsulforidazine was the only metabolite that was present in quantifiable amounts in the serum of patients medicated with sulforidazine (Axelsson 1977). In other studies of sulforidazine treated patients the metabolites identified in urine were the diastereomers of sulforidazine ring sulfoxide and 7-hydroxysulforidazine while the latter metabolite and 7-hydroxy-N-desmethylsulforidazine were found in faeces. Additionally tentative identification was made of the lactam of sulforidazine ring sulfoxide in urine (Papadopoulos et al. 1985, Papadopoulos and Crammer 1986). Finally the diastereomers of sulforidazine ring sulfoxide have been quantified also in dog plasma (Ganes and Midha 1987). There are no published reports to the metabolism of sulforidazine in rat.

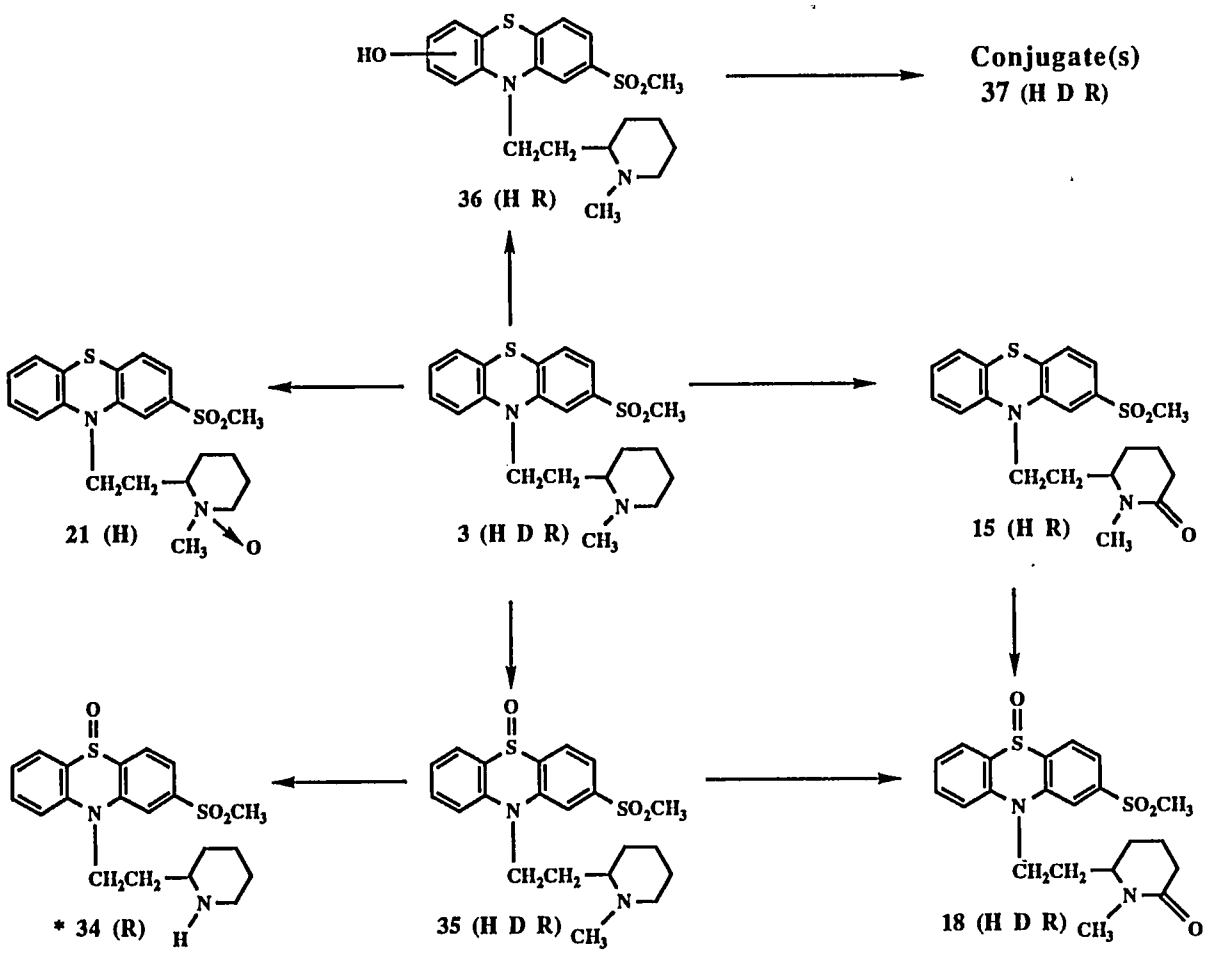
Plasmaspray HPLC-mass spectrometry Plasmaspray HPLC-MS, a technique of recent interest (Mills et al. 1988, Shindo et al. 1990) was utilized to analyze underivatized

extracts of human urine and derivatized extracts of urine of all three species. This technique has advantages over that used to examine the underivatized extracts of rat and dog urine, where the metabolites were separated by conventional HPLC and individually collected prior to direct probe MS analysis. The latter technique is very labour intensive and time consuming, especially in the case of metabolites present in low concentration as with the lactam of sulforidazine ring sulfoxide in extracts of dog urine (Figure 9(D)). The direct and relatively rapid technique of HPLC-MS is sensitive such that in the present work even the lactam of sulforidazine ring sulfoxide in dog urine was identified when derivatized extracts were examined (data not shown). Also in the analysis of compounds with thermally labile groups, for example, the N-oxide and S-oxide metabolites encountered in the present work (Hall et al. 1982, Midha et al. 1991), the use of such a nonthermal on-line technique is advantageous.

The total ion chromatograms of crude urinary extracts obtained by plasmaspray HPLC-MS analysis did not demonstrate complete separation of metabolites (Figure 10(RTIC)), since the analyzer responds to all organic molecules eluting from the chromatographic column including the compounds which do not absorb UV light that do not appear in HPLC-UV analysis. However, by means of computer enhancement to give

reconstructed ion chromatograms based on the m/z values of suspected protonated molecular ions, much clearer chromatograms were obtained that allowed tentative assignment of the structures of analytes (Figure 10). Each reconstructed ion peak was then systematically analyzed by MS. Plasmaspray MS usually exhibit intense protonated molecular ions (MH^+) and few fragment ions of the suspected metabolites (Figure 11), and, therefore, enable tentative assignment of the structures of analytes. In the present work, however, the availability of authentic standards enabled the structures of the metabolites to be confirmed.

Qualitative metabolite profiles The availability of authentic reference standards allowed definitive identification of sulforidazine and some of its metabolites. The proposed metabolic pathways of sulforidazine in three species are shown in Scheme 8. The compounds definitively identified in the urine of all three species were sulforidazine, two diastereomers of sulforidazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide. However, the lactam of sulforidazine was present only in rat and human, while N-desmethylsulforidazine ring sulfoxide diastereomers and sulforidazine N-oxide were identified only in rat and human, respectively. Finally, tentative identification was made of the phenolic metabolites in that the unconjugated form of a phenolic derivative of



Scheme 8. The proposed metabolic pathways of sulforidazine in rat (R), dog (D) and human (H).
3, Sulforidazine; **15**, lactam of sulforidazine; **18**, lactam of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **34**, N-desmethylsulforidazine ring sulfoxide; **35**, sulforidazine ring sulfoxide; **36**, unconjugated hydroxylated sulforidazine; **37**, conjugated hydroxylated sulforidazine. *N-Desmethylsulforidazine (**25**) (synthetic standard available), a possible intermediate in **3** → **34** conversion was not detected.

sulforidazine was detected in both rat and human urine, while the conjugated form(s) of a phenolic derivative of sulforidazine was present in all three species.

Sulfoxide metabolites S-Oxidation is a major route of metabolism of phenothiazine antipsychotic agents (Jørgensen 1986). Three of the identified metabolites were sulfoxides of the phenothiazine ring. The lactam of sulforidazine ring sulfoxide and the two diastereomers of sulforidazine ring sulfoxide were definitively identified and quantified in all three species. In fact, for all three species the combined excretion of these latter diastereomers was the greatest of all the analytes measured.

It is well established that the ring sulfoxide metabolites of piperidine-type phenothiazine antipsychotic drugs, including sulforidazine ring sulfoxide, can be chromatographically separated into their diastereomers (Poklis et al. 1982, Hale and Poklis 1985, Papadopoulos and Crammer 1986, Watkins et al. 1986, Ganes and Midha 1987). In the present work, this chromatographic separation was observed in the cases of sulforidazine ring sulfoxide and N-desmethylsulforidazine ring sulfoxide. In fact, the diastereomers of sulforidazine ring sulfoxide were separately quantified and the mean ratio of the concentrations of the fast eluting diastereomer to that of the slow eluting diastereomer was determined to be 1.24±0.05

(n = 5), 1.37±0.04 (n = 3) and 1.42±0.04 (n = 3) for rat, dog and human, respectively. These data indicate that there is need to reconsider the generally held belief that there is lack of stereoselectivity in the ring S-oxidation of piperidine-type phenothiazine antipsychotic agents (Poklis et al. 1982, Hale and Poklis 1985, Papadopoulos and Crammer 1986, Watkins et al. 1986, Ganes and Midha 1987). Also a recent report indicates that for patients (n = 11) medicated with thioridazine the mean concentration of the fast eluting diastereomer of thioridazine ring sulfoxide was greater than that of the slow eluting diastereomer in both plasma (1.37 fold) and urine (1.54 fold) (Eap et al. 1991). The lack of detection of marked differences between two diastereomeric ring sulfoxides of piperidine-type phenothiazines in earlier studies may have been due to their racemization and photolysis during analysis (Watkins et al. 1985, Eap et al. 1991).

In the present work care was exercised when undertaking analysis of sulfoxide metabolites, for in GLC analysis of ring sulfoxide metabolites of phenothiazine antipsychotic agents decomposition readily occurs (Hall et al. 1982). Such a decomposition phenomenon was found also in the present study. For example, sulforidazine ring sulfoxide decomposed to sulforidazine at injection port temperatures of 310°C and 275°C to the extents of 79% and 9%,

respectively. Moreover the lactam of sulforidazine ring sulfoxide quantitatively decomposed to the lactam of sulforidazine at both these temperatures and even when a cold on-column injection technique (35°C) was utilized the extent of decomposition was 91%. Therefore, when GLC-MS was employed to confirm the existence of the lactam of sulforidazine in rat urinary extracts, so as to ensure the absence of the corresponding sulfoxide metabolite, analysis was performed on the appropriate fraction obtained from purification by HPLC separation rather than the crude extract itself.

Since it is well established that the direct probe positive ion EI MS of the ring sulfoxide metabolites of phenothiazine antipsychotic agents generally give weak molecular ions, the direct probe negative ion mode was also investigated in the case of studies of rat (Tables 3 and 4) and dog (data not presented) urine. All three sulfoxide metabolites isolated gave the respective molecular ion as the base peak in the negative ion mode, whereas for the positive ion mode the molecular ion was very weak. Also each diastereomer of N-acetyl-N-desmethylsulforidazine ring sulfoxide gave a weak molecular ion only in the negative ion mode. On the other hand, for all these ring sulfoxide compounds, the positive ion spectrum gave more diagnostic fragment ions than did the negative ion mode. Therefore,

the two ionization modes complemented one another in the identification of compounds containing a sulfoxide group.

Lactam metabolites In the only previous report on the lactam metabolites of piperidine-type phenothiazine antipsychotic agents, there was tentative identification of such metabolites in urine of patients (Papadopoulos and Crammer 1986). In the present study the availability of synthetic standards of lactam analogues allowed definitive identification of these metabolites in all three species examined. The lactam of sulforidazine ring sulfoxide was found in all three species and in the cases of both rat and human it represented a large proportion (18.1% and 28.1%, respectively) of the compounds that were quantified. Also the lactam of sulforidazine was identified in both rat and human urine, for the first time. However it was present in trace quantities in each case. The present results are the first definitive evidence in any species of lactam formation with piperidine-type phenothiazine antipsychotic agents and indicate that further study of the metabolism of the piperidine ring of these drugs is warranted.

N-Oxide metabolites Metabolic oxidation of the heteroatoms of phenothiazine antipsychotic agents occurs not only at the ring sulfur atom but also at the side chain nitrogen atom(s). The identification of sulforidazine N-oxide as a metabolite of sulforidazine in human is the first

report on the N-oxidation of this drug in any species. However, no N-oxide metabolite was detected in the urine of dog or rat. N-Oxide metabolites are known to be unstable in various stages of sample preparation and analysis (Hubbard et al. 1985, Midha et al. 1991). The availability of synthetic samples of the N-oxide and N,S-dioxide derivatives of sulforidazine enabled it to be proven that there was no such decomposition involved in the extraction and analytical techniques employed in the present work. Also a nonthermal on-line technique, plasmaspray HPLC-MS, was employed in the present work.

N-Demethylated metabolites Despite the availability of a reference sample of N-desmethylsulforidazine it could not be detected as a metabolite of sulforidazine in any species examined. This is somewhat surprising in that the diastereomeric N-desmethyl ring sulfoxide metabolites were detected in the rat. However, further studies are required to establish whether N-desmethylsulforidazine ring sulfoxides are preferentially produced from sulforidazine by S-oxidation followed by N-demethylation rather than vice versa.

Hydroxylated metabolites Initially phenolic metabolites of sulforidazine could not be detected as such in the crude extracts as they did not chromatograph as distinct peaks on the conventional HPLC or HPLC-MS systems.

However, preparation of the tert.-butyldimethylsilyl derivatives allowed their chromatography and identification by the HPLC-MS system in all three species. The conjugated form(s) of a phenolic metabolite of sulforidazine was present in each of the three species, while the unconjugated form(s) was observed in rat and human urine. The position(s) of substitution of the phenolic group was not determined in any species. Generally with phenothiazine antipsychotic agents hydroxylation occurs predominantly in the 3- and 7-positions of the phenothiazine ring (Papadopoulos et al. 1985, Jørgensen 1986). However, the presence of the 2-sulfonyl substituent makes 3-hydroxylation unlikely and only 7-hydroxy metabolites were found as metabolites of sulforidazine in humans (Papadopoulos et al. 1985). The extent of aromatic hydroxylation was not determined, but it is well established that in general for phenothiazine antipsychotic agents this route is a major route of metabolism (Jørgensen 1986).

Interspecies comparisons Comparison can be made among rat, dog and human in terms of the metabolites of sulforidazine found in urine after oral administration of this drug, and the data are summarised in Scheme 8 and Table 6. Firstly sulforidazine was extensively metabolised in all three species. The mean excretion of sulforidazine in urine was between 2.3-7.2% of the administered dose. Also a

common feature of the metabolism in all three species was that the sulfur atom of the phenothiazine ring 2-substituent was fully oxidised in each metabolite. This is consistent with previous observations that in general the sulfone group is metabolically stable and reduction to the sulfoxide is at most a minor route of metabolism (Renwick 1989). However, these studies demonstrated that the metabolic profile for human was both qualitatively and quantitatively more similar to that for rat than for dog. Thus, whereas three metabolites were detected in human urine that were not found in dog, the only difference between human and rat was that the N-oxide and N-desmethyl ring sulfoxide metabolites were found only in human and rat, respectively. Moreover, the lactam of sulforidazine ring sulfoxide was a minor metabolite in dog but a major metabolite in both human and rat and the lactam of sulforidazine was detected only in human and rat.

4.2.3. Urinary metabolites of mesoridazine

4.2.3.1. Characterization of metabolites of mesoridazine in rat

The urine from rat was examined first of the urine samples obtained from the three species administered mesoridazine. The preliminary screen of the metabolites present in the underivatized urinary extracts of rats was

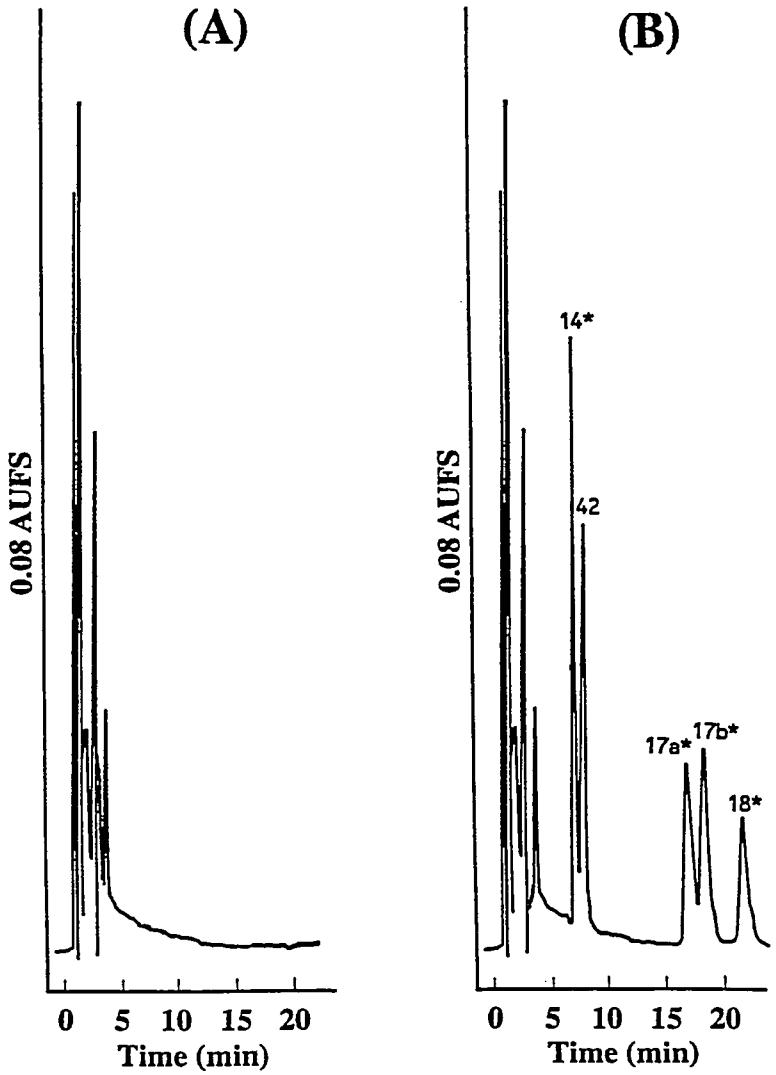


Figure 12. HPLC chromatograms of urinary extracts of (A) blank human urine, (B) blank human urine with added authentic standards, (C) urine from a rat dosed with mesoridazine, (D) urine from a dog dosed with mesoridazine and (E) urine from a man dosed with mesoridazine.

Mobile phase D: 82% 2,2,4-trimethylpentane, 10% dichloromethane, 8% methanol, 0.01% diethylamine. Quantified compounds are indicated by an asterisk. **2**, Mesoridazine; **3**, sulforidazine; **14**, lactam of mesoridazine; **17a**, fast eluting diastereomer of lactam of mesoridazine ring sulfoxide; **17b**, slow eluting diastereomer of lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **33**, N-desmethylmesoridazine ring sulfoxide; **35a** and **35b**, diastereomers of sulforidazine ring sulfoxide; **38a** and **38b**, diastereomers of mesoridazine ring sulfoxide; **42**, prochlorperazine ring sulfoxide (internal standard).

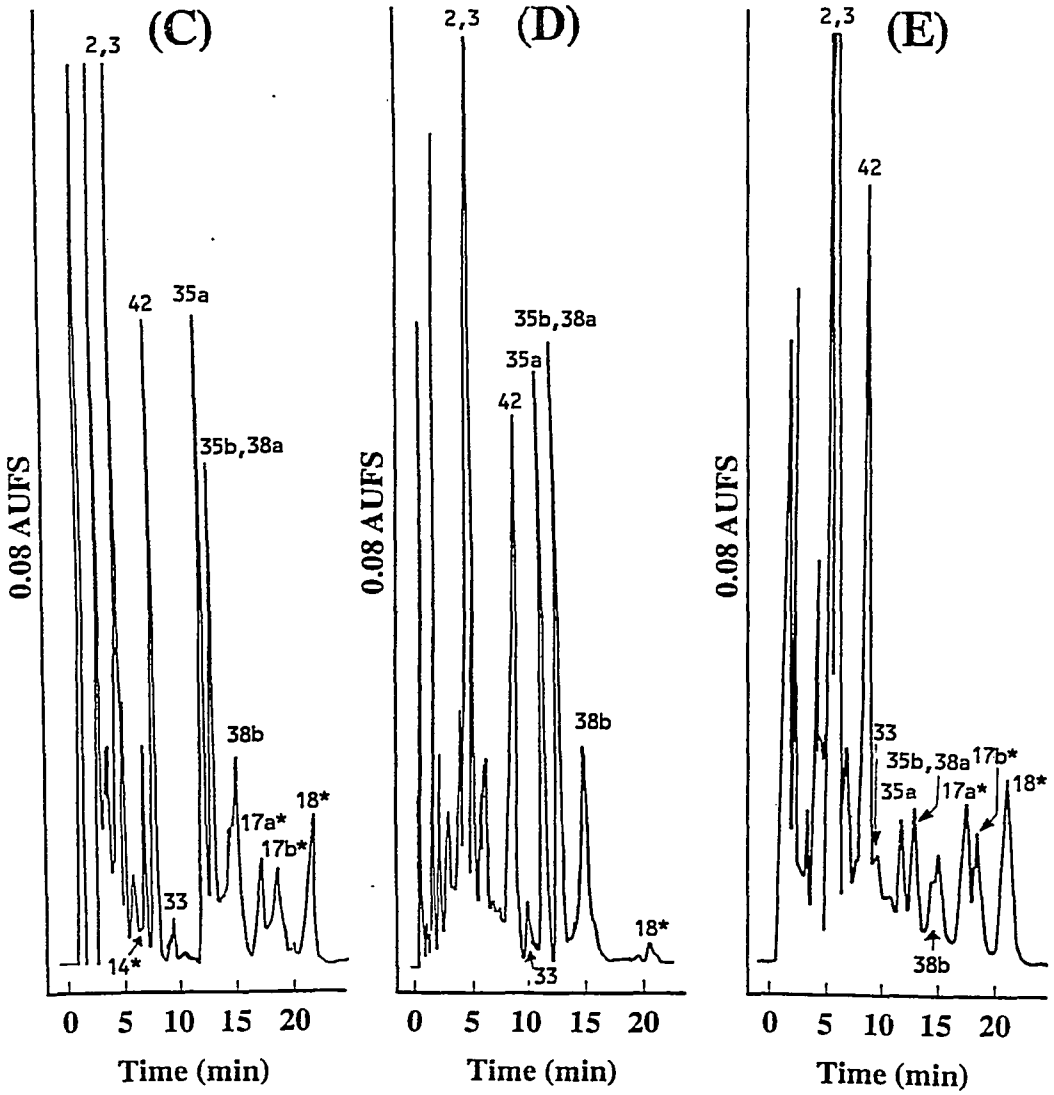


Figure 12. Continued.

carried out by conventional HPLC prior to direct insertion probe plasmaspray MS. The HPLC chromatogram of the underivatized urinary extract contained nine peaks (Figure 12(C)) which were not present in the chromatogram of an extract from control rat urine. Each individual peak was collected and analyzed by using direct insertion probe MS in the positive plasmaspray ionization mode. However, as illustrated in the chromatograms (Figure 12), the apparent quantities (based solely on their relative peak height using UV absorption detection) of the metabolites varied to a large extent. Some were present in only trace amounts in urinary extracts. Therefore, especially in these cases, this method of metabolite identification was a time consuming process due to the need for repeat collections to obtain enough analyte for direct probe MS analysis. Consequently in subsequent work the plasmaspray HPLC-MS technique used in the study of the identification of the metabolites of sulforidazine in human (section 4.2.2.3.) was utilized. The combination of HPLC and MS provided an efficient chromatographic separation and informative MS. Hence based on direct comparison of its order of elution, retention time and MS data between the reference standard and the detected metabolite obtained from both conventional HPLC prior to MS (Table 7) and on-line HPLC-MS techniques (Table 8) seven of the eight compounds were identified,

namely unchanged mesoridazine (**2**), sulforidazine (**3**), the lactam of mesoridazine (**14**), the lactam of mesoridazine ring sulfoxide diastereomers (**17a** and **17b**), the lactam of sulforidazine ring sulfoxide (**18**), sulforidazine ring sulfoxide diastereomers (**35a** and **35b**) and mesoridazine ring sulfoxide diastereomers (**38a** and **38b**).

The identity of the unknown compound (Figure 12(C), retention time 8.8 min) was established as follows. Its direct insertion probe plasmaspray MS gave a protonated molecular ion (MH^+) at m/z 389 and a base peak at m/z 373 corresponding to the loss of an oxygen atom. The more informative positive ion EI MS exhibited ions which were diagnostic of N-desmethylmesoridazine ring sulfoxide (**33**) (m/z 388, $M^{+\cdot}$, 7%; m/z 372, M-16, 12%; m/z 84, protonated 1,2-dehydropiperidinium ion, 100%) as illustrated in Figure 13. Attempts to prepare a synthetic reference sample of this compound were unsuccessful; however, a small quantity of a reference sample of N-acetyl-N-desmethylmesoridazine ring sulfoxide (**30**) was synthesized as outlined in the Experimental (Section 3.2.19.). Consequently a portion of the compound isolated via HPLC separation using mobile phase D was treated with acetic anhydride. Plasmaspray HPLC-MS analysis indicated that the retention times (Figure 14, (A_1) and (B_1)) and MS (Figure 14, (A_2) and (B_2)) of the synthetic sample and the acetylated derivative of the metabolite were

Table 7. The HPLC (conventional system) retention times and direct probe plasmaspray mass spectral data obtained for mesoridazine and its metabolites isolated from rat urine spiked with reference standards or obtained from dosed rats.

Compound number	Identity	HPLC R_t value (min)[a]		MH^+ m/z value (intensity %)	
		Standard	Metabolite	Standard	Metabolite
2	Mesoridazine[b]	5.0	5.0	387(100)	387(100)
3	Sulforidazine[b]	5.1	5.1	403(100)	403(100)
35	Sulforidazine ring sulfoxide[c,d]	11.0, 13.0	11.2, 13.2	419(100)	419(100)
18	Lactam of sulforidazine ring sulfoxide[c]	20.6	20.7	433(100)	433(100)
38	Mesoridazine ring sulfoxide[c,d]	13.2, 15.3	13.2, 15.5	403(100)	403(93)
14	Lactam of mesoridazine	7.8	7.7	401(100)	401(52)
17	Lactam of mesoridazine ring sulfoxide[c]	16.1, 17.6	16.2, 17.8	417(25)	417(25)
33	N-Desmethyl-mesoridazine ring sulfoxide[e]		8.8		389(32)

[a] Mobile phase D: 82% 2,2,4-trimethylpentane, 10% dichloromethane, 8% methanol, 0.01% diethylamine.

[b] Mesoridazine and sulforidazine co-eluted with each other.

[c] Diastereomers.

[d] The slow eluting diastereomer of sulforidazine ring sulfoxide and the fast eluting diastereomer of mesoridazine ring sulfoxide co-eluted with each other.

[e] Reference standard unavailable.

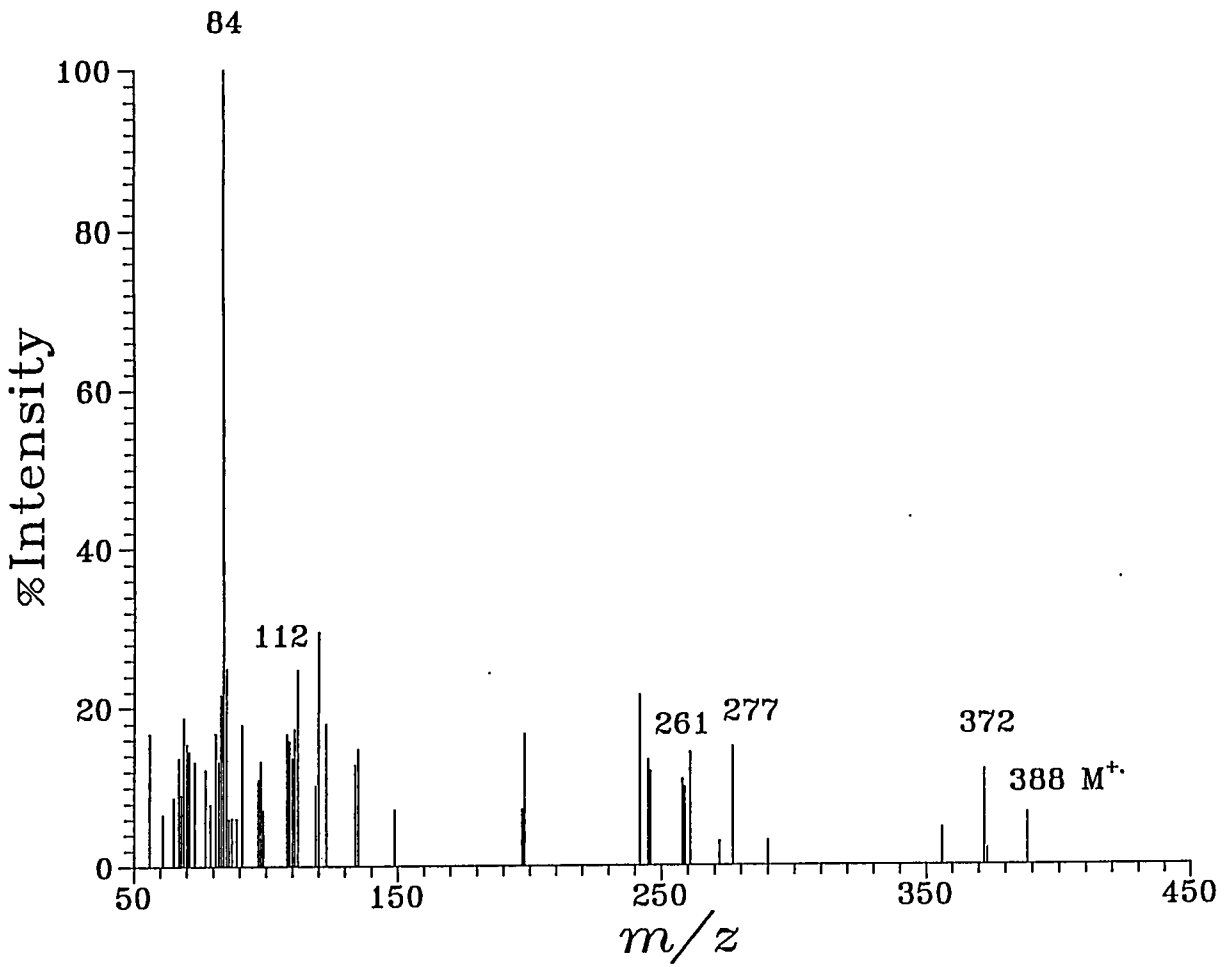


Figure 13. Positive ion electron impact mass spectrum of N-desmethylmesoridazine ring sulfoxide.

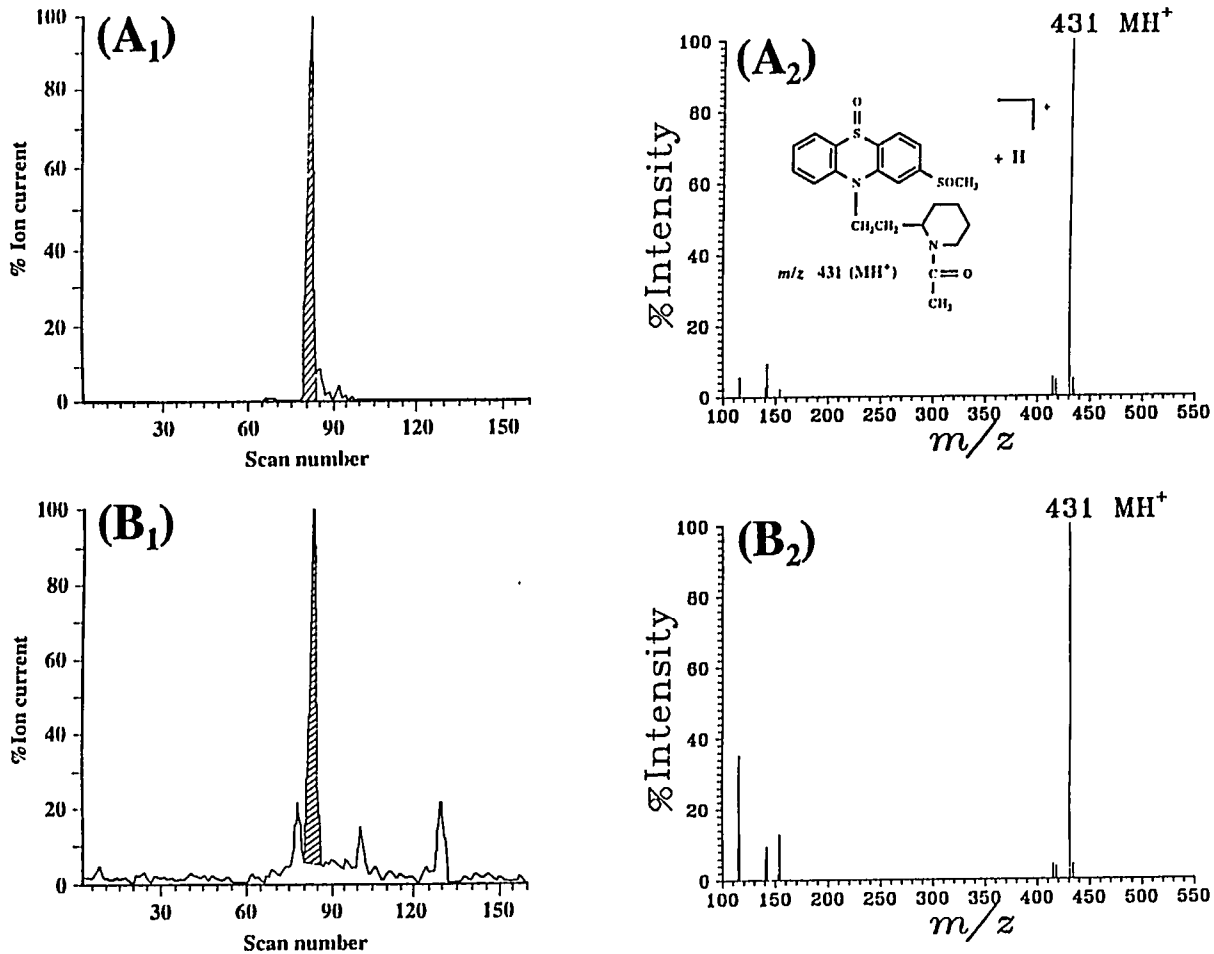


Figure 14. Plasmaspray HPLC-mass spectrometric analysis of (A) authentic N-acetyl-N-desmethylmesoridazine ring sulfoxide and (B) acetic anhydride treated collected HPLC fractions that were in turn obtained from extracts of urine from a rat dosed with mesoridazine.

A_1 and B_1, reconstructed ion chromatograms for m/z 431 (protonated molecular ion of N-acetyl-N-desmethylmesoridazine ring sulfoxide); A_2 and B_2, positive ion mass spectra for scan 82 of A_1 and B_1, respectively.

identical.

For the identification of phenolic metabolites the extracts of rat urine obtained with and without prior enzymic hydrolysis were silylated with MTBSTFA and then analyzed by plasmaspray HPLC-MS. For example, the positive ion plasmaspray MS of the two peaks observed for the enzymically treated extracts gave highest mass ions at m/z 517 and 533 that corresponded to the protonated molecular ions of the tert.-butyldimethylsilylated phenolic analogues of mesoridazine (**41**) and sulforidazine (**37**), respectively as illustrated in Figures 15 and 16. Similar examination of the non-enzymically treated extracts indicated that the free form of the phenolic derivative of sulforidazine (**36**) was also present (Table 8). The position of the aromatic hydroxyl group(s) in each of these metabolites could not be assigned as no appropriate authentic synthetic standards were available.

4.2.3.2. Characterization of metabolites of mesoridazine in dog

The use of similar techniques that including plasmaspray HPLC-MS analysis enabled identification of the following compounds in the underivatized and/or derivatized extracts of dog urine: the lactam of sulforidazine ring sulfoxide (**18**), sulforidazine (**3**), sulforidazine N-oxide

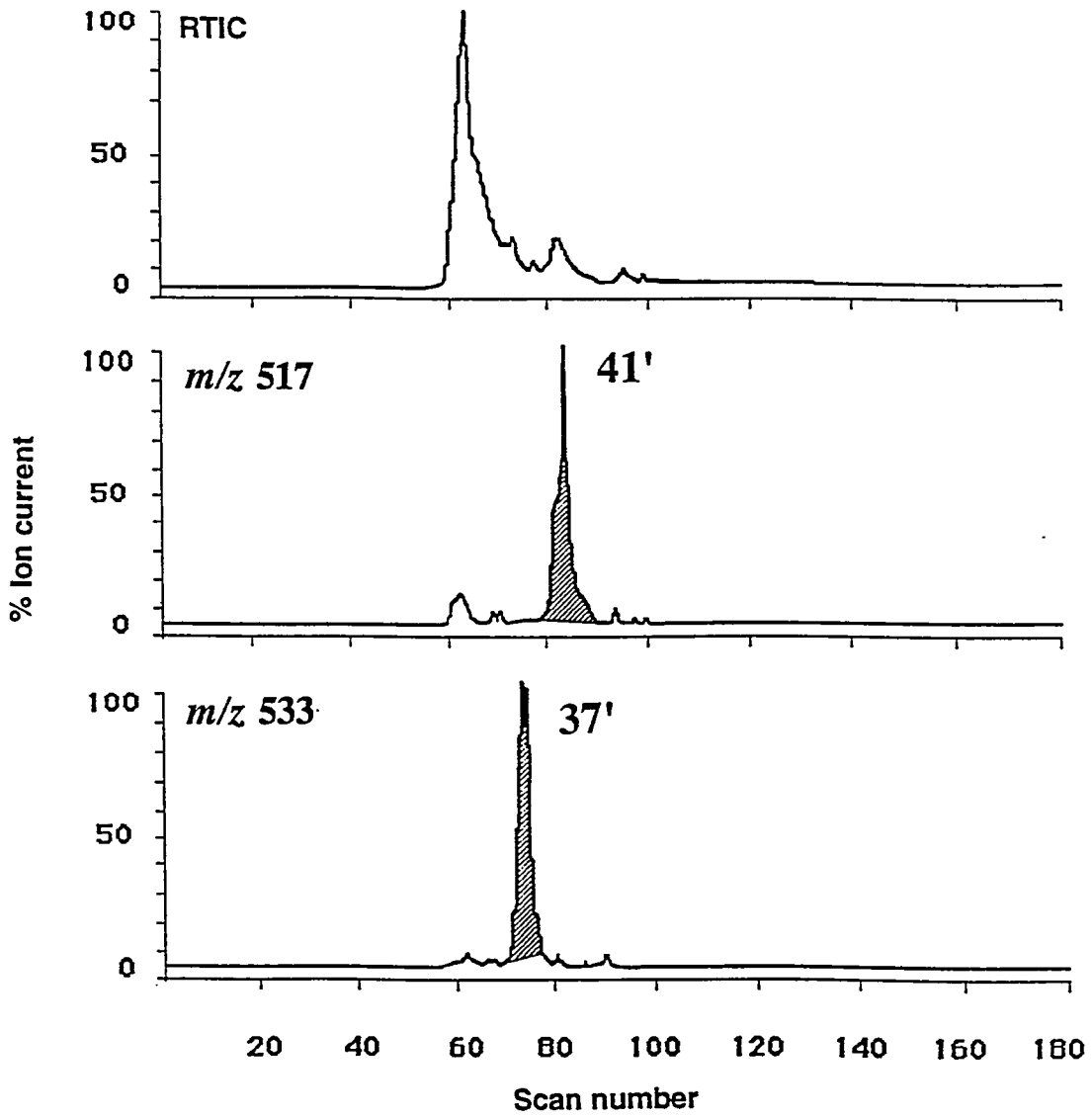


Figure 15. Reconstructed total ion chromatogram and reconstructed ion chromatograms of plasmaspray HPLC-mass spectrometric analysis of the enzymically treated and silylated urinary extract of a rat dosed with mesoridazine.

RTIC, Reconstructed total ion current. **37'**, tert.-Butyldimethylsilyl derivative of hydrolyzed conjugated hydroxylated sulforidazine; **41'**, tert.-butyldimethylsilyl derivative of hydrolyzed conjugated hydroxylated mesoridazine.

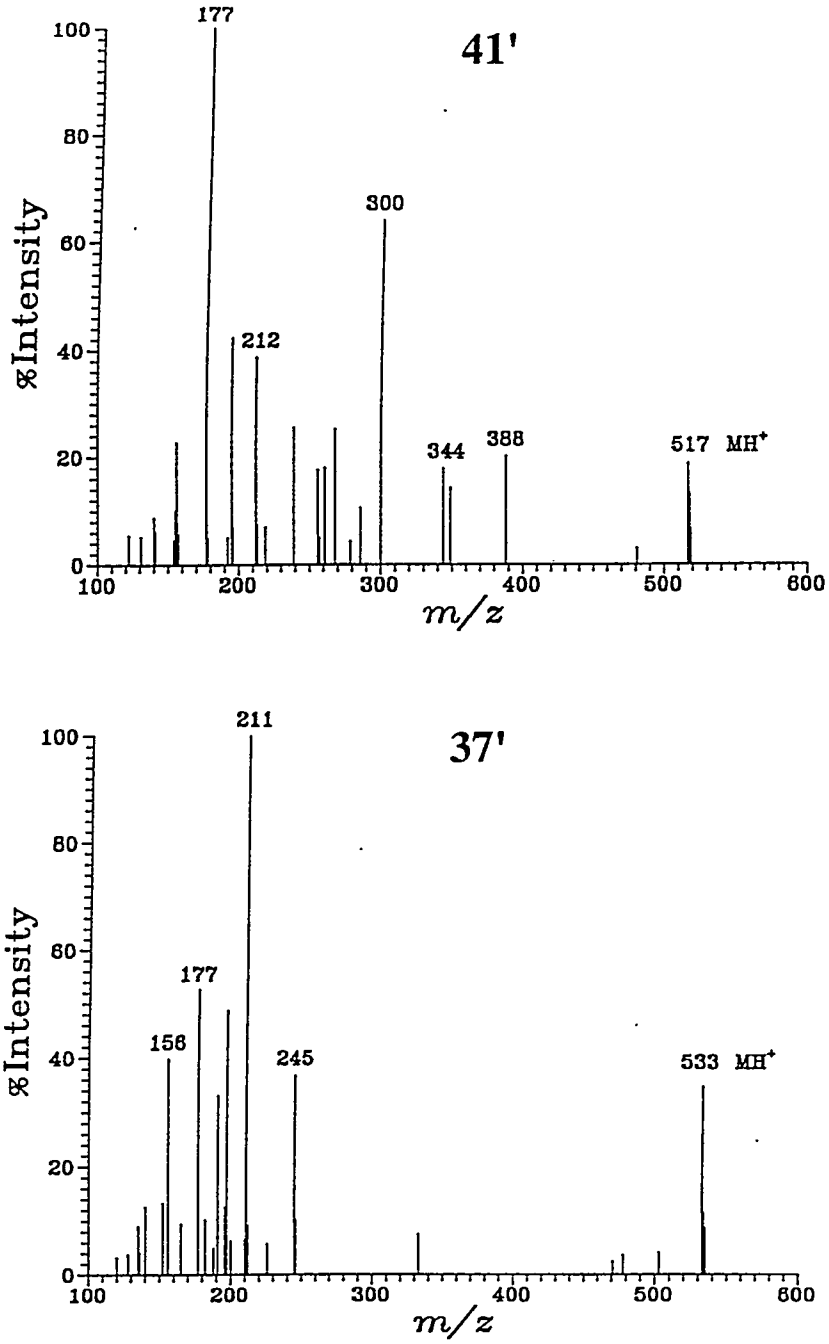


Figure 16. Plasmaspray mass spectra of each silylated phenolic metabolite corresponding to the appropriate reconstructed ion chromatogram in Figure 15.

The identities of the compounds are indicated in Figure 15.

(**21**), N-desmethylmesoridazine ring sulfoxide (**33**), sulforidazine ring sulfoxide (**35**), mesoridazine (**2**), mesoridazine ring sulfoxide (**38**), the unconjugated (**36**) and conjugated (**37**) forms of phenolic derivative(s) of sulforidazine and the unconjugated form of the phenolic derivative of mesoridazine (**40**). The retention times and MS data of all identified compounds by plasmaspray HPLC-MS analysis are summarized in Table 8.

4.2.3.3. Characterization of metabolites of mesoridazine in human

The HPLC-MS techniques used in the urinalysis of rat and dog were applied also to the analyses of urine extracts obtained from human urine. For example, typical ion chromatograms and plasmaspray MS obtained by this technique are illustrated in Figures 17 and 18, respectively. The total ion chromatogram (Figure 17(RTIC)) obtained from the on-line plasmaspray HPLC-MS analysis of a urinary extract from a volunteer dosed with mesoridazine showed very broad peaks. The reconstructed ion chromatograms (Figure 17) based upon a diagnostic quasi-molecular ion of each suspected metabolite gave clearer indication of the metabolite(s) present. The plasmaspray MS obtained for each of the peaks in the reconstructed ion chromatograms indicated that nine drug-related compounds were present

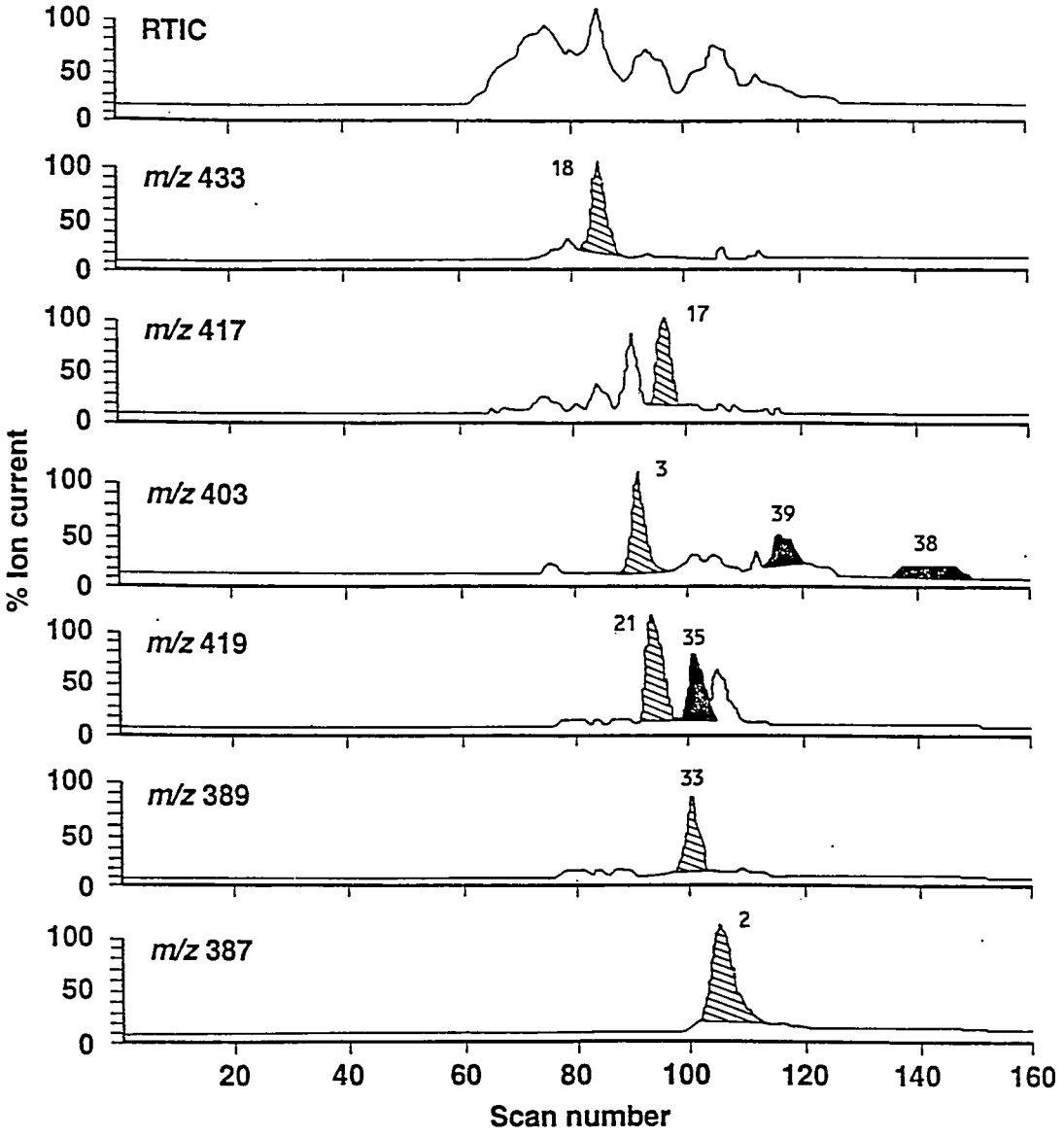


Figure 17. Reconstructed total ion chromatogram and reconstructed ion chromatograms of plasmaspray HPLC-mass spectrometric analysis of extracts of urine of a man dosed with mesoridazine.

RTIC, reconstructed total ion current. **2**, Mesoridazine; **3**, sulforidazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **33**, N-desmethylmesoridazine ring sulfoxide; **35**, sulforidazine ring sulfoxide; **38**, mesoridazine ring sulfoxide; **39**, mesoridazine N-oxide.

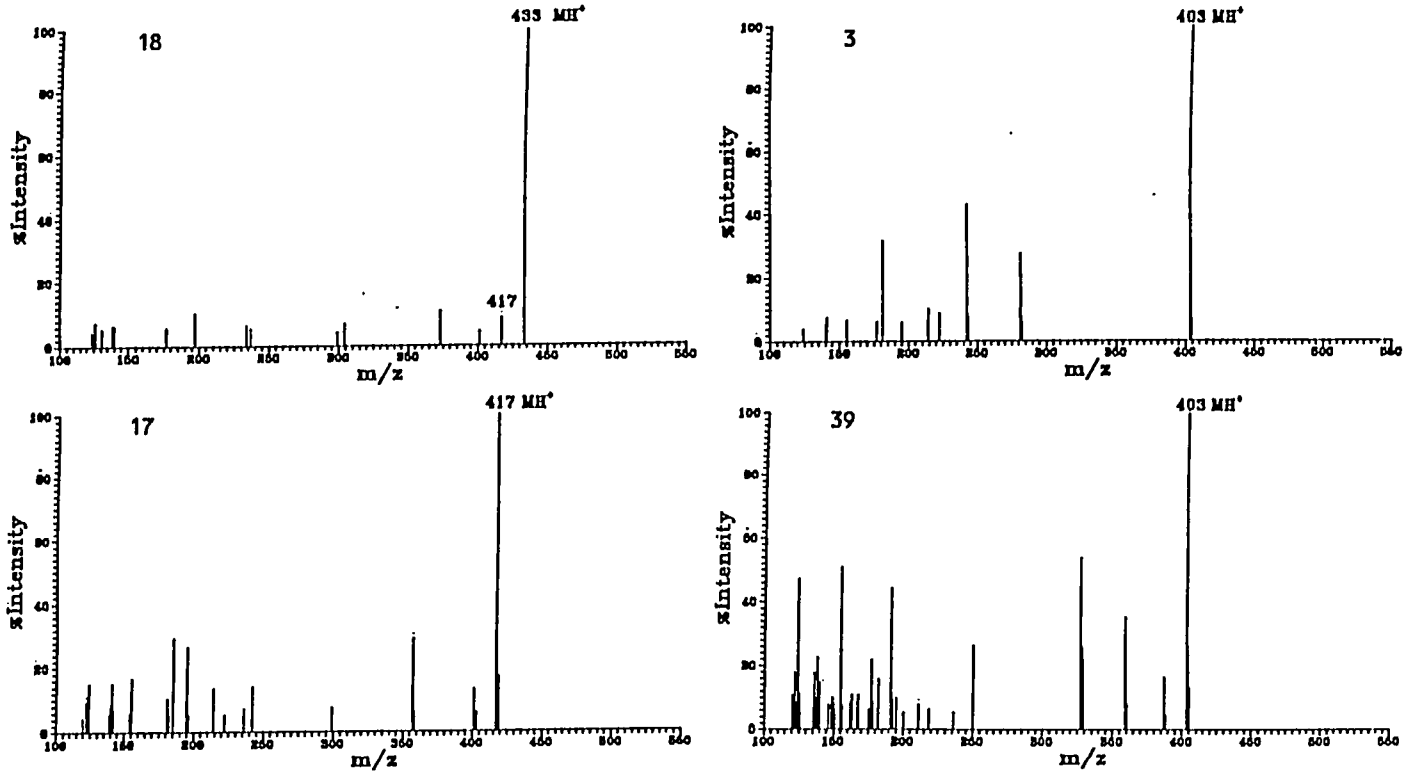


Figure 18. Plasmaspray mass spectra of each individual compound corresponding to the appropriate reconstructed ion chromatogram in Figure 17.
The identities of the compounds are indicated in Figure 17.

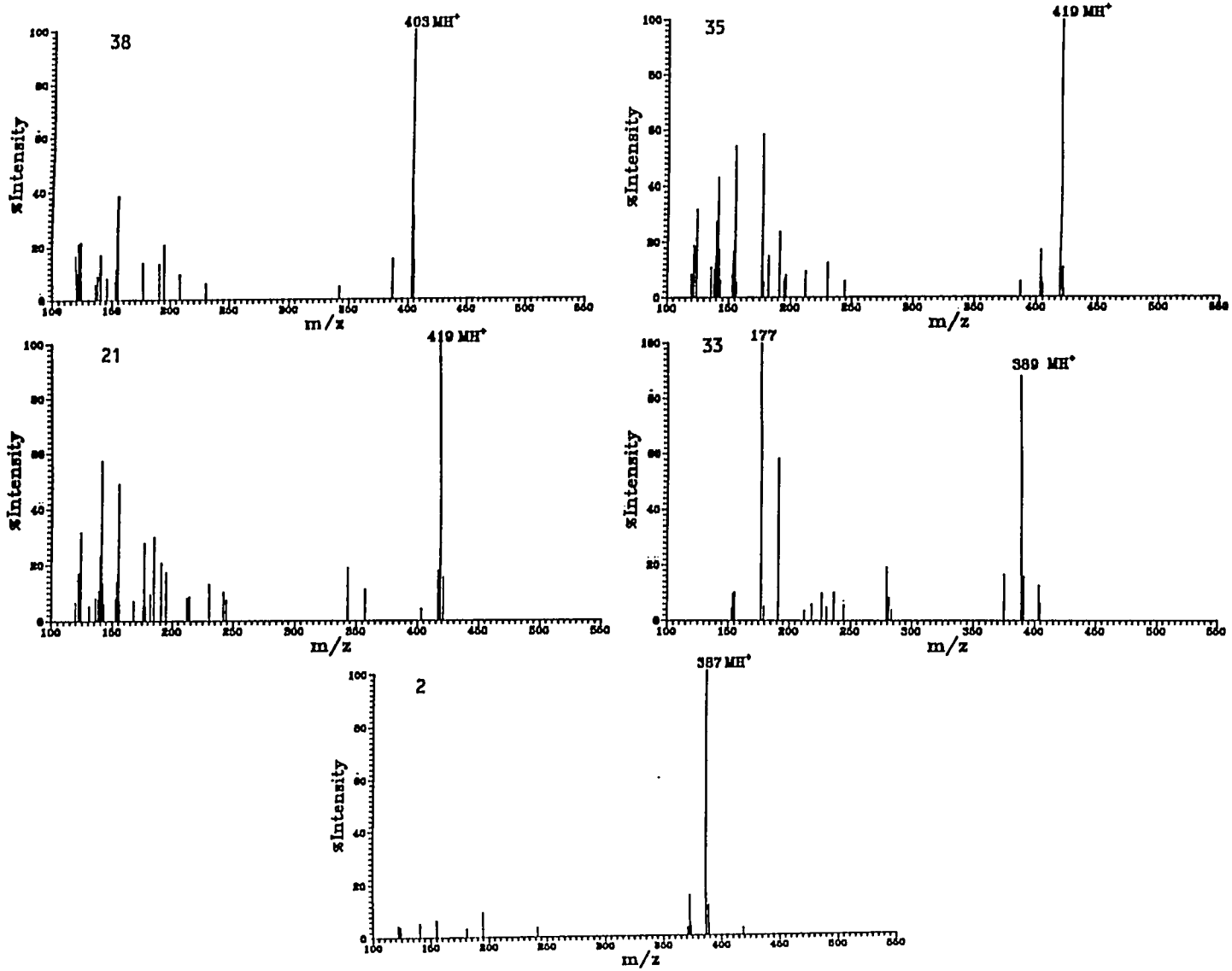


Figure 18. Continued.

(Figure 18). In order of elution these compounds were the lactam of sulforidazine ring sulfoxide (**18**), sulforidazine (**3**), sulforidazine N-oxide (**21**), the lactam of mesoridazine ring sulfoxide (**17**), N-desmethylmesoridazine ring sulfoxide (**33**), sulforidazine ring sulfoxide (**35**), mesoridazine (**2**), mesoridazine N-oxide (**39**) and mesoridazine ring sulfoxide (**38**). The identities of all these metabolites, with the exceptions of N-desmethylmesoridazine ring sulfoxide and mesoridazine N-oxide, were confirmed by direct comparison of their order of elution, retention time and MS data with those obtained for extracts of urine spiked with authentic standards (Table 8). The plasmaspray MS of each analyte demonstrated the presence of an intense protonated molecular ion. In the case of N-desmethylmesoridazine ring sulfoxide its identity was confirmed by the same techniques that were employed in its identification in rat urinary extracts (Section 4.2.5.1.). Mesoridazine N-oxide was only tentatively identified due to the lack of an authentic reference standard. Finally, the unconjugated phenolic derivatives of both sulforidazine (**36**) and mesoridazine (**40**) were detected in derivatized human urinary extracts (Table 8).

4.2.3.4. Quantification of metabolites of mesoridazine in three species

Table 8. The retention times and mass spectral data obtained by plasmaspray HPLC-mass spectrometric analysis of extracts of urine spiked with reference standards or obtained from rats, dogs and healthy volunteers administered oral doses of mesoridazine[a].

Compound number	Identity	R_t value (min)				MH^+ m/z value (intensity %)			
		Standard	Rat	Dog	Human	Standard	Rat	Dog	Human
14	Lactam of mesoridazine	4.6	4.6	nd	nd	401 (100)	401 (100)	nd	nd
18	Lactam of sulforidazine ring sulfoxide	4.7	4.6	4.6	4.7	433 (100)	433 (18)	433 (33)	433 (100)
3	Sulforidazine	5.0	5.1	5.1	5.1	403 (100)	403 (100)	403 (100)	403 (100)
21	Sulforidazine N-oxide	5.2	nd	5.3	5.2	419 (100)	nd	419 (92)	419 (100)
17	Lactam of mesoridazine ring sulfoxide	5.2	5.2	nd	5.1	417 (100)	417 (50)	nd	417 (100)
33	N-Desmethyl-mesoridazine ring sulfoxide		5.3	5.3	5.4		389 (51)	389 (88)	389 (100)
35	Sulforidazine ring sulfoxide	5.5	5.4	5.5	5.6	419 (100)	419 (100)	419 (100)	419 (100)
2	Mesoridazine	5.7	5.7	5.7	5.7	387 (100)	387 (100)	387 (100)	387 (100)
39	Mesoridazine N-oxide		nd	nd	6.2		nd	nd	403 (100)
38	Mesoridazine ring sulfoxide	7.2	7.3	7.2	7.2	403 (100)	403 (66)	403 (100)	403 (100)
36	Unconjugated hydroxylated sulforidazine		4.5	4.6	4.7		533 (5)	533 (12)	533 (6)
37	Conjugated hydroxylated sulforidazine		4.5	4.6	nd		533 (35)	533 (8)	nd
40	Unconjugated hydroxylated mesoridazine		nd	5.0	5.1		nd	517 (10)	517 (5)
41	Conjugated hydroxylated mesoridazine		5.0	nd	nd		517 (19)	nd	nd

[a] Mobile phase, 83% acetonitrile and 17% 0.047 M ammonium acetate. nd, Not detected. Mass spectral data show the appropriate protonated molecular ion (m/z) with the corresponding relative abundance (%) in parentheses. Where no reference standard was available only data for the metabolite are shown.

Since attempts to find a suitable mobile phase in which the peaks due to mesoridazine, sulforidazine, ring sulfoxide metabolites and the three lactam metabolites could be resolved from one another and endogenous interfering peaks were unsuccessful, two mobile phases were employed. Mobile phase D enabled quantification of all three lactam metabolites including each of the two diastereomers of the lactam of mesoridazine ring sulfoxide (Figure 12), while mobile phase E allowed quantification of mesoridazine, sulforidazine, mesoridazine ring sulfoxide and the two diastereomers of sulforidazine ring sulfoxide (Figure 19). The extraction efficiencies of measured analytes either were described previously with respect to the metabolism of sulforidazine (Section 4.2.4.) or are later discussed with respect to the metabolism of thioridazine (Section 4.2.9.), where the mean recoveries of the same or similar compounds were ≥80%. Two calibration curves were required for each analyte as the concentrations of the analytes were far lower in human urine than in dog or rat urine. The ranges of these calibration curves, their statistical relationships and other details are summarized in Table 9. The overall mean coefficient of variation for the five standard solutions over each range of the standard curve for each analyte varied between 3.1-8.3%. Moreover, the coefficient of variation at the lowest quantifiable limit of each

analyte (i.e. the lowest value of each standard curve range) never exceeded 15%.

For each of the three species the mean excretion of each of the measured compounds, where combined totals are given for diastereomers, is given in Table 10. The mean urinary excretion of the lactam of sulforidazine ring sulfoxide in the order of rat (n = 5), dog (n = 3) and human (n = 3) was found to be 0.2±0.1, 0.02±0.01 and 0.4±0.2%, respectively. Also the excretion of the lactam of mesoridazine ring sulfoxide in rat and human was 0.2±0.1 and 0.4±0.2%, respectively, while the corresponding excretion of the lactam of mesoridazine in rat was 0.05±0.05%. Finally the mean total excretions of the measured analytes in rat, dog and human were 2.6±1.1, 29.1±11.7 and 6.3±4.7%, respectively.

The mean diastereomeric ratios of the concentrations of the fast eluting diastereomer to that of the slow eluting diastereomer of sulforidazine ring sulfoxide for rat, dog and human were 1.13±0.02, 0.90±0.10 and 1.48±0.13, respectively, while in the case of the lactam of mesoridazine ring sulfoxide these ratios were 0.98±0.18 and 1.09±0.06 for rat and human, respectively.

4.2.3.5. Discussion of the results

There has been no systematic investigation of the

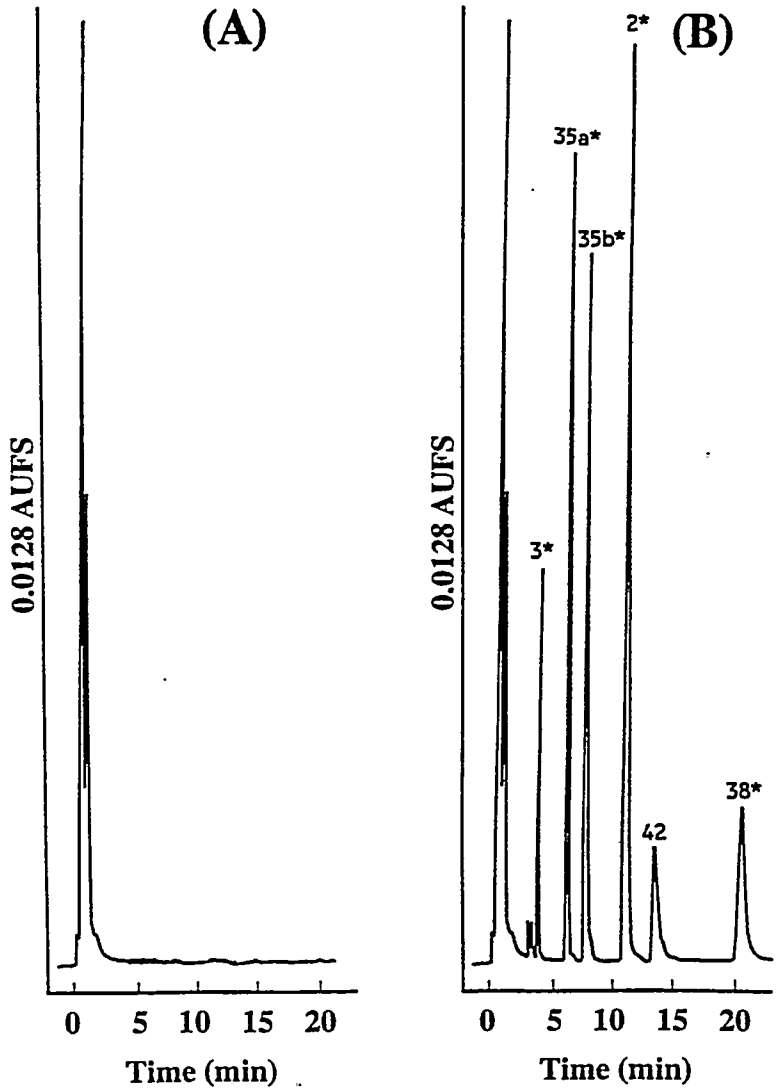


Figure 19. Chromatograms of the HPLC-UV assays for the quantification in urinary extracts of mesoridazine (**2**), sulforidazine (**3**), diastereomers of sulforidazine ring sulfoxide (**35a** and **35b**) and mesoridazine ring sulfoxide (**38**). (A) Blank human urine, (B) blank human urine with added reference standards, (C) urine of a rat dosed with mesoridazine, (D) urine of a dog dosed with mesoridazine and (E) urine of a healthy volunteer dosed with mesoridazine.

Mobile phase E: 92% acetonitrile, 8% 0.05 M ammonium acetate, 0.05% diethylamine. Quantified compounds are indicated by an asterisk. **14**, Lactam of mesoridazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **42**, prochlorperazine ring sulfoxide (internal standard).

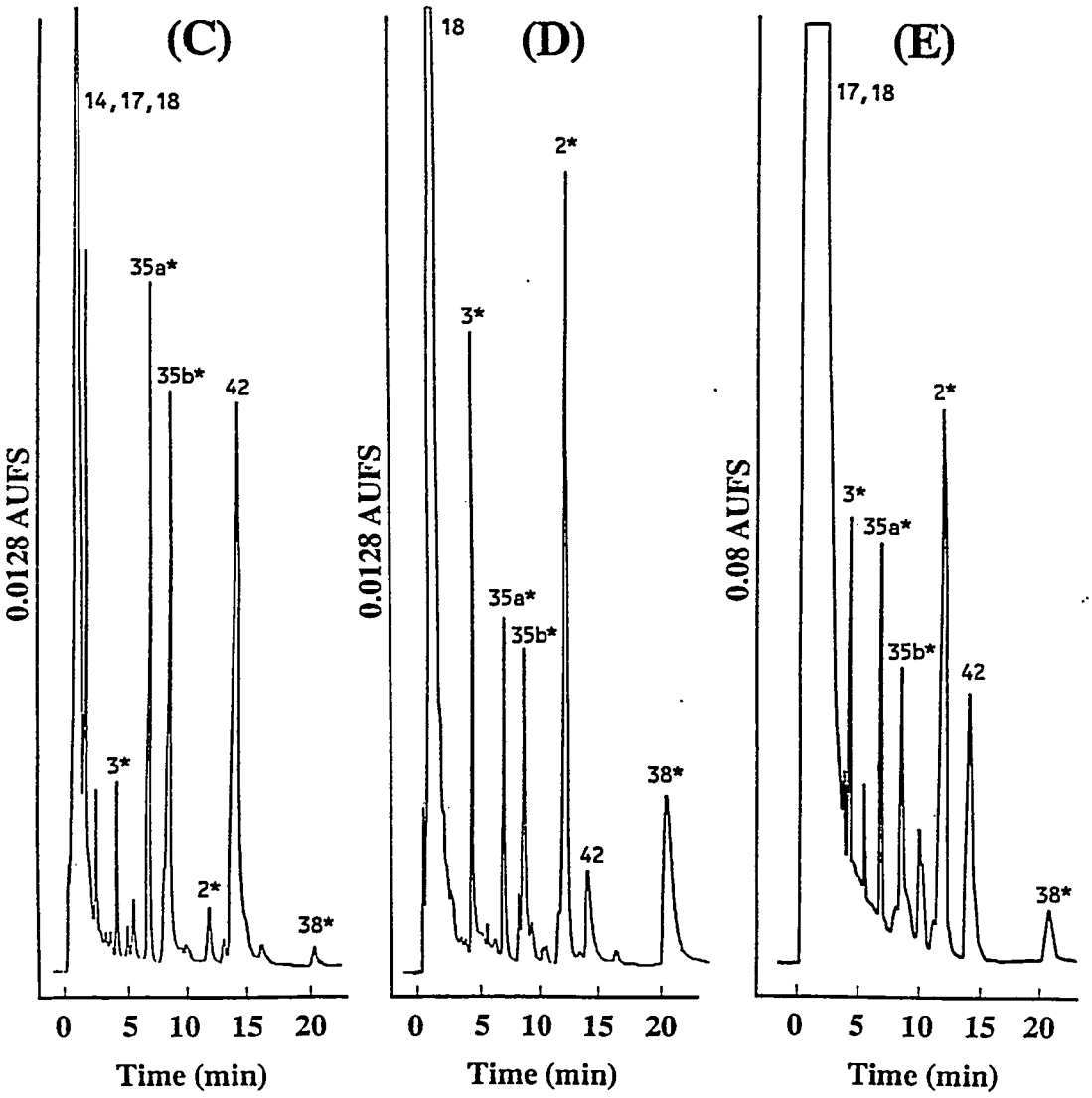


Figure 19. Continued.

Table 9. Calibration curve data for the quantification of mesoridazine and some of its metabolites in rat, dog and human urine by HPLC-UV procedures.

Analyte (compound number)	Mobile phase[a]	HPLC R_t value (min)	Species[b]	Standard curve (r^2)	Concentration range (µg/ml)[c]	Mean % CV[d]
Mesoridazine (2)	E	11.3	H	$y = 0.042x+0.0454$ (0.99)	0.01 - 0.6	4.4
			R,D	$y = 0.038x^2+0.166x+1.857$ (0.98)	1.0 - 20.0	3.1
Sulforidazine (3)	E	4.8	H	$y = -0.000223x^2+0.07x+0.159$ (0.98)	0.005 - 0.1	3.9
			R,D	$y = 0.125x^2+1.98x-0.033$ (0.98)	0.5 - 5.0	5.3
Sulforidazine ring sulfoxide, fast eluting diastereomer (35a)	E	7.0	H	$y = 0.022x+0.727$ (0.98)	0.01 - 5.0	4.5
			R,D	$y = -0.019x^2+0.905x-0.667$ (0.98)	1.0 - 20.0	4.9
Sulforidazine ring sulfoxide, slow eluting diastereomer (35b)	E	8.3	H	$y = 0.02x+0.625$ (0.98)	0.01 - 0.5	5.1
			R,D	$y = -0.017x^2+0.79x-0.574$ (0.98)	1.0 - 20.0	4.9
Lactam of sulforidazine ring sulfoxide (18)	D	20.6	H	$y = 0.002x-0.000104$ (0.98)	0.005 - 0.1	7.7
			R,D	$y = -0.027x^2+0.177x-0.008$ (0.96)	0.1 - 2.5	7.5
Mesoridazine ring sulfoxide (38)	E	18.3	H	$y = 0.008x+0.027$ (0.98)	0.02 - 0.3	6.7
			R,D	$y = 0.152x-0.096$ (0.98)	1.0 - 20.0	6.6
Lactam of mesoridazine (14)	D	7.8	R	$y = 0.765x+0.007$ (0.98)	1.0 - 2.0	6.1

Table 9. Continued.

Analyte (compound number)	Mobile phase[a]	HPLC R_t value (min)	Species[b]	Standard curve (r^2)	Concentration range (μg/ml)[c]	Mean % CV[d]
Lactam of mesoridazine ring sulfoxide, fast eluting diastereomer (17a)	D	16.1	H	$y = 0.0000112x^2+0.002x+0.0000342$ (0.98)	0.005 - 0.1	7.0
			R	$y = 0.203x+0.005$ (0.99)	0.1 - 2.0	5.8
Lactam of mesoridazine ring sulfoxide, slow eluting diastereomer (17b)	D	17.6	H	$y = 0.00000786x^2+0.002x-0.002$ (0.96)	0.005 - 0.1	7.2
			R	$y = 0.231x-0.002$ (0.99)	0.1 - 2.0	8.3

[a] Mobile phase D: 82% 2,2,4-trimethylpentane, 10% dichloromethane, 8% methanol, 0.01% diethylamine; mobile phase E: 92% acetonitrile, 8% 0.05 M ammonium acetate, 0.05% diethylamine.

[b] Calibration curve data used in the analysis of samples obtained from H, human; R, rat; D, dog.

[c] Five concentrations over each standard curve range were prepared in triplicate.

[d] The overall mean coefficient of variation of the mean values (n = 5) obtained at each concentration point of the standard curve range.

Table 10. The mean renal excretion of mesoridazine and some of its major metabolites in rats (n = 5), dogs (n = 3) and healthy male volunteers (n = 3) administered oral doses of mesoridazine.

Compound number	Identity	% Dose excretion for[a]		
		Rat	Dog	Human
2	Mesoridazine	0.2±0.2	9.6±7.0	3.4±4.4
3	Sulforidazine	0.1±0.1	3.7±1.2	0.5±0.1
14	Lactam of mesoridazine	0.05±0.05	nd	nd
17	Lactam of mesoridazine ring sulfoxide	0.2±0.1	nd	0.4±0.2
18	Lactam of sulforidazine ring sulfoxide	0.2±0.1	0.02±0.01	0.4±0.2
35	Sulforidazine ring sulfoxide	1.6±0.2	10.0±5.1	0.9±0.4
38	Mesoridazine ring sulfoxide	0.04±0.02	5.8±3.3	0.7±0.6

[a] nd, Not detected. The data are expressed as means±S.D. Dose of mesoridazine besylate was 28.1 mg/kg, 70.3 mg over 30 h and 35.2 mg in the case of rat, dog and human, respectively. Urine was collected over the periods 0-24 h, 0-72 h and 0-48 h for rat, dog and human, respectively.

metabolism of mesoridazine in any species, however, there are a number of reports to its metabolites in patients medicated with the drug. Thus the metabolites identified in human urine include sulforidazine, mesoridazine ring sulfoxide, sulforidazine ring sulfoxide, 7-hydroxy-mesoridazine and 7-hydroxysulforidazine (Papadopoulos et al. 1985, Papadopoulos and Crammer 1986), while those identified in faeces include 7-hydroxythioridazine, 7-hydroxy-N-desmethylthioridazine, 3-hydroxythioridazine, 7-hydroxy-sulforidazine and 7-hydroxy-N-desmethylsulforidazine (Papadopoulos et al. 1985). Finally the compounds quantified in plasma or serum include mesoridazine, thioridazine, sulforidazine, mesoridazine ring sulfoxide, thioridazine ring sulfoxide and N-desmethylthioridazine (Axelsson 1977, Kinon et al. 1979, Green et al. 1980, Sakalis et al. 1980).

Qualitative metabolite profiles The present work is the first report on the identification of the metabolites of mesoridazine in the urine of dog or rat. The systematic investigation of the urinary metabolic profile of mesoridazine in three species was greatly facilitated by the use of an on-line plasmaspray HPLC-MS technique, the advantages of which were previously discussed in this thesis (Section 4.2.4.). The compounds definitively identified in the urine of all three species were mesoridazine,

sulforidazine, mesoridazine ring sulfoxide, sulforidazine ring sulfoxide, N-desmethylmesoridazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide. In the case of N-desmethylmesoridazine ring sulfoxide, since only a reference sample of the N-acetyl derivative was available, its presence was authenticated by converting the metabolite to this derivative. The other compounds identified definitively but exhibiting species selectivity were the lactam of mesoridazine in rat, the lactam of mesoridazine ring sulfoxide in human and rat, and sulforidazine N-oxide in dog and human.

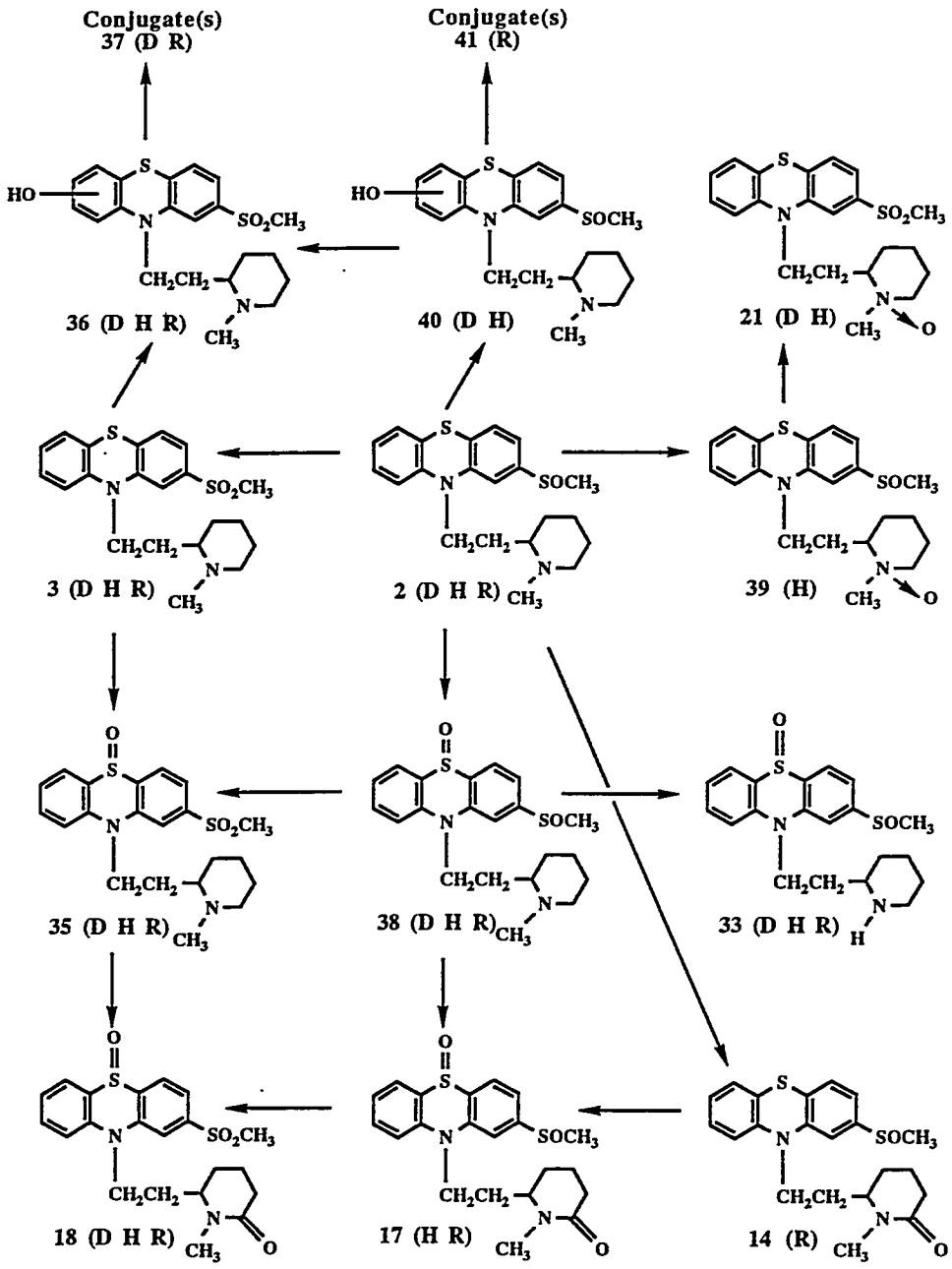
Tentative identification was made of mesoridazine N-oxide in human. A synthetic reference sample was not available and, in fact, selective chemical oxidation of the side chain nitrogen atom rather than the sulfur atoms of mesoridazine would be difficult. Tentative identification was made also in all three species of phenolic derivatives of mesoridazine and sulforidazine. However, only the unconjugated form of the phenolic derivative(s) of sulforidazine was(were) identified in the urine of all three species. Thus the conjugated form(s) of the phenolic derivative(s) of sulforidazine was(were) identified only in dog and rat, while the phenolic derivative(s) of mesoridazine was(were) identified in the urine of dog and human only in the unconjugated form and in the urine of rat

only in the conjugated form(s).

The proposed metabolic profiles of mesoridazine in the three species examined are shown in Scheme 9, where for the sake of simplicity not all possible routes to each metabolite are depicted.

Sulfoxide metabolites S-Oxidation of mesoridazine can occur at two sites; the 5-position of the phenothiazine ring and the 2-sulfoxide substituent. At least four metabolites were identified for each species where the 2-sulfoxide substituent had been converted to the 2-sulfone. In no case was a metabolite detected where the 2-sulfoxide substituent had been reduced to the 2-sulfide. This observation is surprising in that in human thioridazine, thioridazine ring sulfoxide and N-desmethylthioridazine have been quantified in the plasma or serum of some patients medicated with mesoridazine (Axelsson 1977, Green et al. 1980, Sakalis et al. 1980).

In each of the species at least four of the identified metabolites had a sulfoxide group at the 5-position of the phenothiazine ring and it was previously mentioned (Section 4.2.4.) that such metabolites can be chromatographically separated into their diastereomers. In the present study although no such separation occurred on plasmaspray HPLC-MS analysis, both pairs of diastereomers of sulforidazine ring sulfoxide and the lactam of mesoridazine ring sulfoxide were



Scheme 9. The proposed metabolic pathways of mesoridazine in rat (R), dog (D) and human (H). All possible routes to each metabolite are not depicted. **2**, Mesoridazine; **3**, sulforidazine; **14**, lactam of mesoridazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **33**, N-desmethylmesoridazine ring sulfoxide; **35**, sulforidazine ring sulfoxide; **38**, mesoridazine ring sulfoxide; **39**, mesoridazine N-oxide; **36**, unconjugated hydroxylated sulforidazine; **37**, conjugated hydroxylated sulforidazine; **40**, unconjugated hydroxylated mesoridazine; **41**, conjugated hydroxylated mesoridazine.

separately quantified using conventional HPLC-UV analysis and mobile phases E (Figure 19) and D (Figure 12), respectively. Although the diastereomers of N-desmethylmesoridazine ring sulfoxide or the lactam of sulforidazine ring sulfoxide were not resolved using either of these mobile phases, the diastereomers of mesoridazine ring sulfoxide were resolved from one another using mobile phase D (Figure 12). However, interfering peaks, including that of other analytes precluded separate analysis of these diastereomers.

There are no reports to stereoselectivity in the metabolism of mesoridazine. The diastereomers of sulforidazine ring sulfoxide were separately quantified in all three species while the diastereomers of the lactam of mesoridazine ring sulfoxide were separately quantified in only human and rat. The mean diastereomeric ratio was determined in each case and only for sulforidazine ring sulfoxide in human (1.48±0.13) did this ratio vary markedly from unity. In fact, this mean ratio is very similar to that previously obtained for the same analytes in urine after administration of sulforidazine to human (1.42 ± 0.04) as shown in Section 4.2.3.

Lactam metabolites The tentative identification of the lactams of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide in the urine of patients is the only report

to the metabolism of the piperidine ring of mesoridazine in any species (Papadopoulos and Crammer 1986). Both of these lactams as well as the lactam of mesoridazine were definitively identified in the present work. In fact, as compared to the other metabolites that were quantified both the lactams of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide were found in appreciable amounts in the urine of both human and rat. However, the lactam of sulforidazine ring sulfoxide was only a minor metabolite in dog while the lactam of mesoridazine was a minor metabolite in rat only.

N-Oxide metabolites Sulforidazine N-oxide identified in the urine of dog and human and mesoridazine N-oxide identified in human urine were not quantified, although inspection of the HPLC-UV chromatograms (data not shown) gave indication that each was present as a minor metabolite. It is noteworthy that N-oxide metabolites have also been found in human and dog with other antipsychotic drugs (Gruenke et al. 1985, Yeung et al. 1987, Jaworski et al. 1990).

N-Demethylated metabolites N-Desmethylmesoridazine ring sulfoxide was the only metabolite formed via N-demethylation that was identified in the urine of each of the three species. N-Desmethylmesoridazine could not be detected as a metabolite of mesoridazine despite the

availability of a reference sample. Nevertheless further studies are necessary to establish whether N-desmethyl-mesoridazine ring sulfoxide is formed preferentially from mesoridazine by S-oxidation followed by N-demethylation rather than vice versa.

Hydroxylated metabolites The preparation of the tert.-butyldimethylsilyl derivatives and analysis by plasmaspray HPLC-MS gave convincing evidence that unconjugated and/or conjugated forms of the phenols of both mesoridazine and sulforidazine were metabolites of mesoridazine in all three species. The position of substitution of each aromatic hydroxyl group was not determined. Generally with phenothiazine antipsychotic agents aromatic hydroxylation occurs predominantly at the 3- and 7-positions of the phenothiazine ring (Jørgensen 1986). In fact, in the case of humans medicated with mesoridazine, other workers have definitively identified 7-hydroxymesoridazine and 7-hydroxysulforidazine as urinary metabolites (Papadopoulos et al. 1985).

Interspecies comparisons Qualitative and quantitative comparisons can be made from this study between rat, dog and human in terms of the urinary metabolites of mesoridazine after its oral administration, and the data are summarized in Scheme 9 and Tables 8 and 10. Large interspecies variations were observed both qualitatively and

quantitatively with respect to the metabolites of mesoridazine present in urinary extracts. For example, only seven of the ten or more metabolites encountered for each species were common to all three species. However, various routes of metabolism were encountered in each case. Thus S-oxidation was a major route of metabolism in all three species. For example, eight metabolites (although not the same eight) were identified in each of the three species where the phenothiazine ring sulfur atom and/or the sulfoxide group of the 2-substituent was oxidized. Also aromatic hydroxylation of the phenothiazine ring, N-demethylation of the piperidine ring N-substituent and lactam formation of the piperidine ring were common to all three species. However, N-oxide metabolites were found in both human and dog but not rat. On the other hand, it appears from this study that the overall metabolic profile for human more closely resembled rat than dog. Thus the mean excretion of mesoridazine and the mean total excretion of the measured analytes was far less in human (3.4±4.4 and 6.3±4.7%, respectively) and rat (0.2±0.2 and 2.6±1.1%, respectively) than dog (9.6±7.0 and 29.1±11.7%, respectively). Moreover the mean total excretion of lactam metabolites was far more pronounced in human and rat than dog in that their total excretion expressed as percent dose administered and percent drug accounted for were 0.9±0.3 and

11.3±3.4%, 0.5±0.4 and 16.5±0.9%, and 0.02±0.01 and 0.06±0.01%, respectively. Hence it would appear that rat is a more suitable animal than dog to undertake further investigation of the C-oxidation of the piperidine ring of mesoridazine.

4.2.4. Urinary metabolites of thioridazine

4.2.4.1. Characterization of metabolites of thioridazine in human

The examination of the metabolites of thioridazine in human urine was carried out by plasmaspray HPLC-MS techniques. The typical chromatograms obtained in the on-line plasmaspray HPLC-MS analysis of a human underivatized urinary extract are shown in Figure 20. The reconstructed total ion chromatogram (Figure 20(RTIC)) does not show clearly resolved chromatographic peaks; however, when the use of the appropriate quasi-molecular ion of each suspected metabolite was employed to reconstruct the individual chromatograms a much clearer resolution of peaks was obtained (Figure 20). The plasmaspray MS obtained for each of the peaks in the reconstructed ion chromatograms indicated that eight metabolites were present (Figure 21). In order of elution these metabolites were the lactam of sulforidazine ring sulfoxide (**18**), sulforidazine (**3**), sulforidazine N-oxide (**21**), the lactam of mesoridazine ring

sulfoxide (**17**), sulforidazine ring sulfoxide (**35**), mesoridazine (**2**), thioridazine ring sulfoxide (**43**) and mesoridazine ring sulfoxide (**38**). The identities of all detected metabolites were confirmed by direct comparison of their order of elution, retention times and MS data with those obtained for authentic standards from spiked extracts of urine (Table 11). Each plasmaspray MS exhibited an intense protonated molecular ion (MH^+). Intact thioridazine was not detected in the urinary extracts.

For the identification of phenolic metabolites the extracts of human urine obtained with or without prior enzymic hydrolysis were silylated with MTBSTFA prior to on-line HPLC-MS analysis. These analyses showed in both cases that two additional peaks were observed in the reconstructed ion chromatograms compared to those of underivatized peaks. In the case of non-enzymically treated extracts the positive ion plasmaspray spectra of the two peaks gave highest mass ions at m/z 517 and 533 that corresponded to the protonated molecular ions of the tert.-butyldimethylsilylated phenolic analogues of mesoridazine and sulforidazine, respectively. For enzymically treated extracts the analogous highest mass ions of m/z 501 and 517 corresponded to the protonated molecular ions of the tert.-butyldimethylsilylated phenolic derivatives of thioridazine and mesoridazine, respectively (Table 11). The position of the aromatic hydroxyl group in

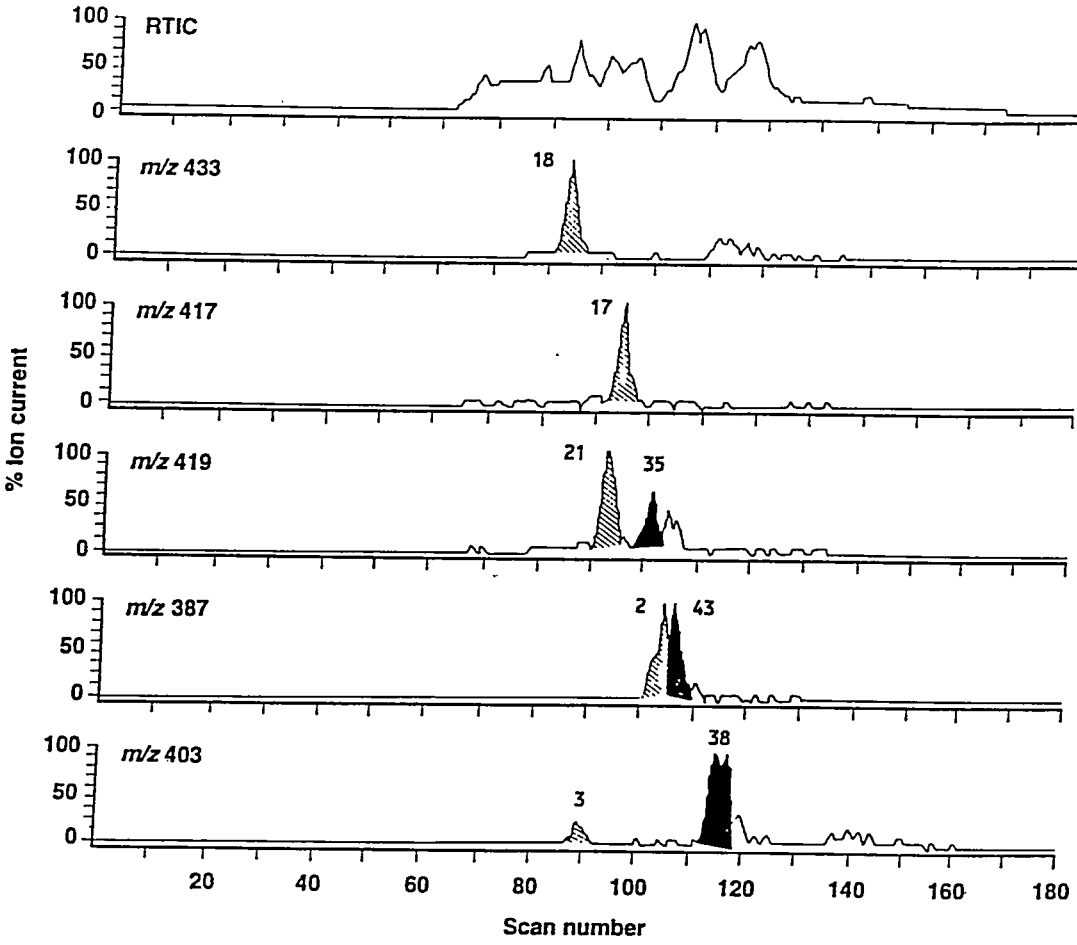


Figure 20. Reconstructed total ion chromatogram and reconstructed ion chromatograms of plasmaspray positive ion HPLC-mass spectrometric analysis of extracts of urine of a man dosed with thioridazine.

RTIC, reconstructed total ion current. **2**, Mesoridazine; **3**, sulforidazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **35**, sulforidazine ring sulfoxide; **38**, mesoridazine ring sulfoxide; **43**, thioridazine ring sulfoxide.

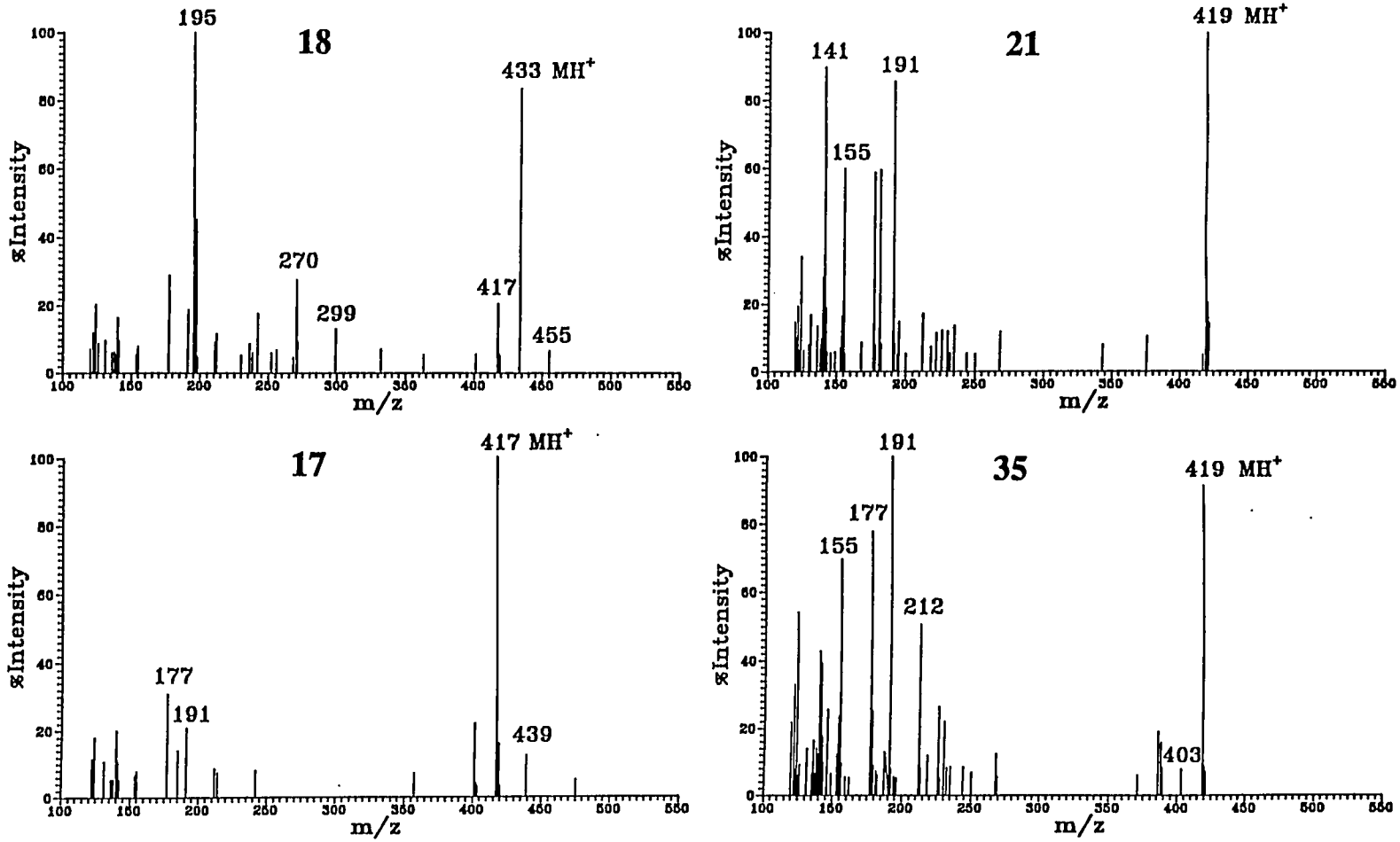


Figure 21. Plasmaspray mass spectra of each individual compound corresponding to the appropriate reconstructed ion chromatogram in Figure 20.
The identities of the compounds are indicated in Figure 20.

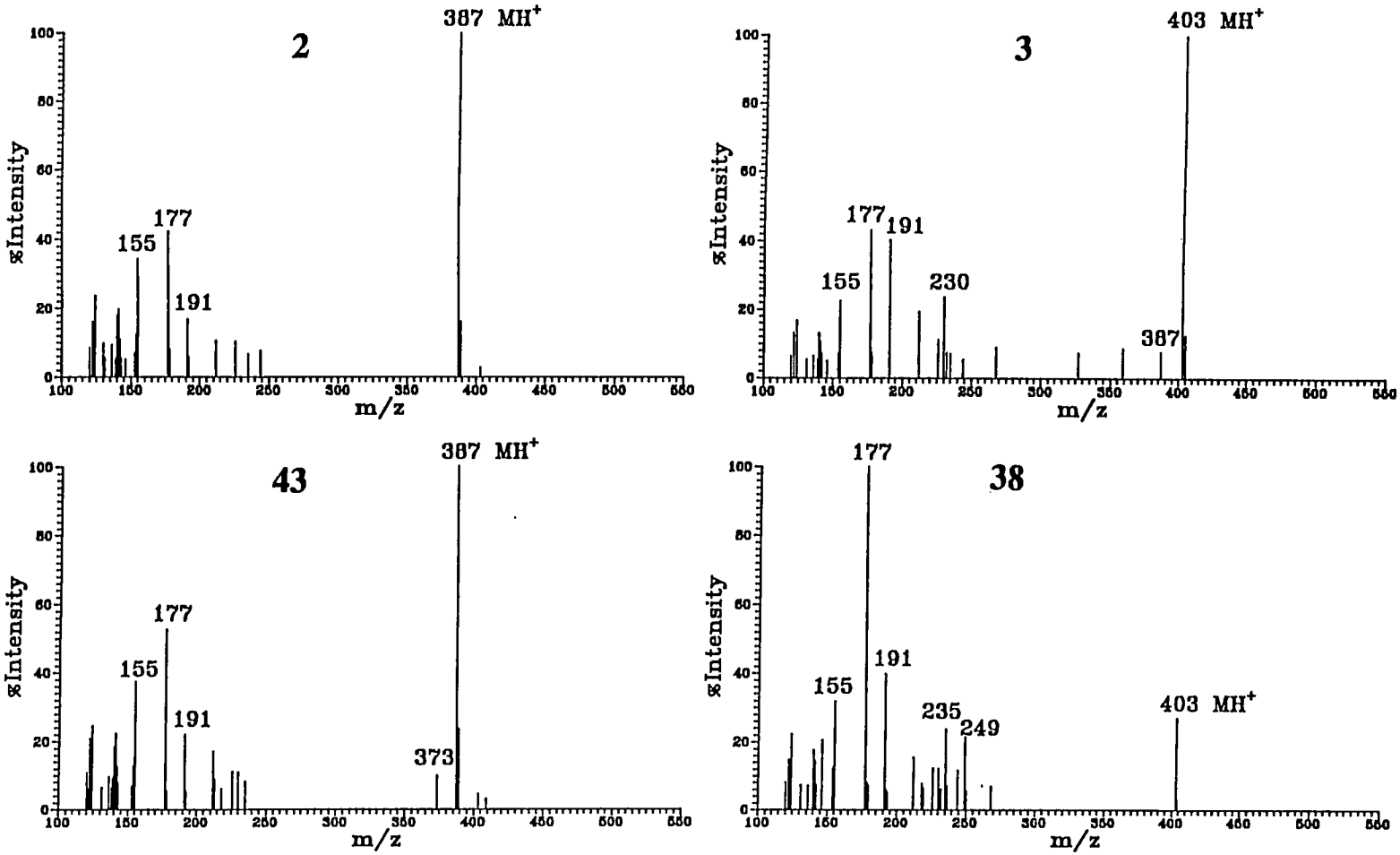


Figure 21. Continued.

each of the phenolic metabolites could not be assigned due to the lack of authentic standards.

4.2.4.2. Characterization of metabolites of thioridazine in rat

The same plasmaspray HPLC-MS techniques used in the urinalysis of human were employed for the investigation of the extracts of rat urine. The compounds identified in the non-derivatized and/or derivatized extracts of urine of rat were thioridazine (**1**), mesoridazine (**2**), sulforidazine (**3**), the lactam of mesoridazine ring sulfoxide (**17**), the lactam of sulforidazine ring sulfoxide (**18**), N-desmethyl-thioridazine ring sulfoxide (**32**), N-desmethylmesoridazine ring sulfoxide (**33**), sulforidazine ring sulfoxide (**35**), mesoridazine ring sulfoxide (**38**), thioridazine ring sulfoxide (**43**), and the unconjugated form of the phenols of sulforidazine (**36**), mesoridazine (**40**) and thioridazine (**44**). The identity of each detected compound from the underivatized extracts, with the exception of both N-demethylated metabolites **32** and **33**, was confirmed by direct comparison of its order of elution, retention time and MS data with that of an authentic standard from spiked extracts of urine as summarized in Table 11. Each plasmaspray MS exhibited a significant protonated molecular ion (MH^+).

In the case of N-desmethylmesoridazine ring sulfoxide

(**33**), a synthetic standard was not available. However, its chromatographic behaviour and mass spectral characteristics were identical to those of the metabolite of mesoridazine unequivocally identified as such in the metabolism of mesoridazine in rat (Section 4.2.5.1.).

Regarding N-desmethylthioridazine ring sulfoxide (**32**), its structural identity was confirmed by its conversion to the N-acetyl derivative and subsequent comparison to an authentic standard. The appropriate fraction isolated from the conventional HPLC system (mobile phase E, retention time 10.2 min) was treated with acetic anhydride. Plasmaspray HPLC-MS analysis indicated that the retention times and MS data of the acetylated derivative of the metabolite (Figure 22(B)) and the synthetic standard (Figure 22(A)) were identical.

4.2.4.3. Characterization of metabolites of thioridazine in dog

Direct plasmaspray HPLC-MS analysis of the underivatized and derivatized extracts of dog urine enabled identification of the following compounds: thioridazine (**1**), mesoridazine (**2**), sulforidazine (**3**), the lactam of mesoridazine ring sulfoxide (**17**), N-desmethylthioridazine ring sulfoxide (**32**), N-desmethylmesoridazine ring sulfoxide (**33**), sulforidazine ring sulfoxide (**35**), mesoridazine ring

Table 11. The retention times and mass spectral data obtained by plasmaspray HPLC-mass spectrometric analysis of extracts of urine spiked with reference standards or obtained from rats, dogs and healthy volunteers administered oral doses of thioridazine[a].

Compound number	Identity	R_t value (min)				MH^+ m/z value (intensity %)			
		Standard	Rat	Dog	Human	Standard	Rat	Dog	Human
1	Thioridazine	5.0	5.0	4.9	nd	371 (100)	371 (42)	371 (100)	nd
18	Lactam of sulforidazine ring sulfoxide	4.7	4.6	nd	4.7	433 (100)	433 (29)	nd	433 (84)
3	Sulforidazine	5.0	4.9	5.1	5.0	403 (100)	403 (40)	403 (100)	403 (100)
21	Sulforidazine N-oxide	5.2	nd	nd	5.1	419 (100)	nd	nd	419 (100)
17	Lactam of mesoridazine ring sulfoxide	5.2	5.2	5.2	5.2	417 (100)	417 (30)	417 (19)	417 (100)
33	N-Desmethyl-mesoridazine ring sulfoxide		5.4	5.4	nd		389 (15)	389 (15)	nd
35	Sulforidazine ring sulfoxide	5.5	5.6	5.6	5.5	419 (100)	419 (100)	419 (100)	419 (100)
2	Mesoridazine	5.7	5.7	5.7	5.6	387 (100)	387 (100)	387 (100)	387 (100)
43	Thioridazine ring sulfoxide	5.9	5.9	5.9	5.8	387 (100)	387 (34)	387 (100)	387 (100)
32	N-Desmethyl-thioridazine ring sulfoxide		6.0	6.0	nd		373 (16)	373 (4)	nd
38	Mesoridazine ring sulfoxide	7.2	7.1	7.1	7.3	403 (100)	403 (23)	403 (14)	403 (24)
44	Unconjugated hydroxylated thioridazine		4.0	nd	nd		501 (15)	nd	nd
45	Conjugated hydroxylated thioridazine		nd	nd	4.1		nd	nd	501 (100)

Table 11. Continued.

Compound number	Identity	R_t value (min)				MH^+ m/z value (intensity %)			
		Standard	Rat	Dog	Human	Standard	Rat	Dog	Human
36	Unconjugated hydroxylated sulforidazine		4.5	4.6	4.5		533 (8)	533 (11)	533 (11)
40	Unconjugated hydroxylated mesoridazine		5.0	4.9	5.0		517 (60)	517 (5)	517 (100)
41	Conjugated hydroxylated mesoridazine		nd	nd	4.9		nd	nd	517 (17)

[a] Mobile phase: 83% acetonitrile and 17% 0.047 M ammonium acetate. nd, Not detected. Mass spectral data show the appropriate protonated molecular ion (m/z) with the corresponding relative abundance (%) in parentheses. Where no reference standard was available only data for the metabolite are shown.

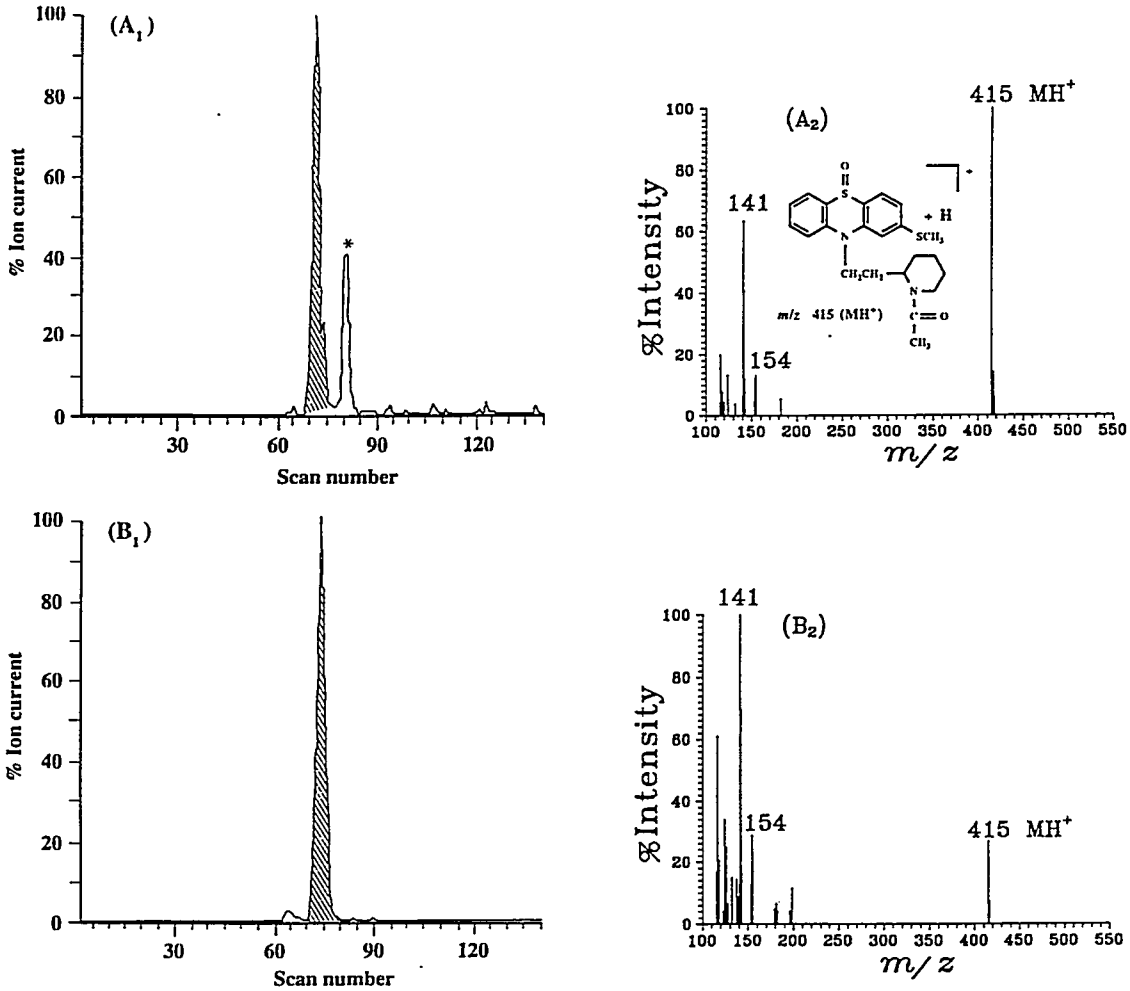


Figure 22. Positive ion plasmaspray HPLC-mass spectrometric analysis of (A) authentic N-acetyl-N-desmethylthioridazine ring sulfoxide and (B) acetic anhydride treated collected HPLC fractions that were in turn obtained from extracts of urine from a rat dosed with thioridazine.

A_1 and B_1, reconstructed ion chromatograms for m/z 415 (protonated molecular ion of N-acetyl-N-desmethyl-thioridazine ring sulfoxide); A_2 and B_2, positive ion mass spectra for scan 71 of A_1 and B_1, respectively. *N-Acetyl-N-desmethylthioridazine (impurity in synthetic standard).

sulfoxide (**38**), thioridazine ring sulfoxide (**43**) and the unconjugated form of phenols of mesoridazine (**40**) and sulforidazine (**36**) (Table 11).

4.2.4.4. Quantification of metabolites of thioridazine in three species

The quantification of both lactam metabolites, thioridazine and some of the other urinary excreted compounds was performed by using HPLC-UV assays. Since a mobile phase could not be found in which the peaks due to all these analytes were resolved from one another and endogenous interfering peaks two mobile phases had to be employed. Mobile phase E allowed quantification of mesoridazine, mesoridazine ring sulfoxide and each of the diastereomers of sulforidazine ring sulfoxide (Figure 23), while mobile phase F enabled determination of both lactam metabolites, including each of the diastereomers of the lactam of mesoridazine ring sulfoxide, and also unchanged thioridazine and each of the diastereomers of thioridazine ring sulfoxide (Figure 24).

The mean extraction efficiencies of each of the analytes (Table 12) as well as the internal standards (lactam of mesoridazine, 87.2±5.3%; prochlorperazine ring sulfoxide, 85.6±8.3%) were determined to be ≥80%, and quality control samples (four or more of the six samples

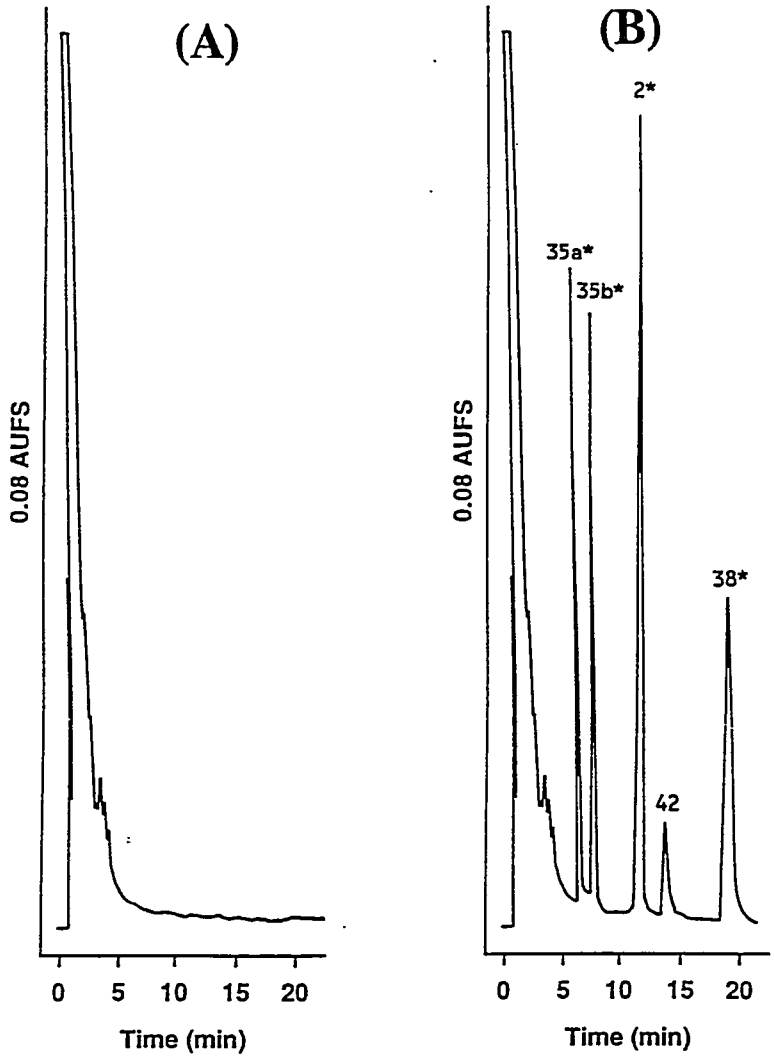


Figure 23. Chromatograms of the HPLC-UV assays for the quantification in urinary extracts of mesoridazine (**2**), diastereomers of sulforidazine ring sulfoxide (**35a** and **35b**) and mesoridazine ring sulfoxide (**38**). (A) Blank human urine, (B) blank human urine spiked with authentic standards, (C) urine of a rat dosed with thioridazine, (D) urine of a dog dosed with thioridazine and (E) urine of a healthy volunteer dosed with thioridazine.

Mobile phase E: 92% acetonitrile, 8% 0.05 M ammonium acetate, 0.05% diethylamine. Quantified compounds are indicated by an asterisk. **1**, Thioridazine; **3**, sulforidazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **32**, N-desmethyl-thioridazine ring sulfoxide; **33**, N-desmethylmesoridazine ring sulfoxide; **43**, thioridazine ring sulfoxide; **42**, prochlorperazine ring sulfoxide (internal standard).

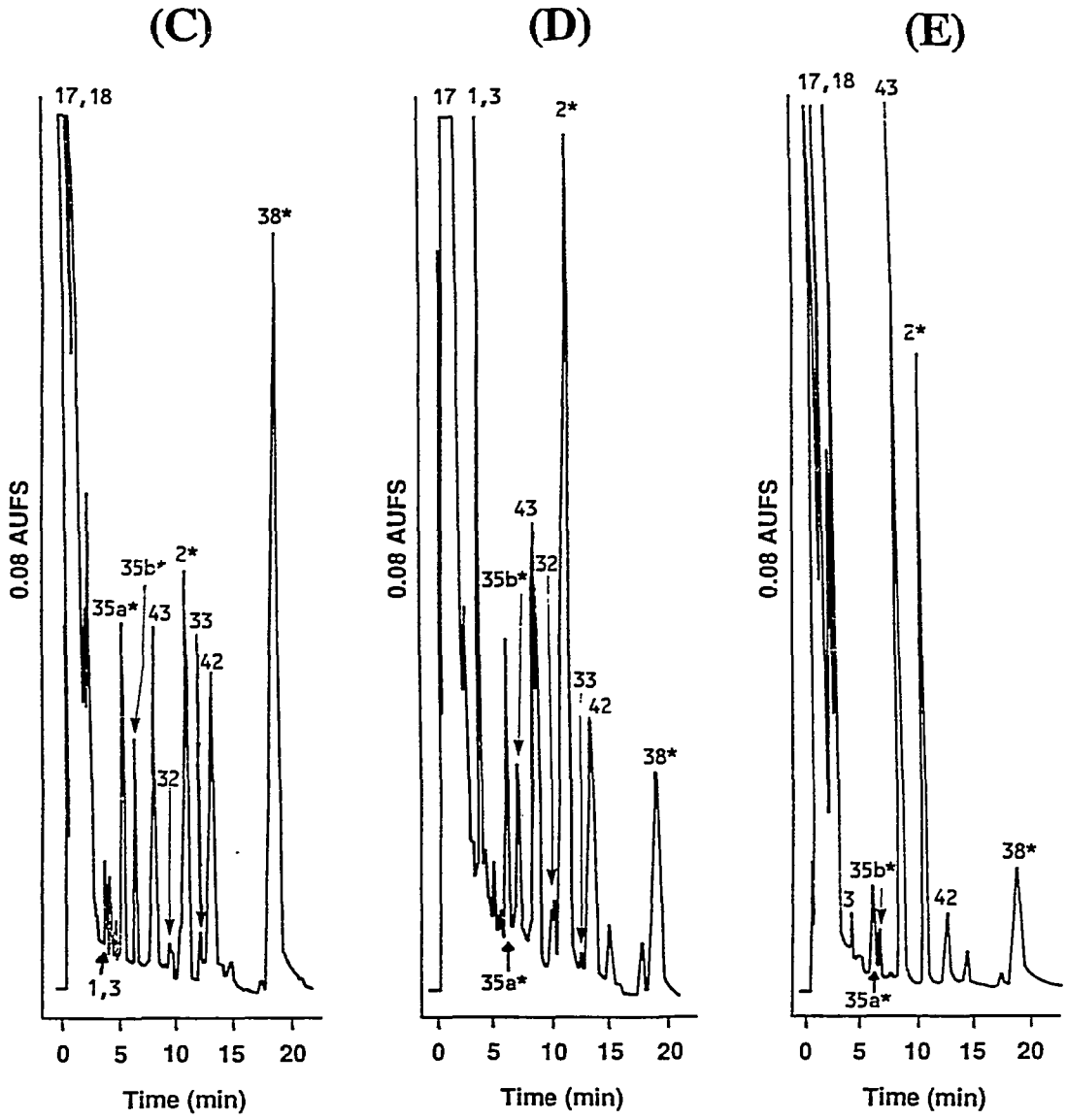


Figure 23. Continued.

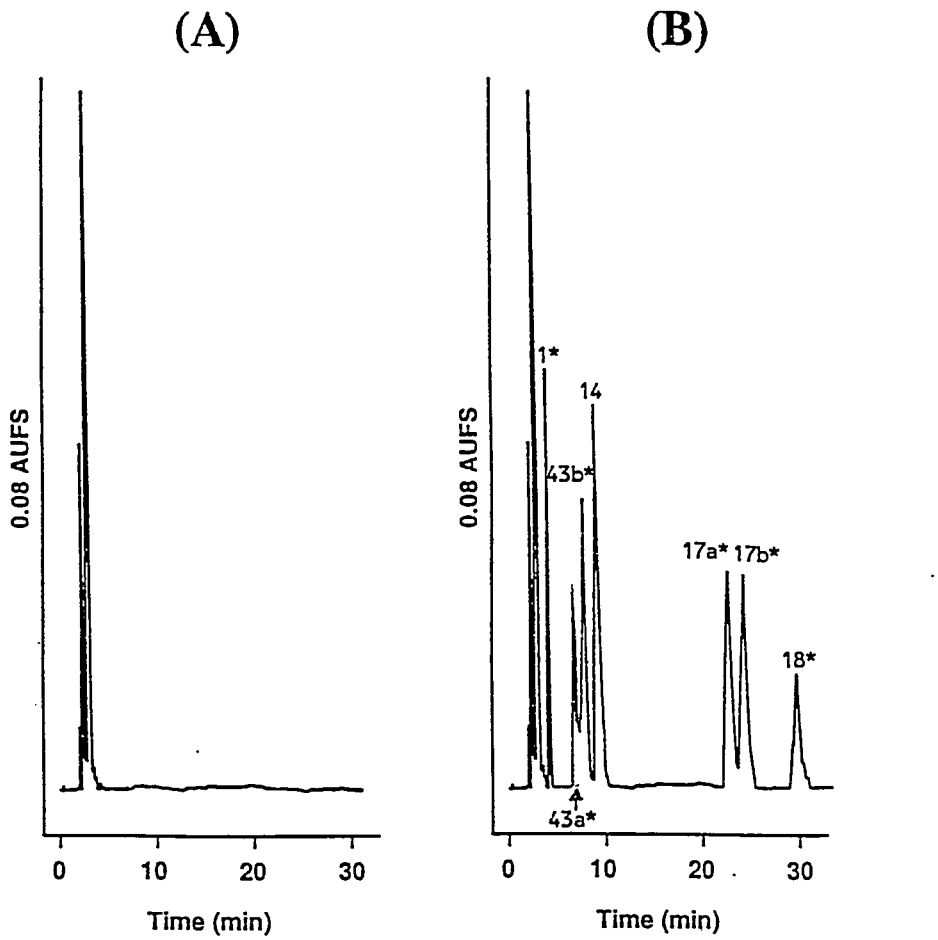


Figure 24. Chromatograms of the HPLC-UV assays for the quantification in urinary extracts of thioridazine (**1**), diastereomers of thioridazine ring sulfoxide (**43a** and **43b**), diastereomers of lactam of mesoridazine ring sulfoxide (**17a** and **17b**) and lactam of sulforidazine ring sulfoxide (**18**). (A) Blank human urine, (B) blank human urine spiked with authentic standards, (C) urine of a rat dosed with thioridazine, (D) urine of a dog dosed with thioridazine and (E) urine from a healthy volunteer dosed with thioridazine.

Mobile phase F: 82% 2,2,4-trimethylpentane, 10% dichloromethane, 8% methanol, 0.1% diethylamine. Quantified compounds are indicated by an asterisk. **2**, Mesoridazine; **3**, sulforidazine; **21**, sulforidazine N-oxide; **35a** and **35b**, diastereomers of sulforidazine ring sulfoxide; **38a** and **38b**, diastereomers of mesoridazine ring sulfoxide; **14**, lactam of mesoridazine (internal standard).

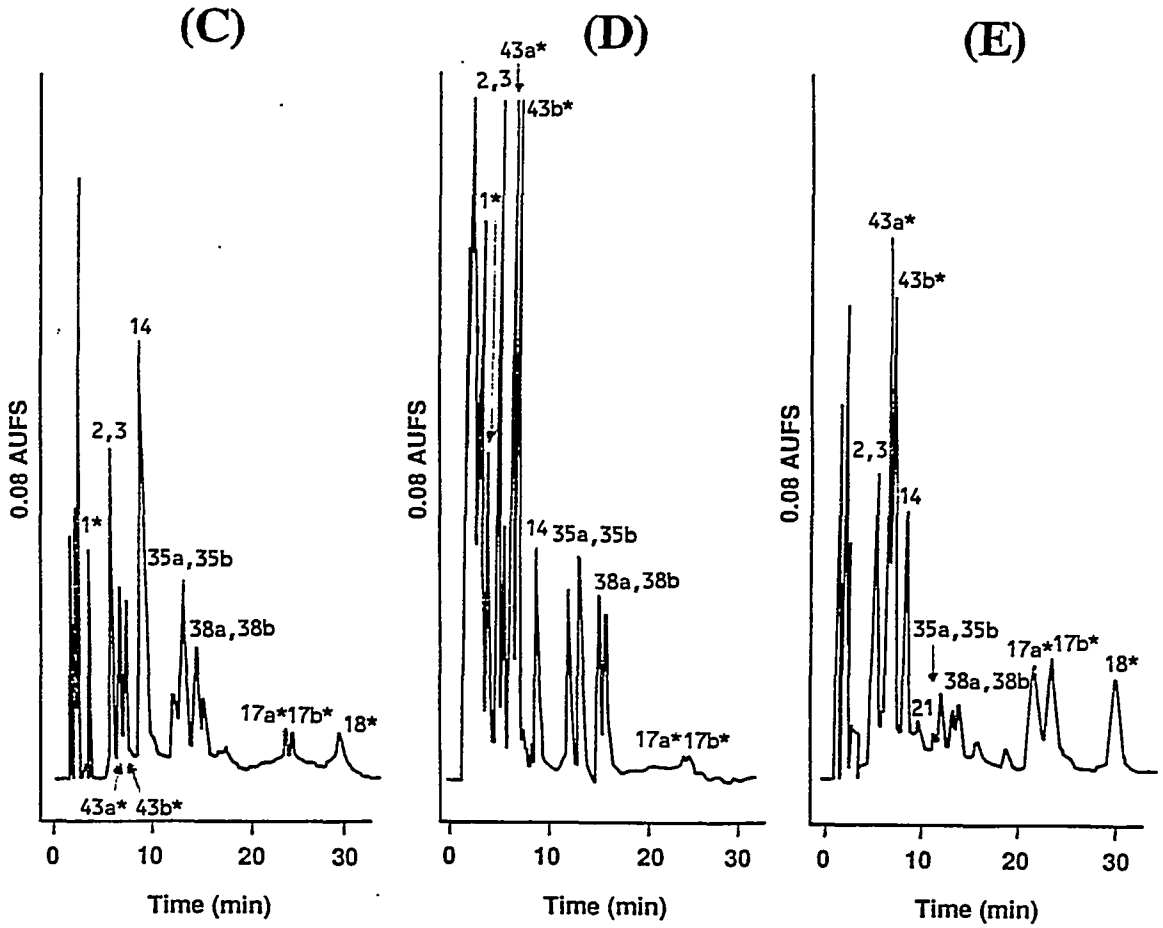


Figure 24. Continued.

were within ±18% of the nominal values) indicated that the assays were accurate and reproducible. The range, statistical relationships and other details of the calibration curve of each analyte are summarized in Table 12. The mean coefficient of variation for the five standard solutions over each range of the standard curve for each analyte varied between 4.0-8.2%. Also the coefficient of variation at the lowest quantifiable limit for each analyte was less than 15%.

The mean urinary excretions of each analyte in human (0-48 h, n = 3), rat (0-24 h, n = 5) and dog (0-72 h, n = 3), where combined totals are given for resolved diastereomers are given in Table 13. The respective mean excretions of the lactam of mesoridazine ring sulfoxide were 0.2±0.2, <0.02 and 1.2±1.0% for rat, dog and human, while the corresponding excretions of the lactam of sulforidazine ring sulfoxide in rat and human were 0.2±0.2 and 0.5±0.4%, respectively. The mean total urinary excretions of the measured compounds in rat, dog and human were determined to be 4.8±1.7, 12.1±5.4 and 4.3±2.9%, respectively.

The mean diastereomeric ratios of the concentrations of the fast eluting diastereomer to that of the slow eluting diastereomer of thioridazine ring sulfoxide and sulforidazine ring sulfoxide for rat, dog and human were found to be 1.39±0.14 and 1.53±0.08, 1.70±0.06 and

Table 12. Calibration curve data for the quantification of thioridazine and some of its metabolites in rat, dog and human urine by HPLC-UV procedures.

Analyte (compound number)	Mobile phase[a]	HPLC R_t value (min)	Extraction efficiency (%)[b]	Standard curve (r^2)	Concentration range (ng/ml)[c]	Mean % CV[d]
Thioridazine (1)	F	2.9	80.0±15.6	y = 0.013x+1.546 (0.99)	50-1000	7.1
Mesoridazine (2)	E	10.9	83.2±5.3	y = 0.020x+0.149 (0.99)	20-800	6.5
Thioridazine ring sulfoxide, fast eluting diastereomer (43a)	F	7.7	89.2±5.1	y = 0.003x+0.121 (0.96)	20-800	6.0
Thioridazine ring sulfoxide, slow eluting diastereomer (43b)	F	8.2	90.2±3.4	y = 0.006x+0.058 (0.96)	20-800	6.4
Mesoridazine ring sulfoxide (38)	E	18.2	80.3±1.6	y = 0.004x+0.208 (0.99)	50-1000	7.6
Sulforidazine ring sulfoxide, fast eluting diastereomer (35a)	E	6.6	88.3±2.3	y = 0.016x+0.323 (0.99)	20-800	8.2
Sulforidazine ring sulfoxide, slow eluting diastereomer (35b)	E	8.0	88.9±1.8	y = 0.015x+0.339 (0.99)	20-800	8.2

Table 12. Continued.

Analyte (compound number)	Mobile phase[a]	HPLC R_t value (min)	Extraction efficiency (%)[b]	Standard curve (r^2)	Concentration range (ng/ml)[c]	Mean % CV[d]
Lactam of mesoridazine ring sulfoxide, fast eluting diastereomer (17a)	F	23.3	84.1±7.3	y =0.001x+0.074 (0.99)	50-500	4.9
Lactam of mesoridazine ring sulfoxide, slow eluting diastereomer (17b)	F	25.0	86.9±2.0	y = 0.001x+0.077 (0.99)	50-500	4.2
Lactam of sulforidazine ring sulfoxide (18)	F	30.5	83.8±5.6	y = 0.001x+0.005 (0.99)	20-500	4.0

[a] Mobile phase E: 92% acetonitrile, 8% 0.05 M ammonium acetate, 0.05% diethylamine; mobile phase F: 82% 2,2,4-trimethylpentane, 10% dichloromethane, 8% methanol, 0.1% diethylamine.

[b] The overall mean±S.D. extraction efficiency of the mean values (n = 3) obtained at three different concentrations over the standard curve range.

[c] Five concentrations over each standard curve range were prepared in triplicate.

[d] The overall mean coefficient of variation of the mean values (n = 5) obtained at each concentration point of the standard curve range.

Table 13. The mean renal excretion of thioridazine and some of its major metabolites in rats (n = 5), dogs (n = 3) and healthy male volunteers (n = 3) administered oral doses of thioridazine.

Compound number	Identity	% Dose excretion for[a]		
		Rat	Dog	Human
1	Thioridazine	0.9±0.8	0.7±0.1	nd
2	Mesoridazine	0.4±0.1	2.3±1.6	0.4±0.3
17	Lactam of mesoridazine ring sulfoxide	0.2±0.2	<0.02	1.2±1.0
18	Lactam of sulforidazine ring sulfoxide	0.2±0.2	nd	0.5±0.4
35	Sulforidazine ring sulfoxide	0.8±0.2	2.2±1.3	0.3±0.2
38	Mesoridazine ring sulfoxide	1.6±0.9	2.4±2.0	0.4±0.3
43	Thioridazine ring sulfoxide	0.7±0.2	4.6±0.8	1.6±1.4

[a] nd, Not detected. The data are expressed as means±S.D. Dose of thioridazine hydrochloride was 21.9 mg/kg, 109.7 mg over 30 h and 54.9 mg in the case of rat, dog and human, respectively. Urine was collected over the periods 0-24 h, 0-72 h and 0-48 h for rat, dog and human, respectively.

1.37±0.03, and 1.86±0.12 and 1.41±0.11, respectively, while in the case of the lactam of mesoridazine ring sulfoxide this ratio was 1.32±0.26 and 0.94±0.07 for rat and human, respectively.

4.2.4.5. Discussion of the results

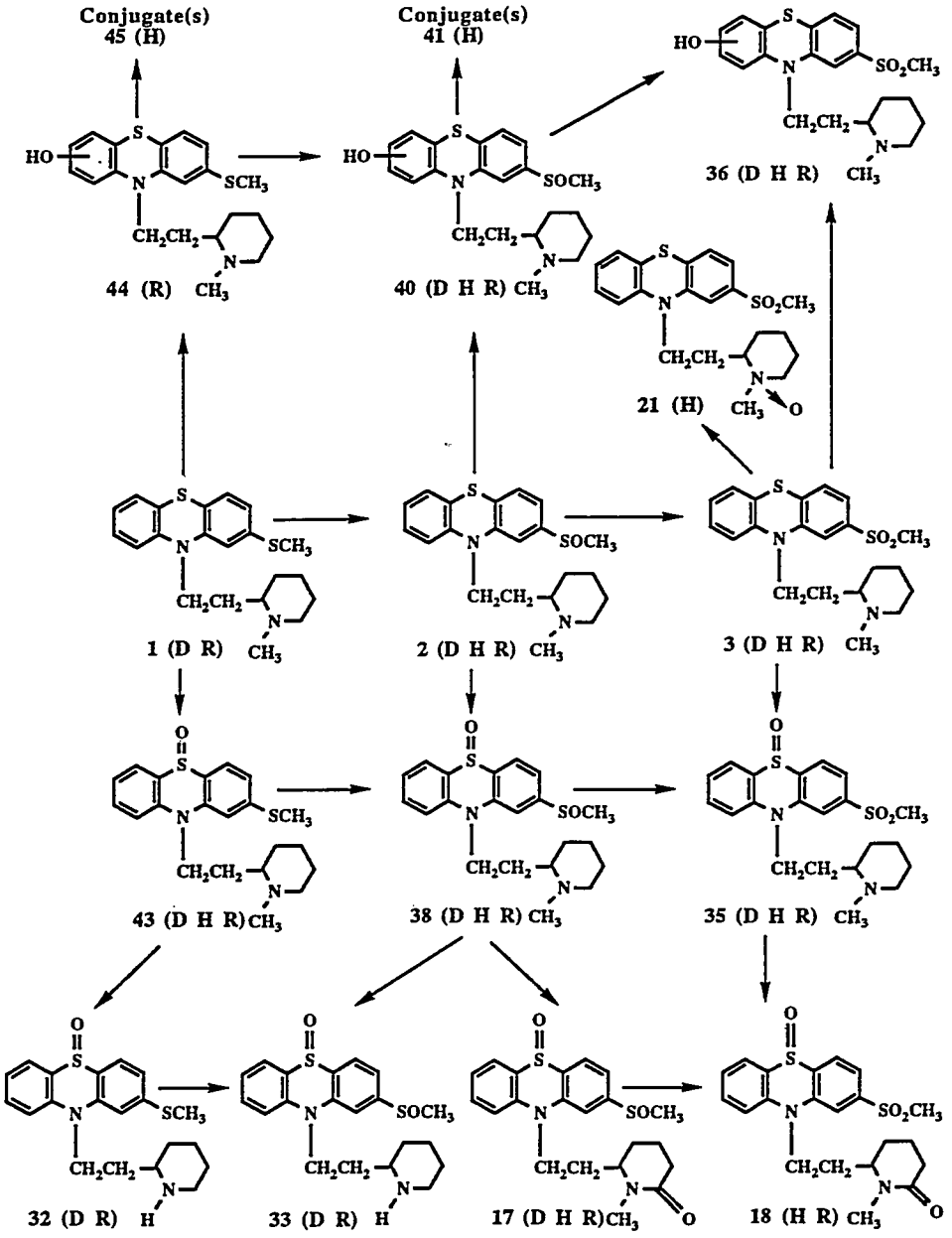
Previous reports to the urinary metabolites of thioridazine have been limited to human (Mårtensson et al. 1975, Papadopoulos et al. 1985, Jørgensen 1986, Papadopoulos and Crammer 1986) and rat (Zehnder et al. 1962, Watkins et al. 1986). Various metabolites have been identified in both species that result from S-oxidation, aromatic hydroxylation and N-demethylation, including various combinations and permutations thereof. Also thioridazine N-oxide has been identified in rat (Watkins et al. 1986), while the lactams of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide have been tentatively identified in human (Papadopoulos and Crammer et al. 1986).

Qualitative metabolite profiles The present investigation of the urinary metabolic profiles of thioridazine in three species was greatly facilitated by use of an on-line plasmaspray HPLC-MS technique. The metabolites definitively identified in the urine of all three species were mesoridazine, sulforidazine, thioridazine ring sulfoxide, mesoridazine ring sulfoxide, sulforidazine

ring sulfoxide and the lactam of mesoridazine ring sulfoxide. Unchanged thioridazine was detected in both dog and rat but not in human. The other compounds identified definitively but exhibiting species selectivity were the lactam of sulforidazine ring sulfoxide found in human and rat, sulforidazine N-oxide detected in human, and both N-desmethylthioridazine ring sulfoxide and N-desmethyl-mesoridazine ring sulfoxide found in rat and dog.

Tentative identification was made in all three species of phenolic derivatives of mesoridazine and sulforidazine. However, while the unconjugated form of both was found in all three species, only the conjugate(s) of the phenolic derivative of mesoridazine was detected in human. Tentative identification was also made of a phenolic derivative of thioridazine in unconjugated and conjugated form(s) in rat and human, respectively. The proposed metabolic profile of thioridazine in rat, dog and human is depicted in Scheme 10, where for the sake of simplicity not all possible routes are shown.

Sulfoxide metabolites It is generally recognised that the major routes of metabolism of thioridazine include S-oxidation at two sites; the phenothiazine ring sulfur atom and the 2-methylthio substituent. In fact, twelve S-oxidized metabolites of thioridazine were identified in both human and rat while ten such metabolites were found in the



Scheme 10. The proposed metabolic pathways of thioridazine in rat (R), dog (D) and human (H)..

All possible routes to each metabolite are not depicted. **1**, Thioridazine; **2**, mesoridazine; **3**, sulforidazine; **17**, lactam of mesoridazine ring sulfoxide; **18**, lactam of sulforidazine ring sulfoxide; **21**, sulforidazine N-oxide; **32**, N-desmethylthioridazine ring sulfoxide; **33**, N-desmethylmesoridazine ring sulfoxide; **35**, sulforidazine ring sulfoxide; **38**, mesoridazine ring sulfoxide; **43**, thioridazine ring sulfoxide; **36**, unconjugated hydroxylated sulforidazine; **40**, unconjugated hydroxylated mesoridazine; **44**, unconjugated hydroxylated thioridazine; **41**, conjugated hydroxylated mesoridazine; **45**, conjugated hydroxylated thioridazine.

case of dog. S-Oxidation of the 2-substituent resulted in at least five metabolites with a 2-sulfoxide function and at least three metabolites with a 2-sulfone function in each species. Moreover each of the species had at least five metabolites with a sulfoxide group at the 5-position of the phenothiazine ring. On the other hand, no detected metabolite of thioridazine had the sulfur atom at the 5-position of the phenothiazine ring as a sulfone function. In previous metabolic studies of thioridazine, thioridazine ring sulfone (Mårtensson et al. 1975, Sakalis et al. 1980) and sulforidazine ring sulfone (Zehnder et al. 1962) were reported or observed in serum and urine of human, and bile and urine of rat, respectively.

In the case of ring sulfoxide metabolites by means of HPLC-UV analysis, each diastereomer of sulforidazine ring sulfoxide was separately quantified using mobile phase E (Figure 23) and both pairs of diastereomers of thioridazine ring sulfoxide and the lactam of mesoridazine ring sulfoxide were separately quantified by use of mobile phase F (Figure 24). The diastereomers of mesoridazine ring sulfoxide were resolved from one another using mobile phase F but other analytes interfered with their analysis (Figure 24). The diastereomers of the lactam of sulforidazine ring sulfoxide, N-desmethylthioridazine ring sulfoxide and N-desmethyl-mesoridazine ring sulfoxide could not be separated by the

mobile phases employed in the present study.

The diastereomers of the three resolved sulfoxide metabolites were separately quantified in all species, however, in the case of dog the urinary levels of the diastereomers of the lactam of mesoridazine ring sulfoxide were below the quantification limits of the assay. The mean diastereomeric ratios were determined in all other cases and except for the lactam of mesoridazine ring sulfoxide in human (0.94±0.07) and rat (1.32±0.26), these ratios varied markedly from unity (1.37-1.86). This latter observation is inconsistent with early reports which noted lack of stereoselectivity in the ring S-oxidation of thioridazine (Poklis et al. 1982, Hale and Poklis 1985, Papadopoulos and Crammer 1986, Watkins et al. 1986). However, a recent report demonstrated that in most of the eleven patients medicated with thioridazine examined the ratio of the concentration of the fast eluting diastereomer to the slow eluting diastereomer of thioridazine ring sulfoxide was greater than unity in both plasma and urine (Eap et al. 1991). In fact, earlier investigations of the lack of stereoselectivity observed in the metabolism of these drugs might have been due to light-induced racemization and photolysis which can occur during analysis and consequently result in false determination of the diastereomeric ratios (Watkins et al. 1986, Eap et al. 1991).

Lactam metabolites The lactams of mesoridazine ring sulfoxide and sulforidazine ring sulfoxide were identified as metabolites of thioridazine. The former was found in all three species, while the latter was detected only in human and rat. Both lactam metabolites were present in appreciable quantities in human and rat urine but the lactam of mesoridazine ring sulfoxide was only a minor metabolite in dog.

N-Oxide metabolites The only previous report to an N-oxide metabolite of thioridazine was to thioridazine N-oxide as a minor metabolite in rat (Watkins et al. 1986). Sulforidazine N-oxide was identified in the urine of human administered thioridazine in the present work. Although it was not quantified, visual inspection of the HPLC-UV chromatograms (Figure 24(E)) indicated that it was a minor metabolite.

N-Demethylated metabolites Both N-desmethyl-thioridazine ring sulfoxide and N-desmethylmesoridazine ring sulfoxide were identified in the urinary extracts of dog and rat. However, neither N-desmethylthioridazine nor N-desmethylmesoridazine were detected as metabolites of thioridazine despite the availability of reference samples. The lack of detection in human urine of a N-desmethyl metabolite was surprising since N-desmethylthioridazine, N-desmethylthioridazine ring sulfoxide, N-desmethyl-

mesoridazine and N-desmethylsulforidazine have been identified as urinary metabolites in patients medicated with thioridazine (Mårtensson et al. 1975, Papadopoulos et al. 1985).

Hydroxylated metabolites Plasmaspray HPLC-MS analysis of extracts treated with MTBSTFA provided convincing evidence for the presence of unconjugated and/or conjugated forms of phenolic derivatives of mesoridazine and sulforidazine in all three species and thioridazine in human and rat. The position of substitution of each aromatic hydroxyl group was not determined. Generally with phenothiazine antipsychotic agents aromatic hydroxylation occurs predominantly at the 3- and 7-positions of the phenothiazine ring (Jørgensen 1986) and, in fact, in the case of humans medicated with thioridazine other workers have definitively identified 7-hydroxymesoridazine and 7-hydroxysulforidazine as urinary metabolites (Papadopoulos et al. 1985).

Interspecies comparisons Thioridazine was extensively metabolized in all three species, and the mean excretion of unchanged drug in urine was between 0-0.9% of the administered dose. S-Oxidation of the phenothiazine ring and/or 2-substituent constituted the predominant pathway in the genesis of most metabolites in all three species. The routes of metabolism observed in human differed from those

common to dog and rat in two regards; N-oxidation was observed only in human and N-demethylation was observed only in dog and rat. However, otherwise the overall metabolic profiles for human more closely resembled rat than dog. Thus the mean total excretion of measured analytes was far less in human (4.3±2.9%) and rat (4.8±1.7%) than dog (12.1±5.4%). Moreover, the same two lactam metabolites were found in the urine of human and rat whereas only one was found in dog urine and the total excretion of these metabolites was far more pronounced in human and rat than dog in that their total excretion when expressed as percent dose administered or percent drug accounted for were 1.7±1.4 and 38.6±9.6%, 0.4±0.4 and 7.0±5.4%, and <0.02 and <0.2%, respectively. Therefore, rat appears to be a more suitable animal than dog to undertake further investigation of the C-oxidation of thioridazine.

4.3. Pharmacological activity evaluation of lactam metabolites

The six available synthetic lactam analogues, namely the lactam of thioridazine, the lactam of mesoridazine, the lactam of sulforidazine, the lactam of thioridazine ring sulfoxide, the lactam of mesoridazine ring sulfoxide and the lactam of sulforidazine ring sulfoxide, were submitted for dopaminergic receptor affinity studies. The radioreceptor

binding assays were carried out by Ms. Kula under the supervision of Dr. Baldessarini (Harvard Medical School, Belmont, MA 02178-9106, USA). The screening data (Appendix B) indicated very low D_1 and D_2 affinities for all lactam metabolites examined. Also the three drugs, thioridazine, mesoridazine and sulforidazine, were tested by the same assays, and as expected they showed high affinities, especially for the D_2 receptor (Appendix B).

5. Conclusions

Synthesis A facile synthetic route to the lactam derivatives of piperidine-type phenothiazine antipsychotic agents via ruthenium tetroxide oxidation was developed. This general approach that involved six steps enabled the successful synthesis of the lactams of thioridazine, mesoridazine and sulforidazine (**13-15**). The lactam ring sulfoxides of thioridazine, mesoridazine and sulforidazine (**16-18**), and also the lactam ring sulfone of sulforidazine (**19**) were obtained by oxidation of the appropriate lactams with either nitrous acid or hydrogen peroxide. The availability of the synthetic reference compounds allowed their definitive identification as metabolites and also quantification of such metabolites present in the urine of man and animals examined.

Analytical techniques Various analytical techniques were employed in the qualitative and quantitative metabolic studies reported in this thesis. Both HPLC separation and collection prior to direct insertion probe MS (in the cases of the studies of sulforidazine in rat and dog, Sections 4.2.2.1. and 4.2.2.2., respectively) and GLC-MS analysis (in the case of the study of sulforidazine in rat, Section 4.2.2.1.) were successfully employed for some of the qualitative studies of the metabolism of sulforidazine. Some disadvantages encountered with both techniques were

overcome by the use of on-line HPLC-MS with a plasmaspray interface (Section 4.2.4.). The HPLC-MS technique provided a relatively rapid, sensitive and selective analysis and also avoided thermal degradation of metabolites such as N-oxides and sulfoxides. Use of this method enabled successful analysis of both underivatized and derivatized urinary extracts obtained from all three species administered each of the three drugs. The quantifications of the unchanged drugs and some of their metabolites present in the urinary extracts were carried out by a HPLC technique with UV detection using various mobile phase systems.

Metabolite identification The urinary metabolic profiles of thioridazine, mesoridazine and sulforidazine in the three species examined were systematically studied for the first time. These three drugs were extensively metabolized in rat, dog and human by various routes that involve S-oxidations of the phenothiazine ring and the ring 2-substituent, aromatic hydroxylation of the phenothiazine ring followed by conjugation, lactam formation of the piperidine ring, N-demethylation of the piperidine ring N-substituent and N-oxidation of the piperidine ring nitrogen atom (not in the case of rat).

The metabolites identified for the first time in any species were N-desmethylmesoridazine ring sulfoxide as the metabolite of thioridazine and mesoridazine, the lactam of

mesoridazine and mesoridazine N-oxide as the metabolites of mesoridazine, the lactam of sulforidazine and N-desmethyl-sulforidazine ring sulfoxide as the metabolites of sulforidazine and sulforidazine N-oxide as the metabolite of all three drugs.

Sulfoxidation and stereoselectivity Sulfoxidations of both ring and 2-substituent sulfur atoms are major routes of metabolism of piperidine-type phenothiazine antipsychotic agents. For all three species the total urinary excretion of all the S-oxidized metabolites accounted for the greatest proportion of all routes of metabolism examined. In the case of thioridazine at least five metabolites with a 2-sulfoxide group and at least three metabolites with a 2-sulfone group were identified in each species, while for mesoridazine at least five metabolites with a 2-sulfone function were detected in each species. On the other hand, no metabolite was found for any of the three drugs in any of the three species where the 2-substituent was reduced. Thus in the case of sulforidazine all the metabolites found in each species had a 2-sulfone functional group. Furthermore in the case of mesoridazine no metabolite with a 2-sulfide substituent was detected in any of the three species. The former observation is compatible with the generally held beliefs regarding the metabolic oxidations of sulfide to sulfoxide, and sulfoxide to sulfone. The oxidation of

sulfoxide to sulfone is a known unidirectional process (Axelsson 1977, Renwick 1989). However, the reduction of sulfoxide to sulfide as a part of the interconversion process between these two types of metabolites was not observed in this study.

In each species at least five, four and two ring sulfoxide metabolites were identified as metabolites of thioridazine, mesoridazine and sulforidazine, respectively. However, no corresponding ring sulfone metabolite was found in any species administered each of the three drugs. These findings are in agreement with previous observations that strained cyclic sulfides such as with the phenothiazine ring are only oxidized as far as the corresponding sulfoxides (Mitchell 1989). Therefore, it is surprising that thioridazine ring sulfone and sulforidazine ring sulfone have been reported to be respective metabolites of thioridazine and sulforidazine in both human and rat (Zehnder et al. 1962, Mårtensson et al. 1975, Sakalis et al. 1980).

Some of the ring sulfoxide metabolites were successfully separated into their diastereomers by HPLC-UV analysis. Therefore, the mean ratios of the concentrations of the fast eluting diastereomer to that of the slow eluting diastereomer were obtained. The resulting data indicated that in some of the cases examined there was

stereoselectivity in the ring S-oxidation of this class of drug, namely sulforidazine ring sulfoxide in human administered thioridazine (1.41±0.11), mesoridazine (1.48±0.13) and sulforidazine (1.42±0.04), and thioridazine ring sulfoxide in rat (1.39±0.14), dog (1.70±0.06) and human (1.86±0.12) dosed with thioridazine. These observations taken together with the recent report that the mean concentration of the fast eluting diastereomer of thioridazine ring sulfoxide was greater than that of the slow eluting diastereomer (1.37 fold in plasma and 1.54 fold in urine) in eleven patients dosed with thioridazine (Eap et al. 1991) suggest that there is need to reconsider the generally held belief that there is lack of stereoselectivity in the phenothiazine ring sulfoxidation of piperidine-type phenothiazine antipsychotic agents (Poklis et al. 1982, Hale and Poklis 1985, Papadopoulos and Crammer 1986, Watkins et al. 1986). Furthermore, it is noteworthy that in human for sulforidazine ring sulfoxide, the ultimate ring sulfoxide metabolite of thioridazine, mesoridazine and sulforidazine, the same diastereomeric ratio was obtained no matter which drug was administered. The consistency of these ratios gives confirmation that there is stereoselectivity in the ring S-oxidation of these drugs.

Lactam metabolites Four lactam metabolites, namely those of mesoridazine, sulforidazine, mesoridazine ring

sulfoxide and sulforidazine ring sulfoxide, were definitively identified, and for each drug in each species at least one lactam metabolite was found in each urinary extract. The present study demonstrates that lactam formation is one of the many biotransformation pathways of piperidine-type phenothiazine antipsychotic agents in all three species examined. Although lactam formation was found to be a minor route of metabolism in dog, this metabolic pathway was important in human and rat. Furthermore except in one instance (lactam of mesoridazine after mesoridazine administration: rat but not human) exactly the same lactam metabolites were found for each drug in human and rat. In each of these two species for each drug at least one lactam metabolite was present in relatively high concentration in the urine. The percentage of the drug excreted in the form of lactam metabolites in human and rat was found to be 39 and 7%, 11 and 16%, and 28 and 18% for thioridazine, mesoridazine and sulforidazine, respectively. Therefore, lactam metabolites account for a significant proportion of the known urinary metabolites of these drugs in human and rat. Moreover since other products of ring oxidation such as ring opened products are commonly found as metabolites of saturated nitrogen-containing heterocyclic ring systems (including piperidine) further investigations of the metabolism of the piperidine ring of this class of

phenothiazine antipsychotic drug are warranted.

The pharmacological activity assays (Appendix B) indicated that all lactam metabolites examined had very low dopaminergic receptor (D_1 and D_2) binding affinities (Appendix B). These results are not surprising since it is widely recognized that a basic amine moiety is essential for antipsychotic activity and the introduction of an α-carbonyl group will destroy this requirement for pharmacological activity (McDowell. 1974, 1975, Lipkowitz et al. 1986, Vida and Tenthorey 1986).

N-Oxidation and N-demethylation Regarding N-oxide metabolites, mesoridazine N-oxide was found in human administered mesoridazine, while sulforidazine N-oxide was identified in human administered all three drugs and in dog dosed with mesoridazine. However, all these N-oxide metabolites were found in trace amounts. These observations indicate that although N-oxidation is more pronounced in human and dog than rat it is at most a minor route of metabolism of piperidine-type phenothiazine antipsychotic agents in the three species examined.

The reported N-demethylated metabolites of this class of drug include N-desmethylthioridazine, N-desmethyl-mesoridazine and N-desmethylsulforidazine (Mårtensson et al. 1975, Papadopoulos et al. 1985). None of these three metabolites was found for any of the three drugs in all

three species despite the fact that all three corresponding ring sulfoxide metabolites were identified in the course of this work. For example, N-desmethylmesoridazine ring sulfoxide was found as a metabolite of mesoridazine in all three species as well as a metabolite of thioridazine in rat and dog. However, further studies are needed to establish whether these N-desmethyl ring sulfoxide metabolites of this class of drug are formed by ring sulfoxidation followed by N-demethylation rather than vice versa.

Phenolic metabolites Tentative identification was made of phenolic metabolites in both free and/or conjugated forms in the case of all three species administered each of the three drugs. The positions of aromatic hydroxylation on the phenothiazine ring could not be assigned due to the unavailability of authentic standards. Hence the amounts of the phenolic metabolites present in the urine of each species could not be quantified. Generally with phenothiazine antipsychotic agents, aromatic hydroxylation occurs predominantly at the 3- and 7-positions of the phenothiazine ring and both free and conjugated forms of phenolic metabolites are present as major metabolites in man and animals (Papadopoulos et al. 1985, Jørgensen 1986). It is interesting to note that whereas aromatic hydroxylation and ring sulfoxidation are major metabolic routes of this class of drug no metabolite that resulted from both these

biotransformations has been identified in any species in the present work or published literature. This observation suggests that prior ring sulfoxidation may prevent the subsequent formation of ring hydroxylated products and vice versa. In fact, in the case of ring sulfoxide metabolites, they are more electron deficient than the parent molecule thereby decreasing their susceptibility to attack by electrophilic reagents (Papadopoulos et al. 1985, Mitchell 1989).

Urinary recoveries The total urinary recoveries as unchanged drugs and their measured metabolites accounted for 12-29, 4-20 and 3-20 % of the administered dose of these drugs in the case of dog, human and rat, respectively. This observation is consistent with previous reports in that in general for phenothiazine antipsychotic agents not a high proportion of the administered dose is accounted for in the urine (Nadeau and Sobolewski 1959, Beckett et al. 1963, Papadopoulos et al. 1985, Papadopoulos and Crammer 1986, Mitchell 1989). These low urinary recoveries are likely due in some measure to enterohepatic circulation and elimination in the faeces (Nadeu and Sobolewski 1959, Papadopoulos et al. 1985). However, the major reason for the relatively small amount of administered dose accounted for in the urine is that phenothiazine antipsychotic agents remain in the body for a relatively long period due to the deep reservoir

compartments. The parent drugs and certain of their metabolites are bound at multiple sites of lipids and proteins. These are thus stored in tissues such as fat. For example, in an early study trace amounts of the ring sulfoxide metabolite of promethazine were detected in the urine for up to two weeks after administration of a single oral dose of this drug and for several months after patients had ceased long-term oral therapy (Cochin and Daly 1963).

Interspecies comparisons Finally interspecies comparisons between rat, dog and human in terms of the metabolism of thioridazine, mesoridazine and sulforidazine indicate that with the exception of N-oxide metabolites the overall metabolic profiles for human more closely resembled rat than dog. Moreover in terms of lactam metabolites, qualitatively and quantitatively human more closely resembled rat than dog, therefore, rat would appear to be a more suitable animal than dog for further studies relating to the C-oxidation of piperidine-type phenothiazine antipsychotic agents.

REFERENCES

Alexander, A. J., and Kebarle, P. 1986, Thermospray mass spectrometry. Use of gas-phase ion/molecule reactions to explain features of thermospray mass spectra. Anal. Chem., 58, 471-478.

Al-Waiz, M., Ayesh, R., Mitchell, S. C., Idle, J. R., and Smith, R. L., 1987, Disclosure of the metabolic retroversion of trimethylamine N-oxide in humans: a pharmacogenetic approach. Clin. Pharmacol. Ther., 42, 608-612.

Anders, M. W., Ratnayake, J. H., Hanna, P. E., and Fuchs, J.A., 1980, Involvement of thioredoxin in sulfoxide reduction by mammalian tissues. Biochem. Biophys. Res. Commun., 97, 846-851.

Anders, M. W., Ratnayake, J. H., Hanna, P. E., and Fuchs, J.A., 1981, Thioredoxin-dependent sulfoxide reduction by rat renal cytosol. Drug Metab. Dispos., 9, 307-310.

Anderson, E. L., Post, A., Staiger, D. S., and Warren, R., 1987, Studies on the oxidation of phenothiazine derivatives. J. Heterocycl. Chem., 17, 597-598.

Arpino, P. J., 1985, Ten years of liquid chromatography-mass spectrometry. J. Chromatogr., 323, 3-11.

Axelsson, R., 1977, On the serum concentrations and antipsychotic effects of thioridazine, thioridazine side-chain sulfoxide and thioridazine side-chain sulfone, in chronic psychotic patients. Curr. Ther. Res., 21, 587-605.

Axelsson, R., and Mårtensson, E., 1977, The concentration pattern of nonconjugated thioridazine metabolites in serum by thioridazine treatment and its relationship to physiological and clinical variables. Curr. Ther. Res., 20, 561-585.

Axelsson, R., and Mårtensson, E., 1980, Side effects of thioridazine and their relationship with the serum concentrations of the drug and its main metabolites. Curr. Ther. Res., 28, 463-389.

Axelsson, R., and Mårtensson, E., 1983, Clinical effects related to the serum concentrations of thioridazine and its metabolites. In Clinical Pharmacology in Psychiatry, edited by L. F. Gram, E. Usdin, S. G. Dahl, P. Kragh-Sorensen, F. Sjoqvist and P. Morselli (London: McMillan), pp. 165-174.

Baldessarini, R. J., 1990, Drugs and the treatment of psychiatric disorders. In The Pharmacological Basis of Therapeutics, 8th edition, edited by A. G. Gilman, A. S. Nies and P. Taylor (New York: Pergamon Press), pp. 383-435.

Baldwin, M. A., and McLafferty, F. W., 1973, Liquid chromatography-mass spectrometry interface. I. The direct introduction of liquid solutions into a chemical ionization mass spectrometer. Org. Mass Spectrom., 7, 1111-1112.

Beckett, A. H., Beavan, M. A., and Robinson, A. E., 1963, Metabolism of chlorpromazine in humans. Biochem. Pharmacol., 12, 779-794.

Beckett, A. H., Essien, E. E., and Smyth, W. F., 1974, A polarographic method for the determination of the N-oxide, N-oxide-sulfoxide and sulfoxide metabolites of chlorpromazine. J. Pharm. Pharmacol., 26, 399-407.

Bickel, M. H., 1972, Liver metabolic reactions: Tertiary amine N-dealkylation, tertiary amine N-oxidation, N-oxide reduction and N-oxide N-dealkylation 1. Tricyclic teriary amine drugs. Arch. Biochem. Biophys., 148, 54-62.

Blakley, C. R., McAdams, M. J., and Vestal, M. L., 1978, Crossed-beam liquid chromatography-mass spectrometer combination. J. Chromatogr., 158, 261-276.

Blakley, C. R., Carmody, J. J., and Vestal, M. L., 1980, A new soft ionization for mass spectrometry of complex molecules. J. Am. Chem. Soc., 102, 5931-5933.

Blakley, C. R., and Vestal, M. L., 1983, Thermospray interface for liquid chromatography/mass spectrometry. Anal. Chem., 55, 750-754.

Breyer-Pfaff, U., Kreft, H., Rassner, H., and Prox, A., 1978, Formation of sulfone metabolites from chlorpromazine and perazine in man. Drug Metab. Dispos., 6, 114-119.

Brookes, L. G., Chao, F. C., Forrest, I. S., Green, D. E., Loeffler, K. O., and Serra, M. T., 1978, New metabolites of thioridazine and mesoridazine. Clin. Chem., 24, 1031.

Bruins, A. P., 1985, Developments in interfacing microbore high-performance liquid chromatography with mass spectrometry (a review). J. Chromatogr., 323, 99-111.

Buckley, J. P., Steenberg, M. L., Barry, H., and Manian, A. A., 1974, In Phenothiazines and Structurally-related Drugs,

edited by I. S. Forrest, C. J. Carr and E. Usdin (New York: Raven Press), pp. 617-631.

Bursey, M. M., Parker, C. E., Smith, R. W., and Gaskell, S. J., 1985, Gas-phase ionization of selected neutral analytes during thermospray liquid chromatography/mass spectrometry. Anal. Chem., 57, 2597-2599.

Bylund, D. B., 1981, Interactions of neuroleptic metabolites with dopaminergic, alpha-adrenergic and muscarinic-cholinergic receptors. J. Pharmacol. Exp. Ther., 217, 81-86.

Caprioli, R. M., 1989, Coupling chromatographic techniques with FABMS for the structural analysis of biological compounds. In Biological Active Molecules, edited by U. S. Schlunegger (Berlin: Springer-Verlag Berlin Heidelberg), pp. 79-96.

Carmosin, R. J., and Carson, J. R., 1987, Preparation of formulation of 3-diphenyl substituted octahydroindolizine analgesic compounds. U.S. Patent 4,683,239, Chem. Abstr., 106, 236507z (1987).

Chakraborty, B. S., 1987, Development and applications of radioimmunoassay procedures for thioridazine and two of its active major metabolites, mesoridazine and sulforidazine. Ph.D. Thesis (Saskatoon: University of Saskatchewan), pp.1-269.

Chakraborty, B. S., Hawes, E. M., and Midha, K. K., 1987a, Development of a radioimmunoassay procedure for mesoridazine and its comparison with a high-performance liquid chromatographic method. Ther. Drug Monit., 9, 464-471.

Chakraborty, B. S., Hawes, E. M., Rosenthaler, J., and Midha, K. K., 1987b, A new radioimmunoassay of thioridazine and its comparison with a high performance liquid chromatographic method and another radioimmunoassay. Ther. Drug Monit., 9, 227-235.

Chakraborty, B. S., Hawes, E. M., and Midha, K. K., 1988, Development of a radioimmunoassay procedure for sulforidazine and its comparison with a high-performance liquid chromatographic method. Ther. Drug Monit., 10, 205-214.

Clement-Cormier, Y., Kebabian, J. W., Petzold, G. L., and Greengard, P., 1974, Dopamine-sensitive adenylate cyclase in mammalian brain: a possible site of action of antipsychotic drugs. Proc. Natl. Acad. Sci., 71, 1113-1117.

Coccia, P. F., and Westerfeld, W. W., 1967, The metabolism of chlorpromazine by liver microsomal enzyme systems. J. Pharmacol. Exp. Ther., 157, 446-458.

Cochin, J., and Daly, J. W., 1963, The use of thin layer chromatography for the analysis of drugs. Identification and isolation of phenothiazine tranquilizers and of antihistamines in body fluids and tissues. J. Pharmacol. Exp. Ther., 139, 160-165.

Covey, T. R., Crowther, J. B., Dewey, E. A., and Henion, J. D., 1985, Thermospray liquid chromatography/mass spectrometry determination of drugs and their metabolites in biological fluids. Anal. Chem., 57, 474-481.

Craig, J. C., and Purushothaman, K. K., 1970, An improved preparation of tertiary amine N-oxides. J. Org. Chem., 35, 1721-1722.

Craig, J. C., Gruenke, L. D., and Lee, S. Y. C., 1978, Synthesis of deuterium-labelled chlorpromazine and chlorpromazine metabolites. J. Labelled Compd. Radiopharm., 15, 31-40.

Creese, I., Burt, D. R., and Snyder, S.H., 1976, Dopamine receptor binding predicts clinical and pharmacological potencies of antipsychotic drugs. Science, 192, 481-483.

Creese, I., and Snyder, S. H., 1977, A simple and sensitive radioreceptor assay for antipsychotic drugs in blood. Nature, 270, 180-182.

Curry, S. H., and Mould, G. P., 1969, Gas chromatographic identification of thioridazine in plasma, and a method for routine assay of the drug. J. Pharm. Pharmacol., 21, 674-677.

Dahl, S. G., 1982, Active metabolites of neuroleptic drugs: possible contribution to therapeutic and toxic effects. Ther. Drug Monit., 4, 33-40.

Dahl, S. G., Hjorth, M., and Hough, E., 1982, Chlorpromazine, methiotrimeprazine and metabolites. Structural changes accompanying the loss of neuroleptic potency by ring sulfoxidation. Mol. Pharmacol., 21, 409-411.

Dajani, R. M., Gorrod, J. W., and Beckett, A. H., 1975, In vitro hepatic and extra-hepatic reduction of (-)-nicotine-1-N-oxide in rats. Biochem. Pharmacol., 24, 109-117.

Damani, L.A., and Crooks, P.A., 1982, Oxidative metabolism of heterocyclic ring systems. In Metabolic Basis of Detoxification, Metabolism of Functional Groups, edited by W.B. Jakoby, J.R. Bend and J. Caldwell (New York: Academic Press), pp. 69-89.

Davis, J. M., 1985, Antipsychotic drugs. In Comprehensive textbook of psychiatry, edited by H. I. Kaplan and B. J. Sadock (Baltimore, MD: Williams and Wilkins), pp. 1481-1513.

Debruyne, O., Moulin, M. A., Camsonne, R., and Bigot, M. C., 1980, Wall-coated open tubular column coupled with nitrogen-selective detector for routine GLC determination of diazepam, meprobamate, phenylbutazone and thioridazine in serum. J. Pharm. Sci., 69, 835-838.

Dinovo, E. C., Gottschalk, L. A., Noble, E. P., and Beinner, R., 1974, Isolation of a possible new metabolite of thioridazine and mesoridazine from human plasma. Res. Commun. Chem. Pathol. Pharmacol., 7, 489-496.

Divono, E. C., Gottschalk, L. A., Nandi, B. R., and Geddes, P. G., 1976, GLC analysis of thioridazine, mesoridazine and their metabolites. J. Pharm. Sci., 65, 667-669.

Eap, C.B., Souche, A., Koeb, L., and Baumann, P., 1991, Light-induced racemization: Artifacts in the analysis of the diastereoisomeric pairs of thioridazine 5-sulfoxide in the plasma and urine of patients treated with thioridazine. Ther. Drug Monit., 13, 356-362.

Edmonds, C. G., Loo, J. A., Barinaga, C. J., Udseth, H. R., and Smith, R. D., 1989, Capillary electrophoresis-electrospray ionization-mass spectrometry. J. Chromatogr., 474, 21-37.

Elsom, L. F., Biggs, S. R., Chasseaud, L. F., Hawkins, D. R., Pulsford, J., and Darrach, A., 1985, Metabolism of the anti-depressant lortalamine. Eur. J. Drug Metab. Pharmacokinet., 10, 209-215.

Fairbrother, J. E., 1979, Analysis of phenothiazine drugs. Pharm. J., 222, 271-275.

Fieser, L. F., and Fieser, M., 1967, Reagents for Organic Synthesis, 1, 135.

Franklin, R. A., Aldridge, A., and White, C. de B., 1976, Studies on the metabolism of meptazinol, a new analgesic drug. Br. J Clin. Pharmacol., 3, 497-502.

Furniss, B. S., Hannaford, A. J., Rogers, V., Smith, P. W. G., and Tatchell, A. R., 1978, In Vogel's Textbook of Practical Organic Chemistry, 4th edition (London: Longman), pp.274.

Games, D. E., 1983, High performance liquid chromatography-mass spectrometry (HPLC-MS). Advan. Chromatogr., 21, 1-39.

Games, D. E., and Martinez, F., 1989, Evaluation of the moving belt as an interface for the high-performance liquid chromatographic-mass spectrometric analysis of the flavonoid aglycones. J. Chromatogr., 474, 372-380.

Ganes, D. A., and Midha, K. K., 1987, Sensitive and specific high-performance liquid chromatographic assay for the quantification of sulforidazine and two diastereomeric sulforidazine-5-sulfoxide metabolites in plasma. J. Chromatogr., 423, 227-237.

Ganes, D. A., 1988, Metabolic and pharmacokinetic studies of thioridazine, mesoridazine and sulforidazine in the dog and man. Ph.D. Thesis (Saskatoon: University of Saskatchewan), pp. 1-345.

Gole, D. J., Pirat, J., Kamenka, J., and Domino, E. F., 1988, Hydroxy metabolites of phencyclidine: Identification and quantitation of two novel metabolites. Drug Metab. Dispos., 16, 386-391.

Gorrod, J. W., Lazarus, C. R., and Beckett, A. H., 1974, Some aspects of the in vitro oxidation of chlorpromazine. Adv. Biochem. Pharmacol., 9, 191-200.

Gorrod, J. W., and Jenner, P., 1975, The metabolism of tobacco alkaloids. In Assays in Toxicology, Volume 6, edited by W. J. Hayes, Jr. (New York: Academic Press), pp. 35-78.

Gottschalk, L. A., Dinovo, E., Biener, R., and Nandi, B., 1978, Plasma concentrations of thioridazine metabolites and ECG abnormalities. J. Pharm. Sci., 67, 155-157.

Gottschalk, L. A., Mennuti, S. A., and Cohn, J. B., 1979, Thioridazine plasma levels and clinical response in five schizophrenic patients receiving daily oral medication: correlations and the prediction of clinical response. In Pharmacokinetics of psychoactive drugs: Further studies, edited by L. A. Gottschalk (New York: Spectrum Publications Inc.), pp. 83-95.

Griffiths, D. C., Hassanali, A., Merritt, L. A., Mudd, A.,

Pickett, J. A., Shah, S. J., Smart, L. E., Wadhams, L. J., and Woodcock, C. M., 1988, Highly active antifeedants against coleopteran pests. Brighton Crop Prot. Conf.-Pests Dis., 1041-1046.

Gruenke, L. D., Craig, J. C., Dinove, E. C., Gottschalk, L. A., Noble, E. P., and Biener, R., 1975, Identification of a metabolite of thioridazine and mesoridazine in human plasma. Res. Commun. Chem. Pathol. Pharmacol., 10, 221-225.

Gruenke, L. D., Craig, J. C., Klein, F. D., Nguyen, T. L., Hitzemann, B. A., Holaday, J. W., Loh, H. H., Braff, L., Fischer, A., Glick, I. D., Hartmann, F., and Bissell, D. M., 1985, Determination of chlorpromazine and its major metabolites by gas chromatography/mass spectrometry: application to biological fluids. Biomed. Mass Spectrom., 12, 707-713.

Hale, P. W., and Poklis, A., 1984a, Determination of diastereomeric pairs of thioridazine-5-sulfoxide by high-performance liquid chromatography. J. Chromatogr., 336, 452-455.

Hale, P. W., and Poklis, A., 1984b, Thioridazine-5-sulfoxide cardiotoxicity in isolated, perfused rat heart. Toxicol. Lett., 21, 1-8.

Hale, P. W., Melethil, S., and Poklis, A., 1985, The disposition and elimination of stereoisomeric pairs of thioridazine-5-sulfoxide in the rat. Eur. J. Drug Metab. Pharmacokinet., 10, 333-341.

Hale, P. W., Jr., and Poklis, A., 1985, Thioridazine-5-sulfoxide diastereoisomers in serum and urine from rats and man after chronic thioridazine administration. J. Anal. Toxicol., 9, 197-201.

Hale, P. W., and Poklis, A., 1986a, The biotransformation of thioridazine to thioridazine-5-sulfoxide stereoisomers in phenobarbital induced rats. Eur. J. Drug Metab. Pharmacokinet., 11, 283-289.

Hale, P. W., and Poklis, A., 1986b, Cardiotoxicity of thioridazine and two stereoisomeric forms of thioridazine-5-sulfoxide in the isolated perfused rat heart. Toxicol. Appl. Pharmacol., 86, 44-55.

Hall, K., Yeung, P. K. F., and Midha, K. K., 1982, Decomposition of sulphoxide metabolites of phenothiazine antipsychotics during gas chromatographic analysis. J.

Chromatogr., 231, 200-204.

Hallström, G., Kammerer, R. C., Nguyen, C. H., Schmitz, D. A., Di Stefano, E. W., and Cho, A. K., 1983, Phencyclidine metabolism in vitro: The formation of a carbinolamine and its metabolites by rabbit liver preparations. Drug Metab. Dispos., 11, 47-53.

Hawes, E. M., Jaworski, T. J., Midha, K. K., McKay, G., Hubbard, J. W., and Korchinski, E. D., 1991, In vivo metabolism of N-oxides. In N-Oxidation of Drugs. Biochemistry, Pharmacology, Toxicology; Hlavica, P.; Damani, L.A., Eds.; Chapman and Hall: London, 1991; pp 263-286.

Heath, A., Svensson, C., and Mårtensson, E., 1985, Thioridazine toxicity: An experimental cardiovascular study of thioridazine and its major metabolites in overdose. Vet. Hum. Toxicol., 27, 100-105.

Heimans, R. L. H., Fennessy, M. R., and Caff, G. A., 1971, Some aspects of the metabolism of morphine-N-oxide. J. Pharm. Pharmacol., 23, 831-836.

Hoag, M. K. P., Trevor, A. J., Kalir, A., and Castagnoli, N. Jr., 1987, Phencyclidine iminium ion: NADPH-dependent metabolism , covalent binding to macromolecules, and inactivation of cytochrome(s) P-450. Drug Metab. Dispos., 15, 485-490.

Hollister, L. E., 1986, Antipsychotic drugs. Drugs of Today, 22, 551-561.

Holsztynska, E. J., and Domino, E. F., 1986, Biotransformation of phencyclidine. Drug Metab. Rev., 16, 285-320.

Honma, S., Iwamura, S., Kobayashi, R., Kawabe, Y., and Shibata, K., 1987, The metabolism of roxatidine acetate hydrochloride: Liberation of deuterium from the piperidine ring during hydroxylation. Drug Metab. Dispos., 15, 551-559.

Horn, A. S., and Snyder, S. H., 1971, Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. Proc. Nat. Acad. Sci. USA, 68, 2325-2328.

Hubbard, J. W., Cooper, J. K., Hawes, E. M., Jenden, D. J., May, P. R. A., Martin, M., McKay, G., Van Putten, T., and Midha, K. K., 1985, Therapeutic monitoring of chlorpromazine I: Pitfalls in plasma analysis. Ther. Drug Monit., 7, 222-

228.

Iwamura, S., Shibata, K., Kawabe, Y., Tsukamoto, K., and Honma, S., 1987, The metabolism of roxatidine acetate hydrochloride in rat and dog liver homogenates. Pharmacobio-Dyn., 10, 229-235.

Jajoo, H. K., Mayol, R. F., LaBudde, J. A., and Blair, I. A., 1990, Structural characterization of urinary metabolites of the antiarrhythmic drug encainide in human subjects. Drug Metab. Dispos., 18, 28-35.

Jaworski, T. J., Hawes, E. M., McKay, G., and Midha, K. K., 1988, The metabolism of chlorpromazine N-oxide in the rat. Xenobiotica, 18, 1439-1447.

Jaworski, T. J., Hawes, E. M., McKay, G., and Midha, K. K., 1990, The metabolism of chlorpromazine N-oxide in man and dog. Xenobiotica, 20, 107-115.

Jaworski, T. J., Sardessai, M. S., Aravagiri, M., Lin, G., Shi, Y. Y., Hawes, E. M., Hubbard, J. W., McKay, G., and Midha, K.K., 1992, Synthesis of the N-oxides of phenothiazine antipsychotic agents. Submitted to J. Pharm. Sci..

Jenner, P., Gorrod, J. W., and Beckett, A. H., 1973, The absorption of nicotine-1-N-oxide and its reduction in the gastro-intestinal tract in man. Xenobiotica, 3, 341-349.

Jørgensen, A., 1986, Metabolism and pharmacokinetics of antipsychotic drugs. In Progress in Drug Metabolism, Volume 9, edited by J. W. Bridges and L. F. Chasseaud (London: Taylor & Francis), pp. 111-174.

Juenge, E. C., Wells, C. E., Green, D. E., Forrest, I. S., and Shooley, J. N., 1983, Synthesis, isolation and characterization of two stereoisomeric ring sulfoxides of thioridazine. J. Pharm. Sci., 72, 617-621.

Kaiser, C., Swagzdis, J. E., Flanagan, T. L., Lester, B. M., Burghard, G. L., Green, H., and Zirkle, C. L., 1972, Metabolism of diphenidol. Urinary products in human and dogs. J. Med. Chem., 15, 1146-1150.

Kametani, T., Kigasawa, K., Hiiragi, M., and Asagi, S., 1968, A synthesis of 4-oxa-9-aza-9-methyl-6,7-benzobicyclic[3,3,1]nonane and attempts to cyclize the piperidone derivatives by reductive cyclization (studies on the synthesis of heterocyclic compounds CCXXVII). Yakugaku

Zasshi, 88, 573-582.

Kamimura, H., Enjoji, Y., Sasaki, H., Kawai, R., Kaniwa, H., Niigata, K., and Kageyama, S., 1987, Disposition and metabolism of indeloxazine hydrochloride, a cerebral activator, in rats. Xenobiotica, 17, 645-658.

Karger, B. L., and Vouros, P., 1985, A chromatographic perspective of high-performance liquid chromatography-mass spectrometry. J. Chromatogr., 323, 13-32.

Kebabian, J. W., Petzold, G. L., and Greengard, P., 1972, Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". Proc. Natl. Acad. Sci., 69, 2145-2149.

Kebabian, J. W., and Calne, D. B., 1979, Multiple receptors for dopamine. Nature, 277, 93-96.

Kinon, G., Sakalis, G., Traficante, L.J., Aronson, M., Bowers, P., and Gershon, S., 1979, Mesoridazine in treatment-refractory schizophrenics. Curr. Ther. Res., 25, 534-539.

Kilts, C. D., Breese, G. R., and Mailman, R. B., 1982, Simultaneous determination of thioridazine and its oxidized and N-demethylated metabolites using high performance liquid chromatography on radialy compressed silica. J. Chromatogr., 231, 377-391.

Kilts, C. D., Knight, D. L., Mailman, R. B., Widerl, V. E., and Breese, G. R., 1984, Effects of thioridazine and its metabolites on dopaminergic function: Drug metabolism as a determinant of the antidopaminergic actions of thioridazine. J. Pharmacol. Exp. Ther., 231, 334-342.

Kitamura, S., Tatsumi, K., Hirata, Y., and Yoshimura, H., 1981, Further studies on sulfoxide-reducing enzyme systems. J. Pharmacobio-Dyn., 4, 528-533.

Kitamura, S., and Tatsumi, K., 1983, A sulfoxide-reducing enzyme consisting of aldehyde oxidase and xanthine oxidase, a new electron transfer system. Chem. Pharm. Bull., 31, 760-763.

Kleinman, J. E., Bigelow, L. B., Rogol, A., Weinberger, D. R., Nasrallah, A., Wyatt, T. R., and Gillin, J. C., 1980, In Phenothiazines and Structurally-Related Drugs: Basic and Clinical Studies, edited by E. Usdin, H. Eckert and I. S. Forrest (Amsterdam: Elsevier), pp. 275-278.

Kobayashi, S., Nakano, M., Goto, T., Kimura, T., and Schaap, A. D., 1986, An evidence of the peroxidase-dependent oxygen transfer from hydrogen peroxide to sulfoxides. Biochem. Biophys. Res. Commun., 135, 166.

Lewis, M. H., Mobilio, J. N., Rissmiller, D. J., and Mailman, R. B., 1984, Thioridazine pharmacodynamics: Clinical effects may depend on drug metabolism. J. Am. Osteopath. Assoc., 84, 124-128.

Lipkowitz, K., Burkett, A., and Landwer, J., 1986, Theoretical studies of psychotropic drugs. Questioning the importance of flex-angle in drug efficacy. Heterocycles, 24, 2757-2769.

Madeschaire, M., 1986, Synthesis of sulfoxides by oxidation of thioethers. Tetrahedron, 42, 5459-5465.

Mannervik, B., 1982, Mercaptans. In Metabolic Basis of Detoxication: Metabolism of Functional Groups, edited by W. B. Jakoby, J. R. Bend and J. Caldwell (Toronto: Academic Press), pp. 171-184.

Mårtensson, E., Nyberg, G., Axelsson, R., and Serck-Hansen, K., 1975, Quantitative determination of thioridazine and nonconjugated thioridazine metabolites in serum and urine of psychiatric patients. Curr. Ther. Res., 18, 687-700.

Masumoto, H., Takeuchi. K., Ohta, S., and Hirobe, M., 1989, Application of chemical P450 model systems to studies on drug metabolism. I. Phencyclidine: A multi-functional model substrate. Chem. Pharm. Bull., 37, 1788-1794.

Masumoto, H., Ohta, S., and Hirobe, M., 1991, Application of chemical cytochrome P-450 model systems to studies on drug metabolism: IV. Mechanism of piperidine metabolism pathways via an iminium intermediate. Drug Metab. Dispos., 19, 768-780.

Mayol, R. F., Jajoo, H. K., Klunk, L. J., and Blair, I. A., 1991, Metabolism of the antipsychotic drug tiospirone in humans. Drug Metab. Dispos., 19, 394-399.

McDowell, J. J. H., 1974, The molecular structures of phenothiazine derivatives. In The phenothiazines and structurally related drugs, edited by I. S. Forrest, C. J. Carr and E. Usdin (New York: Raven Press), pp. 33-54.

McDowell, J. J. H., 1975, The crystal and molecular structure of thioridazine, a derivative of phenothiazine.

Acta Cryst., B31, 2256-2264.

McFadden, W. H., Schwartz, H. L., and Bradford, D. C., 1976, Direct analysis of liquid chromatographic effluents. J. Chromatogr., 122, 389-396.

McKay, G., Cooper, J. K., Gurnsey, T., and Midha, K. K., 1985, A simple, sensitive and simultaneous assay of thioridazine, sulforidazine and mesoridazine in plasma by HPLC. Liq. Chromatogr., 3, 256-258.

McKennis, B. H. Jr., Turnbull, L. B., and Bowman, E. R., 1958, Metabolism of nicotine to (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid. J. Am. Chem. Soc., 80, 6597-6600.

McMahon, R. E., and Sullivan, H. R., 1977, Reduction of levopropoxyphene N-oxide to propoxyphene by dogs in vivo and rat liver microsomal fraction in vitro. Xenobiotica, 7, 377-382.

Meffin, P., Robertson, V. A., Thomas, J., and Winkler, J., 1973, Neutral metabolites of mepivacaine in humans. Xenobiotica, 3, 191-196.

Meltzer, H. Y., Fang, V. S., Simonovich, M., and Paul, S. M., 1977, Eur. J. Pharmacol., 41, 431.

Meltzer, H. Y., 1986, Novel approaches to the pharmacotherapy of schizophrenia. Drug Develop. Res., 9, 23-40.

Meuldermans, W., Peer, A. V., Hendrickx, J., Lauwers, W., Swysen, E., Bockx, M., Woestenborghs, R., and Heykants, J., 1988a, Excretion and biotransformation of cisapride in dogs and humans after oral administration. Drug. Metab. Dispos., 16, 403-409.

Meuldermans, W., Henderickx, J., Lauwers, W., Hurkmans, R., Mostmans, E., Swysen, E., Brackx, J., Knaeps, A., and Heykants, J., 1988b, Excretion and biotransformation of cisapride in rats after oral administration. Drug. Metab. Dispos., 16, 410-419.

Meyer, J. W., Woggon, B., Baumann, P., and Meyer, U. A., 1990, Clinical implications of slow sulphoxidation of thioridazine in a poor metabolizer of the debrisoquine type. Eur. J. Clin. Pharmacol., 39, 613-614.

Midha, K. K., Jaworski, T. J., Hawes, E. M., McKay, G., Hubbard, J. W., Aravagiri, M., and Marder, S. R., 1991,

Radioimmunoassay and other methods for trace analysis of N-oxide compounds. In N-oxidation of drugs: Biochemistry, pharmacology, toxicology, edited by P. Hlavica and L. A. Damani (London: Chapman & Hall), pp. 37-55.

Miller, R. J., Horn, A. S., and Iverson, L. L., 1974, The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3',5'-monophosphate production in rat neostriatum and limbic forebrain. Mol. Pharmacol., 10, 759-766.

Mills, G. A., Walker, V., Clench, M. R., and Parr, V. C., 1988, Analysis of urinary organic acids by plasmaspray™ liquid chromatography/mass spectrometry. Biomedical Environmental Mass Spectrometry, 16, 259-261.

Mitchell, S. C., and Waring, R. H., 1986, The early history of xenobiotic sulfoxidation. Drug Metab. Rev., 16, 255-284.

Mitchell, S. C., 1989, Phenothiazine antipsychotics. In Sulfur-containing Drugs and Related Organic Compounds. Chemistry, Biochemistry and Toxicology, Volume 3, edited by L. A. Damani (New York: John Wiley & Sons), Part A, pp. 19-52.

Mohammad, T., Midha, K. K., and Hawes, E. M., 1988, Synthesis of deuterium labelled thioridazine via ruthenium tetroxide oxidation of the piperidine ring . J. Labelled Compd. Radiopharm., 25, 415-427.

Mohammad, T., Midha, K. K., and Hawes, E. M., 1989, Synthesis of deuterium labelled analogues of S-oxidative metabolites of thioridazine. J. Labelled Compd. Radiopharm., 27, 181-188.

Montzka, T.A., Matiskella, J.D., and Partyka, R.A., 1974, 2,2,2-Trichloroethyl chloroformate: a general reagent for demethylation of tertiary methylamines. Tetrahedron Letters, 1325-1327.

Murphy, P. J., 1973, Enzymatic oxidation of nicotine to nicotine $\Delta^{1'(5')}$ iminium ion: A newly discovered intermediate in the metabolism of nicotine. J. Biol. Chem., 248, 2796-2800.

Muusze, R. G., and Huber, J.F. K., 1973, Evaluation of liquid-liquid systems for the chromatographic separation of the psychotropic drug thioridazine and its metabolites. J. Chromatogr., 83, 405-420.

Muusze, R. G., and Huber, J. F. K., 1974, Determination of

the psychotropic drug thioridazine and its metabolites in blood by means of high pressure liquid chromatography in combination with fluorimetric reaction. J. Chromatogr. Sci., 12, 779-787.

Nadeau, G., and Sobolewski, G., 1959, Estimation of phenothiazine derivatives in urine. II. Efficacy of dosage and conjugation capacity after oral administration of chlorpromazine and levopromazine in man. Can. Med. Assoc. J., 81, 658-659.

Neve, H. K., 1961, The excretion of thioridazine (Mellaril) in urine. Acta Pharmacol. Toxicol., 17, 404-409.

Ng, C. H., and Crammer, J. L., 1977, Measurement of thioridazine in blood and urine. Br. J. Clin. Pharmacol., 4, 173-183.

Niedzwicki, D. M., Mailman, R. B., and Cubeddu, L. X., 1984, Greater potency of mesoridazine and sulforidazine compared with parent compound, thioridazine, on striatal dopamine autoreceptors. J. Pharmacol. Exp. Ther., 228, 636-639.

Norton, T. R., Seibert, R. A., Benson, A. A., and Bergstrom, F. W., 1946, The synthesis of some substituted 8-aminoquinolines. J. Am. Chem. Soc., 68, 1572-1576.

Nyberg, G., Axelsson, R., and Mårtensson, E., 1978, Binding of thioridazine and thioridazine metabolites to serum proteins in psychiatric patients. Eur. J. Clin. Pharmacol., 14, 341-350.

Nyberg, G., Axelsson, R., and Mårtensson, E., 1981, Cerebrospinal fluid concentrations of thioridazine and its main metabolites in psychiatic patients. Eur. J. Clin. Pharmacol., 19, 139-148.

Oae, S., 1977, Sulfoxides and sulfilimines. In Organic Chemistry of Sulfur, (New York: Plenum Press), pp. 383-471.

Oae, S., Mikami, A., Matsuura, T., Ogawa-Asada, K., Watanabe, Y., Fujimori, K., and Iyanagi, T., 1985, Comparison of sulfide oxygenation mechanism for liver microsomal FAD-containing monooxygenase with that for cytochrome P-450. Biochem. Biophys. Res. Commun., 131, 567-573.

Oelschläger, H., and Al Shaik, M., 1985, Metabolic N-oxidation of alicyclic amines. In Biologic Oxidation of Nitrogen in Organic Molecules, Chemistry, Toxicology and

Pharmacology, edited by J.W. Gorrod and L.A. Damani (Chichester: Ellis Horwood, Ltd.), pp. 60-75.

Pacha, W. L., 1969, A method of the fluorimetric determination of thioridazine (Mellaril) or mesoridazine (Lindanil) in plasma. Experientia., 25, 103-104.

Papadopoulos, A. S., Chand, T. G., Crammer, J. L., and Lader, S., 1980, A pilot study of plasma thioridazine and metabolites in chronically treated patients. Br. J. Psychiatry, 136, 591-596.

Papadopoulos, A. S., and Crammer, J. L., 1986, Sulphoxide metabolites of thioridazine in man. Xenobiotica, 16, 1097-1107.

Papadopoulos, A. S., Crammer, J. L., and Cowan, D. A., 1985, Phenolic metabolites of thioridazine in man. Xenobiotica, 15, 309-316.

Parker, C. E., Bursey, M. M., Smith, P. W., and Gaskell, S. J., 1985, Gaseous acidity and basicity scales as guides to chemical ionization in reversed-phase liquid chromatography-mass spectrometry with direct liquid introduction. J. Chromatogr., 347, 61-74.

Peterson, L. A., Trevor, A. J., and Castagnoli, N. Jr., 1987, Stereochemical studies on the cytochrome P-450 catalyzed oxidation of (S)-nicotine to the (S)-nicotine $\Delta^{1'(5')}$-iminium species. J. Med. Chem., 30, 249-254.

Poklis, A., Wells, C. E., and Juenge, E. C., 1982, Thioridazine and its metabolites in post mortem blood, including two stereoisomeric ring sulfoxides. J. Anal. Toxicol., 6, 250-252.

Porter, C. C., Arison, B. H., Gruber, V. F., Titus, D. C., and Vandenheuvel, W. J. A., 1975, Human metabolism of cyproheptadine. Drug Metab. Dispos., 3, 189-197.

Powis, G., Ames, M. M., and Kovach, J. S., 1979, Metabolic conversion of indicine N-oxide to indicine in rabbits and humans. Cancer Res., 39, 3564-3570.

Renwick, A.G., 1989, Sulphoxides and sulphones. In Sulphur-containing drugs and related organic compounds: Chemistry, biochemistry and toxicology, Volume 1: part B, edited by L.A. Damani (Chichester: Ellis Horwood, Ltd.), pp. 133-154.

Renwick, A. G., Evans, S. P., Sweatman, T. W., Cumbaland,

J., and George, C. F., 1982, The role of the gut flora in the reduction of sulphinpyrazone in the rat. Biochem. Pharmacol., 31, 2649-2656.

Renz, J., Bourquin, P., Winkler, H., Brueschweiler, C., and Schwarb., G., 1964, Phenothiazines. Fr. Patent, 1,363,683, Chem. Abstr., 61, 14687d (1964).

Renz, J., Bourquin, J. P., Winkler, H., Gagnaux, P., Ruesch, L., and Schwarb, G., 1966, 3-Substituent 10-[2-(1-methyl-2-piperidyl)ethyl]-phenothiazines. Fr. Patent, 1,459,476, Chem. Abstr., 67, 21923b (1967).

Renz, J., Bourquin, J. P., Winkler, H., Gagnaux, P., Ruesch, L., and Schwarb, G., 1968, Piperidinoethyl phenothiazines. Fr. Patent, 90,868, Chem. Abstr., 70, 96806m (1969).

Reynolds, G. P., 1992, Developments in the drug treatment of schizophrenia. Trends Pharmacol. Sci. Rev., 13, 116-121.

Sakalis, G., Traficante, C. J., and Gershon, S., 1977, Thioridazine metabolism and clinic response: a pilot study. Curr. Ther. Res., 21, 720-724.

Sakalis, G., Traficante, L.J., Gardos, G., and Gershon, S., 1980, Treatment of drug-resistant schizophrenics with mesoridazine. In Phenothiazines and structurally related drugs: Basic and clinical studies, edited by E. Usdin, H. Eckert and I.S. Forrest (New York: Elsevier North Holland, Inc.), pp. 207-214.

Scott, R. P. W., Scott, C. G., Munroe, M., and Hess, J. Jr., 1974, Interface for on-line liquid chromatography-mass spectroscopy analysis. J. Chromatogr., 99, 395-405.

Seeman, P., Lee, T., Wong, C. M., Tedesco, J., and Wong, K., 1976, Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature, 261, 717-719.

Sheehan, J. C., and Tulis, R. W., 1974, Oxidation of cyclic amines with ruthenium tetroxide. J. Org. Chem., 39, 2264-2267.

Shigenaga, M. K., Trevor, A. J., and Castagnoli, N. Jr., 1988, Metabolism-dependent covalent binding of (S)-[5-^{3}H]nicotine to liver and lung microsomal macromolecules. Drug Metab. Dispos., 16, 397-402.

Shindo, N., Yamauchi, N., Murayama, K., Fairbrother, A., and Krolik, S., 1990, Identification of 17-hydroxyprogesterone

and other steroid hormones in saliva from a normal child and patients with congenital adrenal hyperplasia by plasmaspray liquid chromatography/mass spectrometry. Biomed. Chromatogr., 4, 171-174.

Shvartsburd, A., Nwokerfor, V., and Smith, R. C., 1984, Red blood cell and plasma levels of thioridazine and mesoridazine in schizophrenic patients. Psychopharmacol. (Berlin), 82, 55-61.

Sibley, D. R., and Monsma, F. J. Jr., 1992, Molecular biology of dopamine receptors. Trends Pharmacol. Sci. Rev., 13, 61-69.

Skinner, T., Gochnauer, R., and Linnoila, M., 1981, Liquid chromatographic method to measure thioridazine and its active metabolites in plasma. Acta Pharmacol. Toxicol., 48, 223-226.

Smith, R. D., Loo, J. A., Edmonds, C. G., Barinaga, C. J., and Udseth, H. R., 1990, New developments in biochemical mass spectrometry: Electrospray ionization. Anal. Chem., 62, 882-899.

Sokoloff, P., Giros, B., Materes, M-P., Bouthenet, M. L., and Schwartz, J. C., 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature, 347, 146-151.

Strong, H. A., Oates, J., Sembi, J., Renwick, A. G., and George, C. F., 1984, Role of the gut flora in the reduction of sulphinpyrazone in humans. J. Pharmacol. Exp. Ther., 230, 726-732.

Sumita, M., and Nishino, A., 1960, Oxidized derivatives of phenothiazines. Japanese Patent 11, Chem. Abstr., 54, 19726c.

Sunahara, R. K., Guan, H-C., O'Dowd, B. F., Seeman, P., Laurier. L. G., Ng, G., George, S. R., Torchia, J., Van Tol, H. H. M., and Niznik, H. B., 1991, Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature, 350, 614-619.

Svendsen, C. N., and Bird, E. D., 1986, HPLC with electrochemical detection to measure chlorpromazine, thioridazine and metabolites in human brain. Psychopharmacol., (Berlin), 90, 316-321.

Svendsen, C. N., Hrbek, C. C., Casendino, M., Nichols, R.

D., and Bird, E. D., 1988, Concentration and distribution of thioridazine and metabolites in schizophrenic post-mortem brain tissue. Psychiatry Res., 23, 1-10.

Tal'roze, V. L., Karpov, G. V., Gordetskii, I. G., and Skurat, V. E., 1968, Capillary system for the introduction of liquid mixtures into an analytical mass spectrometer. Russ. J. Phys. Chem., 42, 1658-1664.

Tatsumi, K., 1982, Sulfoxide-reducing enzyme systems responsible for activation and deactivation of sulfoxide compounds. In Microsomes, Drug Oxidations, and Drug Toxicity, edited by R. Sato and R. Kato (Tokyo: Japan Scientific Press), pp. 471-477.

Tatsumi, K., Kitamura, S., and Yamada, H., 1982, Involvement of liver aldehyde oxidase in sulfoxide reduction. Chem. Pharm. Bull., 30, 4585-4588.

Tatsumi, K., Kitamura, S., and Yamada, H., 1983, Sulfoxide reductive activities of liver aldehyde oxidase. Biochem. Biophys. Acta, 747, 86-92.

Taylor, K. I., and Ziegler, D. M., 1987, Studies on substrate specificity of the hog liver flavin-containing monooxygenase: anionic organic sulfur compounds. Biochem. Pharmacol., 36, 141-146.

Tomer, K. B., and Parker, C. E., 1989, Biochemical applications of liquid chromatography-mass spectrometry. J. Chromatogr., 492, 189-221.

Traficante, L. J., Siekierski, J., Sakalis, G., and Gershon, S., 1979, Sulfoxidation of chlorpromazine and thioridazine by bovine liver: preferential metabolic pathways. Biochem. Pharmacol., 28, 621-626.

Vanderheeren, F. A. J., and Muusze, R. G., 1977, Plasma levels and half-lives of thioridazine and some of its metabolites: High doses in young acute schizophrenics. Eur. J. Clin. Pharmacol., 11, 135-140.

Van Tol, H. H. M., Bunzow, J. R., Guan, H-C., Sunahara, K. K., Seeman, P., Niznik, H. B., and Civelli, O., 1991, Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature, 350, 610-614.

Vestal, M. L., 1984, High performance liquid chromatography-mass spectrometry. Science, 226, 275-281.

Vida, J. A., and Tenthorey, P. A., 1986, Antipsychotic drugs. In CRC Handbook of CNS Agents and local Anaesthetics, edited by M, Verderame (Boca Raton, FL: CRC Press), pp. 75-123.

von Bahr, C., Movin, G., Nordin, C., Lidén, A., Hammarlund-Udenaes, M., Hedberg, A., Ring, H., and Sjoqvist, F., 1991, Plasma levels of thioridazine and metabolites are influenced by the debrisoquin hydroxylation phenotype. Clin. Pharmacol. Ther., 49, 234-240.

Wakabayashi, T., Watanabe, K., Kato, Y., and Saito, M., 1977, Asymetric synthesis of piperidine derivatives: Application of synthesis of (s)-(-)-sedamine and (s)-(-)-allosedamine. Chem. Letters, 223-228.

Wakabayashi, T., Watanabe, K., and Kato, Y., 1978, (+)- or (-)-2-(2-Haloethyl)-1-methyl-5-oxo-piperidines. Japan Kokai, 63,383. Chem. Abstr., 89, 146778a (1978).

Ward, D., Kalir, A., Baillie, T., and Castagnoli, N. Jr., 1982a, Metabolic formation of iminium species: Metabolism of phencyclidine. J. Med. Chem., 25, 491-492.

Ward, D. P., Trevor, A. J., Adams, K. J. D., Baillie, T. A., and Castagnoli, N. Jr., 1982b, Metabolism of phencyclidine: The role of iminium ion formation in covalent binding to rabbit microsomal protein. Drug Metab. Dispos., 10, 690-695.

Watanabe, Y., Iyanagi, T., and Oae, S., 1982, One electron transfer mechanism in the enzymatic oxygenation of sulfoxide to sulfone promoted by a reconstituted system with purified cytochrome P-450. Tetrahedron Lett., 23, 533-536.

Watkins, G. M., Whelpton, R., Buckley, D. G., and Curry, S. H., 1986, Chromatographic separation of thioridazine sulphoxide and N-oxide diastereoisomers: identification as metabolites in the rat. J. Pharm. Pharmacol., 38, 506-509.

Wells, C. E., Juenge, E. C., and Furman, W. B., 1983, Simultaneous assay of thioridazine and its metabolites in plasma at single dosage levels with a novel report of two ring sulfoxides of thioridazine. J. Pharm. Sci., 72, 622-625.

Whitaker, N. G. G., and Lindstrom, T. D., 1987, Disposition and biotransformation of quinpirole, a new D-2 dopamine agonist antihypertensive agent, in mice, rats, dogs, and monkeys. Drug Metab. Dispos., 15, 107-113.

Whitehouse, C. M., Dreyer, R. N., Yamashita, M., and Fenn, J. B., 1985, Electrospray interface for liquid chromatographs and mass spectrometers. Anal. Chem., 57, 675-679.

Widerl, V. E., Hggerstr, M. J. E., Kilts, C. D., Anderson, U., Breese, G. R., and Mailman, R. B., 1982, Serum concentrations of thioridazine, its major metabolites and serum neuroleptic-like activities in schizophrenics with and without tardive dyskinesia. Acta Psychiatr. Scand. 66, 294-305.

Yamashita, M., and Fenn, J. B., 1984, Negative ion production with the electrospray ion source. J. Phys. Chem., 88, 4671-4675.

Yergey, A. L., Edmonds, C. G., Lewis, I. A. S., and Vestal, M. L., 1989a, Particle beam interfaces. In Liquid Chromatography/Mass Spectrometry: Techniques and Applications, (New York: Plenum Press), pp. 87-88.

Yergey, A. L., Edmonds, C. G., Lewis, I. A. S., and Vestal, M. L., 1989b, Thermospray. In Liquid Chromatography/Mass Spectrometry: Techniques and Applications, (New York: Plenum Press), pp. 31-86.

Yeung, P. K. F., Hubbard, J.W., Korchinski, E. D., and Midha, K. K., 1987, Radioimmunoassay for the N-oxide metabolite of chlorpromazine in human plasma and its application to a pharmacokinetic study in healthy humans. J. Pharm. Sci., 76, 803-808.

Yoshifuji, S., Tanaka, K., Kawai, T., and Nitta, Y., 1985, Chemical conversion of cyclic α-amino acids to α-aminodicarboxylic acids by improved ruthenium tetroxide oxidation. Chem. Pharm. Bull., 33, 5515-5521.

Yoshihara, S., and Yoshimura, H., 1974, Metabolism of prolintane in rats and the effect of prolintane metabolites on general activities in mice. Xenobiotica, 4, 529-535.

Yoshihara, S., and Tastumi, K., 1985, Guinea pig liver aldehyde oxidase as sulfoxide reductase: its purification and characterization. Arch. Biochem. Biophys., 242, 213-224.

Zehnder, K., Kalberer, F., Kreis, W., and Rutschmann, J., 1962, The metabolism of thioridazine (Mellaril®) and one of its pyrrolidine analogues in the rat. Biochem. Pharmacol., 11, 535-550.

Ziegler, D. M., 1980, Microsomal flavin-containing monooxygenase: Oxygenation of nucleophilic nitrogen and sulfur compounds. In Enzymatic Basis of Detoxication, volume I, edited by W. B. Jakoby (New York: Academic Press), pp. 201-227.

Ziegler, D. M., 1982, Organic sulfur compounds. In Metabolic Basis of Detoxication: Metabolism of Functional Groups, edited by W. B. Jakoby, J. R. Bend and J. Caldwell (Toronto: Academic Press), pp. 171-184.

Ziegler, D. M., 1984, Metabolic oxygenation of organic nitrogen and sulfur compounds. In Drug Metabolism and Drug Toxicity, edited by J. R. Mitchell and M. G. Horning (New York: Raven Press), pp. 33-53.

Ziegler, D. M., 1988, Flavin-containing monooxygenase: catalytic mechanism and substrate specificities. Drug Metab. Rev., 17, 2-32.

Ziegler, D. M., 1989, S-Oxygenases, I: Chemistry and biochemistry. In Sulfur-containing Drugs and Related Organic Compounds. Chemistry, Biochemistry and Toxicology, Volume 2, edited by L. A. Damani (New York: John Wiley & Sons), Part A, pp. 53-65.

Ziegler, D. M., 1991, Unique properties of the enzymes of detoxication. Drug Metab. Dispos., 19, 847-852.

Ziegler, D. M., Poulsen, L. L., and Duffel, M. W., 1980, Kinetic studies on mechanism and substrate specifity of the flavin-containing monooxygenase. In Microsomes, Drug Oxidation and Chemical Carcinogenesis, edited by M. J. Coon, A. H. Cooney, R. Estabrook, J. R. Gelboin, J. R. Gillette and P. J. O'Brien (New York: Acdemic Press), pp. 637-645.

Appendix A. Ethics Committee approved forms and protocol for the administration of sulforidazine to humans.

UNIVERSITY ADVISORY COMMITTEE ON ETHICS IN HUMAN EXPERIMENTATION

Name and E.C. File #: Drs. E.D. Korchinski, E.M. Hawes, K.K. Midha 90-58 June 25, 1

Your project entitled: To Study the Metabolism of Sulforidazine

has been approved by the Committee.

1. Therefore you are free to proceed with the project subject to the following conditions:

Approved.

2. Please submit the revisions requested above to the Director of Research Services, Room 50, Murray Building.

3. Any significant changes of your protocol should be reported to the Director of Research Services for Committee consideration in advance of its implementation.

Sincerely,

ROLAND MUIR
Director of Research Services
University of Saskatchewan

Roland Muir

for Dr. E.A. McKenna, Chairman
University Advisory Committee on
Ethics in Human Experimentation

UNIVERSITY OF SASKATCHEWAN ADVISORY COMMITTEE ON
ETHICS IN HUMAN EXPERIMENTATION

OFFICE OF
EARCH SERVICES

ORS USE ONLY
File Number _____

Date Received: _____

RESEARCHER'S SUMMARY

PROJECT TITLE: To study the metabolism of sulforidazine

SUBMITTED BY: Drs. E.D. Korchinski, (Faculty Member) E.M. Hawes and K.K. Midha DEPARTMENT: Colleges of Medicine and Pharmacy

1. Hypothesis (state briefly the proposition the research is seeking to uphold):

Sulforidazine is a therapeutically effective metabolite of the piperidine type phenothiazine antipsychotic agents thioridazine and mesoridazine. It is proposed to investigate the metabolism of sulforidazine in healthy male volunteers. It is hypothesized that the metabolites will include those resulting from oxidation of the piperidine ring such as by α C-oxidation (e.g. lactams) and ring opening.

2. Scientific Validity (provide your own comments and those resulting from peer review. Indicate if any committee or other body has assessed the project's scientific validity.)

It is well established with other drugs and exogenous chemicals that contain a piperidine ring or similar saturated nitrogen heterocyclic ring systems that extensive metabolic oxidation can occur at this site to result in lactams and ring opened compounds. We have synthesised the lactams of these compounds and have proven the existence of sulforidazine ring sulfoxide lactam as a major metabolite of sulforidazine in rat. This methodology can now be applied to humans. It would be preferred to initially study sulforidazine before that of mesoridazine and thioridazine since unlike the former, the metabolism of the two latter drugs is made more complex by the extensive S-oxidation (and reduction) of the phenothiazine ring 2-substituent. Although sulforidazine is not marketed in Canada, it is marketed in other countries including West Germany. It is administered in 50 or 100 mg doses three times a day.

3. Funding (indicate the source of funds supporting the research. If externally funded, state whether the grant or contract is in application or has been awarded.)

Funding is available to carry out this work; including a MRC Program Grant (PG-34).

4. Subjects (classify by requirements of research initiative and by other subject categories, if appropriate):

Six to twelve drug-free healthy males between the ages of 18 to 55 years weighing no more than ±15% from the ideal weight for height, defined by the Metropolitan Life Insurance Company Statistical Bulletin, will be recruited. All subjects will pass a physical examination given by Dr. Korchinski including blood chemistry, hematology and urine tests as well as a medical questionnaire.

5. Procedures (clearly identify the medical and other procedures to be followed in obtaining research data):

One single oral dose of sulforidazine (50-100 mg) will be administered to overnight-fasted volunteers. A blood sample (0 hr) and a blank urine sample will be collected prior to dosing. Not more than 16 blood samples (15 ml each) will be drawn by venipuncture over the 72 hr period following drug administration. All urine and faeces will be collected over this time period. The volunteers will remain under medical supervision for 8 hr following dosing and blood pressure will be monitored over the first 6 hr and longer, if necessary.

6. Consent Form (indicate whether a consent form is to be used and give reasons, if not):

A consent form is to be used and a copy is attached.

7. Other Comments (include further information or details which might assist the Ethics Committee in understanding the nature and purpose of the research):

See Attached

The Research Protocol has been reviewed and is recommended for approval.

Department Head

Note: to be supplied in 12 copies

Other comments:

The study of sulforidazine will not be limited to investigation of the metabolism of the piperidine ring. For example, there was indication from our previous work that the 2-substituent of piperidine type phenothiazine drugs is metabolised via novel routes and such resultant metabolites will be looked for in the present study. Also not only the excreta will be examined for metabolites, but also the plasma. Thus attempts will be made to quantitate metabolites in the plasma which are found in the excreta.

This research group has had experience in undertaking pharmacokinetic and metabolic studies in healthy male volunteers with many antipsychotic agents, including the related drugs mesoridazine and thioridazine. For example, in the case of mesoridazine, single (or divided) oral (12.5-50 mg) and intramuscular (12.5 mg) doses were given to healthy male volunteers in order to study the effect of dose and route of administration on the bioavailability of mesoridazine (Ethics Committee: 83-24).

A Investigational New Drug Application (IND) is being submitted separately to the Health Protection Branch which describes the synthesis of sulforidazine carried out in our laboratories. Capsules containing 50-100 mg will be prepared extemperaneously as per "secondum artem" and will be administered in this study.

APPENDIX 1

College of Pharmacy
University of Saskatchewan

TO: Students, Staff and Faculty of all Colleges and Departments

FROM: College of Pharmacy

SUBJECT: Volunteers for a metabolic study of the drug sulforidazine

DATE:

The study will consist of not more than one single dose of the drug. Sulforidazine is itself used as a drug and is also a metabolite of the drugs thioridazine and mesoridazine. All three of these drugs are used mainly in the treatment of psychotic conditions. Other than transient sedation and sometimes hypotension, untoward effects are normally absent following a single dose in healthy volunteers. Six to twelve volunteers will be required. Each volunteer will be asked to ingest not more than one single oral dose not exceeding 100 mg of sulforidazine.

Not more than seventeen (15 ml) blood samples over the 72 hr of the study will be collected from each volunteer. All urine and faeces will be collected over this period. An honorarium will be paid for participation in this study.

Any male person between the ages of 18 and 55 years interested in participating should contact Margaret Campbell, Drug Metabolism and Drug Disposition Group at Room 301, College of Pharmacy, Thorvaldson Building, Phone 966-6371.

I am interested in taking part in the drug metabolism study involving sulforidazine and I would like to obtain more details.

NAME:

DEPARTMENT:

PHONE NO.:

_____
Signature

APPENDIX 2

SULFORIDAZINE STUDY

MEDICAL SCREENING FORM

Those persons interested in taking part in the study are asked to complete this form. Information will be confidential and seen only by a physician. The final selection of volunteers will be made on the basis of this information and physical examination by the supervising physician.

1. Name:

2. Home Address:

3. Telephone No. (i) Work
   (ii) Home

4. Date of Birth:

5. Social Insurance Number:

6. Health Care Registration Number:

7. Have you ever been seen by anyone in the Family Medicine Unit before?

8. Next of Kin, Relationship and Address:

9. Are you in good general health?

10. Have you ever had your urine examined and found to be abnormal? If so when?

11. Have you ever suffered from any serious disease? If so, please specify. Particular note should be made of gastrointestinal problems such as ulcers and ulcerative colitis, kidney and liver problems, respiratory problems, cardiovascular problems including heart condition and high blood pressure, pheochromocytoma, epilepsy, glaucoma, parkinsonism, hypo or hyperglycemia, hyperthyroidism and blood disorders such as anemia.

12. Have you a history of drug allergy or reaction, e.g., to aspirin or penicillin or phenothiazines or antihistamines?

13. Do you have any other known allergies? If so, please specify.

14. Do you take any drugs regularly? If so, please specifiy. Note that this includes such things as Valium®, aspirin, antacids, vitamins and marijuana.

15. Do you smoke? How much?

16. Race (ethnic origin)

_____ _____
Date Signature

APPENDIX 3

SULFORIDAZINE STUDY

BIOCHEMICAL TESTS

Tests to be done pretrial and after completion of the study:

Chemistry	total bilirubin, SGOT (AST), alkaline phosphatase, uric acid, total protein, albumin, calcium, phosphorus, glucose, LDH, BUN
	Na^+, K^+, Cl^-, CO_2, creatinine
	Complete urinalysis
Hematology:	WBC, RBC, hemoglobin, PCV, differential, platelet estimate.

APPENDIX 4

SULFORIDAZINE STUDY

PHYSICIAN'S REPORT FORM - VOLUNTEER SELECTION

Personal Information on Volunteer

Name _____

Social Insurance No. _____ Date of Birth _____

Weight _____ Height _____

Remarks _____

_____

Physical Examination

Heart Sounding _____ Pulse Rate _____

Blood Pressure _____ Abdomen _____

Breathing _____ Tongue _____

Throat _____

General Assessment (include pretrial lab report comment)

_____

Physician's Report

I _____ have examined this subject and find him to be in good physical health and apparent sound mind. He has been told the nature of the drugs to be administered to him for the purpose of the experimental human study, the expected duration of the effects, the methods and means by which the drugs are to be administered and the methods, techniques and procedures to be used to assess the effects. In light of this information, the volunteer has freely decided to participate in the study, and understands that he is free to drop out of the study whenever he wishes.

Signed: _____

SULFORIDAZINE STUDY

SPECIAL CONSENT TO USE OF DRUGS FOR EXPERIMENTAL HUMAN STUDIES

Volunteer:_____ Date:_____

Time:_____ a.m. p.m.

I have been told the nature of the drug to be administered to me for the purpose of Experimental Human Studies, the expected duration of its effects the methods and means by which it is to be administered, and the methods, techniques and procedures to be used to assess its effects. A brief summary of these particulars is as follows:

Sulforidazine is a tranquilizer used mainly in the treatment of schizophrenia. Sulforidazine is not marketed in Canada, however, it is available as a prescription medicine in other countries including West Germany. It is administered in doses of 50 to 100 mg three times a day. The time taken for the highest blood levels to fall to half of their highest value is, on average, in a group of individuals, probably about 24 hours. One single oral dose of sulforidazine will be administered (50 to 100 mg of sulforidazine). A transient sedation can be expected at the dose level being used. No other unusual effects are expected, but possible adverse reactions are given below. All urine and faeces will be collected over the 72 hour period of the study and also 'blank' samples if they are requested. Also, in addition to the 0 hour sample, not more than 16 (14-16 ml each) blood samples will be taken by venipuncture over the 72 hours following ingestion of the drug. Finally, samples for clinical blood and urine tests will be required before the trial and on completion of the study.

I am fully aware of the experimental nature of the tests being performed, and the risks and potential risks involved with this type of drug in an experimental study of this nature. The known risks were outlined to me and are as follows:

The majority of adults tolerate the dosage used in this study without adverse drug effects apart from a transient sedation. If any other adverse drug reactions occur, they would usually be expected to be transient, because of the limited dosage used, the fact that only one dose is to be administered and the supervision provided. The reactions usually associated with chronic administration include postural hypotension (a rapid fall in blood pressure on changing from lying down to standing up causing one to feel dizzy, especially when done rapidly), cardiac effects such as bradycardia (a slow heart beat), involuntary movements characteristic of an extrapyramidal syndrome, anticholinergic effects (which include a dry mouth, nasal congestion, constipation, urinary retention, blurring of vision, and alteration in the extent of sweating), pigmentary retinopathy (a visual disorder), and sensitivity rections (including rash, itching and blood disorders).

I have been cautioned that for the 24 hours after ingestion of the drug, I must not drive any motor powered vehicle, such as a car or snowmobile, or operate any machinery, whether small such as a power drill or power saw, or

heavy such as a farm tractor or farm machinery. I will not take any drug (including such things as aspirin and medications for colds) for the two weeks prior to the study and for 72 hours after drug ingestion. If in an emergency, it becomes necessary for me to take any drug, I will inform the physician.

I have been told of the serious nature of a drug interaction with alcohol. As a consequence, I have been cautioned that I must abstain from alcohol for the 24 hours before drug administration and until at least 24 hours after the last blood sample has been taken.

I have also been informed of interactions of sulforidazine, which depresses the central nervous system, with other drugs which affect the central nervous system. These include such drugs as alcohol, marijuana, tranquilizers such as Valium® and Librium®, amphetamines, anesthetics, hypnotics and sleep aids, antidepressants, barbiturates, narcotics and monoamine oxidase inhibitors (the marketed monoamine oxidase inhibitors are phenelzine-Nardil®, tranylcypromine-Parnate®, isocarboxazid-Marplan® and selegiline-Eldepryl®. Other drugs which may have monoamine oxidase effects are debrisoquine-Declinax®, isoniazid-Isotamine®, Rimifon®, metoclopramide-Maxeran®, Reglan® and procarbazine-Natulan®).

Also, sulforidazine is related to the drug chlorpromazine, which has been reported to give photosensitivity reactions, and therefore, as a result, I agree to avoid excessive exposure to direct sunlight or the use of sun lamps or UV tanning lights both during the study and for the 48 hours after the last biological sample of the study.

Additionally, I will refrain from exercise on the day of drug administration, and if, at any time, I feel dizzy or weak, I will lie down immediately. I have also agreed to an overnight fast and that I will not eat until the standardized meal supplied at 4 1/2 hours after dosing (or a light breakfast 1 hour after dosing).

I have agreed to abstain from caffeine for 12 hours after drug administration. This includes coffee, tea, chocolate and some cola beverages.

I have agreed to remain under medical supervision for 8 hours after drug administration. Supervision may be lengthened if necessary.

I have been informed that there are possible unknown reactions from an experimental study of this kind, and I acknowledge that no guarantees have been made to me concerning the effects of the drugs to be administered. In light of this information, which I have considered and fully understand. I have agreed to participate in the study with the understanding that I am free to drop out at any time.

I have been informed that if I have any problems I may contact Dr. Korchinski at 966-7859.

_____ _____

Witness Volunteer

APPENDIX 6

SULFORIDAZINE STUDY

PHYSICIANS REPORT FORM - STUDY RECORD

VOLUNTEER #:_____ NAME:_____

DATE:_____ Time of Drug Administration_____

1. Blood Pressure B.P. TIME

0 hr (pre-dose)		
1.0 hr		
2.0 hr		
3.0 hr		
4.0 hr		
6.0 hr		

Additional B.P.'s and comments:

2. Note with respect to an adverse reaction:

a) Nature of adverse reaction

b) Severity (grade as mild, moderate, severe)

c) Relationship of adverse reaction to sulforidazine

d) Onset time of occurrence

e) Duration of reaction

f) Corrective measures taken

g) Other comments

APPENDIX 7

REGIMEN FOR VOLUNTEERS WILLING TO PARTICIPATE IN A STUDY OF SULFORIDAZINE

To participate in the study the volunteers must agree with the following conditions:

1. All volunteers must pass a physical. A post drug study physical is also necessary before receiving payment. All physicals are done by Dr. Korchinski in the Family Medicine Unit of the University Hospital.

2. The volunteer must avoid all drugs (including aspirin and cold preparations) for two weeks prior to the study and during the trial.

3. No alcohol should be consumed for 24 hours prior to ingestion of the dose of sulforidazine and for 24 hours after the last biological sample of the study.

4. The volunteer must fast overnight from 9:00 p.m. before administration of the drug. Nothing is ingested for the first 4.5 hours following the dose other than a 7-Up two hours after dosing or a light breakfast 1 hour after dosing. A standardized lunch will be provided.

5. The volunteer must not drive any motor powered vehicle 24 hours after ingestion of the drug. A transportation allowance will be provided.

6. The volunteer must avoid excessive exposure to direct sunlight or excessive tanning lights during and for two days following the study.

7. The volunteer must refrain from exercise on the first day of the study.

8. Payment will be made by cheque upon completion of the post-study physical.

9. The volunteers must spend the first 8 hours following drug administration under medical supervision in the Family Medicine Unit. They are not allowed to leave the unit during this time.

10. Smoking is not allowed during confinement in the Family Medicine Unit.

11. An iron supplement may be prescribed at the completion of the study if indicated by the post-study physical results.

12. The volunteer should not donate blood to the Red Cross for at least 3 months after completion of the study.

13. The volunteer must abstain from caffeine during the fasting period and for 12 hours after drug administration. This includes coffee, tea, chocolate and some cola beverages.

Appendix B. Dopamine receptor binding affinities of piperidine-type phenothiazine antipsychotic agents and some of their lactam analogues.

Compound	No.	IC_{50} (nM) ±SEM D_1	D_2
Thioridazine	**1**	33.9±3.2	6.5±0.3
Mesoridazine	**2**	62.7±2.7	10.7±1.6
Sulforidazine	**3**	8.1±1.1	3.6±0.3
Lactam of thioridazine	**13**	>10,000	4,890±870
Lactam of mesoridazine	**14**	>100,000	>10,000
Lactam of sulforidazine	**15**	>100,000	6,080±580
Lactam of thioridazine ring sulfoxide	**16**	>50,000	>10,000
Lactam of mesoridazine ring sulfoxide	**17**	>30,000	>10,000
Lactam of sulforidazine ring sulfoxide	**18**	>50,000	>10,000

Affinities were examined by competition assays of test compounds with rat striatal homogenates in This-HCl buffer (pH 7.4) containing 150 mM NaCl, at 30°C. The D_1 assay (30 min) used ^{3}H-SCH-23390 (0.3 nM) as ligand and cis-flupenthixol (0.3 μM) as blank. The D_2 assay (90 min) used ^{3}H-YM-09151-2 (0.065 nM) with (+)-butaclamol (1 μM) as blank. Test agents were included at ≥6 concentrations, above and below IC_{50}, in duplicate, with three replications, and resulting data were curve-fit with the ALLFIT program. Independently determined ligand affinites (Kd) were 0.34 nM (D_1) and 0.045 nM (D_2).