JOURNAL OF
MEDICINAL
CHEMISTRY



Volume 36, Number 16 August 6, 1993

## Articles

# Anticonvulsant Activities of Some Arylsemicarbazones Displaying Potent Oral Activity in the Maximal Electroshock Screen in Rats Accompanied by High Protection Indices

J. R. Dimmock,*,† K. K. Sidhu,† R. S. Thayer,† P. Mack,† M. J. Duffy,†,‡ R. S. Reid,‡ J. W. Quail,‡ U. Pugazhenthi,‡ A. Ong,§ J. A. Bikker,§ and D. F. Weaver§

*College of Pharmacy and Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada, and Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada*

*Received January 20, 1993*

Various semicarbazones derived from aryl aldehydes, phenylalkyl aldehydes, and phenylalkyl ketones as well as some related compounds were evaluated for anticonvulsant activity. Most of the compounds displayed anticonvulsant activity in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens accompanied by neurotoxicity when given to mice by the intraperitoneal route. However quantitative data revealed protection indices ($TD_{50}$/$ED_{50}$) of less than 4 in general. Oral administration of the compounds to rats led to excellent potency in the MES screen accompanied by high protection indices while virtually no activity in the scPTZ test was displayed. These observations support the theory that one large hydrophobic group (in this case the aryl ring) and two electron donor atoms (present in the semicarbazono group) are requirements for protection in the MES screen. In general, the semicarbazones had rapid onsets of action, and one of the ways in which these compounds displayed their anticonvulsant activity is likely to be interaction with chloride channels. Empirical and semiempirical conformational calculations indicated that certain molecular fragments and hydrophobicity of these molecules affect bioactivity.

## Introduction

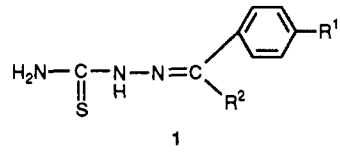
The structural requirements for activity in the maximal electroshock (MES) anticonvulsant screen, which is claimed to identify compounds with efficacy against generalized tonic-clonic ("grand mal") seizures,[1] have been stated to be two electron donor atoms close to a bulky hydrophobic group.[2] Similarly, in the subcutaneous pentylenetetrazole (scPTZ) test, which is thought to detect compounds with usefulness in treating generalized absence ("petit mal") seizures,[1] two electron donor atoms are also considered to be necessary for bioactivity, but close to a smaller, less hydrophobic group than is present in compounds active in the MES screen.[2]

Three previous studies by Dimmock *et al.* have produced synthetic anticonvulsants which incorporated these molecular features and yet were structurally dissimilar from many common monocyclic anticonvulsants containing the dicarboximide function (CONRCO) which may contribute to toxic side effects.[3] In the first study, a series of thiosemicarbazones and semicarbazones of arylidene methyl ketones were prepared and evaluated in the MES, scPTZ, and neurotoxicity screens.[4] Seventeen of the 22 compounds examined were active in the MES and/or scPTZ screens when given by the intraperitoneal route in mice (phase I screening, Antiepileptic Drug Development Program, NIH[5]). Both neurotoxicity and lethality in mice were higher in the thiosemicarbazones than the semicar-

* To whom inquiries should be directed.
† College of Pharmacy, Saskatoon.
‡ Department of Chemistry, Saskatoon.
§ Department of Chemistry, Kingston.

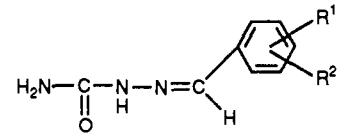
0022-2623/93/1836-2243$04.00/0 

**Chart I**



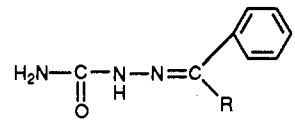
1

a: $R^1 = R^2 = H$
b: $R^1 = H$; $R^2 = CH_3$
c: $R^1 = Cl$; $R^2 = CH_3$
d: $R^1 = OCH_3$; $R^2 = CH_3$



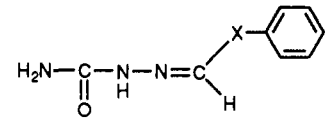
2

a: $R^1 = R^2 = H$
b: $R^1$ = 2-Cl; $R^2 = H$
c: $R^1$ = 3-Cl; $R^2 = H$
d: $R^1$ = 4-Cl; $R^2 = H$
e: $R^1$ = 2-Cl; $R^2$ = 3-Cl
f: $R^1$ = 2-Cl; $R^2$ = 4-Cl
g: $R^1$ = 2-Cl; $R^2$ = 6-Cl
h: $R^1$ = 3-Cl; $R^2$ = 4-Cl
i: $R^1 = 4\text{-}OCH_3$; $R^2 = H$



3

a: $R = CH_3$
b: $R = CH_2CH_3$
c: $R = CH(CH_3)_2$
d: $R = (CH_2)_3CH_3$
e: $R = (CH_2)_4CH_3$
f: $R = C_6H_5$



4

a: X = $CH_2$
b: X = $CH_2CH_2$
c: X = CH=CH

bazones. Since the two most active compounds were both thiosemicarbazones, development of this series of derivatives rather than the corresponding semicarbazones was considered more likely to produce potent anticonvulsants. A second study[6] involved principally variation of the alkyl groups of some thiosemicarbazones of arylidene ketones and aldehydes. Activity was found in eight of the 14 compounds in the phase I screen. In addition, removal of the olefinic double bond led to the formation of aryl alkyl ketone thiosemicarbazones and in the case of **1b**, this compound displayed activity in the MES test not only by the intraperitoneal route in mice ($ED_{50}$ = 18.97 mg/kg) but also when given orally to rats (phase VI screening, see Table II). A third study[7] revealed that 25 of 28 various aryl alkyl ketone thiosemicarbazones and related compounds were active in the phase I screen. Many of these derivatives, including **1a-c,3a** (Chart I), demonstrated activity in the MES but not scPTZ screens when given orally to rats.

The objectives of this present study are 3-fold: (i) The first objective is the "synthetic chemistry objective" and involves the selection and synthesis of a group of semicarbazone analogs; (ii) the second objective is the "molecular modeling objective" and involves the use of empirical and semiempirical conformational calculations to evaluate the structure–activity properties of the semicarbazone analogs from objective i; and (iii) the third objective is the "pharmacologic mechanism objective" and involves and evaluates the mechanism(s) of action of the semicarbazone analogs against chemoconvulsant-induced seizures.

The first objective was the preparation of some semicarbazones of various aryl aldehydes and ketones and related compounds for evaluation in the MES and scPTZ screens. These analogs were selected with a particular goal of finding compounds with oral activity. The decision to prepare semicarbazones was made on the basis that neurotoxicity and death were noted previously with thiosemicarbazones not semicarbazones when these compounds were given by the intraperitoneal route to mice.[4,6,7] Hence semicarbazones may possess anticonvulsant properties accompanied by less toxic symptoms than the corresponding thiosemicarbazones. In addition, the present investigation showed that while **3a** had similar anticonvulsant activity in the phase VI MES screen to its isostere **1b** (Table II), the protection index (PI, i.e. $TD_{50}/ED_{50}$) was markedly higher in the case of the semicarbazone **3a**. The reasons leading to the synthesis of series **2–4** were as follows. Previous work with thiosemicarbazones had revealed a higher PI in the phase VI MES screen with the aldehyde thiosemicarbazone **1a** rather than the corresponding *homo* derivative **1b**.[6,7] Also substitution in the aryl ring by chloro atoms such as **1c** gave potent orally active anticonvulsants in contrast to compounds with electron-releasing substituents such as **1d** which were only weakly active in the phase VI MES screen. It was therefore of interest to observe whether a similar observation would be made with the analogous semicarbazones in series **2**. In the case of series **3**, variation in the nature of the alkyl or aryl R groups should have a marked effect on the hydrophobicity of the molecules which may be reflected in changes in bioactivity. Furthermore variation in the $E_s$ values of the R group may affect the percentage of *E* and *Z* isomers in solution which in turn could alter the anticonvulsant properties of the molecules. In series **4**, the different spacer groups employed may indicate the importance of both the distance between the phenyl ring and the carbimino (C=N) group and also the nature of the spacer group itself in conferring anticonvulsant activity. The first objective therefore was to prepare and screen the semicarbazones in series **2–4** which represent novel departures from todays anticonvulsants.

The second objective was to employ molecular modeling calculations to evaluate the semicarbazone structure–activity properties. The use of molecular mechanics and semiempirical quantum mechanics techniques was proposed with a view to discerning the electronic and steric properties of the molecules which are associated with anticonvulsant activities. X-ray crystallography of selected molecules was also considered in order to compare this data with the predictions made by the use of empirical and nonempirical (molecular orbital) calculations.

The third objective was to determine the mode(s) of action of representative compounds by examining whether protection would be afforded against various chemically-induced seizures.

In summary, the objectives of this study were to produce a series of novel anticonvulsants ideally possessing oral activity, to find the structural features associated with this bioactivity, and to study their pharmacologic mechanism(s) of action. The results of this logical sequence of studies may permit the rational design of additional novel anticonvulsants.

## Results

**A. Chemistry.** The synthesis of **1** and **3a** has been described previously[7] and the semicarbazones **2**, **3b–f**, and **4** were prepared by reacting the appropriate aryl aldehyde, aryl alkyl ketone, or aralkyl aldehyde with semicarbazide.

**Table I.** Protection against Seizures Induced in the MES and scPTZ Screens and Also Neurotoxicity by Various Compounds after Intraperitoneal Injection in Mice

| | MES screen | | | scPTZ screen | | | toxicity screen | | | PI | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | *t* (h) | $ED_{50}$ (mg/kg) (95% CI) | slope (SE) | *t* (h) | $ED_{50}$ (mg/kg) (95% CI) | slope (SE) | *t* (h) | $TD_{50}$ (mg/kg) (95% CI) | slope (SE) | $\left(\frac{TD_{50}}{ED_{50}}\right)_{MES}$ | $\left(\frac{TD_{50}}{ED_{50}}\right)_{scPTZ}$ |
| **1c** | 0.5 | 60.43 (44.01–102.72) | 3.99 (1.30) | 0.25 | 69.64 (46.30–102.94) | 3.15 (0.88) | 24 | 23.77 (20.05–27.67) | 10.64 (3.36) | 0.39 | 0.34 |
| **2a** | 0.25 | 69.71 (53.87–84.79) | 5.64 (1.64) | 0.25 | 127.32 (71.07–201.82) | 2.39 (0.65) | 0.25 | 204.12 (149.51–306.49) | 3.19 (0.89) | 2.93 | 1.60 |
| **2c** | 1.00 | 90.36 (70.99–126.53) | 4.85 (1.74) | 1.00 | 177.23 (102.42–292.92) | 2.76 (0.84) | 0.25–24 | >500 | | >5.53 | >2.82 |
| **2d** | 1.00 | 74.84 (64.53–85.09) | 8.90 (2.50) | 0.25 | 135.1 (71.83–220.5) | 2.26 (0.65) | 2.00 | 252.2 (143.7–357.9) | 3.46 (1.18) | 3.37 | 1.87 |
| **2h** | 4.00 | 145.75 (104.55–192.30) | 4.96 (1.61) | 4.00 | >500 | | 0.25–24 | >500 | | >3.43 | |
| **3a** | 0.25 | 35.10 (33.21–36.80) | 34.53 (10.91) | 0.25 | 78.49 (62.54–94.59) | 7.31 (2.41) | 0.25 | 82.28 (75.93–89.42) | 18.94 (5.28) | 2.34 | 1.05 |
| **3d** | 0.25 | 58.27 (46.11–68.25) | 6.84 (2.01) | 0.25 | 108.43 (85.75–137.22) | 6.52 (1.95) | 0.25 | 105.63 (91.72–117.84) | 16.94 (5.68) | 1.81 | 0.97 |
| **3f** | 0.25 | 46.98 (40.16–58.25) | 13.12 (4.99) | 0.25 | 76.16 (47.41–123.98) | 2.63 (0.80) | 0.25 | 89.71 (64.88–110.06) | 6.95 (2.22) | 1.91 | 1.18 |
| **4a** | 0.25 | 45.50 (43.97–48.10) | 48.82 (17.37) | 0.25 | 127.88 (112.51–142.26) | 13.21 (3.92) | 0.25 | 119.64 (106.26–138.41) | 10.32 (3.13) | 2.63 | 0.94 |
| **4b** | 0.25 | 47.00 (43.67–51.98) | 26.55 (10.00) | 0.25 | 78.64 (70.73–86.20) | 13.49 (3.48) | 0.25 | 72.26 (55.80–86.50) | 6.69 (1.81) | 1.54 | 0.92 |
| **4c** | 0.25 | 48.89 (40.20–56.04) | 8.55 (2.34) | 0.25 | 84.70 (60.16–110.89) | 4.58 (1.12) | 0.25 | 98.21 (77.70–128.42) | 4.52 (1.26) | 2.01 | 1.16 |

The average yield was 62% ranging from 78% in the synthesis of **2a** to 14% in preparing **3c**. Compounds **2a–h** and **3a,d** were dissolved in deuterated dimethyl sulfoxide and incubated at 37 °C until the times of peak effect (TPE) were reached. These figures are indicated in Table II for the oral MES screen. $^1H$ NMR spectra recorded as soon as possible after dissolution and at the TPE were identical. The X-ray structures of **2a,b,d** were obtained.

**B. Molecular Modeling.** The calculational strategies employed are described in the Experimental Section. All analogs from series 2, 3, and 4 were studied for which $ED_{50}$ figures have been generated. Each analog was described by 39 descriptors (listed in Table IV). A subset of descriptors which correlated with oral anticonvulsant activity ($ED_{50}$) in the MES test (Table II) was selected from the full descriptor array using stepwise and forward-selective regression procedures at the 95% and 85% confidence levels. At the 95% level, none of the descriptors were significant. At the 85% level, eight descriptors were significant; these are listed in Table V.

**C. Pharmacology.** Phase I screening consisted of administering doses of 30, 100, and 300 mg/kg of the compounds in series 1–4 to mice by the intraperitoneal route and at the end of 0.5 and 4 h evaluating whether protection in the MES and scPTZ screens occurred and also whether neurotoxicity was demonstrated. Compounds **1a–d, 2a,c,d,h, 3a–f** and **4a–c** were active in the MES screen while compounds **1a–c, 2a,c,d, 3a,c,e,** and **4c** afforded protection in the scPTZ test. Neurotoxicity was noted in compounds **1a–c, 2a,d, 3a–f,** and **4a-c**. In addition, neurotoxic symptoms, other than inability to stay on a rotorod, were noted in all members of the thiosemicarbazone series 1, whereas among the semicarbazones this property was found only with **3a** and **4a,b**.

Table I indicates the quantitative anticonvulsant evaluation of selected compounds in all three screens after intraperitoneal injection in mice. In addition, compounds **2b,f** were given to mice at a dose of 100 mg/kg by both the intraperitoneal and oral routes. No protection in the MES screen was observed by these two compounds except that half of the mice were protected when **2f** was given intraperitoneally. Compounds **2a,b** and **4c** were administered orally to mice. The $ED_{50}$ values for these compounds in the MES test were 40.27, 57.74, and 77.85 mg/kg respectively and 54.86, 68.67, and 147.22 mg/kg in the scPTZ screen. The $TD_{50}$ figures for **2a,d** and **4c** were 87.67, 252.66, and 377.18 mg/kg respectively. Table II displays the evaluation of all of the compounds in series 1–4 in the MES and neurotoxicity screens after oral administration to rats. In the scPTZ screen, at the maximum doses employed (250 mg/kg or higher doses on occasions) compounds **1a–c, 2a–d,f–h, 3a–f,** and **4a–c** were inactive while **1d** and **2i** protected one in four animals at a dose of 300 mg/kg. Intraperitoneal injection of **1c** into rats revealed an $ED_{50}$ value in the MES test and $TD_{50}$ figure of 7.46 and 20.45 mg/kg, respectively. In addition, intraperitoneal injection of **3a** (75 mg/kg) and **4c** (100 mg/kg) demonstrated neurotoxicity in 8/8 and 5/8 rats, respectively.

Ten semicarbazones were examined for their ability to prevent seizures induced by bicuculline, picrotoxin, and strychnine. The results of this evaluation using bicuculline and picrotoxin are presented in Table III; at the maximum doses employed, no protection against seizures induced by strychnine was noted except for marginal activity in the case of **4a**. The ability of **2a,d** and **3b,e** to displace radiolabeled flunitrazepam and γ-aminobutyric acid from their respective receptors was examined. At the maximum concentrations of compounds employed, **2a,d** caused small but statistically significant increases in the binding of [$^3H$]flunitrazepam to its receptors rather than lowering the amount of bound ligand. Compounds **3b,e** had no effect in this assay. Both **2a** and **3e** inhibited the binding of [$^3H$]γ-aminobutyric acid to GABA receptors while **2d** and **3b** were inactive in this test. In an assay measuring the alteration in the uptake of [$^3H$]adenosine into mouse whole-brain synaptosomes, **2a** inhibited the uptake of the ligand, **3e** gave erratic results, and **2d** and **3b** had no effect. Compounds **2a,d** and **3b,e** increased the times prior to the first focal seizures and also chronic convulsions in mice

Table II. Evaluation of Various Compounds in the MES and Neurotoxicity Screens after Oral Dosing in Rats

| | MES screen | | | | | neurotoxicity screen | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | t (h) | $ED_{50}$ (mg/kg) | 95% CI | slope | SE | t (h) | $TD_{50}$ (mg/kg) | 95% CI | slope | SE | PI |
| 1a[a] | 2 | 12.84 | 8.98–16.64 | 4.77 | 1.24 | 0.25–24 | >200 | | | | >15.58 |
| 1b[b] | 1 | 16.89 | 13.60–22.44 | 7.65 | 2.69 | 1 | 73.04 | 48.75–112.08 | 4.06 | 1.21 | 4.33 |
| 1c | 1 | 15.51 | 10.67–22.12 | 4.78 | 1.40 | 8 | 118.94 | 84.68–150.41 | 5.79 | 1.81 | 7.67 |
| 1d[c] | | | | | | | | | | | |
| 2a | 1 | 22.50 | 18.04–31.35 | 6.48 | 2.03 | 1 | 254.3 | 185.3–331.2 | 4.70 | 1.56 | 11.30 |
| 2b | 0.5 | 38.78 | 33.02–45.72 | 7.83 | 2.48 | 0.25–24 | >500 | | | | >12.89 |
| 2c | 1 | 24.38 | 16.41–34.90 | 4.44 | 1.28 | 0.25–24 | >500 | | | | >20.51 |
| 2d | 1 | 18.57 | 14.67–25.53 | 6.16 | 1.95 | 0.25–24 | >500 | | | | >26.93 |
| 2e[d] | | | | | | | | | | | |
| 2f | 4 | 43.11 | 27.60–59.24 | 3.96 | 1.27 | 0.25–24 | >500 | | | | >11.60 |
| 2g | 1 | 58.96 | 43.11–86.92 | 4.33 | 1.38 | 4 | 128.8 | 82.86–190.3 | 3.70 | 1.05 | 2.18 |
| 2h | 6 | 20.18 | 15.44–24.25 | 6.40 | 1.85 | 0.25–24 | >500 | | | | >24.78 |
| 2i[e] | | | | | | | | | | | |
| 3a | 0.25 | 20.25 | 13.79–24.52 | 6.07 | 1.94 | 1 | 268.3 | 181.1–450.1 | 4.48 | 1.57 | 13.25 |
| 3b | 0.25 | 32.80 | 29.12–37.07 | 14.45 | 4.45 | 0.5 | 404.4 | 262.9–620.9 | 3.47 | 1.06 | 12.33 |
| 3c | 0.25 | 43.31 | 30.08–55.23 | 4.76 | 1.35 | 1 | 245.12 | 175.01–370.02 | 4.05 | 1.32 | 5.66 |
| 3d | 0.25 | 59.47 | 42.34–75.21 | 5.79 | 1.81 | 1 | >500 | | | | >8.41 |
| 3e | 0.50 | 32.61 | 22.75–44.27 | 5.06 | 1.40 | 0.25–24 | >500 | | | | >15.33 |
| 3f | 0.25 | 22.88 | 16.35–27.71 | 7.75 | 2.56 | 1 | 235.48 | 173.60–301.98 | 6.74 | 1.90 | 10.29 |
| 4a | 0.5 | 42.78 | 31.95–52.21 | 6.04 | 2.01 | 0.25–24 | >500 | | | | >11.69 |
| 4b | 0.25 | 36.43 | 23.85–49.61 | 3.70 | 0.93 | 0.25 | 333.67 | 288.56–386.11 | 8.54 | 2.47 | 9.16 |
| 4c | 1 | 11.49 | 7.56–14.91 | 3.97 | 1.21 | 0.25–24 | >500 | | | | >43.52 |

[a] Data taken from ref 7 and reproduced with permission. [b] Data taken from ref 6 and reproduced with permission. [c] At a dose of 300 mg/kg, 1/2 animals were protected 0.5 h after administration but after 0.25, 1, 2, and 4 h no anticonvulsant activity was demonstrated. At this dose, no neurotoxicity was noted in 0/2 animals 0.25, 0.5, 1, 2, and 4 h after oral dosage. [d] At a dose of 500 mg/kg, the number of animals (out of four) protected at the end of 0.25, 1, 4, 6 and 8 h were 0, 0, 1, 2, and 1, respectively. By using these time intervals and the same number of animals and dose of the compound, no neurotoxicity was noted. [e] Administration of 300 mg/kg of 2i revealed protection in 1/2, 2/2, 2/2, 2/2, and 2/2 rats but no neurotoxicity at the end of 0.25, 0.5, 1, 2, and 4 h, respectively. No activity or neurotoxicity was noted at these times when a dose of 50 mg/kg was employed.

Table III. Evaluation of Selected Compounds for Protection against Seizures Induced by Subcutaneous Injections of Bicuculline and Picrotoxin in Mice

| | sc bicuculline screen | | | | | sc picrotoxin screen | | | |
|---|---|---|---|---|---|---|---|---|---|
| compd | t (h) | $ED_{50}$ (mg/kg) | 95% CI | slope | SE | $ED_{50}$ (mg/kg) | 95% CI | slope | SE |
| 2a | 0.25 | 116.0 | 87.36–145.8 | 5.44 | 1.59 | 103.6 | 72.98–134.0 | 5.91 | 1.66 |
| 2c | 1.00 | >500 | | | | 200.19 | 126.05–317.71 | 3.20 | 0.94 |
| 2d | 0.25 | >300 | | | | 146.1 | 72.0–530.4 | 1.80 | 0.75 |
| 2h | 4.0 | >500 | | | | >500 | | | |
| 3a | 0.25 | >100 | | | | 60.78 | 48.02–72.49 | 9.30 | 2.86 |
| 3b | 0.25 | >100 | | | | 127.9 | 95.39–166.2 | 5.34 | 1.77 |
| 3e | 0.25 | 362.7 | 227.5–737.8 | 3.31 | 1.38 | 230.3 | 197.6–286.9 | 8.45 | 2.68 |
| 3f | 0.25 | >100 | | | | >100 | | | |
| 4a | 0.25 | 59.63 | 23.19–105.92 | 1.76 | 0.57 | >150 | | | |
| 4b | 0.25 | >120 | | | | >120 | | | |

induced by the intravenous administration of pentylenetetrazole. The semicarbazones **2a,d** and **3b,e** increased the times prior to the first focal seizures and also chronic convulsions in mice induced by the intravenous administration of pentylenetetrazole. The semicarbazones **2a,d** and **3e** protected mice against clonic seizures and also forelimb tonic extensions induced by *N*-methyl-D-aspartate.

## Discussion

**A. Analog Syntheses and Biological Evaluation Studies.** A recent report indicated that the phase I evaluation of a number of thiosemicarbazones, which included **1a–d**, displayed anticonvulsant activity but in most cases these derivatives displayed neurotoxicity.[7] In addition, all of the compounds in series 1 demonstrated other neurotoxic symptoms such as continuous seizure activity. The semicarbazones **2–4** displayed anticonvulsant activity and toxicity in 72 and 61% of the compounds, respectively; however, in contrast to the thiosemicarbazones, no other neurotoxic symptoms were noted except in the cases of **4a,b**. In general therefore, anticonvulsant activity is retained in the semicarbazones while toxicity is diminished relative to the related thiosemicarbazones.

This initial favorable result suggested that quantitation of representative compounds should be undertaken in mice (phase II screening) in order to have precise indications of their potencies, to compare their activities with that of existing drugs, and to evaluate the protective index (PI) values of the compounds where possible. This information is presented in Table I for 10 semicarbazones and data for a representative thiosemicarbazone namely **1c** is included for comparison. The TPE determinations revealed that for seven of the 10 semicarbazones, the TPE was 0.25 h and hence in general the compounds are rapidly acting anticonvulsants. Phenobarbital, a drug used in treating generalized tonic-clonic seizures, has an $ED_{50}$ value in the phase II MES screen in mice of 21.8 mg/kg.[1] Hence these semicarbazones range in potency from 62% (**3a**) to 15% (**2h**) that of the reference drug in this screen. In the scPTZ test, the semicarbazones had $ED_{50}$ values which were higher than the figures obtained in the MES screen. However in comparison with ethosuximide which has an $ED_{50}$ value of 130 mg/kg in this screen,[1] **3a** and **4b** were more potent,

Table IV. Descriptors Used in the Molecular Modeling Studies of **2a-d, 2f-h, 3a-f,** and **4a-c**

| | | | |
|---|---|---|---|
| 1 | bond length: C8–N3 | 24 | molecular dipole moment |
| 2 | bond length: N2–C8 | 25 | Randic index 1 ($x-1$) |
| 3 | bond length: N1–N2 | 26 | Randic index 2 ($x-2$) |
| 4 | bond length: C7–N1 | 27 | Randic index 3 ($x-3$) |
| 5 | bond angle: N2–C8–N3 | 28 | Randic index 4 ($x-4$) |
| 6 | bond angle: C7–N1–N2 | 29 | Kier–Hall index 1 ($xv-1$) |
| 7 | torsional angle: C7–N1–N2–C8 | 30 | Kier–Hall index 2 ($xv-2$) |
| 8 | torsional angle: Ar–C7–N1–N2 | 31 | Kier–Hall index 3 ($xv-3$) |
| 9 | interatomic distance: C1–N3 | 32 | Kier–Hall index 4 ($xv-4$) |
| 10 | interatomic distance: C4–O | 33 | Zagreb index 1 (M1) |
| 11 | volume of the aryl ring | 34 | Zagreb index 2 (M2) |
| 12 | volume of atom or group attached to carbimino function i.e. hydrogen (series *2* and *4*) or R (series *3*) | 35 | Platt index (F) |
| | | 36 | molecular weight of the molecule |
| | | 37 | number of carbon atoms in the molecule |
| 13 | combined volumes from 11 and 12 | | |
| 14 | atomic charge on atom N3 | 38 | number of atoms in the molecule |
| 15 | atomic charge on atom C8 | 39 | log *P* |
| 16 | atomic charge on atom N2 | geometric descriptors | 1–13 |
| 17 | atomic charge on atom N1 | electronic descriptors | 14–24 |
| 18 | atomic charge on atom C7 | topological descriptors | 25–38 |
| 19 | atomic charge on atom 0 | graph theory | (25–35) |
| 20 | atomic charge on atom C2 | ad hoc | (36–38) |
| 21 | atomic charge on atom C3 | physicochemical descriptors | 39 |
| 22 | atomic charge on atom C4 | | |
| 23 | atomic charge on atom C6 | | |

Table V. Significant Descriptors at the 85% Confidence Level

| |
|---|
| molecular dipole |
| log *P* |
| atomic charge on N3 |
| atomic charge on C2 |
| C7–N1–N2–C8 torsional angle |
| Randic 3 index |
| C4–O distance |
| C7–N1–N2 angle |

and **2a,c,d, 3d,f,** and **4a,c** were equipotent with this drug. Table I indicates that the protection indices of the semicarbazones were greater than that of the thiosemicarbazone **1c** and that for each compound the figure obtained in the MES screen was approximately twice that found in the scPTZ test. In the MES screen, the most promising compounds in regard to the separation of activity and neurotoxicity were **2c,d,h** with PI values greater than or comparable to phenobarbital (PI = 3.17).[1] The PI of ethosuximide is 3.39;[1] hence only **2c** has a protection index comparable to this established drug. In summary, phase II screening suggests that the semicarbazones have greater activity in the MES rather than the scPTZ screens both in terms of potencies and protection indices.

Of considerable interest is the oral activity of many of the semicarbazones **2–4** in the MES screen in rats. In addition their low neurotoxicity resulted in high protection indices. This information is presented in Table II along with the data for four thiosemicarbazones in series **1**. At the maximum doses administered ($\nleq$ 250 mg/kg), **1a–c, 2a–d,f–h, 3a–f,** and **4a–c** were inactive in the scPTZ screen while **1d** and **2i** displayed only marginal activity in this test at 300 mg/kg. Thus a noteworthy MES-selective protection was demonstrated by these compounds when given orally. A comparison of the data in Tables I and II indicate that in general, potency in the MES screen was increased and neurotoxicity decreased when the compounds were given orally to rats rather than by intraperitoneal injection into mice. Hence much higher PI values are recorded in Table II. This phenomenon is likely due to differences in species of animals and routes of administration. Thus administration of three representative compounds namely **2a,d** and **4c** to both rats and mice by the oral route indicated that while **2a** had the same $ED_{50}$ value in the MES screen whether given orally to either mice or rats, both **2d** and **4c** displayed greater potency in rats. The $TD_{50}$ figures of all three compounds were higher in rats and hence higher PI figures were obtained by oral dosing in rats. The route of administration appears unimportant in mice since lower $ED_{50}$ and $TD_{50}$ figures were recorded for **2a** when given orally and for **4c** when given by the intraperitoneal route while **2d** had the same bioactivity when given by either route of administration. On the other hand, intraperitoneal injection of **1c, 3a,** and **4c** to rats produced neurotoxicity at lower doses than when the compounds were given orally and the $ED_{50}$ value in the MES screen of **1c** was reduced. In addition, a dose of 100 mg/kg of **2e** given intraperitoneally into rats gave protection in half of the animals in the MES screen. In other words, intraperitoneal injection gave higher bioactivities than when the compounds were administered orally to rats.

Table II reveals that in series **1, 1a–c** have the same potency and are clearly superior to **1d** while the thiosemicarbazone derived from an aryl aldehyde namely **1a** had the highest PI. Series **2** comprising a number of semicarbazones of various aryl aldehydes revealed that high potency was retained in this group of compounds, and in general, protection indices comparable to or greater than **1a** were obtained. Compounds **2c,d,h** were equipotent with **1a** but in the case of these three semicarbazones, no neurotoxicity was noted at a dose of 500 mg/kg. The presence of a 4-methoxy group in the aryl ring **2i**, like the analogous thiosemicarbazone **1d**, had relatively low activity. The $ED_{50}$ values in series **2** appear to be influenced by the positions of the substituents in the aryl ring rather than their electronic and hydrophobic properties. Thus greater anticonvulsant properties were noted with both the unsubstituted semicarbazone **2a** and also compounds containing a 3-chloro and/or 4-chloro substituents (**2c,d,h**) rather than derivatives with a 2-chloro atom (**2b,f,g,e**). This ortho substitution would cause a large interplanar angle ($\theta$) to be formed between the aryl ring and the adjacent azomethine bond,[8,9] and it is conceivable therefore that small $\theta$ values are associated with marked oral activity in the MES screen. In order to examine this possibility, X-ray data for **2a,b,d** revealed that the aryl rings com-

prising carbon atoms 1–6 made angles of 5.1°, 7.6°, and 9.1°, respectively with the adjacent C7-N1-N2 plane (see Figure 1 for numbering scheme). Hence in the crystal form at least, ortho substitution in these three compounds did not change the $\theta$ values as predicted.

The potencies in the oral MES screen in series **2** and **3** were similar being 32.4 and 35.2 mg/kg respectively using the data of the compounds for which $ED_{50}$ values were computed. However neurotoxicity was noted at lower doses in series **3**. Thus while six of the nine compounds in series **2** had $TD_{50}$ figures in excess of 500 mg/kg, only two of the six semicarbazones in series **3** were free from neurological deficit at this dose. This phenomenon contributed to protection indices being somewhat lower in series **3** than **2**; hence in general alkyl groups attached to the azomethine group are disadvantageous. The most promising lead compound in series **3** is **3e** with a PI of greater than 15. In order to seek correlations between the potencies of **2a** and **3a–f** and variations in the hydrophobic, steric and electronic properties of the hydrogen atom (in **2a**) or group R (in **3**) attached to the azomethine bond, linear plots of the $ED_{50}$ figures versus the fragment constant ($f$), molar refractivity (MR), resonance ($\mathcal{R}$) and field ($\mathcal{F}$) constants were made. Using the test for zero correlation,[10] anticonvulsant potency was significantly correlated with $f$ and $\mathcal{R}$ values ($p < 0.05$) as well as the $\mathcal{F}$ constants ($p < 0.10$) but not with the MR figures ($p > 0.10$).

The semicarbazones **4b,c** possessing two carbon atom spacer groups between the aryl ring and azomethine carbon atom had similar and greater potencies, respectively, than **2a**. Compounds **2a** and **4c** are more active than **4a** in which a methylene group insulates the phenyl ring from the azomethine linkage. However **4a** is less neurotoxic than **2a** resulting in a higher protection index (>11.69) than is found in **2a** (PI = 11.30). Of the two compounds possessing two carbon atom spacer groups, an unsaturated linkage is preferable both in terms of potency and PI. In fact the PI of **4c** is singularly noteworthy, and the marked difference to phase II screening is of interest.

The average times of peak effects in series **2–4** were approximately 2, 0.25, and 0.6 h, respectively. The time required for maximum penetration to a site of action may be influenced by the hydrophobic properties of the molecules. Hence a linear plot of the TPE values of the rat oral MES screen of the compounds in series **2–4** (except **2e,i**) versus the fragment constants ($f$) of the variable atoms and/or groups on the carbon atom on the carbimino function i.e. the non-carbon aryl substituents and either a proton (series **2** and **4**) or a R group (series **3**) was performed. No correlation was observed ($r = 0.00$).

Mephenytoin and phenobarbital have $ED_{50}$ figures in the oral MES screen in rats of 18.1 and 9.1 mg/kg respectively;[5] hence **2a,c,d,h**, **3a,e,f**, and **4b,c** are equipotent with mephenytoin and **4c** has the same activity as phenobarbital. However the PI values of mephenytoin and phenobarbital are 4.73 and 6.71, respectively. These figures are exceeded by 14 of the18 compounds in series **2–4**; in fact **2c,d,h** and **4c** have protection indices greater than 20. Thus these compounds represent templates for future molecular modification with a goal to providing novel, potent anticonvulsant drugs in which the differential between doses which are effective therapeutically and those causing neurotoxicity is greater than is found with currently available medication.

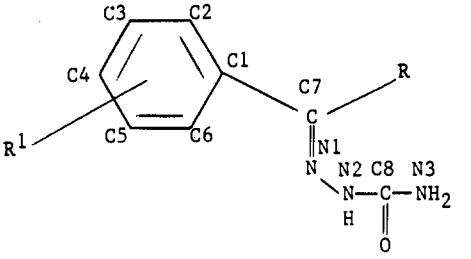



**Figure 1.** Numbering scheme of semicarbazones used in the molecular modeling and X-ray studies.

Certain anticonvulsants have proconvulsant properties. In order to determine the likelihood of the compounds prepared in this report having convulsant activities, pentylenetetrazole was infused into the tail veins of mice and the times taken for the appearances of the first focal seizures and also clonic seizures were recorded. The experiment was repeated using varying concentrations of **2a,d** and **3b,e** and in each case the times prior to seizures occurring were increased and hence they appear to be bereft of proconvulsant properties.

A previous $^1H$ NMR study of thiosemicarbazones of compounds similar to series **1**, except that there was an olefinic bond between the aryl ring and the carbimino group, revealed that the percentage of compounds with the $E$ configuration of the carbimino group was influenced by the size of the $R^2$ group.[6] As the size of the $R^2$ group increased so the percentage of the $E$ isomers diminished. In the present study, the spectra of **2a–h** in deuterated dimethyl sulfoxide were recorded as rapidly as possible after dissolution and at the times of peak effect in the oral MES screen in rats. The spectra at both time intervals were identical and indicated the presence of one isomer only. The absorptions of the methine protons for compounds containing an ortho halogen (**2b,e,f,g**) were at 8.09–8.25 ppm while for **2a,c,d,h** which have no substituent in the ortho position the resonances of the methine protons was displayed in the range of 7.80–7.89 ppm. Since X-ray crystallography revealed that compounds with either a halogen atom in the ortho position (**2b**) or no ortho substituents (**2a,d**) had the $E$ configuration, it is likely that the compounds in series **2** possess this stereochemistry. Since the TPE of **3a–d** was 0.25 h, only one spectrum was recorded as rapidly as possible after dissolution. Observations of the methyl, methylene, and methine protons revealed the presence of only one isomer which was assumed to have the $E$ configuration. It is likely therefore that the anticonvulsant activity of the compounds described in this report possess this stereochemistry when isomerism is possible.

**B. Molecular Modeling Studies.** These semicarbazone anticonvulsants may be considered as bifunctional molecules, possessing a lipophilic moiety (substituted phenyl ring) and a hydrogen bonding moiety (the semicarbazono portion, N1–N2–C8(O)–N3). Figure 1 indicates the numbering of these molecules. The eight statistically significant descriptors (Table V) suggest that the geometric distance between the hydrogen bonding and lipophilic moieties is important for activity (C4–O distance) and that the orientation between the lipophilic and hydrogen bonding moieties is important (C7–N1–N2–C8 torsional). The geometry of the hydrogen bonding moiety (C7–N1–N2 angle) is important as is the charge on the N3 atom. Activity is also influenced by the phenyl substitution pattern (charge on the C2 atom of the phenyl ring).

**Table VI.** Comparison of Crystal and AM1 Geometics for Compound **2d**

| | Crystal | AM1 | | Crystal | AM1 |
|---|---|---|---|---|---|
| | | Distances (Å) | | | |
| C1–C2 | 1.371 | 1.404 | C7–N1 | 1.281 | 1.309 |
| C2–C3 | 1.385 | 1.391 | N1–N2 | 1.387 | 1.327 |
| C3–C4 | 1.369 | 1.399 | N2–C8 | 1.375 | 1.433 |
| C4–C5 | 1.367 | 1.398 | C8–O | 1.240 | 1.253 |
| C5–C6 | 1.383 | 1.394 | C8–N3 | 1.336 | 1.379 |
| C1–C6 | 1.386 | 1.401 | C4–Cl | 1.740 | 1.699 |
| C1–C7 | 1.466 | 1.465 | | | |
| | | Angles (deg) | | | |
| C1–C7–N1 | 121.6 | 122.03 | N2–C8–N3 | 117.2 | 121.92 |
| C7–N1–N2 | 114.9 | 121.07 | O–C8–N3 | 124.2 | 121.89 |
| N1–N2–C8 | 119.6 | 122.09 | Cl–C4–C3 | 118.9 | 119.73 |
| N2–C8–O | 118.6 | 116.19 | Cl–C4–C5 | 118.6 | 119.85 |
| | | Torsional Angles (deg) | | | |
| C2–C1–C7–N1 | 8.4 | −169.76 | N1–N2–C8–O | 178.0 | −168.45 |
| C1–C7–N1–N2 | 182.3 | 177.15 | N1–N2–C8–N3 | −3.2 | 11.94 |
| C7–N1–N2–C8 | −169.5 | 168.23 | | | |

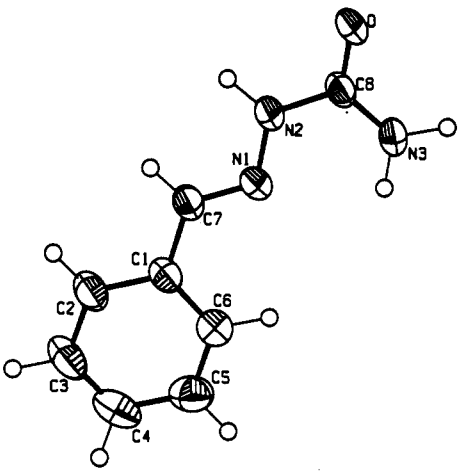



**Figure 2.** ORTEP diagram of **2a**.

Therefore, the molecular modeling calculations suggest that the activity of semicarbazone anticonvulsants is dependent on both pharmacokinetic and pharmacodynamic considerations. First the molecule must reach the receptor microenvironment (significance of log *P*); next, it must interact with its receptor. The receptor may have a lipophilic pocket and a hydrogen bonding surface to interact with the lipophilic and hydrogen bonding moieties of the semicarbazone, respectively. The separation and orientation of the lipophilic pocket and hydrogen bonding surface influence activity.

To ensure the ability of the MM2/AM1 calculational strategy used in this study to reproduce experimental geometries, three calculated structures were compared with three structures (compounds **2a,b,d**) determined by X-ray crystallography. The root-mean-square (RMS) for the fit of the crystal geometries to the AM1-optimized geometries was 0.213 for the three compounds. This low RMS value demonstrates the ability of the AM1 Hamiltonian to reproduce experimental aryl semicarbazone geometries. A comparison between X-ray and AM1-optimized geometries for compound **2d** is presented in Table VI. The X-ray structures of compounds **2a,b,d** are presented in Figures 2–4.

**C. Mechanisms of Pharmacologic Action Study.** A number of common anticonvulsants inhibit clonic seizures and forelimb tonic extension (FTE) in mice induced by the excitory amino acid *N*-methyl-D-aspartate (NMDA).[11] Compounds **2a,d** and **3e** afforded protection in both tests.

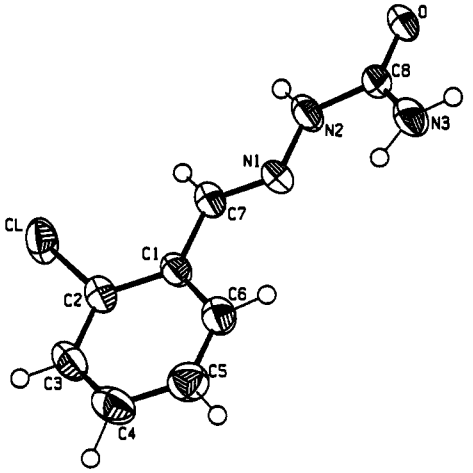



**Figure 3.** ORTEP diagram of **2b**.

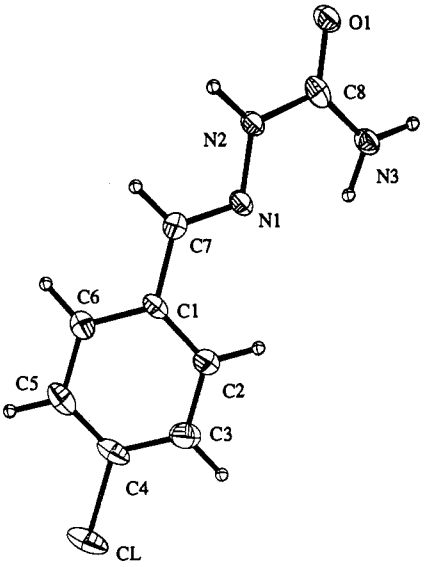



**Figure 4.** ORTEP diagram of **2d**.

In contrast to valproic acid, the semicarbazones **2a** and **3e** were more active in protecting against clonic seizures while in the FTE screen, **2d** was more potent and **2a** and **3e** were equipotent with this antiepileptic drug. In both tests, the three compounds were less active than phenytoin and phenobarbital. Attempts were made to gain some understanding in the possible sites of action of these compounds. Anticonvulsants which block seizures induced by bicuculline, picrotoxin, and strychnine do so by acting on γ-aminobutyric acid (GABA) receptors, chloride channels, and glycine receptors respectively.[11] The data in Table III reveal that **2a**, **3e**, and **4a** interfere with the binding of bicuculline to the GABA receptors while six of the 10 derivatives interfere with the binding of picrotoxin to the chloride channels. No significant protection was noted by any of the compounds listed in Table III against seizures induced by strychnine. Thus the data suggest that GABA receptors and chloride channels may be sites of action of these anticonvulsants.

Second, **2a,d** and **3b,e** were investigated in three *in vitro* receptor-binding tests.[11] Compounds **2a,d**, but not **3b,e**, caused an increase in the binding of [$^3$H]flunitrazepam to benzodiazepine receptors in mouse brain synaptic membranes. Since none of these derivatives displaced the radioligand from the homogenate, no interactions of **2a,d** and **3b,e** with the benzodiazepine receptor site occurs. On the other hand, **2a** and **3e** inhibited the binding of

[$^3$H]GABA to gabaergic receptors in synaptic membranes and hence these two compounds bind at GABA receptors. Compound **2a** inhibited the uptake of radiolabeled adenosine into synaptosomes. Since adenosine is thought to be involved in the central actions of benzodiazepines,[12] a number of compounds binding at the benzodiazepine receptor may inhibit the uptake of adenosine into synaptosomes. However in the case of the four compounds examined, no such correlation was observed.

## Conclusion

Most of the compounds described in this report have significant activity in protecting convulsions in the MES screen when they were administered orally to rats. High protection indices were found in the majority of cases; in fact no neurotoxicity was displayed at the maximum doses administered in many compounds. The presence of a large hydrophobic group (aryl ring) near four electron donor atoms (in the semicarbazono group) fulfills the structural requirements for activity in the MES screen which may account for the selective protection in this test in contrast to the scPTZ screen when the compounds were given orally. In general these semicarbazones are rapidly acting compounds and the data generated suggest that a common mode of action may be interaction with chloride channels. The fragments of the semicarbazone molecules associated with oral activity in rats in the MES screen were obtained using empirical and semiempirical conformational calculations which also suggest that the lipophilicity of the molecules was an important feature.

## Experimental Section

A. **Chemistry.** Melting points are uncorrected. Elemental analyses (C, H, N) were undertaken on **2a–i**, **3b–f**, and **4a–c** by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan, and were within 0.4% of the calculated values except for **3d** (calcd for $C_{12}H_{17}N_3O$: C, 65.72. Found: C, 66.21). The melting points (°C) and yields (%) of **2e–h** are as follows: **2e**, 260, 77; **2f**, 252, 60; **2g**, 228–229, 77; **2h**, 238–239, 75. The remaining compounds had melting points in accord with literature values. The $^1$H NMR studies were undertaken using a Bruker AM 300 FT NMR instrument equipped with an Aspect 3000 Computer.

(i) **Synthesis of Compounds.** The preparation of compounds **1a–d** and **3a** have been described previously.[7] The general method for the preparation of the other semicarbazones is as follows. A solution of the appropriate aldehyde (0.01 mol) in methanol (10 mL) was added to a solution of semicarbazide hydrochloride (0.01 mol) and sodium acetate (0.01 mol) in water (10 mL). The mixture was stirred at room temperature for 1 h; the precipitate was collected, dried and recrystallized from 95% ethanol (**2a,d,i** and **3b,f**), absolute ethanol (**2b,c, 3c–e**, and **4a,b**), 1-propanol (**2e–g**), 1-butanol (**2h**), or acetic acid (**4c**). Minor modifications to this general procedure were as follows. In the preparation of **3c,e** solutions of the reactants were stirred at room temperature for 3.5 and 2 h, respectively, followed by refrigeration overnight and 1 h, respectively. The synthesis of **3f** entailed heating the reaction mixture under reflux for 25.5 h and on cooling it was refrigerated overnight.

(ii) **Use of Physicochemical Constants.** The $f$, MR, $\mathcal{R}$, and $\mathcal{F}$ constants were taken from the literature.[13] The fragment constants were calculated using $f_H$, $f_C$, $f_{C_6H_5}$, $f^o_H$, and $f^o_{Cl}$ figures of 0.23, 0.20, 1.90, 0.23, and 0.94 respectively. The $f$ values for the ethyl, isopropyl, $n$-butyl, and $n$-pentyl groups were obtained using a bond factor ($F_b$) of –0.12 applied $n$ – 1 times where $n$ is the number of bonds in the group. In the case of the isopropyl function, a one-time chain branch factor ($F_{cBr}$) of –0.13 was used. The olefinic group in the styryl function of **4c** required the use of a double bond factor ($F^o_=$) of –0.44.

(iii) **$^1$H NMR Studies of 2a–h and 3a–d.** $^1$H NMR spectra of 10 mM solutions of **2a–h** and **3a-d** in deuterated dimethyl sulfoxide at 37 °C were obtained. Acquisition of the spectra was obtained with 16K TD and 16K SI and the resolution was 0.513 Hz/Pt. The pulse angle was about 30° giving 1.95-s acquisition and the relaxation delay was 2 s. The total number of scans were 128. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane as the internal standard. The absorption of the methine protons were as follows: **2a**, 7.84; **2b**, 8.24; **2c**, 7.89; **2d**, 7.82; **2e**, 8.25; **2f**, 8.18; **2g**, 8.09; and **2h**, 7.80. The alkyl absorptions for **3a–d** were as follows: **3a**, 2.18 ($CH_3$); **3b**, 2.73 ($CH_2$, $J$ = 7.6 Hz), 1.01 ($CH_3$, $J$ = 7.6 Hz); **3c**, 3.31 (CH, $J$ = 7.1 Hz), 1.10 [$(CH_3)_2$, $J$ = 7.1 Hz]; and **3d**, 2.73 ($CH_2$), 1.37 ($CH_2CH_2$), 0.88 ($CH_3$).

(iv) **X-ray Crystallographic Studies on 2a,b,d.** All three compounds were crystallized from a mixture of methanol and 2-propanol. The data for **2a** was as follows: $C_8H_9N_3O$, Mr = 162.09, thin platelets, 0.45 x 0.28 x 0.025 mm, $a$ = 11.946(1), $b$ = 5.4543(5), c = 12.952(1) Å, $\beta$ = 102.20(1)°, $V$ = 824.9(1) Å$^3$, $Z$ = 4, space group = $P2_1/c$, monoclinic, $D_m$ (by flotation) = 1.284 g cm$^{-3}$, $D_x$ = 1.314 g cm$^{-3}$ (Cu K$\alpha$) = 1.5418 Å, $\mu$ = 0.076 mm$^{-1}$, $F$(000) = 343.9, $T$ = 287 K. Lattice parameters are determined from 25 reflections in the $\theta$ range 22.01–44.38° and 1704 unique reflections, $[(\sin\theta)/\lambda]_{max}$ = 0.6265 Å$^{-1}$, $-14 \le h \le 14$, $-4 \le k \le 6$, $-2 \le l \le 16$. Merging $R$ is based on intensities 0.0167 for 796 replicate reflections, $R(F)$ = 0.062, $R_w$ = 0.045, $S$ = 2.968 for 1501 observed reflections. Parameters refined = 111 [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.2517. $\Delta\rho$ in final difference map within +0.2 and –0.3 e Å$^{-3}$.

The data for **2b** was as follows: $C_8H_8ClN_3O$, Mr = 197.54, colorless needles, 0.725 × 0.1 × 0.05 mm, $a$ = 17.475(2), $b$ = 3.8744(4), c = 13.719(1) Å, $\beta$ = 110.20(1)°, $V$ = 871.8(2) Å$^3$, $Z$ = 4, space group = $P2_1/c$, monoclinic, $D_m$ (by flotation) = 1.566 g cm$^{-3}$, $D_x$ = 1.506 g cm$^{-3}$, (Cu K$_\alpha$) = 1.5418 Å, $\mu$ = 0.33 mm, $F$(000) = 407.91, $T$ = 287 K. Lattice parameters are determined from 25 reflections in the $\theta$ range 15.39–31.59° and 1765 unique reflections, $[(\sin\theta)/\lambda]_{max}$ = 0.6265 Å$^{-1}$, $-21 \le h \le 20$, $-2 \le k \le 4$, $0 \le l \le 16$. Merging $R$ is based on intensities 0.0101 for 195 replicate reflections. $R(F)$ = 0.041, $R_w$ = 0.040, $S$ = 2.717 for 1628 observed reflections. Parameters refined = 119 [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.1271. $\Delta\rho$ in final difference map within +0.24 and –0.23 e Å$^{-3}$.

The data for **2d** was as follows: $C_8H_8ClN_3O$, Mr = 197.54, colorless needles, 0.5 × 0.1 × 0.1 mm, $a$ = 11.8943(15), $b$ = 4.4480(5), c = 17.072(5) Å, $\beta$ = 92910(16)°, $V$ = 902.0(3) Å$^3$, $Z$ = 4, space group = $P2_1/a$, monoclinic, $D_m$ (by flotation) = 1.440 g cm$^{-3}$, $D_x$ = 1.455 g cm$^{-3}$, (Mo K$_\alpha$) = 0.70930 Å, $\mu$ = 0.38 mm$^{-1}$, $F$(000) = 408.67, $T$ = 287 K. Lattice parameters are determined from 25 reflections in the $2\theta$ range 18.61–37.44° and 1246 unique reflections $[(\sin\theta)/\lambda]_{max}$ = 0.5486 Å$^{-1}$, $-12 \le h \le 12$, $0 \le k \le 4$, $0 \le l \le 18$. Merging $R$ is based on intensities 0.014 for 175 replicate reflections, 695 reflections with $I > 2.5\sigma(I)$ were used in refinements. $R(F)$ = 0.050, $R_w$ = 0.049 [$w = 1/\sigma^2(F)$], $S$ = 4.18. Parameters refined = 118; final $(\Delta/\sigma)_{max}$ = 0.002. $\Delta\rho$ in final difference map within +0.24 and –0.25 e Å$^{-3}$.

The angles (esd in parentheses) between the aryl rings and the adjacent C7–N1–N2 plane of **2a,b,d** were 5.1(1), 7.6(2), and 9.1(7) respectively.

With all three compounds, an Enraf-Nonius CAD-4 diffractometer was used for data collection and the structures were solved by direct methods using XTAL 3.0[14] for **2a,b** and NRCVAX[15] for **2d** whereby all non-hydrogen atoms were found on the $E$ map and refined anisotropically. The hydrogen atoms were calculated and not refined. Atomic scattering factors were taken from ref 16. All calculations were performed on a VAX 8650 computer at the University of Saskatchewan.

B. **Molecular Modeling.** For molecular mechanics calculations, two force-field equations were employed: Dreiding, as implemented in BIOGRAF 3.0, and MM2(85).[17] Semiempirical molecular orbital quantum mechanics calculations were performed using the AM1 Hamiltonian[18] as implemented in MOPAC 5.01.[19] For statistical calculations the SAS package of statistical programs, version 6.06, was used.[20] Molecular mechanics and semiempirical molecular orbital conformational calculations were performed on IBM RS/6000 550 and 320H RISC computers operating under AIX in the Queen's University/IBM Molecular

Modeling Laboratory; statistical calculations were performed on an IBM ES/9129 computer operating under VM/CMS.

To ascertain the conformation of a given molecule, the following calculational strategy was used. First, for each analog 50 different starting conformations were fully optimized using the Dreiding force field and a first derivative minimization procedure. These analogs were selected by varying dihedral angles. Next, the 10 lowest energy conformations from the Dreiding calculations were optimized using the MM2(85) force field and a second-derivative Newton-Raphson minimization procedure. The lowest energy conformer from the MM2 calculations was then mimized using the AM1 semiempirical molecular orbital Hamiltonian. The AM1-optimized conformation and geometries were used for the structure–activity relationship studies.

For structure–activity studies, each analog was described by four series of descriptors: (i) geometric descriptors to represent three-dimensional properties and to reflect aspects of molecular size and shape (e.g. bond lengths, torsional angles, interatomic distances, substituent volumes[21]); (ii) electronic descriptors to represent variable electron distribution throughout the molecular framework (e.g. atomic charge densities, molecular dipole); (iii) topological descriptors encoding aspects of molecular composition and connectivity (e.g. Randic indices, Keir–Hall indices[22,23]); and (iv) physicochemical descriptors describing molecular lipophilicity (e.g. log $P$). The geometric and electronic descriptors were obtained from the optimized AM1 calculational results. Topological descriptors were divided into two groups: graph theory descriptors and ad hoc. The graph theory topological descriptors were determined from graph theory calculations. The physicochemical descriptor was calculated using the approach of Ghose and Crippen.[24] Descriptors are listed in Table IV.

Each compound was described by 39 descriptors. Regression and discriminant statistical analyses were performed to establish a relationship between the molecular descriptors and biological activity and to ascertain the minimal number of descriptors for identifying optimal bioactivity.

**C. Pharmacology.** The evaluation of the compounds for anticonvulsant activities was undertaken by the National Institute of Neurological Disorders and Stroke, National Institutes of Health, using their reported procedures.[5] In the phase II screen, the $ED_{50}$ values (95% CI) of phenobarbital, phenytoin, and ethosuximide in the MES test are as follows: 21.8 (15.0–25.5), 9.50 (8.13–10.4), and >1000 mg/kg respectively.[1] The figures in the scPTZ screen are 13.2 (5.87–15.9), inactive, and 130 (111–150) mg/kg, respectively.[5] In the neurotoxicity screen, the figures for these three drugs are 69.0 (62.8–72.9), 65.5 (52.5–72.1), and 441 (383–485) mg/kg, respectively.[1] After oral administration to rats, the $ED_{50}$ values for phenobarbital and mephenytoin in the MES screen are 9.1 (7.58–11.86) and 18.1 (14.07–24.91) mg/kg, respectively[5] and the $TD_{50}$ figures for these two compounds are 61.1 (43.72–95.85) and 85.7 (69.88–93.70) mg/kg, respectively.[5,25] The 95% confidence intervals for a number of derivatives mentioned in the text are as follows namely compound (route of administration and animals), screen, $ED_{50}$ or $TD_{50}$ values (mg/kg), **1c** (intraperitoneal injection to rats): MES, 7.46 (4.14–13.58); $TD_{50}$, 20.45 (11.57–33.41), **2a** (oral administration to mice): MES, 40.27 (31.04–53.08); scPTZ, 54.86 (39.50–87.10); $TD_{50}$, 87.67 (72.00–101.92), **2d** (oral administration to mice): MES, 57.74 (52.16–65.79); scPTZ, 68.67 (54.61–94.96); $TD_{50}$, 252.66 (95% CI is unavailable since slope for the compound is very flat); **4c** (oral administration to mice): MES, 77.85 (66.45–95.23); scPTZ, 147.22 (103.10–202.86); $TD_{50}$, 377.18 (261.92–558.25).

**(i) Evaluation of 2a,d and 3b,e in Benzodiazepine and γ-Aminobutyric Acid Receptor Binding Assays Using Synaptic Membranes.** In the benzodiazepine receptor binding assay,[26] concentrations of 3, 10, 30, and 100 μM of **2a** gave percentage increases ($p$ value) in the binding of [$^3$H]flunitrazepam to membranes isolated as a $P_2$ fraction from mouse whole-brain homogenates of 11.8 (<0.05), 13.8 (<0.05), 16.4 (<0.01) and 23.0 (<0.01). In the case of **2d**, a significant increase of 9.1% ($p$ < 0.01) in [$^3$H]flunitrazepam binding to the receptor at a concentration of 100 μM was noted. No significant effect ($p$ > 0.05) on the displacement of the labeled ligand to the receptors was obtained using concentrations up to and including 100 μM of **3b,e**.

Compounds **2a** and **3e** inhibited the binding of [$^3$H]γ-aminobutyric acid to GABA receptors in mouse whole brain $P_2$ pellets[27,28] by 20.7–28.7% ($p$ < 0.05) and 22.8–24.1% ($p$ < 0.05) using concentrations of 0.05–10 and 0.1–10 μM of **2a** and **3e** respectively. No significant effect ($p$ > 0.05) was observed with **2d** and **3b** using concentrations up to and including 10 μM.

**(ii) Effect of 2a,d and 3b,e on Adenosine Uptake into Synaptosomes.** Use of a literature procedure[29] revealed that **2a** inhibited the uptake of [$^3$H]adenosine into mouse whole-brain synaptosomes by 15.5% ($p$ < 0.05) at a concentration of 100 μM. Compound **3e** caused percentage enhancement of binding of 3.6 ($p$ < 0.05), 8.7 ($p$ < 0.01), 9.4 ($p$ < 0.01), and 7.7 ($p$ < 0.01) at concentrations of 0.3, 1, 10, and 30 μM, respectively, while there was no significant effect at 3 and 100 μM. No inhibition of adenosine uptake ($p$ > 0.05) was obtained using concentrations up to and including 100 μM with **2d** and **3b**.

**(iii) Evaluation of 2a,d and 3b,e in the Timed Intravenous Pentylenetetrazole Test.** The compounds in methylcellulose solution (0.5%) were administered by the intraperitoneal route to mice using 10 animals per dose (nine animals for the 135 mg/kg dose of **2d**). After 0.25 h, a solution of 0.5% pentylenetetrazole in heparinized 0.9% sodium chloride solution was infused into the tail veins of mice and the times of the first appearance of both the focal seizure and also clonic seizure were recorded. These times were compared to values obtained using 10 control animals (nine in the case of **2d**). The times of the first focal seizures (dose in milligrams per kilograms, time in seconds, $p$ value) were as follows: (**2a**) 50, 33.9, 0.10; 100, 38.4, 0.0004; (**2d**) 75, 37.4, 0.04; 135, 42.6, 0.002; (**3b**) 50, 38.1, 0.003; 100, 37.7, 0.03; (**3e**) 50, 33.1, 0.33; 100, 35.8, 0.02. The times of the clonic seizures (dose in milligrams per kilograms, time in seconds, $p$ value) were as follows: (**2a**) 50, 47.3, 0.008; 100, 53.5, 0.0002; (**2d**) 75, 62.4, 0.004; 135, 60.5, 0.001; (**3b**) 50, 46.8, 0.008; 100, 50.5, 0.002; (**3e**) 50, 40.5, 0.26; 100, 45.7, 0.005.

**(iv) Protection of 2a,d and 3e against Seizures Induced by *N*-Methyl-D-Aspartate.** Various doses of compounds **2a,d** and **3e** in 0.5% methyl cellulose were injected by the intraperitoneal route into mice. After 0.25 h (**2a** and **3e**) or 1 h (**2d**) an intracerebroventricular injection of *N*-methyl-D-aspartate (0.2 μg/5 μL) was administered and the number of animals protected was noted. Five to 10 animals per dose were utilized. The $ED_{50}$ values (95% CI, slope, SE) for the protection against clonic seizures were as follows: (**2a**) 64.86 mg/kg (39.37– 99.41, 3.20, 0.94); (**3e**) 208.6 mg/kg (125.8–332.5, 2.9, 0.93). The protection afforded by **2d** at doses of 5, 10, 20, 40, and 80 mg/kg was 1/5, 2/6, 3/10, 4/6, and 6/10 animals, and an $ED_{50}$ was unable to be calculated. The experiment was repeated using a concentration of 3.0 μg/5 μL of *N*-methyl-D-aspartate and protection against forelimb tonic extensions in mice by **2a,d** and **3e** expressed as $ED_{50}$ values (95% CI, slope, SE) was as follows: (**2a**) 28.62 mg/kg (17.92–44.68, 2.20, 0.51); (**2d**) 12.9 mg/kg (8.6–18.4, 4.65, 1.36); (**3e**) 59.67 mg/kg (30.5–172.08, 1.38, 0.42).

The $ED_{50}$ values (95% CI) of phenobarbital, phenytoin, and ethosuximide in protecting against clonic seizures in this test are 2.75 (0.17–4.90), 8.59 (6.64–14.10), and 408.09 (341.68–487.96) mg/kg, respectively.[11] The same drugs have $ED_{50}$ values in protecting against forelimb tonic extension of 3.09 (1.44–4.67), 0.60 (0.34–0.95), and 82.58 (30.94–131.87) mg/kg, respectively.[11]

**(v) Evaluation of 2a,c,d,h, 3a,b,e,f, and 4a,b for Protection against Seizures Induced by Bicuculline, Picrotoxin and Strychnine.** Various doses of the compounds were administered by the intraperitoneal route to mice. Bicuculline (2.7 mg/kg), picrotoxin (2.5 mg/kg), or strychnine (1.8 mg/kg) were administered subcutaneously and the protection afforded by the semicarbazones noted. Where $ED_{50}$ values were obtained, eight animals per dose were used and in the remaining cases two, seven, or eight mice per dose were employed. No protection was afforded by the compounds in the subcutaneous strychnine test using doses up to and including 80 (**4b**), 100 (**3a,f**), 150 (**4a**), 200 (**3b**), 300 (**2a,d**), 500 (**2c,h** and **3e**) although in the case of **4a**, one in eight mice was protected at a dose of 150 mg/kg. The $ED_{50}$ values for mephenytoin, valproic acid, and phenobarbital in the sc bicuculline test are 124.1, 360.0, and 37.7 mg/kg, respectively,[5] and in the sc picrotoxin screen the figures are 101.0, 387.2, and 27.5 mg/kg, respectively.[5]

The X-ray data for **2a,b,d** are available from J. W. Quail or J. R. Dimmock upon request. The information on these

compounds are the atomic anisotropic displacement parameters, hydrogen positional and isotropic displacement parameters, atomic positional and equivalent isotropic displacement parameters, and the bond distances and bond angles.

**Acknowledgment.** The authors thank Mr. J. P. Stables, Antiepileptic Drug Development Program, NIH for his extraordinary assistance in obtaining the biological data described in this work. In addition, thanks are extended to the Medical Research Council for funds which provided summer student employment to Mr. R. S. Thayer and to Mrs. S. D. Tumber who prepared compound **4c**.

**Supplementary Material Available:** Atomic anisotropic displacement parameters, hydrogen positional and isotropic displacement parameters, atomic positional and equivalent isotropic displacement parameters, and bond distances and angles for **2a,b,d** (10 pages). Ordering information is given on any current masthead page.

## References

(1) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard; E. A. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **1978**, *19*, 409–428.
(2) Jones, G. L.; Woodbury, D. M. Anticonvulsant structure-activity relationships: historical development and probable causes of failure. *Drug Dev. Res.* **1982**, *2*, 333–355.
(3) Kadaba, P. K. Triazolines. XIII: $\Delta^2$-1,2,3-Triazolines, a new class of anticonvulsants. *J. Pharm. Sci.* **1984**, *73*, 850–852.
(4) Dimmock, J. R.; Smith, D. C.; Brenner, J. M.; Jonnalagadda, S. S.; Sardessai, M. S.; Wood, J. D.; Bigam, G. E. Antiepileptic and antileukemic thiosemicarbazones and semicarbazones of 4-aryl-3-buten-2-ones. *Eur. J. Med. Chem.* **1986**, *21*, 187–192.
(5) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. Antiepileptic drug development program. *Cleveland Clin. Q.* **1984**, *51*,293–305.
(6) Dimmock, J. R.; Jonnalagadda, S. S.; Hussein, S.; Tewari, S.; Quail, J. W.; Reid, R. S.; Delbaere, L. T. J.; Prasad, L. Evaluation of some thiosemicarbazones of arylidene ketones and analogues for anticonvulsant activities. *Eur. J. Med. Chem.* **1990**, *25*, 581–588.
(7) Dimmock, J. R.; McColl, J. M.; Wonko, S. L.; Thayer, R. S.; Hancock, D. S. Evaluation of the thiosemicarbazones of some aryl alkyl ketones and related compounds for anticonvulsant activities. *Eur. J. Med. Chem.* **1991**, *26*, 529–534.
(8) Dimmock, J. R.; Brenner, J. M.; Phillips, O. A. Evaluation of some benzenesulphonylhydrazones of aryl aldehydes and ketones as antiepileptic agents. *Pharmazie* **1987**, *42*, 376–378.
(9) Dimmock, J. R.; Baker, G. B.; Taylor W. G. Aroylhydrazones-Part II. The ultraviolet spectroscopy and antimicrobial evaluation of some substituted aroylhydrazones. *Can. J. Pharm. Sci.* **1973**, *7*, 100–103.
(10) Bolton, S. *Pharmaceutical Statistics*; Dekker: New York, 1984; p 207.
(11) Kupferberg, H. J. Antiepileptic drug development program: a cooperative effort of government and industry. *Epilepsia* **1989**, *30*, S51-S56.
(12) Phillis, J. W.; O'Regan, M. H. The role of adenosine in the central actions of benzodiazepines. *Progr. Neuro-Psychopharmacol. Biol. Psychiatry* **1988**, *12*, 389–404.
(13) Hansch, C.; Leo, A. J. Substituent constants for correlation analysis in chemistry and biology; John Wiley and Sons Inc.: New York, 1979.
(14) Hall, S. R.; Stewart, J. M. The XTAL system of Crystallographic Programs. Universities of Western Australia, Australia, and Maryland, 1990.
(15) Gabe, E. J.; LePage, Y.; Charland, J.-P.; Lee, F. L.; White, P. S. An interactive program system for structure analysis. *J. Appl. Cryst.* **1989**, *22*, 384–387.
(16) *International tables for X-ray crystallography*; Kynoch Press: Birmingham, 1974; Vol. IV.
(17) Biograf 3.0. Molecular Simulations Inc., Pasadena, CA, 1991.
(18) Dewar, M. J. S.; Zoebisch, E. G.; Healey, E. F.; Steward, J. J. P. Development and use of quantum mechanical molecular models. 76. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
(19) Stewart, J. J. P. *Quantum Chem. Prog. Exchange Bull.* **1989**, *9*, QCEP no. 581.
(20) SAS Institute Inc., Version 6.06; Cary: North Carolina, 1991.
(21) Motoc, I; Marshall, G. R. Van der Waals fragmental volume constants. *Chem Phys. Lett.* **1985**, *116*, 415–419.
(22) Randic, M. On characterization of molecular branching. *J. Amer. Chem. Soc.* **1975**, *97*, 6609–6615.
(23) Kier, L. B.; Hall, L. H. Molecular connectivity in chemistry and drug research, *Medicinal Chemistry;* Academic Press: New York, 1976; Vol. 14, pp 33–60.
(24) Ghose, A. K.; Crippen, G. M. Atomic physicochemical parameters for three-dimensional structure-directed quantitative structure-activity relationships: partition coefficients as a measure of hydrophobicity. *J. Comput. Chem.* **1986**, *7*, 565–577.
(25) National Institute of Neurological Disorders and Stroke, National Institutes of Health. Private communication.
(26) Braestrup, C.; Squires, R. F. Specific benzodiazepine receptors in rat brain characterized by high affinity [$^3$H]diazepam binding. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3805–3809.
(27) Zukin, S. R.; Young, A. B.; Snyder, S. H. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4802–4807.
(28) Enna, S. J.; Snyder, S. H. Properties of gamma-aminobutyric acid (GABA) receptor bindings in rat brain synaptic membrane fractions. *Brain Res.* **1975**, *100*, 81–97.
(29) Phyllis, J. W.; Wu, P. H.; Bender, A. S. Inhibition of adenosine uptake into rat brain synaptosomes by the benzodiazepines. *Gen. Pharmacol.* **1981**, *12*, 67–70.