EVALUATION OF SOME THIOSEMICARBAZONES
OF ARYL ALKYL KETONES AS
CANDIDATE ANTICONVULSANT AGENTS

A Thesis
Submitted to the Faculty of Graduate Studies
and Research in Partial Fulfilment of the Requirements
For the Degree of
Master of Science
in Pharmacy

by
Janice Marian McColl, B.S.P.
Saskatoon, Saskatchewan

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ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. J.R. Dimmock for his guidance, encouragement and personal concern throughout the course of this project. I also acknowledge the helpful suggestions given by the members of my research advisory committee.

Many thanks to the College of Graduate Studies and Research for the graduate scholarship award and the National Institute of Neurological and Communicative Disorders and Stroke, U.S.A., for providing the anticonvulsant screening data.

I wish to thank all who in one way or the other have contributed to the successful completion of this project.
Dedicated

To

My Husband David

without whose love and understanding

this could not have been
ABSTRACT

Thiosemicarbazones have been found to possess a wide range of biological activity including anticonvulsant activity. Structural requirements for anticonvulsant activity are thought to be electron donating groups in the presence of aryl or alkyl functional groups. Earlier work from the laboratories of Dr. J.R. Dimmock have established that some thiosemicarbazones of nuclear substituted 4-aryl-3-buten-2-ones and aryl-substituted acetophenones possess anticonvulsant properties and that electron donating substituents on the aryl ring were associated with bioactivity.

The aim of the present investigation was the synthesis of a number of thiosemicarbazones of aryl-substituted acetophenones and some related compounds to be evaluated as potential anticonvulsant agents. In particular, the following were examined, namely, the effect on bioactivity of various electron donor substituents on the aryl ring of some aryl-substituted acetophenones, the nature of the linkage between the aryl ring and the carbon bearing the thiosemicarbazono and methyl functions, replacement of the aryl ring by alkyl functions and replacement of the thiosemicarbazono moiety with related functional groups. Thiosemicarbazones can exist in the E or Z configuration and in some cases different proportions of the geometrical isomers were observed.
Although most of the thiosemicarbazones and related derivatives examined possessed some anticonvulsant activity none possessed significant anticonvulsant activities over acetophenone thiosemicarbazone. No clear correlation between chemical structure and anticonvulsant activity could be discerned based on the anticonvulsant screening results which were available.
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1.0.0 INTRODUCTION

1.1.0 Classification and treatment of epilepsies

Epilepsy is a common chronic neurological disorder characterized by recurrent, usually transient, seizures having a sudden onset and a spontaneous resolution (Parker, 1984). Epilepsy affects approximately 1% of the population and estimates suggest that from 40-50% of patients are not controlled with available therapy (Porter and Pitlick, 1982; Clark, 1988). Thus, there exists a need for new compounds to increase the percentage of patients whose epilepsies may be controlled by drugs.

Epilepsies are due to the activation or inactivation of cerebral neurons which exhibit an abnormal and sudden degree of electrical discharge. This synchronous electrical discharge results in an area of aberrant tissue called the focus or focal lesion. The majority of epilepsies are primary or idiopathic in which no identifiable cause can be determined. A second smaller group is comprised of the secondary or organic epilepsies in which case seizures exist in conjunction with an identifiable precipitating factor. Many precipitating factors can trigger abnormal electrical discharges in susceptible individuals and subsequently cause seizures. Precipitants of seizures include metabolic disorders, head injury, central nervous system infections, degenerative diseases such as Alzheimer's disease and multiple sclerosis, drug overdose and abrupt drug withdrawal.
Epileptic seizures are classified on the basis of the affected cerebral area and the subsequent clinical symptomatology (Bancaud et al., 1981; Parker, 1984; Dreiffus et al., 1985). A simplified version (Porter et al., 1984) is given in Table 1.1, distinguishing the two main types of seizures, namely partial and generalized seizure. Virtually all epileptic seizures can be classified as either partial or generalized seizures (Porter, 1982). Partial seizures are distinguished from generalized seizures by local onset which may be determined clinically or electroencephalographically. In rare cases, some seizures may not be classifiable as being partial or generalized.

<table>
<thead>
<tr>
<th>I. Partial seizures (beginning locally)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Simple partial seizures (consciousness not impaired)</td>
</tr>
<tr>
<td>1. With motor symptoms</td>
</tr>
<tr>
<td>2. With somatosensory or special sensory symptoms</td>
</tr>
<tr>
<td>3. With autonomic symptoms</td>
</tr>
<tr>
<td>4. With psychic symptoms</td>
</tr>
<tr>
<td>B. Complex partial seizures (with impairment of consciousness)</td>
</tr>
<tr>
<td>1. Beginning as simple partial seizures and progressing to impairment of consciousness</td>
</tr>
<tr>
<td>a. With no other features</td>
</tr>
<tr>
<td>b. With features as in simple partial seizures</td>
</tr>
<tr>
<td>c. With automatisms</td>
</tr>
<tr>
<td>2. With impairment of consciousness at onset</td>
</tr>
<tr>
<td>a. With no other features</td>
</tr>
<tr>
<td>b. With features as in simple partial seizures</td>
</tr>
<tr>
<td>c. With automatisms</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Generalized seizures (bilaterally symmetrical without local onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 1. Absence seizures</td>
</tr>
<tr>
<td>2. Atypical seizures</td>
</tr>
<tr>
<td>B. Myoclonic seizures</td>
</tr>
<tr>
<td>C. Clonic seizures</td>
</tr>
<tr>
<td>D. Tonic seizures</td>
</tr>
<tr>
<td>E. Tonic-clonic seizures</td>
</tr>
<tr>
<td>F. Atonic seizures</td>
</tr>
</tbody>
</table>

| III. Unclassified epileptic seizures (data inadequate or incomplete) |
--------------------------------------------------------------------|

\(^a\) Taken from Porter et al. (1984) and reproduced with permission of the copyright owner.
Partial or focal seizures result if the primary electrical discharge remains localized. However, if the primary discharge spreads and involves the entire cerebrum with no evidence of localized onset generalized seizures result. The seizures' clinical expression is dependent on the location of the primary discharge and the pattern of spread from the site of initiation. Once the seizure activity has peaked, a decrease in the frequency of neuronal discharge occurs resulting in cessation of hypersynchronous discharge and seizure activity.

The degree of success obtained with antiepileptic drugs is largely dependent on the type of seizure and the extent of associated neurological abnormalities. Simple absence (petit mal) and tonic-clonic (grand mal) epilepsies are two types of generalized seizures which respond well to antiepileptic drug therapy. Grand mal epilepsy is the most common seizure disorder occurring in about 90% of epileptics (Parker, 1984). However, many of these may be partial seizures which have secondarily generalized.

It is unlikely that a wide variety of epileptic seizures could be managed successfully with just one drug since more than one mechanism may be responsible for the various seizures and drugs useful for one seizure type may actually aggravate other types. Drugs useful in the treatment of the two major types of seizures, namely partial and generalized, are quite distinct in their clinical effects. In some cases, however, partial seizures respond to the same drugs used for generalized tonic-clonic seizures (Porter, 1982). This coincidence may reflect the secondary origin of most generalized tonic-clonic seizures.
Antiepileptic drugs useful against absence seizures elevate the minimal seizure threshold but have little or no ability to prevent the spread of seizures. In contrast, those drugs effective against generalized tonic-clonic (GTC) seizures prevent the spread of seizures and may or may not increase the minimal seizure threshold. Experimental models of seizures and initial screening of potential anticonvulsant agents is designed to detect compounds that either elevate the minimal seizure threshold (which correlates with effectiveness against petit mal epilepsy) or prevent the spread of seizures (detects agents which are effective against grand mal epilepsy), and to provide some insight as to the mechanisms of action of potential anticonvulsant agents. Drugs useful in the treatment of petit mal and grand mal epilepsy are given in Table 1.2 (Parker, 1984).
Table 1.2: Treatment of generalized tonic-clonic and absence seizures a

<table>
<thead>
<tr>
<th></th>
<th>Generalized Tonic-clonic</th>
<th>Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First choice(s)</strong></td>
<td>Phenobarbital or Phenytme</td>
<td>Ethosuximide or</td>
</tr>
<tr>
<td></td>
<td>or Phenobarbital</td>
<td>Valproic acid</td>
</tr>
<tr>
<td><strong>Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Primary</strong></td>
<td>Combination b</td>
<td>Combination b</td>
</tr>
<tr>
<td></td>
<td>Carbamazepine</td>
<td>Clonazepam</td>
</tr>
<tr>
<td></td>
<td>Primidone</td>
<td>Valproic acid</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td>Clonazepam</td>
<td>Acetazolamide</td>
</tr>
<tr>
<td></td>
<td>Ethotoin</td>
<td>Methsuximide</td>
</tr>
<tr>
<td></td>
<td>Mephenytoin</td>
<td>Trimethadione</td>
</tr>
<tr>
<td></td>
<td>Mephobarbital</td>
<td></td>
</tr>
</tbody>
</table>

a Adapted from Parker (1984) and reproduced with permission of the copyright owner.

b Many clinicians recommend combination therapy with first choice drugs before adding alternative agents.
\[
\frac{5 \text{ pounds}}{11 \text{ oz}} = \frac{2300}{520} = \frac{2620}{2620}
\]

Phenobarbital
Primidone
Mysoline
Clonazepam
Nitrazepam
Valproate
Phenytoin
Ethosuximide
Succinamides
Methsuximide
Valproate Na
Valproic acid
Mechanisms of action of antiepileptic agents are only poorly understood. Most anticonvulsants have been shown to have multiple physiological, biochemical, and pharmacological actions and clear correlations of these effects with anticonvulsant activity are often difficult. Considering the elementary process of synaptic transmission and neurotransmitter function three broad categories of events may determine the occurrence of a seizure:

1) An increase in excitatory synaptic influences.
2) A decrease in inhibitory synaptic influences.
3) An alteration in normal neuronal membrane characteristics.

Thus an effective anticonvulsant drug should stabilize neuronal membranes, enhance inhibitory processes or suppress excitation. The mechanisms of action most often correlated with anticonvulsant activity include effects on membrane function, particularly ionic conductances and membrane permeability and effects on the metabolism and/or disposition of neurotransmitters such as γ-aminobutyric acid (GABA), glycine, aspartic acid, acetylcholine and norepinephrine. In general, antiepileptic drugs effective against partial seizures, for example phenytoin, alter ionic transport across excitable membranes.

The complexity of the central nervous system, the variety of seizure types and the selectivity of drugs used to treat such seizures provide ample justification for the hypothesis of multiple antiepileptic mechanisms. Of the major anticonvulsant drugs in use, most are structurally similar. The general structure of anticonvulsant drugs is given in Figure 1.1. Some specific examples of anticonvulsant drugs and their structures are given in Table 1.3.
<table>
<thead>
<tr>
<th>Nature of X</th>
<th>Class of Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = CONH</td>
<td>Barbiturates</td>
</tr>
<tr>
<td>X = NH</td>
<td>Hydantoins</td>
</tr>
<tr>
<td>X = O</td>
<td>Oxazolidinediones</td>
</tr>
<tr>
<td>X = CH₂</td>
<td>Succinimides</td>
</tr>
<tr>
<td>X = NH₂</td>
<td>Acetylureas</td>
</tr>
</tbody>
</table>

Figure 1.1: General structure of anticonvulsant drugs.
### Table 1.3: Major classes of anticonvulsants

<table>
<thead>
<tr>
<th>Barbiturates</th>
<th>X R¹ R² R³ R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenobarbital</td>
<td>Ph Et H H</td>
</tr>
<tr>
<td>Mephobarbital</td>
<td>Ph Et H Me</td>
</tr>
<tr>
<td>Metharbital</td>
<td>Et Et H Me</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hydantoins</th>
<th>X R¹ R² R³ R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>Ph Ph H -</td>
</tr>
<tr>
<td>Ethotoin</td>
<td>Ph H Et -</td>
</tr>
<tr>
<td>Mephenytoin</td>
<td>Et Ph Me -</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oxazolidinediones</th>
<th>X R¹ R² R³ R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethadione</td>
<td>Me Me Me -</td>
</tr>
<tr>
<td>Paramethadione</td>
<td>Et Me Me -</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Succinimides</th>
<th>X R¹ R² R³ R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethosuximide</td>
<td>Et Me H -</td>
</tr>
<tr>
<td>Phensuximide</td>
<td>Ph H Me -</td>
</tr>
<tr>
<td>Methsuximide</td>
<td>Ph Me Me -</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deoxybarbiturates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primidone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acetylureas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacemide</td>
</tr>
</tbody>
</table>
Three other antiepileptic drugs, namely valproic acid, \(2\), carbamazepine, \(3\), and the benzodiazepines (for example diazepam, \(4a\), and clonazepam, \(4b\)) have unrelated chemical structures.

![Chemical structures](image)

\[ \text{CH}_3\text{CH}_2\text{CH}_2\]
\[\text{CH} - \text{COOH}\]
\[\text{CH}_3\text{CH}_2\text{CH}_2\]

![Chemical structures](image)

\[\text{R}^1 \quad \text{R}^2 \quad \text{R}^3\]

\[4a: \text{Me} \quad \text{Cl} \quad \text{H}\]

\[4b: \text{H} \quad \text{NO}_2 \quad \text{Cl}\]
The chemical diversity of structures known to possess anticonvulsant properties makes prediction of anticonvulsant activity difficult. Convulsions produced in experimental animals by a variety of widely used convulsants involve interference with major inhibitory amino acid neurotransmitters in the brain and spinal cord, most notably glycine and γ-aminobutyric acid (GABA) (Aird et al., 1984). Of the chemical convulsants, strychnine antagonizes the effects of glycine and causes a reduction of inhibitions mediated by glycine. Other chemical convulsants such as bicuculline, picrotoxin and penicillin are potent antagonists of the inhibitory action of GABA. Thiosemicarbazide and semicarbazide produce convulsions by antagonizing the action of pyridoxal phosphate, a cofactor for glutamic acid decarboxylase (GAD). By inhibiting GAD in presynaptic regions of neurons the synthesis of GABA is also decreased thereby producing convulsions. Many of the convulsant agents affect other processes as well, for example alteration of membrane properties producing an increased excitability of neurons. Although the mechanisms of action of many of the convulsive agents is not completely understood, interruption of the inhibitory process is a significant factor in effecting convulsant activity.

Two main in vivo test systems are employed in the routine screening of potential anticonvulsant compounds. The maximal electroshock (MES) test is used to detect compounds effective against grand mal epilepsy whereas the subcutaneous pentylenetetrazole-induced threshold (scMET) test detects those compounds effective versus absence seizures. These test systems are discussed in detail in Section 1.3.0.
1.2.0 Approaches to the development of anticonvulsant drugs

The biological action of a drug is a function of its structural, chemical, and physical properties. Gradual change in structure may lead to a quantitative change in the biological activity of drugs. Molecular manipulation or modification of drug compounds takes into account the significance of certain chemical groups in the drug molecule, or alternatively, may consider the drug molecule as a whole, with emphasis on overall physicochemical properties such as lipid/water-solubility and charge distribution effects. Several correlations and approaches may be utilized in the design and development of biologically active drug compounds.

1.2.1 The Hammett correlation

The Hammett correlation expresses the quantitative relationship between chemical reactivity and the electronic nature of a substituent on an aromatic ring for a homologous series. The Hammett relationship is expressed as in equation 1.1 (Hammett, 1937) where $K_H$ and $K_X$ are the rate or ionization constants for the unsubstituted and substituted compounds, respectively, sigma ($\sigma$) is the Hammett substituent constant which quantifies the electronic effect (electron donating or withdrawing properties) of a substituent group X on the aromatic ring of the compound and rho ($\rho$) is the reaction constant characteristic of the reaction under investigation.

$$\log \left( \frac{K_H}{K_X} \right) = \sigma \rho \quad \ldots \ldots \quad 1.1$$
The substituent constant, \( \sigma \), represents a combination of inductive and resonance effects for meta and para substituents. Both the sign and magnitude of \( \sigma \) and \( \rho \) provide information about the reaction being examined. Sigma (\( \sigma \)) is positive for electron withdrawing groups and negative for electron donating groups. Accordingly \( \rho \) will be positive for reactions that are favored by electron withdrawing groups and negative for reactions that are favored by electron donating groups.

1.2.2 Physicochemical Parameters

Certain parameters which represent the physicochemical properties of drugs may be utilized to correlate chemical structure with pharmacological activity. Lipophilic or hydrophobic parameters and steric parameters are examples of such parameters. The Hansch hydrophobic substituent constant or \( \pi \) value correlates biological activity with the partition coefficient and is defined by equation 1.2.

In this equation \( \pi \) is the measure of contribution of the substituent to solubility and \( P_H \) and \( P_X \) are the partition coefficients for the unsubstituted and substituted compounds respectively. If \( \pi \) (\( \pi \)) is positive the substituent group increases the solubility of the compound in nonpolar solvents (increases lipid solubility) and if it is negative the substituent group increases the solubility of the compound in polar solvents. Substituent (\( \pi \)) constants permit estimation of partition coefficients for most structures. Groups which have essentially equivalent contributions to the partition coefficient may be referred to as isoliphilic groups. If lipophilicity is important in determining the biological activity then substitution of one isolipophilic group for another may allow retention of activity.
Log \( \frac{P_X}{P_H} \) = \( \pi_X \) \quad \ldots \ldots 1.2

Steric parameters reflect the form and size of the substituent introduced in a parent molecule and may represent width, bulk, topology or dimensions in defined directions. The Taft \( E_S \) values are measures of the van der Waals radii of a substituent; the bulkier the substituent the more negative is the steric parameter \( E_S \). The Taft \( E_S \) parameter is considered since the interaction of a drug with its site of action involves the mutual approach of molecules. Taft steric parameters are utilized to quantitate the role of the size of substituents with the biological potency of the compounds. If the steric parameter is a dominating factor and is responsible for drug interaction with a particular receptor then only groups with similar \( E_S \) values would be bio-isosteric, that is their exchange would result in drug compounds with comparable or improved biological activity.

The biological activity of a compound is due to a combination of all the physicochemical properties of a molecule. Therefore it is not usually possible to obtain perfect correlation of biological action if only one parameter or a reduced number of parameters are considered, unless such parameters play a predominant role in determining biological activity. The biological activity of a drug is multiconditional based on a sequence of complicated physicochemical events, where conditions for a myriad of processes must be fulfilled (Ariens, 1971).
Properties optimal for one step may be contradictory or incompatible with other individual processes. In some cases detection of a relationship between physicochemical properties of the drug molecules and their biological action is not possible.

1.2.3 Topliss approaches to drug design

The Topliss decision-tree approach to drug design is one of the non-mathematical and relatively simple approaches for the optimization of activity of a lead compound. The approach can be applied to aromatic ring substitutions and side chain modifications and involves the introduction of predictable changes in certain parameters (i.e. $\sigma$, $\pi$ and $E_s$ values, classification of the biological activity and interpretation of the results. The decision-tree approach for aromatic substitution is shown in Figure 1.2 (Topliss, 1972). First the 4-chloro analog is prepared and its biological activity compared to that of the parent unsubstituted compound. If the 4-chloro derivative is more active, then the 3,4-dichloro derivative will be synthesized, since this will further increase the magnitude of both the $\pi$ ($\pi$) and sigma ($\sigma$) values. Alternatively the 4-methoxy derivative is prepared if the 4-chloro derivative is less active than the parent compound and the 4-methyl analog is prepared if the 4-chloro derivative is equiactive to the parent compound. Similarly, the decision-tree scheme for side chain alkyl groups is presented in Figure 1.3. Stepwise synthesis and evaluation of the new derivatives according to the operational decision-tree approach is followed until optimum activity is attained.
Figure 1.2: Operational scheme for aromatic substitution. The descending lines indicate the sequence of compounds to be prepared; the brackets indicate alternative compounds. L: less active; E: equiactive; M: more active compound. Taken from Topliss (1972) and reproduced with permission of the copyright owner.
Figure 1.3: Operational scheme for side chain alkyl groups. The descending lines indicate the sequence of compounds prepared. L: less active; E: equiactive; M: more active. Taken from Topliss (1972) and reproduced with permission of the copyright owner.
An alternative approach proposed by Topliss (1977) is based on the initial synthesis and biological evaluation (potency order) of five substituted derivatives, namely the unsubstituted, 4-chloro, 3,4-dichloro, 4-methoxy and 4-methyl derivatives. The dependency of the series in terms of $\sigma$ and $\pi$ values and various combinations of these parameters is determined by comparison with Table 1.4. Consultation with Table 1.5 then determines which substituents should be investigated next. Thus, information about the parameter dependence can be utilized to generate optimally active (lead) compounds. This approach overcomes the difficulty of the stepwise nature of the first Topliss decision-tree approach. Structural modification of a lead compound and analog development will be presented in Section 2.1.0.
### Table 1.4: Potency order for various parameter dependencies

<table>
<thead>
<tr>
<th>Substituents</th>
<th>Parameters</th>
<th>π</th>
<th>$2π-π^2$</th>
<th>σ</th>
<th>$-σ$</th>
<th>$π+σ$</th>
<th>$2π-σ$</th>
<th>$π-σ$</th>
<th>$π-2σ$</th>
<th>$π-3σ$</th>
<th>$E_4^B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Cl₂</td>
<td>3-CF₃, 4-Cl; 3-CF₃, 4-NO₂; 4-CF₃; 2,4-Cl₂; 4-c-C₆H₅; 4-C₂H₅</td>
<td>1</td>
<td>1-2</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1-2</td>
<td>3-4</td>
<td>5</td>
<td>2-5</td>
</tr>
<tr>
<td>4-Cl</td>
<td>4-CH(CH₃)₂; 4-C(CH₃)₃; 3,4-(CH₃)₂; 4-O(CH₂)₃CH₃; 4-OCH₂Ph; 4-N(C₂H₅)₂</td>
<td>2</td>
<td>1-2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2-3</td>
<td>3</td>
<td>3-4</td>
<td>3-4</td>
<td>2-5</td>
</tr>
<tr>
<td>4-CH₃</td>
<td>4-N(C₆H₅)₂; 4-N(CH₃)₂; 4-NH₂; 4-NHC₆H₅; 4-OH; 4-OCH(CH₂)₃; 3-CH₃; 4-OCH₃</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2-3</td>
<td>1-2</td>
<td>1</td>
<td>1</td>
<td>2-5</td>
</tr>
<tr>
<td>4-OCH₃</td>
<td>4-Br; 3-CF₃; 3,4-(CH₃)₂; 4-C₂H₅; 4-O(CH₂)₃CH₃; 3-CH₃; 4-Cl</td>
<td>4-5</td>
<td>4-5</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2-5</td>
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<td>H</td>
<td>4-5</td>
<td>4-5</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>3-4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Taken from Topliss (1977) and reproduced with permission of the copyright owner.

*b* Unfavorable steric effect from 4-substitution.

### Table 1.5: New substituent selection

<table>
<thead>
<tr>
<th>Probable operative parameters</th>
<th>New substituent selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>π, π+σ, σ</td>
<td>3-CF₃, 4-Cl; 3-CF₃, 4-NO₂; 4-CF₃; 2,4-Cl₂; 4-c-C₆H₅; 4-c-C₂H₅</td>
</tr>
<tr>
<td>π, 2π-σ, π-σ</td>
<td>4-CH(CH₃)₂; 4-C(CH₃)₃; 3,4-(CH₃)₂; 4-O(CH₂)₃CH₃; 4-OCH₂Ph; 4-N(C₂H₅)₂</td>
</tr>
<tr>
<td>π-2σ, π-3σ, -σ</td>
<td>4-N(C₆H₅)₂; 4-N(CH₃)₂; 4-NH₂; 4-NHC₆H₅; 4-OH; 4-OCH(CH₂)₃; 3-CH₃; 4-OCH₃</td>
</tr>
<tr>
<td>$2π-π^2$</td>
<td>4-Br; 3-CF₃; 3,4-(CH₃)₂; 4-C₂H₅; 4-O(CH₂)₃CH₃; 3-CH₃; 4-Cl</td>
</tr>
<tr>
<td>Ortho effect</td>
<td>2-Cl; 2-CH₃; 2-OCH₃; 2-F</td>
</tr>
<tr>
<td>Other</td>
<td>4-F; 4-NHCOC₅H₅; 4-NHSO₂CH₃; 4-NO₂; 4-COCH₃; 4-SO₂CH₃; 4-CNH₂; 4-SO₂NH₂</td>
</tr>
</tbody>
</table>

*a* Taken from Topliss (1977) and reproduced with permission of the copyright owner.
1.2.4 Conformational and stereochemical features of potential anticonvulsant agents

Compounds that exhibit a common biological effect may be divided into two basic classes, namely structurally diverse and structurally similar compounds. Structurally similar compounds may be involved with specific interactions with common receptors. Such receptors may interact with the drugs via one or more of the following mechanisms (Chu, 1980; Stenlake, 1979; Ariens, 1971).

1) Electrostatic effects, for example charge-dipole interactions.
2) Hydrophobic interactions.
3) Secondary force interactions, such as hydrogen bonding.
4) Charge transfer reactions such as π - π interactions.
5) Intramolecular bonds.

Structurally diverse chemicals can exhibit a common biological effect by totally different mechanisms or by the same or similar mechanisms of action in a number of instances (Chu, 1980; Lien, 1970), for example the following.

1) Possession of common physical properties, for example partition coefficient, degree of ionization, size or electronic properties.
2) Metabolism to a "common" intermediate.
3) Similar topography and geometric characteristics which allows interaction with a common receptor.
When pharmacological activities of the major anticonvulsant compounds are compared with a variety of independent variables correlations between anticonvulsant activity and the partition coefficient, molecular weight and dipole moment have been observed (Jones and Woodbury, 1982; Lien, 1970). In general anticonvulsants are of low molecular weight, possess relatively high lipid solubility, are weak acids or bases and are poorly water soluble.

Structural characteristics of molecules may be divided into topological (two-dimensional) and topographical (three-dimensional) patterns. Topological patterns may include descriptors such as the following molecular features (Chu, 1979).

1) Types and number of atoms and bonds.
2) Types of functional groups.
3) Size and shape of ring nuclei.
4) Connections between groups, for example the carbon path between heteroatoms.

Topographical molecular patterns involve a group of atoms and their interatomic distances. These patterns or pharmacophores may correlate well with the biological activity displayed by certain compounds. The pharmacophoric moiety may be defined as that portion of the molecule which imparts pharmacological action to the drug. Structure activity relationships involve correlation of the contribution of specific moieties in a molecule to particular aspects of drug action or toxicity and recognizing critical and noncritical moieties in the drug molecule.
Among the chemically and structurally diverse anticonvulsant agents, certain structural requirements have been found to be necessary for molecules to be effective against generalized tonic-clonic (GTC) seizures and absence seizures (Jones and Woodbury, 1982). At least two electron donor atoms (usually oxygen or nitrogen) in some proximity to a hydrophobic site are required for activity against both MES- and scMET-induced seizures. For activity against MES-induced seizures, the hydrophobic group should be a bulky phenyl or other aryl group. Smaller alkyl groups confer activity against scMET-induced seizures. The incorporation of both aryl and alkyl substituents in the presence of two electron donor atoms has produced agents effective against both petit mal and grand mal epilepsies.

Preferred conformations of molecules can be determined and many apparently different molecules may possess similar interatomic distances between like atoms (Chu, 1979). Camerman and Camerman (1977) demonstrated the conformational similarity between a number of anticonvulsant agents, many of which are chemically unrelated, and have shown that the distance between alkyl and aryl groups is critical in order for compounds to exhibit anticonvulsant activity. Thus the interatomic distances between the two electron-donor atoms thought to be involved in anticonvulsant activity and the distance between these atoms and the hydrophobic groups has been measured in a number of anticonvulsants. The distance between the two electronegative carbonyl oxygens in phenytoin is 4.56Å. When the phenyl groups of diazepam and phenytoin are superimposed the carbonyl oxygen and the trigonal
nitrogen at the 4-position of the azepine ring approximate to the positions of the two carbonyl oxygen atoms of phenytoin. The interatomic distance in this case is 3.35 Å. Furthermore, Camerman and Camerman proposed that a critical lower limit of approximately 2.4 Å must be exceeded before a particular compound will possess significant anticonvulsant activity (Jones and Woodbury, 1982). However, on comparison of interatomic distances with anti-MES and anti-scMET potencies for various anticonvulsants no quantitative correlation can be demonstrated.

Although it is well established that a phenyl or similar aromatic group is required for anti-MES activity the stereochemical dependence proposed by Camerman and Camerman seems to apply only to anticonvulsants effective against generalized tonic-clonic seizures. For example, trimethadione and similar alkyl-substituted compounds that possess selective action against absence seizures do not conform to the structural requirements proposed. Although diazepam and similar 1,4-benzodiazepines have two bulky hydrophobic groups they are actually more potent when tested in the scMET model than in the MES test system. This inconsistency may be explained by the existence of a fundamentally different mechanism of action for the benzodiazepines based on evidence from receptor binding studies (Jones and Woodbury, 1982; Caccia and Garattini, 1985).
Straight chain analogues of GABA have been found to exhibit anticonvulsant activity in a variety of test systems (Vida, 1977). The pharmacophoric pattern necessary for GABA-like action requires a fully extended zwitterionic molecule in which the distance between the onium group and the carboxylate oxygen atoms is ideally 5 - 6 Å as depicted below (Murray and Kier, 1977; Kier et al., 1974).

The four prominent structural classes of antiepileptics, the hydantoins \(5\), the oxazolidinediones \(6\), the succinimides \(7\) and the barbiturates \(8\) are metabolized to compounds resembling GABA as depicted in Scheme 1.1. The conformations of the open chain acids, \(9\), \(10\), \(11\) and \(12\) fit the predicted GABA pharmacophore and thus these metabolites may have anticonvulsant activity via GABA-like effects.
Scheme 1.1: Metabolism of antiepileptic drugs.

5: $X = \text{NH}$, hydantoins
6: $X = \text{O}$, oxazolidinediones
7: $X = \text{CH}_2$, succinimides

8: barbiturates

9: $X = \text{NH}$
10: $X = \text{O}$
11: $X = \text{CH}_2$

12
1.3.0 Screening of potential anticonvulsant agents

Screening of potential anticonvulsant agents is designed *inter alia* to evaluate four aspects of the drug action:

1) The existence and specificity of anticonvulsant activity.

2) Toxicity, especially to the central nervous system.

3) Potency of the drug and the protective index.

4) The time course of the activity.

Almost all marketed antiepileptic agents have been investigated and developed based on results obtained from experimental models of epilepsy. The two common experimental models of epilepsy are the maximal electroshock seizure (MES) test and the subcutaneous pentylenetetrazole (Metrazole) seizure threshold (scMET) test. The rotorod ataxia test is routinely performed to evaluate toxicity of the test compound. The MES test detects the ability of the compound to prevent the spread of seizure discharge through neural tissue and correlates with effectiveness against grand mal seizures. Activity versus scMET-induced seizures indicates the ability of a compound to prevent threshold seizures. Succinimides and oxazolidinediones, drugs active against absence (petit mal) seizures, are particularly effective anticonvulsants in this test.

Pentylenetetrazole (Metrazole) injected subcutaneously to mice produces seizures in more than 97% of animals. This is called the convulsive dose 97 or CD$_{97}$. The CD$_{97}$ in rats is 70mg/kg. Pentylenetetrazole activates excitatory pathways due to a decrease in the threshold of neurons. The scMET test measures the ability of
anticonvulsant drugs to provide complete protection against threshold (clonic) seizures induced by the subcutaneous injection of the CD₉₇ of pentylentetrazole. Protection is defined as failure to observe even a threshold seizure (a single episode of clonic spasms at least five seconds in duration) during the thirty minute observation period and indicates anticonvulsant activity in the test compound.

The MES test is performed according to the method described by Swinyard and co-workers (1982) and produces a maximal seizure in normal mice. Animals are stimulated through corneal electrodes by a 60-cycle alternating current (50mA in mice and 150mA in rats) applied for 0.2-0.3 seconds. Electroshock seizures are characterized by tonic limb flexion (1-2 seconds), followed by tonic limb extension (10-12 seconds) and finally generalized clonic movements for approximately 12 seconds. Protection is defined as abolition of the hind limb extension and indicates that the compound possesses anticonvulsant activity. The animals are challenged at certain time intervals following drug administration. The intervals at which the greatest percentage of animals are protected are recorded as the time of peak activity. The MES test is useful to select drugs likely to be effective in grand mal and psychomotor seizures.

The rotorod ataxia test, designed to detect minimal neurotoxicity, consists of placement of the animal on a one inch diameter rod rotating at 6 rpm. Normal mice can remain indefinitely on the rod, but the failure of the animal to maintain equilibrium on the rod for at least one minute, in each of three trials, is defined as neurological deficit (for example ataxia, sedation, hyperexcitability).
The rotorod ataxia test is used to evaluate central nervous system toxicity, and correlates well with the clinical assessment of minimal neurotoxicity.

The anticonvulsant evaluation of compounds described in this thesis was carried out by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, Maryland, U.S.A., using their protocols (Porter et al., 1984). The Anticonvulsant Screening Project (ASP) testing protocol is comprised of seven phases as described in Table 1.6. If results are favorable the compounds are tested further in the Toxicology Project and, in turn, clinical investigations may be conducted if warranted.
Table 1.6: Test phases in the Anticonvulsant Screening Project

| Phase 1: Anticonvulsant identification to determine the level of activity |
| [active (≤100 mg/kg) to inactive (>300 mg/kg)] (mice, i.p.) |
| 1. Maximal electroshock (MES) test - seizure spread |
| 2. Subcutaneous pentylenetetrazole (scMET) test - seizure threshold |
| 3. Rotorod ataxia test - neurotoxicity |
| Phase 2: Anticonvulsant quantification to determine the level of activity at the ED50, TD50, and protective index (TD50/ED50) (mice, i.p.) |
| 1. Maximal electroshock (MES) test - seizure spread |
| 2. Subcutaneous pentylenetetrazole (scMET) test - seizure threshold |
| 3. Rotorod ataxia test - neurotoxicity |
| Phase 3: Toxicity profile to assess general behavior and selected pharmacologic response at toxic doses (mice, i.p.) |
| 1. Median lethal dose (LD50) |
| 2. Median hypnotic dose (HD50) |
| Phase 4: Anticonvulsant quantification to measure activity by the usual clinical route of administration and indicate the absorption and metabolic characteristics of the compound (mice, p.o.) |
| 1. Maximal electroshock (MES) test - seizure spread |
| 2. Subcutaneous pentylenetetrazole (scMET) test - seizure threshold |
| 3. Rotorod ataxia test - neurotoxicity |
| Phase 5: Antiepileptic drug differentiation and comparison with known effective drugs to help determine the mechanism of action (mice, i.p.) |
| 1. Pentylenetetrazole seizure threshold test |
| 2. Picrotoxin seizure threshold test |
| 4. Strychnine seizure threshold test. |
| 5. Special in vitro receptor binding studies on selected candidate compounds |
| Phase 6: Anticonvulsant quantification to measure activity in another species at the ED50, TD50, and protective index (TD50/ED50) (rats, p.o.) |
| 1. Maximal electroshock (MES test) |
| 2. Subcutaneous pentylenetetrazole (scMET) test |
| 3. Positional sense test - neurotoxicity |
| 4. Gait and stance test - neurotoxicity |
| Phase 7: Estimation of minimal lethal dose (LD3) and effect of prolonged administration on anticonvulsant activity (rats, p.o.) |
| 1. Estimated LD3 in male and female rats following administration once a day for 5 days. |
| 2. Administration for 5 days - tolerance |
| 3. Hexobarbital sleep time test - tolerance |
| 4. Microsomal enzyme studies in vitro - tolerance |

a Taken from Porter et al. (1984) and reproduced with permission of the copyright owner.
Phase 1 in the ASP (anticonvulsant identification in mice; compounds administered by the intraperitoneal route) estimates the potency and neurotoxicity of active compounds and eliminates from further consideration toxic or inactive compounds. Testing is carried out at doses of 30, 100 and 300 mg/kg (using four mice at each dose level) 30 minutes and four hours after compound administration. Compounds tested fall into one of four categories:

1) Compounds with no anticonvulsant activity at doses up to 300 mg/kg are not tested further.

2) Compounds with activity at 100 mg/kg are generally advanced to further testing.

3) Compounds active at 300 mg/kg may be tested further depending on the novelty of the structure.

4) Compounds with activity and/or toxicity at 30 mg/kg are usually retested and may be tested further.

Phase 2 quantitates the anticonvulsant activity and neurotoxicity of the compounds. The median effective dose ($ED_{50}$) is the dose of a compound that elicits an anticonvulsant response in 50% of the animals, and when compared to that required for a standard of reference the $ED_{50}$ provides information relating to potency and potential therapeutic value. $ED_{50}$ values can be misleading unless determined at the time of peak activity of the test compound which presumably correlates with brain concentration of the drug or an active metabolite. The $ED_{50}$ may be influenced by a variety of factors, such as the species, strain, sex and age of the animal and the route of administration.
Toxic effects are measured by the median toxic dose (TD$_{50}$) and median lethal dose (LD$_{50}$). The TD$_{50}$ is the dose required to produce neurological deficit and the LD$_{50}$ is the dose which causes death in 50% of the animals. The ED$_{50}$ is determined in mice using the MES and scMET tests at the time of peak effect and the median toxic dose (TD$_{50}$) is determined at the time of peak neurological deficit. Compounds failing to produce a minimal neurological deficit are tested up to doses ten times their lowest anticonvulsant ED$_{50}$ when possible. The protective index (P.I.) of the compound is defined as the TD$_{50}$ /ED$_{50}$ and is a rough measure of the safety of the drug.

Compounds evaluated in screen 1 and 2 of the Anticonvulsant Screening Project may be classified on the basis of their anticonvulsant activity as follows.

1) Potent compounds exhibit activity at 30 or 100 mg/kg.
2) Moderately potent compounds possess activity only at 300 mg/kg.
3) Impotent compounds show no activity at any dose level.

In phase 3 the median hypnotic dose (HD$_{50}$) and the median lethal dose (LD$_{50}$) are determined to establish the compounds' toxicity profile. The test compound is administered to mice by the intraperitoneal route at the TD$_{50}$, twice the TD$_{50}$, and four times the TD$_{50}$ (two mice at each dose level are utilized). The mice are tested at 10, 20 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours post administration and observed for the onset, intensity and nature of overt toxicity.
Phase 4 testing is similar to phase 2 except that the compounds are administered orally as opposed to intraperitoneally to uncover the absorption and metabolic characteristics of the test compound.

The *in vivo* and *in vitro* antiepileptic potential of the test compounds is determined in phase 5 of the protocol. Selective chemical convulsants, namely pentylenetetrazole, strychnine, picrotoxin and bicuculline are administered subcutaneously. The CD$_{97}$ of the convulsant is injected into mice at the time of peak effect of the test compound. Each of these convulsants induce seizures by different mechanisms and the resulting ED$_{50}$s may reflect the activity profile of the test compound. Anticonvulsant activity of the compounds can be compared with clinically effective antiepileptic drugs. Receptor binding of the test compound is correlated with its anticonvulsant activity in the *in vitro* test.

Phase 6 consists of anticonvulsant quantification in rats and verifies the anticonvulsant activity and neurotoxicity in another rodent species. After oral administration the ED$_{50}$s in the MES and scMET tests are determined. The positional sense, gait and stance test are used to evaluate neurotoxicity. Compounds found active in mice are generally active in rats although the therapeutic-or protective indices may differ.

Phase 7 of the protocol determines the minimum lethal dose (LD$_3$) and the effect of prolonged administration on anticonvulsant activity of the compound administered orally in rats. Both male and female rats are given the estimated minimal lethal dose for 5 days.
Development of tolerance to the anticonvulsant effect is determined by administration of the ED$_{50}$ to one group of male rats for five consecutive days, with anticonvulsant activity at the time of peak effect being determined on day five using the MES or scMET test. A second group receives only saline or suspension media for four days, followed by the ED$_{50}$ on day five using the MES or scMET test at the time of peak effect. The third group receives saline or suspension media for five days and is tested on the fifth day. Development of tolerance is indicated if the rats in group one experience a greater number of seizures than those in group two. In addition tolerance may be determined by the hexobarbital sleep time test and microsomal enzyme studies in vitro.

If results from all seven phases of the ASP are favorable compounds may undergo further testing in the Toxicology Project which identifies potential sites of toxicity prior to clinical trials of the test compound. Selection of compounds for toxicity studies is based on the following criteria.

1) Adequate absorption after oral administration to mice and rats.
2) Adequate protective indices following oral and intraperitoneal administration to mice and rats.
3) Novel chemical structure.
4) Absence of tolerance to the anticonvulsant effects.

The most commonly used anticonvulsant drugs have been evaluated in phases 2 to 6 of the Anticonvulsant Screening Project and the results are given in Table 1.7.
<table>
<thead>
<tr>
<th>Compound</th>
<th>MES ED$_{50}$ (mg/kg)</th>
<th>scMET ED$_{50}$ (mg/kg)</th>
<th>Rot. TD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>9.5</td>
<td>not active</td>
<td>65.5</td>
</tr>
<tr>
<td>Mephenytoin</td>
<td>60.5</td>
<td>30.5</td>
<td>153.8</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>21.8</td>
<td>13.2</td>
<td>69.0</td>
</tr>
<tr>
<td>Primidone</td>
<td>11.4</td>
<td>58.6</td>
<td>679.7</td>
</tr>
<tr>
<td>Trimethadione</td>
<td>627.5</td>
<td>300.5</td>
<td>819.1</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>&gt;1000</td>
<td>130.4</td>
<td>440.8</td>
</tr>
<tr>
<td>Methsuximide</td>
<td>76.3</td>
<td>68.3</td>
<td>187.6</td>
</tr>
<tr>
<td>Diazepam$^c$</td>
<td>19.1</td>
<td>0.17</td>
<td>7.3</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>86.6</td>
<td>0.02</td>
<td>0.18</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>8.8</td>
<td>not active</td>
<td>71.6</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>271.7</td>
<td>148.6</td>
<td>425.8</td>
</tr>
</tbody>
</table>

$^a$ Adapted from Porter et al. (1984) and reproduced with permission of the copyright owner.

$^b$ MES = Maximal electroshock test, scMET = subcutaneous pentylene-tetrazole (Metrazole) test, Rot. = rotorod ataxia test; ED$_{50}$ = Median effective dose, TD$_{50}$ = Median toxic dose.

$^c$ Adjunct only.
2.0.0.0 RATIONALE OF THE PRESENT INVESTIGATION

2.1.0 Introduction

Most anticonvulsant agents have been investigated and developed based on results obtained from screening of potential drugs. A "lead" or prototype compound of known biological activity is subjected to systematic structural modification (analog development) in an effort to improve efficacy and decrease toxicity. The following chemical approaches can be utilized in structural modification (Korolkovas and Burckhalter, 1976; Craig, 1980).

1) The preparation of a series of homologous compounds or modification of compounds by chain branching which causes changes in lipophilicity and structural features.

2) The preparation of isosteres which involves the insertion of substituents with similar steric, electronic or other physical or chemical properties. For example, groups that possess similar electron-donating or electron-withdrawing properties may be considered to be isoelectronic groups.

3) Resolution of isomeric mixtures.

4) The concept of disjunction or molecular simplification which involves the synthesis and evaluation of simpler and simpler analogues of the prototype compound.

5) Preparation of topological analogs i.e. retention of general topological relationships while making alterations in the reference or lead compound.
Structural modifications of a lead compound are designed to achieve specific goals such as the following improvements over the prototype molecule.

1) The development of more potent analogs.
2) To eliminate or minimize toxic effects.
3) To discover the pharmacophoric moiety and to identify and separate the molecular features responsible for the desired activity and the undesirable or toxic effects.
4) Modification of the pharmacokinetic properties of the compound.

The application of some of these chemical approaches to structural modification will be presented.

Various semicarbazones, thiosemicarbazones and related compounds have been found to exhibit a wide range of biological activities including anticonvulsant activity. Examples of compounds chemically and structurally related to hydrazone derivatives which exhibit anticonvulsant activity include 4-substituted benzenesulfonamides, \textsuperscript{13}, phenylureas, \textsuperscript{14}, and acylureas, \textsuperscript{15}.
Numerous semicarbazones, thiosemicarbazones, hydrazone and hydrazide derivatives have been found to possess anticonvulsant activity (Popp, 1977). Many anticonvulsant agents contain an amide, imide or urea subunit and the fragment shown below, which is chemically similar to the semicarbazone and thiosemicarbazone moieties, has been proposed as being necessary for anticonvulsant activity (Murray and Kier, 1977).
The present project involves the synthesis and anticonvulsant evaluation of a number of thiosemicarbazones of aryl-substituted acetophenones and some related derivatives with the following aims in mind.

1) To seek correlations between chemical structure, anticonvulsant activity and toxic effects.

2) To obtain predictive values from the above studies that could be useful in designing further series of compounds.
2.1.1 Previous Work Undertaken in these Laboratories

The preparation and anticonvulsant evaluation of some thiosemicarbazones and semicarbazones of 4-aryl-3-buten-2-ones has been previously undertaken (Dimmock et al., 1986). These compounds possess a small hydrophobic methyl group and an arylidene function on the carbon atom alpha to the groups containing the electron donor atoms viz the thiosemicarbazono and semicarbazono functions. Thus, the structural requirements proposed to be necessary for compounds to possess anticonvulsant activity against MES and scMET induced seizures (Jones and Woodbury, 1982) have been fulfilled. These compounds may have advantages over clinically used barbiturates and related bioisosteric heterocycles since they do not possess a dicarboximide function (-CO-NH-CO-), which may be associated with toxicity and side effects, such as sedation, of these drugs (Kadaba, 1984; Andrews, 1969). A majority of the thiosemicarbazones and semicarbazones in the series showed some anticonvulsant activity. The thiosemicarbazones (16a) and (16b) and the semicarbazones (17a–c) were of particular interest.

![Chemical Structure](image)

16a: $R = H$

b: $R = CH_3$
All compounds were screened in phase 1 of the Anticonvulsant Screening Project (ASP). Compound 16a exhibited notable anticonvulsant activity in the MES test. Compound 16b demonstrated marked potency in the scMET test with an ED\textsubscript{50} of 6.96 mg/kg and a P.I. of 10.37. This is of comparable or higher activity than clinically used anticonvulsants (see Table 1.7 for comparison).

Of the semicarbazones 17a exhibited partial protection against scMET-induced seizures and displayed no neurotoxicity at doses up to and including 2000mg/kg. Compound 17b demonstrated activity in both the MES and scMET screens, with ED\textsubscript{50} values of 229.1 and 746 mg/kg respectively which compares favorably with standard anticonvulsants.
Phase 4 screening involved oral administration of the compounds to mice. Compared to intraperitoneal injection oral administration of 16a effected a 17-fold reduction in activity in the scMET screen. Compound 17a displayed activity by the oral route in the MES test in contrast to the intraperitoneal route where no activity was noted in a dose of 1000mg/kg. No neurotoxicity was evident in doses up to and including 2000mg/kg. Compound 16b was examined in phase 5 of the Anticonvulsant Screening Project. The in vivo test in phase 5 examines the ability of the compounds to prevent seizures induced by bicuculline, picrotoxin, and strychnine. Bicuculline exerts its convulsant activity by blockade of GABA receptors whereas picrotoxin is a noncompetitive GABA antagonist at presynaptic sites. Strychnine blocks postsynaptic inhibition mediated by glycine. Compound 16b afforded partial protection against bicuculline-induced seizures. The ED₅₀ value against picrotoxin-induced seizures was 21.22 mg/kg which is comparable to the ED₅₀ value of 27.5 mg/kg for phenobarbital in this test. The anticonvulsant properties of 16b may be associated with potentiating the action of GABA.

Receptor binding of the compound is correlated with anticonvulsant activity in the in vitro phase 5 test. Benzodiazepines are known to facilitate transmission of GABA in the central nervous system and if a drug compound acted at the benzodiazepine receptor site the action of GABA may be potentiated. However, compound 16a was not observed to interact at the benzodiazepine receptor. Compound 16b was also administered orally to Sprague-Dawley rats in phase 6 of the ASP. No protection in the MES or scMET screens and no neurotoxicity at doses ranging from 400 to 1000 mg/kg was noted for this compound in phase 6.
In order to develop structure–activity relationships, Dimmock et al. (1989) synthesized compounds in series 18 and 19 in which the methyl group attached to the C–N double bond was replaced with other atoms or groups, namely hydrogen, ethyl, iso-propyl, phenyl and cyclohexyl.

\[ \begin{align*}
\text{a: } R &= \text{CH}_3 \\
\text{b: } R &= \text{H} \\
\text{c: } R &= \text{C}_2\text{H}_5 \\
\text{d: } R &= \text{CH(CH}_3)_2 \\
\text{e: } R &= \text{C}_6\text{H}_5 \\
\text{f: } R &= \text{c-C}_6\text{H}_{11}
\end{align*} \]
In series 18, only 18a exhibited anticonvulsant activity in phase 1 screening. Activity at 30 mg/kg in the scMET screen was noted at the end of 4 hours for this compound. In series 19, compounds 19a, 19b and 19e demonstrated anticonvulsant activity against both MES- and scMET-induced seizures. Compound 19d showed activity in the MES screen at the end of 0.5 hours and compounds 19c and 19f were inactive in the screens.
Phase 2 screening of compounds 18a, 19a, 19b and 19e involved quantification of the anticonvulsant activity. Compound 18a had an $ED_{50}$ value of 6.96 mg/kg and a $TD_{50}$ value of 72.18 mg/kg against pentylenetetrazole-induced seizures at the end of 4 hours. Compounds 19a, 19b and 19e with $ED_{50}$ values of 39.35 mg/kg, 44.19 mg/kg and 191.03 mg/kg in the MES screen compare favorably with clinically used antiepileptic drugs.

Molecular simplification of the thiosemicarbazones of the aryldene ketones (series 18 and 19) led to the synthesis and anticonvulsant screening of some thiosemicarbazones of aryl-substituted acetophenones, series 20 and 21. Compounds in series 20 include the unsubstituted compound 20a and the other four derivatives required for a Topliss analysis.

$$\text{C-CH}_3$$
$$\text{N-N-C-NH}_2$$
$$\text{R}^1, \text{R}^2$$

20

a: $R^1 = R^2 = H$
b: $R^1 = \text{CH}_3; R^2 = H$
c: $R^1 = \text{OCH}_3; R^2 = H$
d: $R^1 = R^2 = \text{Cl}$
e: $R^1 = \text{Cl}; R^2 = H$
The nuclear substituents chosen in series 20 have Hammett values ranging from -0.27 to +0.60 (Lewis, 1986). In addition, compounds in series 21 where the aliphatic function (the methyl group) has been replaced by another atom or group (namely by a hydrogen atom, an ethyl group, an iso-propyl group and a phenyl moiety) have been previously prepared.

The presence of one methyl group, or none, or the presence of bulkier alkyl or aryl groups on the carbon atom bearing the thiosemicarbazono group may have both electronic and steric influences. The Taft steric parameter ($E_s$) for the hydrogen, methyl, ethyl, iso-propyl, cyclohexyl and phenyl moieties are 0.00, -1.24, -1.31, -1.71, -2.03 and -2.42 respectively (Hansch and Leo, 1979).
Results of the anticonvulsant screening of compounds in series 20 and 21 is presented in Table 2.1. The phase 1 screen involves intraperitoneal administration of the compounds in polyethylene glycol 400 (30%) into Carworth number 1 mice at doses of 30, 100 and 300 mg/kg. Four animals at each dose level are normally used.

Acetophenone and thiosemicarbazide have been evaluated in the Anticonvulsant Screening Project and the results are given in Table 2.1.

Phase 2 involves administration of compounds by the intraperitoneal route to mice and evaluation of the anticonvulsant activity at 0.25, 0.5, 1, 2 and 4 hours after administration. Acetophenone had an ED₅₀ of 79.24 mg/kg in the MES screen 0.25 hours after injection. No neurotoxicity was noted between 21.25 and 170 mg/kg.

Phase 6 screening involves oral administration of the compounds to Sprague-Dawley rats. Phase 6a evaluates animals at 0.25, 0.5, 1, 2 and 4 hours after administration. Phase 6b involves quantification of the anticonvulsant activity and neurotoxicity in rats. The ED₅₀ in the MES and scMET tests and the TD₅₀ are determined at the time of peak effect. Data obtained from phase 6 screening of compounds in series 20 and 21 are summarized in Tables 2.2 and 2.3.
Table 2.1: Phase 1 screening of compounds 20a-e, 21a-d, acetophenone and thiosemicarbazide

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES screen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>scMET screen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Neurotoxicity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5h 4h</td>
<td>0.5h 4h</td>
<td>0.5h 4h</td>
</tr>
<tr>
<td>20&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>100 100&lt;sup&gt;ic,d&lt;/sup&gt;</td>
<td>300 100&lt;sup&gt;ic,d&lt;/sup&gt;</td>
<td>(T(100) T(100))</td>
</tr>
<tr>
<td>b</td>
<td>1 1</td>
<td>30 30</td>
<td>(T(100) T(100))</td>
</tr>
<tr>
<td>c</td>
<td>300 I</td>
<td>I I</td>
<td>N.T. N.T.</td>
</tr>
<tr>
<td>d</td>
<td>300 300</td>
<td>I I</td>
<td>N.T. T(30)</td>
</tr>
<tr>
<td>e</td>
<td>100 I&lt;sup&gt;e&lt;/sup&gt;</td>
<td>300&lt;sup&gt;f&lt;/sup&gt; 30&lt;sup&gt;g&lt;/sup&gt;</td>
<td>T(300) T(30)</td>
</tr>
<tr>
<td>21&lt;sup&gt;a&lt;/sup&gt;a</td>
<td>30 100</td>
<td>30 100</td>
<td>(T(100) T(100))</td>
</tr>
<tr>
<td>b</td>
<td>100 100</td>
<td>I h</td>
<td>N.T. T(300)&lt;sup&gt;d,i&lt;/sup&gt;</td>
</tr>
<tr>
<td>c</td>
<td>100 100</td>
<td>I I</td>
<td>T(300) T(300)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>d</td>
<td>100 100</td>
<td>I&lt;sup&gt;j&lt;/sup&gt; I&lt;sup&gt;j&lt;/sup&gt;</td>
<td>T(300) T(300)&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetophenone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 I</td>
<td>I I</td>
<td>T(300) N.T.</td>
</tr>
<tr>
<td>Thiosemicarbazide&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300 I</td>
<td>I&lt;sup&gt;k&lt;/sup&gt; I&lt;sup&gt;k&lt;/sup&gt;</td>
<td>(T(300)&lt;sup&gt;l&lt;/sup&gt; T(300)&lt;sup&gt;l&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The figures refer to the minimum dose (mg/kg) giving protection or causing neurotoxicity. The letters I, N.T. and T refer to inactive, nontoxic and toxic respectively.

<sup>b</sup>Data taken from Dimmock et al. (1989).

<sup>c</sup>After 4 hours, no protection in the MES or scMET screens was found up to and including doses of 100mg/kg.

<sup>d</sup>The animals receiving a dose of 300mg/kg died between 0.5 and 4 hours after injection.

<sup>e</sup>Delayed tonic extension.

<sup>f</sup>At a dose of 100mg/kg protection was found in 4/5 animals.

<sup>g</sup>At doses of 100mg/kg and 300mg/kg continuous myoclonic jerking (ataypical seizures) was noted.

<sup>h</sup>After 30 minutes, protection was found in 3/5 animals at a dose of 100mg/kg. At a dose of 300mg/kg clonic seizure followed by continuous seizure activity was observed.

<sup>i</sup>At a dose of 100mg/kg toxicity was found in 3/4 animals.

<sup>j</sup>Continuous seizure activity at a dose of 300mg/kg was noted in the scMET screen. Animals died before 4 hours test at a dose of 300mg/kg.

<sup>k</sup>Tonic extension and death occurred at doses of 30, 100 and 300mg/kg.

<sup>l</sup>Neurotoxicity was found in 3/8 (100mg/kg) and 4/4 (300mg/kg) mice with one mouse dead at 300mg/kg after 0.5 hours. Between 0.5 and 4 hours after receiving doses of 30, 100 and 300mg/kg of thiosemicarbazide animals had multiple clonic seizures followed by death.
Table 2.2: Phase 6a screening of compounds 20a,c-e, 21a-d

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES screen</th>
<th>scMET screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 0.5 1 2 4</td>
<td>0.25 0.5 1 2 4</td>
</tr>
<tr>
<td>20a</td>
<td>0 2 3 1 1</td>
<td>Not tested</td>
</tr>
<tr>
<td>c</td>
<td>0 0 0 0 0</td>
<td>Not tested</td>
</tr>
<tr>
<td>d</td>
<td>0 1 1 0 0</td>
<td>Not tested</td>
</tr>
<tr>
<td>e</td>
<td>4 3 3 3 1</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>21a</td>
<td>3 4 4 4 3</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>b</td>
<td>3 1 2 1 3</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>c</td>
<td>0 0 0 0 1</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>d</td>
<td>3 3 4 2 2</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Compounds were administered orally to Sprague-Dawley rats at a dose of 50mg/kg except for 20a which was administered at a dose of 100 mg/kg. Numbers refer to the number of animals protected out of 4 animals tested except in the case of 20a where 8 animals were tested. No neurotoxicity was noted (4 animals tested) at 0.25, 0.5, 1, 2 and 4 hours after administration of the compounds, except for 20a. At 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours after administration of 20mg/kg of 20a neurotoxicity was noted in 0/8, 1/8, 5/8, 3/8, 1/8, 1/8, 0/8 and 0/8 animals tested.
Table 2.3: Phase 6b quantification of 20a,e and 21a,b,d in the MES, scMET and neurotoxicity tests.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES</th>
<th>scMET</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$</td>
<td>Time (mg/kg)</td>
<td>ED$_{50}$</td>
</tr>
<tr>
<td>20a</td>
<td>16.89(13.60-22.44)</td>
<td>1</td>
<td>&gt;500</td>
</tr>
<tr>
<td>20e</td>
<td>15.51(10.67-22.12)</td>
<td>1</td>
<td>&gt;250</td>
</tr>
<tr>
<td>21a</td>
<td>12.84(8.98-16.64)</td>
<td>2</td>
<td>&gt;400</td>
</tr>
<tr>
<td>b</td>
<td>34.18(22.54-47.27)</td>
<td>0.50</td>
<td>&gt;500</td>
</tr>
<tr>
<td>d</td>
<td>39.01(22.97-58.54)</td>
<td>1</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

$^a$ Time of test = 1 hour.

$^b$ At a dose of 50mg/kg toxicity was found in 0/8, 0/8, 0/8, 0/8, 1/8, 1/8, 4/8 and 0/8 animals at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours.

$^c$ At a dose of 100mg/kg no toxicity was found in animals tested at 0.25 through 24 hours. At a dose of 400mg/kg toxicity was found in 5/8, 7/8, 8/8, 8/8, 8/8, 8/8, 8/8 and 4/8 animals at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours.

$^d$ At a dose of 250mg/kg toxicity was found in 2/8, 3/8, 3/8, 2/8, 2/8, 2/8 and 3/8 animals at 0.25, 0.5, 1, 2, 4, 6 and 24 hours.

$^e$ Time of test = 2 hours.
2.1.2 Structural modifications attempted in this study

The following structural variations were undertaken in an attempt to discern the molecular features responsible for anticonvulsant activity.

1) Variations of the electronic nature of the substituent groups on the aryl ring. This modification emphasizes the importance of both the Hammett sigma (σ) and the Hansch hydrophobic (π) substituent constants.

2) Variation of the distance and the nature of the linkage between the aryl ring and the carbon bearing the thiosemicarbazono group.

3) Utilization of the isosteric principle.

4) Replacement of the benzene ring with alkyl moieties.

2.2.0 Thiosemicarbazones of aryl-substituted acetophenones

Thiosemicarbazones of aryl-substituted acetophenones have been found to display a wide variety of biological activities. These properties include antibacterial, antifungal (Runti et al., 1968), tuberculostatic (Buu-Hoi et al., 1953; Donovick et al., 1950; Bernstein et al., 1951; Hamre et al., 1950) and growth regulating properties for some plants (Pratt et al., 1952).
I

a) \( R^2 = R^4 = H; R^1 = R^3 = \text{CH}_3 \)
b) \( R^1 = R^4 = H; R^2 = R^3 = \text{CH}_3 \)
c) \( R^1 = R^3 = R^4 = \text{CH}_3; R^2 = H \)
d) \( R^2 = R^4 = H; R^1 = R^3 = \text{OCH}_3 \)
e) \( R^1 = R^4 = H; R^2 = R^3 = \text{OCH}_3 \)
f) \( R^2 = R^3 = H; R^1 = R^4 = \text{OCH}_3 \)
g) \( R^1 = R^4 = H; R^2 = R^3 = -\text{OCH}_2^- \)

II

a) \( R^1 = \text{CH}_2\text{CH}_3 \)
b) \( R = \text{c-C}_6\text{H}_{11} \)
c) \( R = \text{OCH}_2\text{CH}_3 \)
d) \( R = \text{OC}_6\text{H}_5 \)
e) \( R = \text{C-O-CH}_2\text{CH}_3 \)
The anticonvulsant properties of thiosemicarbazones of a wide variety of structures has been reported. Various anticonvulsant thiosemicarbazides, semicarbazides, and hydrazine derivatives have been shown to inhibit monoamine oxidase and to possess antihemolytic activity (Verma et al., 1974; Misra et al., 1974; Dwivedi et al., 1974) but no correlation between these properties and the anticonvulsant activity could be demonstrated. Many hydrazones are highly active anticonvulsant agents effective against MES convulsions, with little or no effect against seizures induced by subcutaneous administration of pentylentetrazole (Murray and Kier, 1977).

Biological molecules may form chelate structures by forming a ring structure with a metal through coordinate covalent and covalent bonds. The nitrogen, oxygen or sulfur atoms of thiosemicarbazones and semicarbazones serve as electron donating groups whereas cupric ion or other bivalent or trivalent metals, particularly those of the transition group, serve as electron acceptors. Various copper complexes have been shown to possess antimalarial (Klayman et al., 1983), antiinflammatory, antulcer and anticonvulsant activity (Sorenson et al., 1980; Sorenson et al., 1979). This supports the hypothesis that the active forms of antiepileptic drugs are copper complexes which facilitate normal central nervous system function (Sorenson et al., 1979). Thiosemicarbazones are known for their chelating ability, and their ability to form complexes, for example with copper (Mishra and Mohapatra, 1983).
Compound 20a, acetophenone thiosemicarbazone, is useful as a prototype anticonvulsant agent and since previous work with the related 4-aryl-3-buten-2-one thiosemicarbazones showed that electron-donating substituents are associated with bioactivity (Dimmock et al., 1986) the synthesis of some aryl-substituted acetophenones, namely Ia-g and IIa-e was therefore undertaken.

The synthesis and screening of series I and II permits the examination of the relative contributions of the inductive and/or field effects. Compound IIe was contemplated since the presence of an ester function may enable rapid metabolism of the compound to occur. As such, compound IIe may be considered to be a soft analog of compound 20a. Soft analogs are close structural analogs of bioactive compounds which have a specific metabolically sensitive part built into their structures which directs the metabolism (Bodor, 1984).

Compound Ig was prepared since compound 22 was shown to be active in the MES and scMET anticonvulsant screening tests (Dimmock et al., 1986).
Stiripentol, compound 23, is a related derivative and has been found to be an efficacious and nontoxic anticonvulsant agent.

\[
\begin{align*}
\text{O} & \quad \text{CH}_3 \\
\mid & \\
\text{CH} = \text{CH} & \quad \text{CH} - \text{C} - \text{CH}_3 \\
\mid & \\
\text{CH}_3 & 
\end{align*}
\]

23

Stiripentol has been found to inhibit drug metabolism, an effect which is probably related to the methylenedioxyphenyl moiety (Vincent, 1986). The mechanism of inhibition involves the formation of a stable metabolic intermediate cytochrome P-450 complex. The methylenedioxy function is able to delocalize an odd electron resulting from the homolytic removal of a methylene hydrogen. Abstraction of a hydrogen by a microsomal enzyme from a compound containing the methylenedioxyphenyl (MDP) group could generate a relatively stable free radical which could act as an inhibitor of a free-radical generating enzyme responsible for oxidation of C-H bonds in lipophilic drugs. A relatively stable radical, if sufficiently lipophilic, could remain bound to an enzyme, thus inhibiting its further action.
In addition, Hodgson and Casida (1960) found that certain methylenedioxyphenyl compounds exert synergistic insecticidal and toxic properties because they serve as alternate substrates sparing the insecticidal chemical from detoxification or reacting with another site in the mixed function system preventing oxidative detoxification. Interactions of MDP compounds with other drugs metabolized by microsomal enzymes may increase or decrease the toxicity of a chemical, depending on the shift in the balance of the competing activation or detoxification reactions caused by their presence.

Anticonvulsant activity is found in other structurally related compounds which possess the methylenedioxyphenyl moiety (Li and Zhuo, 1980; Vallet, 1978).
The synthesis of series IIIa-c was proposed to examine the nature of the linkage between the aryl ring and the carbon bearing the thiosemicarbazono and methyl functions as follows.

\[
\begin{array}{c}
\begin{array}{c}
\text{N-N-C-NH}_2 \\
\text{H} \\
\text{S}
\end{array} \\
\text{X-C-CH}_3
\end{array}
\]

IIIa: X = CH\(_2\)
b: X = CH\(_2\)CH\(_2\)
c: X = OCH\(_2\)

1) To investigate the differences in anticonvulsant activities in a series of compounds in which the distance between the carbon bearing the thiosemicarbazono group and the aryl ring varied.

2) The application of the isosteric principle in which O is substituted for the CH\(_2\) group (i.e. compounds IIIb,c).

Numerous anticonvulsant agents exist where the aryl or substituted aryl ring is directly attached to the carbon atom bearing the electron donor group or moiety. These include thiosemicarbazones, semicarbazones (Dimmock et al., 1986), sulfonamides, phenylureas, carbamates, ureas and ketones (Murray and Kier, 1977). When the aryl ring and the electron donor group are linked together by a methylene function anticonvulsant activity may be retained or increased in some cases. For example phenacemide 24, and other acetylureas are potent anticonvulsant agents. Similarly, compounds containing an ethylene linkage between two hydrophobic groups such as the carbamate 25 and the amide 26 have been found to exhibit anticonvulsant activity (Murray and Kier, 1977). Thus, compounds IIIa and IIIb were prepared and screened for anticonvulsant activity.
Isosteric replacement of an ethylene linkage by an -OCH₂- function may modify the biological activity of drug compounds. Mexiletine, 27, which incorporates an -OCH₂- linkage possesses marked anticonvulsant properties (Vida, 1977). Compound IIIc was therefore synthesized and evaluated for anticonvulsant activity.
2.3.0 Thiosemicarbazones of alkyl ketones

\[
\begin{align*}
\text{R-C-CH}_3 \\
\text{H} \\
\text{N-N-C-NH}_2 \\
\text{H} \\
\text{S}
\end{align*}
\]

IVa: \( R = \text{C(CH}_3\text{)}_3 \)

b: \( R = (\text{CH}_2\text{)}_3\text{CH}_3 \)

Replacement of the aryl ring of acetophenone thiosemicarbazone by alkyl groups may change bioactivity since steric and electronic influences will be different. Compounds exist which possess only alkyl groups as hydrophobic moieties yet exhibit potent anticonvulsant activity. Acids such as valproic acid 28 and diethylacetic acid 29a-c possess marked anticonvulsant properties.

\[
\begin{align*}
\text{CH}_3\text{CH}_2 \\
\text{\_} \\
\text{CH-COOH} \\
\text{\_/} \\
\text{CH}_3\text{CH}_2
\end{align*}
\]

28

\[
\begin{align*}
\text{R-O-C-NH}_2 \\
\text{H} \\
\text{O}
\end{align*}
\]

29a: \( R = \text{C}_3\text{H}_7 \)

b: \( R = \text{C}_4\text{H}_9 \)

c: \( R = \text{i-C}_4\text{H}_9 \)
The type of anticonvulsant activity demonstrated in compounds in series IV may be altered since, in general, bulky aryl or aromatic groups exhibit optimal activity against generalized (GTC) seizures whereas alkyl substituents impart efficacy against absence (petit mal) seizures. Representative straight-chain and branched-chain alkyl functions were chosen. In addition, since anticonvulsant properties are influenced by hydrophobicity (Jones and Woodbury, 1982), the transportation of IVa and b to sites of actions may be different than in the case of compound 20a.

Correlations between lipophilicity and relative anticonvulsant potency has been investigated (Lehmann and Pedro, 1979). Results indicate that for compounds of low lipophilicity ED$_{50}$ values will also be at toxic concentrations whereas compounds of high lipophilicity will be associated with a high therapeutic margin.

The $\pi$ values for the phenyl, t-butyl and butyl groups are 1.96, 1.98 and 2.13 respectively (Hansch and Leo, 1979) and the $E_{s}$ values are -2.42, -2.78 and -1.63 respectively (Hansch and Leo, 1979; Lowry and Richardson, 1976). Comparison of the biological activity of compounds IVa and b with acetophenone thiosemicarbazone may provide insight as to the relative importance of steric, electronic and hydrophobic factors.
2.4.0 **1,4-Diacetylbenzene bis(thiosemicarbazone)**

![Chemical Structure]

The synthesis and screening of compound V was contemplated for the following reasons.

1) The contribution of specific moieties in a molecule to particular aspects of drug action and the concept of critical and non-critical moieties in a drug molecule is of significance. Transport of drugs is strongly dependent on certain carrier moieties in the drug molecule such as the presence of nonpolar alkyl or aryl groups (Ariens, 1971). If the biological activity of aryl-substituted acetophenone thiosemicarbazones is associated with the side chain and the aryl ring acts as a carrier, then the incorporation of two such groups as in compound V may improve activity.

2) The concept of molecular replication or "doubling the molecule" involves the incorporation of identical moieties in a drug molecule. The presence of two symmetrical or related functional groups in a drug molecule may be advantageous. This can be illustrated with the increased anticonvulsant activity demonstrated with benzenedisulfonamides compared to the benzenesulfonamides (Murray and Kier, 1977). Many of the disulfonamides had an ED$_{50}$ value less than 30 mg/kg, for example N-isopropyl 4-benzenedisulfonamide, 30, with an ED$_{50}$ against MES-induced seizures of 21 mg/kg.
Biological molecules may form chelate structures by forming a ring structure with a metal through coordinate covalent and covalent bonds. Thiosemicarbazones are known to possess the ability to chelate heavy metals and this may be related to their anticonvulsant activity (Sorenson, 1980). Bis(thiosemicarbazones) are very powerful and selective metal ion chelating agents (French and Freedlander, 1958) and if chelation is important in conferring biological activity then compound V may demonstrate increased biological activity compared to 20a. A large number of enzymes including pyridoxal enzymes contain, require or can utilize trace heavy metals for their actions.
2.5.0 Derivatives of acetophenone

\[
\begin{align*}
\text{C-CH}_3 & \\
\text{H} & \\
\text{N-N-C-NH}_2 & \\
\text{H} & \\
\text{X} & \\
\end{align*}
\]

VIa: \( X = 0 \)

b: \( X = NH \)

Acetophenone thiosemicarbazone exhibits marked anticonvulsant activity. Isosteric replacement of S by O and NH yields acetophenone semicarbazone (VIa) and acetophenone guanylhydrazone (VIb) respectively. Compounds VIa and b were prepared in order to develop structure-activity relationships.

Various semicarbazones have been found to exhibit anticonvulsant properties (Popp, 1977; Dimmock et al., 1986).

Guanidines also have been found to possess various biological properties including adrenergic neuronal blocking ability, antidiabetic, antibacterial, antimalarial, antiviral, anticancer and effects on mitochondria (Stenlake, 1979). Guanidines and related compounds are of metabolic interest since the guanidino moiety is found in the amino acid arginine which is formed in the urea cycle.
Guanidine and its derivatives compete with magnesium ions for phosphate groups on mitochondrial binding sites and are capable of inhibiting the formation of phosphate bonds in mitochondria. The \(-C(=N)-N-\) moiety is thought to be essential for specific virus inhibiting activity (Tamm and Eggers, 1963).

The symmetry of the guanidinium ion and its resonance properties makes guanidine itself a very strong base. Isosteric replacement of the NH of guanidine by O yields urea. Both guanidine hydrochloride and urea disrupt noncovalent interactions and are useful denaturing agents. Acetylureas such as phenacemide, \(25\), and cyclic ureides such as phenobarbital are well known antiepileptic drugs. Certain guanidine derivatives have been found to possess anticonvulsant activity (Chapleo et al., 1987; Raman et al., 1979; Joshi and Parmar, 1978).

\[\text{C - CH}_3\]
\[\text{N}\]
\[\text{OH}\]

\(\text{VII}\)

Various oximes and oxime ethers have been found to possess a wide range of biological and pharmacological properties (St. Georgiev et al., 1987). Oxime ether derivatives of substituted acetophenones exhibit both analgesic and anticonvulsant activity (Buzas et al., 1972). The synthesis and anticonvulsant properties of acetophenone oxime, VII, was therefore contemplated.
3.0.0 DESCRIPTION OF THE EXPERIMENTAL WORK

3.1.0 Materials and methods

Chromatography

Thin-layer chromatography (TLC) was performed on Eastman chromatogram sheets, type 13181 (silica gel with fluorescent indicator), type 13252 (alumina with fluorescent indicator) and on Kieselgel 60 F254 (silica gel) from Merck. Spots were observed under short-wave ultraviolet light or in an iodine chamber. All compounds reported were homogeneous by TLC unless otherwise stated in the text.

Drying and purification of solvents and reagents

Drying and purification of solvents and reagents was carried out according to literature procedures (Vogel, 1978a). Filtration and evaporation to dryness were carried out at water pump pressure. Organic extracts were washed with water and dried over anhydrous magnesium sulfate.

Elemental analysis

Analyses were performed by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan. All compounds were dried over phosphorous pentoxide at ~65°C or at room temperature in an Abderhalden drying pistol before analysis.
Melting points

Melting points were determined on the Gallenkemp MF-370 and Mettler FP61 melting point apparatuses and are uncorrected.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were obtained using a Varian T-60 spectrometer. High resolution NMR spectra were determined on a Bruker AM-300 FT NMR spectrometer. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DDS) was used as the internal standard for samples dissolved in deuterium oxide. Tetramethylsilane (TMS) was used as the internal standard for spectra recorded in deuterochloroform, carbon tetrachloride, dimethyl sulphoxide-\textsubscript{d\textsubscript{6}} and methanol-\textsubscript{d\textsubscript{4}}. Chemical shifts (\delta) are reported in ppm downfield from the internal standard. The following abbreviations are used: s(singlet), d(doublet), t(triplet), q(quartet) and m(multiplet).

Infrared (IR) spectroscopy

Infrared spectra were recorded on a Beckman Acculab TM 4 spectrophotometer.

Weighings of compounds

Weighings were carried out on an analytical Mettler AE 100 balance from Fisher Scientific.
Antiepileptic evaluation

Evaluation of the anticonvulsant activity of the compounds was undertaken by the Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland, U.S.A. according to their protocols (Porter et al., 1984).

3.2.0 General procedure for the preparation of the thiosemicarbazones (Ia-g, IIa-e, IIIa-c and IVa,b)

A mixture of thiosemicarbazide (0.01 mole) in ethanol (20ml) was added slowly with stirring to a solution of the appropriate ketone (0.01 mole) in a mixture of ethanol:37% w/v aqueous hydrochloric acid (20ml:2ml). The mixture was stirred at room temperature or heated under reflux for the length of time indicated below. The reaction mixture was monitored for the disappearance of unreacted ketone by thin-layer chromatography (TLC) on silica gel using chloroform:methanol 7:1 or 9:1 as the developing solvents. On cooling, the resultant precipitate was filtered, dried and purified by recrystallization from the appropriate solvent.
Reaction mixtures were generally heated under reflux for 72 hours with the following exceptions: Ia (room temperature for 18 hours), Ib (room temperature for 15 minutes), Ic (room temperature for 60 hours or heated under reflux for 9 hours), If (room temperature for 24 hours), Ig (heated under reflux for 48 hours), IIIb (room temperature for 60 hours or heated under reflux for 22 hours), IIIc (room temperature for 120 hours or heated under reflux for 48 hours) and IVa (heated under reflux for 96 hours). The compounds were recrystallized from 95% ethanol or absolute ethanol with the following exceptions: Id, IIa (ethanol-water), Ie (ethanol-acetone) and IIe (methanol). The structures of the compounds were confirmed by NMR spectroscopy and elemental analysis.

The physical data for compounds Ia-g, IIa-e, IIIa-c and IVa,b are given in Tables 3.1, 3.2, 3.3 and 3.4 respectively.
Table 3.1: Physical data of some aryl-substituted acetophenone thiosemicarbazones (Ia-g)

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>Yield (%)</th>
<th>Melting point (°C)</th>
<th>Molecular formula</th>
<th>Calculated Elemental Analysis</th>
<th>Found Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
<td>37</td>
<td>167.5ᵃ</td>
<td>C₁₁H₁₅N₃S</td>
<td>59.70 6.83 18.99</td>
<td>59.51 7.02 18.82</td>
</tr>
<tr>
<td>b</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>63</td>
<td>190.₀ᵇ</td>
<td>C₁₁H₁₅N₃S</td>
<td>59.70 6.83 18.99</td>
<td>59.65 6.91 19.23</td>
</tr>
<tr>
<td>c</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>54</td>
<td>160.5ᶜ</td>
<td>C₁₂H₁₇N₃S</td>
<td>61.24 7.28 17.85</td>
<td>61.52 7.46 17.63</td>
</tr>
<tr>
<td>d</td>
<td>OCH₃</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>32</td>
<td>157.1</td>
<td>C₁₁H₁₅N₃O₂S</td>
<td>52.16 5.97 16.59</td>
<td>52.27 5.88 16.24</td>
</tr>
<tr>
<td>e</td>
<td>H</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>47</td>
<td>227 (dec.)</td>
<td>C₁₁H₁₅N₃O₂S</td>
<td>52.16 5.97 16.59</td>
<td>52.14 5.75 16.33</td>
</tr>
<tr>
<td>f</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>OCH₃</td>
<td>55ᵈ</td>
<td>101.⁹ᵉ</td>
<td>C₁₁H₁₅N₃O₂S</td>
<td>52.16 5.97 16.59</td>
<td>51.92 5.94 16.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48ᶠ</td>
<td>137.2</td>
<td></td>
<td></td>
<td>52.26 6.28 16.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51ᵍ</td>
<td>157.2</td>
<td></td>
<td></td>
<td>51.94 5.97 16.83</td>
</tr>
<tr>
<td>g</td>
<td>H</td>
<td>-OCH₂⁻</td>
<td>H</td>
<td></td>
<td>39</td>
<td>186.₀ʰ</td>
<td>C₁₀H₁₁N₃O₂S</td>
<td>50.62 4.67 17.71</td>
<td>50.88 4.80 17.54</td>
</tr>
</tbody>
</table>

ᵃ lit. m.p. = 158 (Buu-Hoi et al., 1956)
ᵇ lit. m.p. = 190 (Furukawa and Ueda, 1960)
ᶜ lit. m.p. = 170 (Buu-Hoi et al., 1956)
ᵈ m.p. range 98.0 - 101.9
ᵉ lit. m.p. = 163 (Runti et al., 1968)
ᶠ m.p. range 128.0 - 137.3
ᵍ m.p. range 143.5 - 157.2
ʰ lit. m.p. = 181.5 (Runti et al., 1968)
Table 3.2: Physical data of some 4-substituted acetophenone thiosemicarbazones (IIa-e)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
<th>Melting point (°C)</th>
<th>Molecular formula</th>
<th>Elemental Analysis Calculated</th>
<th>Elemental Analysis Found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%C %H %N</td>
<td>%C %H %N</td>
</tr>
<tr>
<td>a</td>
<td>CH₂CH₃</td>
<td>52</td>
<td>137.8</td>
<td>C₁₁H₁₅N₃S</td>
<td>59.70 6.83 18.99</td>
<td>59.55 7.02 19.04</td>
</tr>
<tr>
<td>b</td>
<td>c-C₆H₁₁</td>
<td>69</td>
<td>166.0</td>
<td>C₁₅H₂₁N₃S</td>
<td>65.42 7.69 15.26</td>
<td>65.33 7.70 15.19</td>
</tr>
<tr>
<td>c</td>
<td>OCH₂CH₃</td>
<td>34</td>
<td>159.7</td>
<td>C₁₁H₁₅N₃O₂S</td>
<td>55.67 6.37 17.71</td>
<td>55.85 6.57 17.76</td>
</tr>
<tr>
<td>d</td>
<td>OC₆H₅</td>
<td>49</td>
<td>170.2</td>
<td>C₁₅H₁₅N₃O₂S</td>
<td>63.13 5.30 14.73</td>
<td>63.32 5.53 14.53</td>
</tr>
<tr>
<td>e</td>
<td>C-OC₆H₅</td>
<td>53</td>
<td>215.8</td>
<td>C₁₂H₁₅N₃O₂S</td>
<td>54.32 5.70 15.84</td>
<td>54.51 5.68 15.68</td>
</tr>
</tbody>
</table>

a lit. m.p. = 133.5-135.5 (Bernstein et al., 1951)
b lit. m.p. = 158-9 (Bernstein et al., 1951); 154-5 (Libermann et al., 1953)
c lit. m.p. = 149 (Buu-Hoi et al., 1956)
Table 3.3: Physical data of the thiosemicarbazones of phenylacetone, benzylacetone and phenoxyacetone (IIIa-c)

![Chemical structure of thiosemicarbazone]

<table>
<thead>
<tr>
<th>Compound III</th>
<th>X</th>
<th>Yield (%)</th>
<th>Melting point(°C)</th>
<th>Molecular formula</th>
<th>Elemental Analysis Calculated</th>
<th>Elemental Analysis Found</th>
</tr>
</thead>
</table>
|              |      |           |                   |                   | %C   | %H   | %N   | %C   | %H   | %N   |%
| a            | CH₂  | 41        | 145.8⁻           | C₁₀H₁₃N₃S         | 57.94 | 6.32 | 20.27| 58.06| 6.51 | 20.30|
| b            | CH₂CH₂| 50        | 100.8⁻ - 102.6   | C₁₁H₁₅N₃S         | 59.70 | 6.83 | 18.99| 59.51| 6.65 | 18.69|
| c            | OCH₂ | 32        | 135.0⁻           | C₁₀H₁₃N₃OS        | 53.79 | 5.87 | 18.82| 53.90| 5.70 | 18.56|

a lit. m.p. = 145-7 (Bernstein et al., 1951)
b lit. m.p. = 136-8 (Sah and Daniels, 1950); 137 (Landquist, 1970)
Table 3.4: Physical data of the thiosemicarbazones of 3,3-dimethyl-2-butanone and 2-hexanone (IVa,b)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
<th>Melting point (°C)</th>
<th>Molecular formula</th>
<th>Calculated Elemental Analysis</th>
<th>Found Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td>C(CH₃)₃</td>
<td>25</td>
<td>125.2 - 125.9</td>
<td>C₇H₁₅N₃S</td>
<td>48.52 8.73 24.25</td>
<td>48.26 8.80 23.99</td>
</tr>
<tr>
<td>IVb</td>
<td>C₄H₉</td>
<td>28</td>
<td>52.5 - 53.0</td>
<td>C₇H₁₅N₃S</td>
<td>48.52 8.73 24.25</td>
<td>48.05 8.48 24.90</td>
</tr>
</tbody>
</table>
3.3.0 **Procedure for the synthesis of 1,4-diacetylbenzene bis(thiosemicarbazone) (V)**

A solution of thiosemicarbazide (0.0050 mole) in ethanol (10ml) was added slowly with stirring to a solution of 1,4-diacetylbenzene (0.0025 mole) in a mixture of ethanol:40% w/v aqueous hydrochloric acid (10ml:1ml). The mixture was heated under reflux for 96 hours during which time a precipitate was deposited in the reaction mixture. The crude product was recrystallized from a mixture of dimethylsulfoxide:chloroform to give the title compound in 43% yield, m.p. 200 - 202 °C (dec.).

Anal. Calc. for C_{12}H_{16}N_{5}S_{2}: C 46.73 H 5.23 N 27.25
Found : C 47.32 H 5.44 N 26.88
3.4.0 Procedure for the synthesis of acetophenone semicarbazone

(VIa)

A mixture of semicarbazide (0.01 mole) in ethanol (20 ml) was added slowly with stirring to a solution of acetophenone (0.01 mole) in a mixture of ethanol:37% w/v aqueous hydrochloric acid (20 ml: 2 ml). The mixture was heated under reflux for 96 hours and then cooled. The product which crystallized was filtered, dried and recrystallized from ethanol-water to give the title compound. The physical data of the compound is presented in Table 3.5.

3.5.0 Procedure for the synthesis of acetophenone guanylhydrazone

(VIb)

A mixture of aminoguanidine bicarbonate (0.01 mole) and acetophenone (0.01 mole) in ethanol:50% aqueous hydrochloric acid (20 ml: 2 ml) was heated under reflux for one hour. The reaction mixture was monitored by TLC on silica gel using chloroform:methanol:acetic acid 7:1:0.5 as the developing solvent. On cooling, the solution was basified and extracted with ether. After addition of an ethanol-water mixture to the ether-extract, the reaction mixture was cooled in the refrigerator. The resultant colorless needles or plates were purified by recrystallization from ethanol-water to give acetophenone guanylhydrazone. The physical data of the compound is presented in Table 3.5.
Table 3.5: Physical data of acetophenone semicarbazone (VIa) and acetophenone guanylhydrazone (VIb)

![Chemical structure of acetophenone semicarbazone and acetophenone guanylhydrazone]

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Yield (%)</th>
<th>Melting point (°C)</th>
<th>Molecular formula</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>0</td>
<td>66</td>
<td>201.6(^a)</td>
<td>C(^9)H(^11)N(^3)O</td>
<td>%C 61.00  %H 6.26  %N 23.71</td>
</tr>
<tr>
<td>b</td>
<td>NH</td>
<td>73</td>
<td>179-180 (dec.)(^b)</td>
<td>C(^9)H(^12)N(^4)</td>
<td>%C 61.34  %H 6.86  %N 31.79</td>
</tr>
</tbody>
</table>

\(^a\) lit. m.p. = 198 (Skoldinov and Koecheshkov (1942); 198-9 (DeBenneville, 1941); 202 (Ramart-Lucas and Bruzau, 1934)

\(^b\) lit. m.p. = 182.5-183 (Pearson et al., 1953)
3.6.0 Procedure for the synthesis of acetophenone oxime (VII)

The preparation of this compound was carried out in analogy to a literature procedure (Vogel, 1978b) with some modifications. To a mixture of hydroxylamine hydrochloride (2.0 g) and sodium acetate (4.0 g) in water was added acetophenone (1.0 g). The mixture was warmed on a water bath at 35-40°C and enough ethanol was added dropwise to obtain a clear solution. On cooling the reaction mixture was basified with aqueous sodium hydroxide solution and extracted with ether. A mixture of ethanol-water was added to the ether extract and the solution was cooled in the refrigerator. The resultant colorless crystals were filtered, dried and purified by repeated recrystallization from distilled water to give the title compound in 74% yield, m.p. 59-60°C. The reported melting point of this compound is 59.5-60.5°C for the E-oxime and 81-3°C for the Z-oxime (Buckingham, 1982).

Anal. Calc. for \( \text{C}_8\text{H}_9\text{NO} \): C 71.09 H 6.71 N 10.36

Found : C 70.87 H 6.74 N 10.36
4.0.0 RESULTS AND DISCUSSION

4.1.0 Thiosemicarbazone derivatives

Thiosemicarbazide and other hydrazine derivatives add to the carbonyl group of ketones resulting in formation of the corresponding thiosemicarbazone or hydrazone.

The chemical structure of some hyrazones are shown below.

\[
\begin{align*}
\text{Hydrazone} & \quad \text{C} = \text{N} - \text{NH}_2 \\
\text{Semicarbazone} & \quad \text{C} = \text{N} - \text{NH} - \text{C} - \text{NH}_2 \\
\text{Thiosemicarbazone} & \quad \text{C} = \text{N} - \text{NH} - \text{C} - \text{NH}_2 \\
\text{Guanylhydrazone} & \quad \text{C} = \text{N} - \text{NH} - \text{C} - \text{NH}_2
\end{align*}
\]

The general reaction showing the formation of imine derivatives involves addition of the amino compound to form an aminohydrin followed by elimination to form the imine.
Addition involves attack of an amino compound on the carbonyl group producing a high-energy, unstable zwitterionic intermediate which is converted to the aminohydrin by two proton transfers. Strong nucleophiles such as hydroxylamine will attack an unactivated carbonyl carbon but weaker nucleophiles such as semicarbazide or phenylhydrazine require acid catalysis to activate the carbonyl group, as depicted in Scheme 4.1.

![Chemical Reaction Diagram](#)

**Scheme 4.1: Formation of an aminohydrin: addition phase of hydrazone formation.**

Protonation of the carbonyl oxygen makes the carbonyl carbon more susceptible to nucleophilic attack and the addition will be favored by high acidity. However the hydrazine, NH$_2$-X, can also undergo protonation to form the ion, +NH$_3$-X, which lacks unshared electrons and is no longer nucleophilic. As the concentration of the free base falls with increasing acidity, the amine becomes progressively more protonated and the reaction is retarded or prevented.
Considering the amine, the addition is favored by low acidity. The solution therefore must be acidic enough for an appreciable fraction of the carbonyl compound to be protonated but not so acidic that the concentration of the free hydrazine is too low, as indicated in Scheme 4.2.

\[
\begin{align*}
\text{C} & \xrightleftharpoons{\text{H}^+} \text{C} + \text{H}_2\text{O} + \text{H}^+ \\
\text{H}_2\text{N} - \text{X} \quad & \xrightleftharpoons{\text{H}^+} \quad \text{H}_2\text{N} - \text{X} + \text{NH}_3 - \text{X}
\end{align*}
\]

Scheme 4.2: Effect of acidity on the formation of an aminohydrin.

The stability of the tetrahedral intermediate and the extent to which the reaction proceeds to form the product rather than reverting to starting materials governs the reversibility of the reaction. Structural features of the ketones affect reactivity towards the nucleophile and determine, in part, the position of equilibria in reversible additions to carbonyl compounds. Alkyl or aryl substituents of ketones increase the steric repulsion to the attacking nucleophile. Electron-pair and van der Waals repulsions between the substituents increase in going from the ground state to the transition state as the carbon atom changes from sp\(^2\) hybridization (~120° bond angles) to sp\(^3\) hybridization (~109.5° bond angles) during the rate-determining step.
The elimination or dehydration step may proceed with acid or base catalysis depending on the base strength. Strong base aminohydrins may dissociate without catalytic assistance or with acid catalysis whereas less strongly basic aminohydrins may require basic catalysis, as represented mechanistically in Scheme 4.3.

A) \( R{-}\text{NH-C-OH} \xrightleftharpoons{\text{H}^+} \text{RNH} - C - \overset{\ddagger}{\text{OH}}_2 \xrightarrow{\text{R-N=H}} \text{R-N=C} + H_2O \)

B) \( \text{R-N} \xrightleftharpoons{\text{OH}} \text{H}_2O + \text{RN=C} + \overset{\ddagger}{\text{OH}} \)

C) \( \text{R-N=H} \xrightarrow{\text{RN=C}} + \overset{\ddagger}{\text{OH}} \)

Scheme 4.3: Elimination phase of imine formation

A) Elimination with acid catalysis
B) Elimination with base catalysis
C) Uncatalyzed elimination

Thus the reaction rates for imine formation vary with pH in a characteristic manner, which is a consequence of a change in the rate limiting step. At neutral and alkaline pH the dehydration step is generally slow and rate limiting. As the solution is made more acidic, the rate increases as a result of the acid catalysis of the dehydration until the maximum rate is attained, generally at a pH between 2 and 5.
A decrease of rate occurs on further acidity since although the dehydration is facilitated, the addition step is inhibited because only the unprotonated amine is reactive. At low pH the first step then becomes rate-determining. Thus the rate of formation of hydrazone derivatives of carbonyl compounds tends to exhibit pH optima. The entire mechanism is represented in Scheme 4.4.

Scheme 4.4: General mechanism for formation of imine derivatives.
Discrepancies in literature melting points and observing different melting points for thiosemicarbazones are due to the geometrical isomerism of these compounds. Thiosemicarbazones, hydrazones and related derivatives exhibit geometrical isomerism on account of the restricted rotation about the carbon-nitrogen double bond (Smith, 1983). These types of compounds can exist in the E or Z configuration as shown in Figure 4.1 for acetophenone thiosemicarbazone.

![Figure 4.1: E and Z configurations of acetophenone thiosemicarbazone.](image)

Stereoisomeric composition is generally evaluated by spectroscopy of the compounds in solution. Isomers interconvert rapidly in solution but the composition of the compounds in the solid state is not always known and may be difficult to determine. Single isomers generally exhibit sharp melting points whereas mixtures of E and Z isomers melt over wide ranges and at lower temperatures (Karabatsos et al., 1964).
Stereoisomeric constitution of a compound, whether in the crystalline state or in solution, may be influenced by several factors, such as the following.

1) Steric effects.
2) Hydrogen bonding.
3) The size and electronic nature of the groups attached to the carbon of the imine moiety.
4) Solubility.

Steric interactions in the transition state would predict formation of the thermodynamically more stable isomer. Isolation of a single isomer implies kinetically controlled formation of the more stable isomer, or rapid isomer equilibration and precipitation of the less soluble isomer.

Generally the $E$ isomer is favored over the $Z$ isomer. With aryl-substituted acetophenone thiosemicarbazones and related derivatives substituents on the aromatic ring may interact with the thiosemicarbazono group, favoring formation of the $Z$ isomer, represented by Figure 4.2.

![Figure 4.2: Interaction favoring Z-isomer formation.](image)
Internal hydrogen bonding has allowed for the isolation of pure stereoisomers in some cases (Karabatsos et al., 1962). Such interactions between groups are especially important in solvents which can cause protonation of the thiosemicarbazono moiety.

Thiosemicarbazones and other N-containing derivatives of carbonyl compounds can also exhibit configurational isomerization i.e. they can exist in different tautomeric forms, as depicted in figure 4.4 for acetophenone thiosemicarbazone (Raevskii et al., 1968).

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\setminus & \quad \setminus \\
C = N - NH - C - NH_2 & \quad C - NH - NH - C - NH_2 \\
/ & \parallel \parallel \\
CH_3 & \quad S \\
& \quad \text{S} \\
\text{I} & \quad \text{I} \\
\end{align*}
\]

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\setminus & \quad \setminus \\
C = N - N = C - NH_2 & \quad C - NH - N = C - NH_2 \\
/ & \parallel \parallel \\
CH_3 & \quad \text{SH} \\
& \quad \text{SH} \\
\text{III} & \quad \text{IV} \\
\end{align*}
\]

Figure 4.3: Tautomeric forms of acetophenone thiosemicarbazone.

Form I is generally favored over forms II, III and IV but depending on the derivatives studied, different tautomeric forms have been proposed (Scovill et al., 1982; Raevskii et al., 1968).
The tautomeric form of a compound will depend, in part, upon acidity, solvent effects and the nature of the atoms or groups attached to the carbon and nitrogen of the imino moiety.

In some cases biological properties may only be exhibited by one of the isomers. Certain thiosemicarbazones containing heterocyclic ring systems possess antitumor activity and this may be due to chelation with the ions of the transition metals (Antonini et al., 1979). Only thiosemicarbazones with an E-configuration will be effective chelating agents as illustrated in Figure 4.4.

![Figure 4.4: N-heterocyclic thiosemicarbazones possessing chelating properties.](image)

Structure-activity relationships in some dimethylcinnamamides (Balsamo et al., 1977) show that geometrical isomers act differently on the central nervous system; the E derivatives displayed CNS depressant and anticonvulsant activity, whereas the Z isomers possessed CNS stimulant activity.
The aryl-substituted acetophenone thiosemicarbazones and related compounds examined in this study were probably mixtures of E and Z isomers and this may affect the biological and anticonvulsant activities which were observed.

The thiosemicarbazones Ia-g, IIa-e, IIIa-c and IVa,b were prepared from a mixture of equimolar quantities of the appropriate ketone and thiosemicarbazide, a catalytic amount of aqueous hydrochloric acid and ethanol either at room temperature or heated under reflux for an appropriate length of time. Under the reaction conditions employed, the reactions went to completion between 0.25 and 72 hours, as indicated by the disappearance of unreacted ketone. An increase in reaction temperature from room temperature to heating under reflux generally resulted in a shorter reaction time being required and higher yields being observed.

Compound If was observed to exhibit polymorphism, presumed to be due to mixtures of E and Z isomers in varying proportions. Three different crystalline solids melting at temperatures ranging from 101.9°C to 157.2°C were observed. In one experiment compound If was recrystallized from ethanol and the first precipitate melted at 101.9°C. Subsequent partial evaporation of the solvent led to formation of crystals with a melting point of 137.2°C. Compound IIe was observed to be sensitive to light.

Compounds IVa,b were prepared in relatively low yields only after heating under reflux for prolonged periods of time. This may be due, in part, to electronic or steric factors.
Acetophenone may be thought of as a relatively rigid system because of the partial double bond character of the link between the carbonyl and phenyl groups. Pinacolone, the starting ketone in the case of IVa, on the other hand, possesses a large number of internal degrees of freedom and is of lower reactivity. The transition state for thiosemicarbazone formation possesses a relatively rigid structure, so that a carbonyl compound containing many degrees of freedom loses most of these on activation whereas a more rigid carbonyl compound cannot. The transition state system would also be expected to be a strongly polar one with a charge of significant magnitude in the area of the carbonyl carbon atom.

Increasing bulk size as with the tertiary butyl group in the case of compound IVa will produce a highly crowded transition state (as the bond angles approach ~109°) and the rate of reaction will subsequently decrease. Thus the relative ease and rate of reaction for thiosemicarbazone formation may reflect the rigidity, size and the electronic nature of the carbonyl system involved in the reaction.

For the bis derivative, V, one molar quantity of 1,4-diacetylbenzene and two molar quantities of thiosemicarbazide, a catalytic amount of hydrochloric acid and ethanol were heated under reflux for 96 hours.
4.2.0 Related derivatives

Semicarbazones, oximes and guanylhydrazone are formed by the same general reaction mechanism as shown in Scheme 4.4 for thiosemicarbazone formation. The effect of structural changes upon reactivity for carbonyl reaction systems has been investigated by Fiarman and Gettler (1962). A parallelism between semicarbazone, thiosemicarbazone, oxime and guanylhydrazone formation is evident in that all reactions are second order kinetically. Disparity between the reaction characteristics is also apparent. The formation of the semicarbazones and almost all of the thiosemicarbazones that were examined exhibited reversible characteristics, whereas oxime formation has been found to be irreversible in all cases. Where comparable data exist, the rates of formation follow the sequence: oximation > semicarbazone formation > thiosemicarbazone formation.

When rate constants are examined (Fiarman and Gettler, 1962) a marked parallelism exists between thiosemicarbazone, semicarbazone and oxime formation which arises partly because of an implied similarity of the transition states of the reactions.

Acetophenone semicarbazone, VIa, was prepared by heating under reflux a mixture of equimolar quantities of acetophenone and semicarbazide hydrochloride with or without a catalytic amount of sodium acetate in ethanol.
Compound VIb, acetophenone guanylhydrazone, was prepared by heating under reflux a mixture of equimolar amounts of aminoguanidine bicarbonate, acetophenone and a catalytic amount of aqueous hydrochloric acid. The mixture was cooled, basified with aqueous sodium hydroxide solution and extracted with ether to obtain the free base.

The preparation of compound VII, acetophenone oxime, was prepared by the method of Vogel (1978b), with some modifications. A mixture of acetophenone, hydroxylamine hydrochloride and sodium acetate was warmed on a water bath to obtain a clear solution according to the proposed method. The mixture was then basified with aqueous sodium hydroxide solution to ensure the product would be in the free base form. Subsequent purification gave the compound in 74% yield. The melting point of the compound agreed with the reported melting point of the E-oxime.

4.3.0 Anticonvulsant screening results

Drugs affecting the central nervous system (CNS) may be classified as follows:

1) General CNS depressants such as sedatives.
2) General CNS stimulants including convulsants such as strychnine and picrotoxin.
3) Selective CNS stimulants or depressants including such CNS depressants as analgesics, anesthetics and anticonvulsants.
In order for a drug molecule to effect a particular biological response it must be delivered to its site of action, and factors such as absorption, distribution and metabolism become important. For agents affecting the CNS, the particular problem of transversing the "blood-brain barrier" into the central nervous system tissue must be overcome. Neutral lipophilic molecules can generally pass through cellular membranes into the CNS, and a number of CNS-active drugs, including those possessing convulsant and anticonvulsant properties, show a parabolic dependence on log P (octanol/water partition coefficient) having an optimal value of approximately 2 (Fukunaga and Berger, 1983). Thus the difficulties arising in trying to obtain a drug compound with a highly selective CNS action becomes apparent.

The lipophilicity of a drug molecule plays a cardinal role in producing convulsive, anticonvulsive and other CNS effects. Slight differences in physicochemical or other properties may cause one series of compounds to possess anticonvulsant activity, whereas another structurally analogous series will be epileptogenic (Fukunago and Berger, 1983). Some anticonvulsant agents, for example thiosemicarbazide, produce convulsions in high doses.

In addition, the chemical properties requisite for different biological actions may coincide to a substantial degree and certain drugs may have simultaneous independent biological effects. Drugs affecting the CNS often display multiple biological activities, for example benzodiazepines, such as diazepam, possess sedative, muscle relaxant and anticonvulsant properties. Specific biological properties are often dose-related.
Antiepileptic drugs are potential general CNS depressants and may cause respiratory depression or anesthesia in high doses. In animals, loss of the righting reflex indicates CNS depression whereas tremors and convulsions indicate stimulation of the central nervous system (Balsamo et al., 1977).

Most of the thiosemicarbazones and related compounds described in this thesis have been found to display CNS-depressant and, in some cases, CNS-stimulant activities. The anticonvulsant screening of the compounds described was undertaken by the Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland, U.S.A. according to their protocols (Porter et al., 1984). The data obtained for the compounds belonging to series 20 and 21 are presented in Tables 2.2 and 2.3.

The aryl-substituted acetophenone thiosemicarbazones in series 20 were selected according to the Topliss approach to structure-activity relationships (Topliss, 1977). The nuclear substituents chosen, namely the unsubstituted, 4-methyl, 4-methoxy, 3,4-dichloro and 4-chloro derivatives have Hammett σ values ranging from -0.27 to +0.60 which permit changes in chemical reactivity which may correlate with biological activity. The unsubstituted compound, 20a was the most active compound in the MES screen with an ED50 value of 15.51 mg/kg. The 4-methyl derivative, compound 20b possessed activity in the scMET screen at doses of 30 mg/kg. The remaining three compounds possessed activity in one or both screens at doses of 100 or 300 mg/kg.
Compounds 20a and 20b exhibited neurotoxicity at doses of 100 mg/kg and compounds 20d and 20e at doses of 30 mg/kg. The 4-methoxy derivative showed no neurotoxicity at the dose levels tested. Since the 4-chloro and 3,4-dichloro derivatives in series 20 exhibited toxicity at doses lower than for the other substituent groups, it is possible that the electron withdrawing influence and/or the greater lipophilicity of the substituents causes this effect.

The biological data obtained indicates that the unsubstituted compound and those possessing electron-donating groups on the aryl ring give rise to derivatives possessing anticonvulsant activity.

Thiosemicarbazones and other derivatives of ketones possess anticonvulsant properties. Thiosemicarbazide exhibits anticonvulsant activity in the MES screen at doses above 30 mg/kg but is lethal within 4 hours (Dimmock et al., 1989). It is conceivable that hydrolysis of thiosemicarbazones to the corresponding carbonyl compounds and thiosemicarbazide in vivo could account, in part, for the anticonvulsant activity manifested by these compounds (Williams, 1959).

Some ketones are known to be potent anticonvulsants. Since ketones are principally metabolized to acidic metabolites an acidotic state may be produced upon the administration of ketones. Systemic acidosis is one of the physiological conditions presumed to prevent the onset of seizures, and this explanation has been invoked to explain the mode of action and anticonvulsant effects displayed by certain ketones (Murray and Kier, 1977).
Dimmock et al. (1989) evaluated the anticonvulsant properties of some ketones and their corresponding thiosemicarbazones. The unsaturated ketones which on reaction with thiosemicarbazide gave 18c-f, 19a-f, 20a,b and 31 were evaluated in the phase 1 screen. Ketones possessing anticonvulsant properties, namely the precursor carbonyl compounds for 19d,e, 20a,b gave rise to thiosemicarbazones which also displayed activity and inactive thiosemicarbazones (18c,d, 19c-f) were derived from inactive ketones. However, the anticonvulsant properties of the thiosemicarbazones 19a,b and 31 cannot be attributed to the corresponding ketones and thiosemicarbazide since the ketones corresponding to these thiosemicarbazones were inactive in the MES and scMET screens. In addition, assuming 100% hydrolysis of thiosemicarbazones 19a,b and 31 at the ED$_{50}$ values in the MES screen, the quantities of thiosemicarbazide liberated would be insufficient to provide protection in the MES screen or although some protection may be provided the amount of thiosemicarbazide would be lethal within 4 hours, which was not observed. Thus, although certain molecular features of the ketones and the corresponding thiosemicarbazones may be associated with activity and inactivity, the anticonvulsant properties of the thiosemicarbazones are probably due to the molecules per se.

\[
\begin{align*}
&\text{CH}_3 \\
&\text{CH} = \text{C} - \text{C} - \text{H} \\
&\text{H} \quad \text{N} - \text{N} - \text{C} - \text{NH}_2 \\
&\text{H} \quad \text{S}
\end{align*}
\]
Compounds in series 21 were prepared and evaluated for anticonvulsant activity in order to examine the effect of replacement of the methyl group attached to the carbon bearing the aryl ring and the thiosemicarbazono function with various other atoms or groups. Replacement of the methyl group by a hydrogen atom or an isopropyl moiety improved anticonvulsant activity whereas compounds possessing ethyl and phenyl moieties exhibited lower anticonvulsant activity. All of the compounds in series 21 demonstrated anticonvulsant activity at doses of 30 or 100 mg/kg and neurotoxicity at doses of 100 or 300 mg/kg. The biological data obtained did not show any clear correlation between in vivo anticonvulsant activity and Hammett $\sigma$ values, Hansch $\pi$ values or Taft $E_S$ values.

The rationale for the synthesis of compounds belonging to series I-III, V - VII has been discussed in Section 2.0.0. The data obtained from the anticonvulsant screening of the compounds is presented in Tables 4.1 and 4.2.
Table 4.1: Phase 1 screening of compounds Ia-g, IIa-e, IIIa-c, V, VIa,b and VII.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES screen(^a)</th>
<th>0.5 h</th>
<th>4 h</th>
<th>scMET screen(^a)</th>
<th>0.5 h</th>
<th>4 h</th>
<th>Neurotoxicity(^a)</th>
<th>0.5 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>I</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>T(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>300</td>
<td>I</td>
<td>100</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>T(300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>300</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>T(300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>300</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td></td>
<td>N.T.</td>
<td>T(300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>100</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td>T(300)</td>
<td>T(100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>300</td>
<td>I</td>
<td>I</td>
<td>300</td>
<td>30</td>
<td>I</td>
<td>N.T.</td>
<td>T(30)</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>300</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>T(300)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>300</td>
<td>300</td>
<td>I</td>
<td>300</td>
<td>300</td>
<td>N.T.</td>
<td>T(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>100</td>
<td>300</td>
<td>100</td>
<td>300</td>
<td>T(300)</td>
<td>T(300)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>I</td>
<td>T(300)</td>
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<td></td>
</tr>
<tr>
<td>c</td>
<td>100</td>
<td>300</td>
<td>300</td>
<td>I</td>
<td>T(300)</td>
<td>T(300)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIa</td>
<td>100</td>
<td>300</td>
<td>100</td>
<td>I</td>
<td>T(300)</td>
<td>T(300)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b(^i)</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>T(30)</td>
<td>T(30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>100</td>
<td>I</td>
<td>300</td>
<td>I</td>
<td></td>
<td></td>
<td>T(300)</td>
<td></td>
<td>N.T.</td>
</tr>
</tbody>
</table>

\(^a\) The figures refer to the minimum doses (mg/kg) giving protection or causing neurotoxicity. The letters I, N.T. and T refer to inactive, nontoxic and toxic respectively.

At a dose of 100mg/kg protection was found in 1/2 animals after 0.25 and 1 hr.

At a dose of 300mg/kg protection was found in 3/4 animals.

At a dose of 30mg/kg continuous tremors followed by tonic extension occurred. The animals receiving a dose of 300mg/kg died between 0.5 and 4 hours after injection.

At a dose of 100mg/kg protection was found in 2/4 animals after 4 hours.

At a dose of 300mg/kg protection was found in 4/5 animals.

At a dose of 100mg/kg protection was found in 2/2 animals after 0.25 hours and 1/2 animals after 1 hour.

Animals were anesthetized.

No protection was found in the MES or scMET screen at 3mg/kg and 10mg/kg after 0.5 and 4 hours. At doses of 100mg/kg and 300mg/kg animals died from respiratory depression.
### Table 4.2: Phase 6a screening of compounds Ia,c, IIa-c, IIIa-c, VIa,b and VII a

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES</th>
<th>scMET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Ia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IIIa</td>
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</tr>
<tr>
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<td>2</td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>VIa</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* a Same as footnote a in Table 2.2.*
Most of the compounds belonging to series I and II, the aryl-substituted thiosemicarbazones, demonstrated activity at a dose of 100 or 300 mg/kg except for 3,4-dimethylacetophenone thiosemicarbazone (Ib) and 2,5-dimethoxyacetophenone thiosemicarbazone (If) where no activity was exhibited at the dose levels tested. The compounds exhibited varying degrees of toxicity, from being nontoxic, toxic at 100 or 300 mg/kg or toxic at 30 mg/kg in the case of 4-cyclohexylacetophenone thiosemicarbazone (IIb). Compound IIb was also the only compound in series I and II which demonstrated anticonvulsant activity at a dose of 30 mg/kg. Compounds Ia,f and IIe exhibited no toxicity in the neurotoxicity screen and compound If was the only compound void of both anticonvulsant activity and neurotoxicity.

In series I and II no clear correlation between biological activity and Hammett $\sigma$ values or Hansch $\pi$ values can be demonstrated. An unfavorable steric effect from substitution on the aryl ring may be operative. In general, derivatives containing aryl substituents with electron donating properties and a small steric parameter were associated with anticonvulsant activity.

Compounds in series III examined the nature of the linkage between the aryl group and the carbon bearing the thiosemicarbazone group. These compounds exhibited anticonvulsant activity against both MES- and scMET-induced seizures and neurotoxicity at doses of 100 or 300 mg/kg. No clear correlation between lipophilicity and anticonvulsant activity could be demonstrated in this series.
Compound IIIc which contains the -OCH\textsubscript{2}- linkage produced anesthetic effects in the mice. This compound can be compared chemically to most anesthetic agents, which consist of a lipophilic group (aromatic ring) connected by an intermediate chain to an ionizable group, commonly an amine moiety. Optimal anesthetic activity requires a balance between the lipophilic and hydrophilic groups. Local anesthetics, like anticonvulsants, produce central nervous system depression but can cause CNS stimulation, producing convulsions, in high doses.

In phase 1 anticonvulsant screening, compound V, 1,4-diacetyl-benzene bis(thiosemicarbazone) was inactive in both the scMET and MES screens and demonstrated no neurotoxicity at the dose levels tested.

Acetophenone semicarbazone (VIa) and acetophenone oxime (VII) displayed anticonvulsant activity against MES- and scMET-induced seizures similar to the activity of acetophenone thiosemicarbazone. While acetophenone thiosemicarbazone produced neurotoxicity at a dose of 100 mg/kg at both 0.5 and 4 hours after administration, the semicarbazone and oxime of acetophenone displayed neurotoxicity only at a dose of 300 mg/kg 0.5 hours after compound administration. Acetophenone semicarbazone caused anesthesia in mice after 0.5 hours at a dose of 300 mg/kg.

Acetophenone guanylhydrazone provided no protection against scMET- or MES-induced seizures at doses of 3 and 10 mg/kg, produced neurotoxicity at a dose of 30 mg/kg and caused death due to respiratory depression at a dose of 100 mg/kg.
The loss of the righting reflex and the respiratory depression noted after administration of VIb indicates that this compound is a very potent general CNS depressant with no selective anticonvulsant activity.

The reduced anticonvulsant activity of acetophenone semicarbazone and acetophenone guanylhydrazone may be explained by comparing the covalent radii and electronegativity of the various isosteres. Both N and O are smaller atoms than S (covalent radii of 0.74 Å for nitrogen and oxygen compared to 1.04 Å for the sulphur atom) (Agrawal et al., 1974) and are more electronegative atoms (electronegativity values for nitrogen and oxygen are 3.0 and 3.5 compared to 2.5 for sulphur) (Agrawal et al., 1974). Higher electronegativity may produce lesser of the resonant forms which may lower biological activity.
4.4.0 Summary

The present study has achieved the following objectives. The synthesis of a variety of aryl-substituted acetophenone thiosemicarbazones and some related derivatives based on the molecular modification of the "lead" compound, 20a, and their anticonvulsant evaluation. The anticonvulsant activities of some of these derivatives was confirmed but none of the compounds evaluated possessed significant anticonvulsant activities over the lead compound. No clear correlation between chemical structure and biological activity could be discerned based on the anticonvulsant screening results which were available. However, based on the results obtained from this study future work could be directed towards investigating other hydrazone derivatives or related compounds which might provide useful information for the further design of potential anticonvulsant agents.
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