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Some aryl semicarbazones possessing anticonvulsant activities

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Summary — A number of aryl semicarbazones displayed anticonvulsant activity in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens when administered intraperitoneally to mice. When given by the oral route to rats, protection was afforded in the MES but not scPTZ tests. Correlations were noted between the σ and σ* values of the aryl substituents, the interplanar angles made by the aryl rings with the adjacent carbimino groups and the shapes of certain semicarbazones determined by X-ray crystallography, and the activities in the rat oral MES screen. Molecular modeling studies revealed a number of statistically significant descriptors which contributed to anticonvulsant activity.

aryl semicarbazone / anticonvulsant activity / X-ray crystallography / molecular modeling / structure–activity relationship

Introduction

A recent report described a series of aryl semicarbazones **1**, which displayed excellent oral activity in the maximal electroshock (MES) screen in rats [1]. These compounds have low neurotoxicity while affording little protection in the subcutaneous pentylenetetrazole (scPTZ) test. These observations support the theory that activity in the MES screen requires one large hydrophobic group (in this case the aryl ring) and at least two electron-donor atoms (found in the semicarbazono group) [2]. The compounds in series **1** are virtually insoluble in water but solutions of the semicarbazones in deuterated dimethylsulfoxide were shown by ^{1}H-NMR spectroscopy to be stable and isomerically pure up to and including the times of peak effects in the MES screen. Since X-ray crystallography of three representative compounds revealed that the semicarbazones had the *E* configuration, it is likely that the anticonvulsant activity is due to the molecules *per se* displaying the *E* configuration. These compounds generally have rapid onsets of action and their anticonvulsant properties are probably due, at least partially, to interactions with chloride channels. Molecular modeling has revealed certain molecular fragments and hydrophobicity affected bioactivity [1].

A previous study [1] revealed that the unsubstituted compound **2a** gave good oral activity in the MES screen in rats accompanied by neurotoxicity. The 4-chloro analog **2b** had similar potency to **2a** but its toxicity was lowered significantly (table I) resulting in a higher protection index (PI *viz* TD_{50}/ED_{50} where TD_{50} and ED_{50} refer to the doses of compounds causing neurotoxicity and protection in 50% of the rats). A subsequent molecular modification led to **2c** possessing similar potency as **2a** and **2b** with an excellent PI value [3]. Compound **2c** was designated the lead compound among the initial series of aryl semicarbazones and a detailed evaluation of its anticonvulsant properties has been undertaken [3].

The objectives of the present study were twofold. First, to develop structure–activity relationships in this novel series of anticonvulsants with particular reference to the prototypic molecule **2c**. Second, to use empirical and semiempirical conformational calculations with a view to determining which portions of the molecules are important in conferring anticonvulsant properties.

The reasons for proposing the various molecular modifications are as follows. Introduction of chloro or bromo substituents into the aryl ring of **1** (R^3 = H) produced compounds with promising activities in contrast to the use of the electron-donating 4-methoxy group [1]. Hence series **2** was extended using bromo (**2d**, **e**), fluoro (**2f–n**), iodo (**2o**) and nitro (**2p**) substituents which are electron-attracting in nature. In order to evaluate further whether the electronic

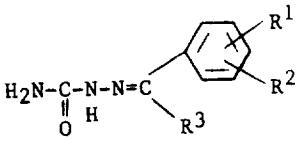
Table I. Evaluation of compounds **2–10** in the MES and neurotoxicity screens after oral dosing in rats.

Compound	*MES screen*					*Neurotoxicity screen*					*PI*[a]
	t(h)	*ED_{50} (mg/kg)*	*95% CI*	*Slope*	*SE*	*t(h)*	*TD_{50} (mg/kg)*	*95% CI*	*Slope*	*SE*	
2b[b]	1	18.57	14.67–25.53	6.16	1.95	0.25–24	> 500	–	–	–	> 26.93
2c[c]	2	22.64	10.28–38.88	2.42	0.88	2	973.93	745.97–1360.24	5.08	1.66	43.02
2d	0.5	50.77	43.60–57.93	11.29	3.53	0.25–24	> 500	–	–	–	> 9.85
2e	1	27.34	18.56–34.97	5.29	1.50	0.25–24	> 500	–	–	–	> 18.29
2f	0.5	19.21	12.71–25.02	4.69	1.37	0.25–24	> 500	–	–	–	> 26.03
2g	0.5	14.33	9.65–20.72	4.26	1.21	2	228.91	144.95–330.65	3.66	1.24	15.97
2h	2	8.77	5.26–13.02	3.42	1.01	2	99.26	65.22–145.13	3.93	1.11	11.32
2i	1	87.55	43.79–175.02	1.80	0.55	0.25–24	> 500	–	–	–	5.71
2a[b]	0.25	22.50	18.04–31.35	6.48	2.03	1	254.3	185.3–331.2	4.70	1.56	11.30
2j	0.5	19.26	10.29–37.34	2.33	0.79	0.25–24	> 500	–	–	–	> 25.96
2k	1	88.72	42.00–171.42	1.54	0.43	0.25–24	> 500	–	–	–	> 5.64
2l	1	31.09	20.24–54.16	2.64	0.77	0.25–24	> 500	–	–	–	> 16.08
2m	2	13.60	9.14–20.06	3.43	0.93	6	168.87	119.80–210.23	6.21	1.90	12.42
2n	1	12.88	8.29–19.03	3.70	1.05	6	674.69	557.00–804.67	8.07	2.63	52.38
2o	2	10.43	6.32–14.78	3.06	0.81	4	> 500	–	–	–	> 47.94
2p	4	29.93	20.33–40.76	4.69	1.28	0.25–24	> 500	–	–	–	> 16.71
2q	1	36.45	24.74–46.63	5.29	1.50	0.25–24	> 500	–	–	–	> 13.72
2r	0.5	28.02	19.61–35.87	5.29	1.59	2	253.43	163.87–337.87	4.25	1.30	9.04
2s	0.5	25.57	20.33–35.97	4.29	1.31	0.25–24	> 1000	–	–	–	> 39.11
2t	0.25	106.98	65.18–152.41	3.61	1.04	0.25–24	> 500	–	–	–	> 4.67
2u	0.25–6	> 90	–	–	–	0.25–6	> 120	–	–	–	–
2v	4	83.66	56.15–112.68	3.27	0.97	0.25–24	> 500	–	–	–	> 5.98
2w	1	> 80[d]	–	–	–	0.25–24	> 500	–	–	–	–
3a[b]	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	21.89	13.02–30.77	3.40	1.26	24	193.4	156.9–226.3	11.58	3.96	8.84
3c	0.5	30.36	18.60–41.67	3.82	1.23	24	200.64	165.61–218.43	17.18	6.50	6.61
3d	0.25	28.61	18.12–41.33	3.66	1.24	0.25–24	> 500	–	–	–	> 17.48
3e	1	69.82	46.18–98.79	3.96	1.12	0.25–24	> 500	–	–	–	> 7.16
3f	2	14.04	6.90–22.73	2.19	0.63	0.25–24	> 500	–	–	–	> 35.61
3g	2	34.83	15.70–46.79	3.85	1.51	0.25–24	> 500	–	–	–	> 14.36
3h	2	64.06	28.05–131.44	1.35	0.44	0.25–24	> 500	–	–	–	> 7.81
3i	0.25	11.76	9.23–14.00	7.47	2.18	2	102.96	67.51–141.97	3.52	0.91	8.76
3j	2	44.76	24.83–81.50	2.56	0.89	0.25–24	> 500	–	–	–	> 11.17
3k	0.25–4	< 300[e]	–	–	–	0.25–4	> 300	–	–	–	–
3l	0.5	166.15	116.06–223.42	4.61	1.57	0.25–24	> 500	–	–	–	> 3.01

Table I. *Continued.*

Compound	*MES screen*					*Neurotoxicity screen*					*PI*[a]
	t(h)	*ED_{50} (mg/kg)*	*95% CI*	*Slope*	*SE*	*t(h)*	*TD_{50} (mg/kg)*	*95% CI*	*Slope*	*SE*	
4a[b]	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
4b	0.5	30.46	21.23–39.73	4.14	1.14	2	188.44	123.78–287.23	3.50	1.01	6.19
5a	0.5	277.10	109.76–93887.36	1.51	0.72	0.25–24	> 500	–	–	–	> 1.80
5b	1	327.91	167.15–2886.28	1.62	0.65	8	192.62	165.79–226.63	8.04	2.41	0.59
5c	4	53.78	37.71–90.09	3.66	1.25	0.25–24	> 500	–	–	–	> 9.30
5d	4	14.44	9.58–19.08	5.03	1.79	0.25–24	> 500	–	–	–	> 34.63
6	4	611.06	384.29–958.95	3.40	1.22	0.25–24	> 1000	–	–	–	> 1.64
7	0.25–4	> 500	–	–	–	0.25–6	> 500	–	–	–	–
8	4	> 500	–	–	–	0.25–6	> 750	–	–	–	–
9	2	> 500	–	–	–	0.25–24	> 500	–	–	–	–
10	0.25, 1, 4	> 500	–	–	–	0.25, 1, 4	> 500	–	–	–	–
Phenytoin	2	23.2	21.4–25.4	15.1	4.28	0.25–24	> 500	–	–	–	> 22
Carbamazepine	1	3.57	2.41–4.72	3.84	1.15	1	361	319–402	11.4	2.96	101
Valproate	0.5	395	332–441	8.13	2.76	0.5	859	719–1148	6.57	2.17	2.2

[a]PI, protection index, *ie* TD_{50}/ED_{50}. [b]Data taken from reference [1] and reproduced by permission of the copyright owner. [c]Data taken from reference [3] and reproduced by permission of the copyright owner. [d]Using doses of 20, 40, 80 and 160 mg/kg, 0/8, 2/8, 3/8 and 0/16 animals were protected. Administration of 50 mg/kg of **2w** to rats by the intraperitoneal route afforded no protection in four rats after 0.25, 0.5, 1, 2 and 4 h. [e]Compound **3k** when evaluated at 300 mg/kg afforded protection in 2/2, 1/2, 1/2, 0/2 and 1/2 rats at the end of 0.25, 0.5, 1, 2 and 4 h respectively. No protection was noted at these times using a dose of 50 mg/kg.



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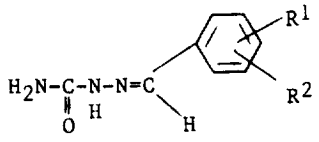
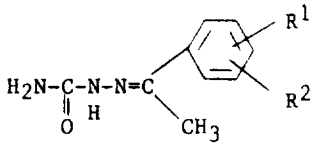


2

a: $R^1=R^2=H$
b: $R^1=4\text{-Cl}$; $R^2=H$
c: $R^1=4\text{-Br}$; $R^2=H$
d: $R^1=2\text{-Br}$; $R^2=H$
e: $R^1=3\text{-Br}$; $R^2=H$
f: $R^1=2\text{-F}$; $R^2=H$
g: $R^1=3\text{-F}$; $R^2=H$
h: $R^1=4\text{-F}$; $R^2=H$
i: $R^1=2\text{-F}$; $R^2=3\text{-F}$
j: $R^1=2\text{-F}$; $R^2=4\text{-F}$
k: $R^1=2\text{-F}$; $R^2=5\text{-F}$
l: $R^1=2\text{-F}$; $R^2=6\text{-F}$

m: $R^1=3\text{-F}$; $R^2=4\text{-F}$
n: $R^1=3\text{-F}$; $R^2=5\text{-F}$
o: $R^1=4\text{-I}$; $R^2=H$
p: $R^1=4\text{-NO}_2$; $R^2=H$
q: $R^1=2\text{-OCH}_3$; $R^2=H$
r: $R^1=2\text{-CH}_3$; $R^2=H$
s: $R^1=3\text{-CH}_3$; $R^2=H$
t: $R^1=4\text{-CH}_3$; $R^2=H$
u: $R^1=2\text{-CH}_3$; $R^2=4\text{-CH}_3$
v: $R^1=2\text{-CH}_3$; $R^2=5\text{-CH}_3$
w: $R^1=4\text{-C}_6\text{H}_5$; $R^2=H$



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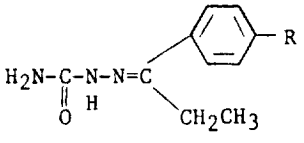

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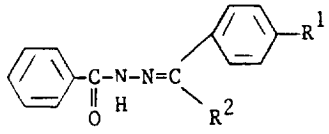


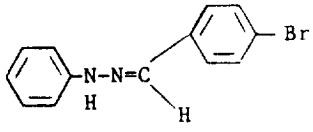
5

a: $R^1=R^2=H$
b: $R^1=4\text{-Cl}$; $R^2=H$
c: $R^1=4\text{-Br}$; $R^2=H$
d: $R^1=2\text{-Br}$; $R^2=H$
e: $R^1=3\text{-Br}$; $R^2=H$
f: $R^1=2\text{-Cl}$; $R^2=H$

g: $R^1=2\text{-Cl}$; $R^2=4\text{-Cl}$
h: $R^1=3\text{-Cl}$; $R^2=4\text{-Cl}$
i: $R^1=4\text{-F}$; $R^2=H$
j: $R^1=4\text{-NO}_2$; $R^2=H$
k: $R^1=4\text{-OCH}_3$; $R^2=H$
l: $R^1=4\text{-CH}_3$; $R^2=H$

a: R = H
b: R = Br

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



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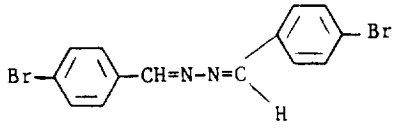

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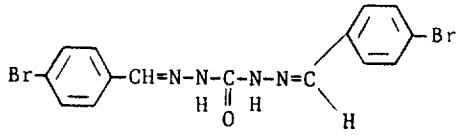

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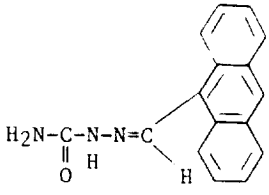

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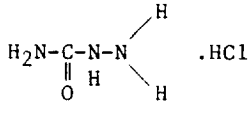

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Structures **1–10**.

Table II. Interplanar angles of some aryl semicarbazones determined by electronic absorption spectroscopy.

Substitution in aryl ring	*Compound*	λ_{max} *(nm)*	*SD of* λ_{max}	$\varepsilon \times 10^4$	*SD of* ε	θ	*SD of* θ
H	**2a**	281.6	0.2	2.32	82	0	–
2-Cl	**1**, R^1 = 2-Cl, R^2 = R^3 = H	284.1	0.2	2.19	76	23.2	0.4
3-Cl	**1**, R^1 = 3-Cl, R^2 = R^3 = H	285.1	0.1	2.27	79	20.3	0.3
4-Cl	**2b**	286.1	0.2	2.55	29	0	–
2-Br	**2d**	284.8	0.1	2.09	110	33.3	0.8
3-Br	**2e**	285.1	0.2	2.18	67	30.9	0.7
4-Br	**2c**	287.3	0.1	2.79	225	0	–
2-F	**2f**	283.6	0.1	2.03	66	23.2	0.4
3-F	**2g**	283.4	0.1	2.25	61	14.2	0.2
4-F	**2h**	277.7	0.2	2.39	53	0	–
2,4-F_2	**2j**	278.1	0	1.81	76	32.0	0.8
2,5-F_2	**2k**	285.0	0.2	1.70	46	34.9	0.9
2-CH_3	**2r**	281.2	0.2	2.04	70	25.5	0.5
3-CH_3	**2s**	283.7	0.2	2.37	336	12.0	0.3
4-CH_3	**2t**	284.2	0.2	2.47	103	0	–
2,4-$(CH_3)_2$	**2u**	283.5	0.2	2.28	62	22.5	0.5
2,5-$(CH_3)_2$	**2v**	282.5	0.2	2.04	83	30.0	0.8
4-I	**2o**	290.6	0.1	2.99	179	0	–

properties of the aryl substituents influence anticonvulsant activity, other groups were planned to be inserted into the aryl rings, namely the electron-donating 2-methoxy substituent (**2q**), methyl group (**2r–t**) or groups (**2u, v**) as well as the 4-phenyl function (**2w**), which is virtually neither an electron donor nor acceptor possessing a Hammett sigma value of –0.01 [4]. In addition, our previous work has revealed that compounds having chloro substituents in the *meta* and/or *para* positions of the aryl ring had greater potencies than the aryl semicarbazones containing one or two *ortho* chloro substituents [1]. This result could be due to the marked interplanar angle (θ) between the aryl ring and the adjacent carbimino (C=N) group in compounds containing *ortho* substituents [5, 6]. Hence measurement of the θ values of a number of representative compounds in solution was suggested in order to seek a correlation between the interplanar angles with potencies in the rat oral MES screen.

Compounds **2a** and **3a** have similar activity (table I) and hence comparison of the anticonvulsant activities in series **3** with the *nor* analogs in series **2** was suggested. This comparison may shed light on the importance of the size of the R^3 group of structures **1** in conferring anticonvulsant properties. For this reason, series **4** was also planned in which ethyl groups are attached to the carbimino function.

Our previous work has indicated that the atomic charge on the terminal nitrogen atom was a significant descriptor at the 85% confidence level [1]. Hence replacement of the terminal amino group of the semicarbazones by an aryl ring producing series **5** may indicate whether the amino group is essential for bioactivity and also whether the change in size of the terminal function is an important factor in eliciting anticonvulsant activity. As a continuation of this approach, replacement of the aminocarbonyl ($CONH_2$) group of **2c** to produce **6** was suggested. If the 4-bromophenylcarbimino group contributes significantly to bioactivity, then the presence of two such

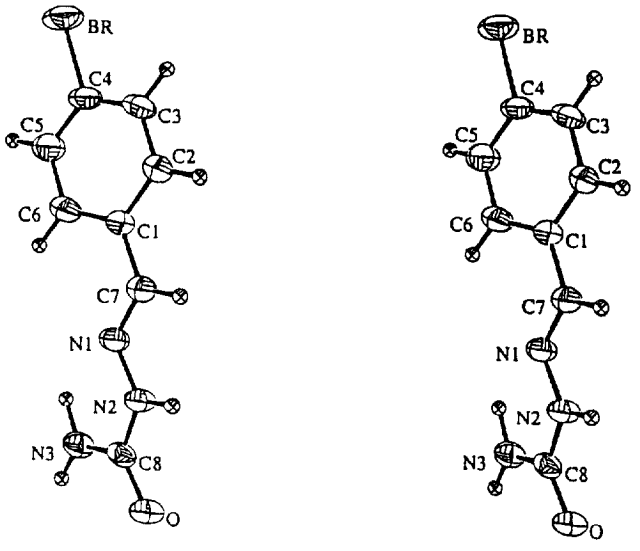

Fig 1. Ortep diagram of **2c**.

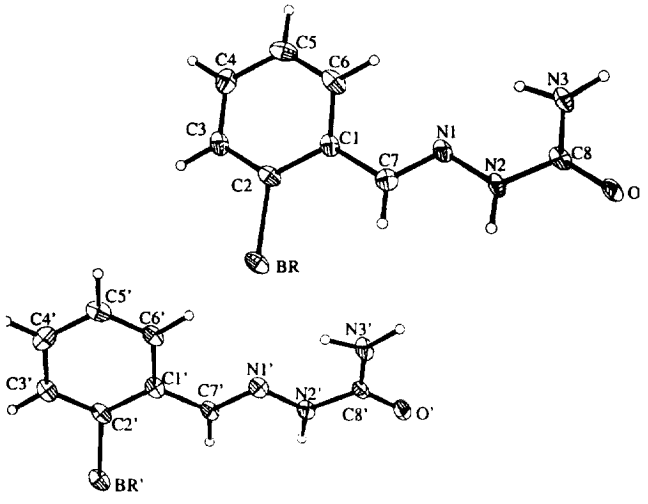

Fig 2. Ortep diagram of **2d**.

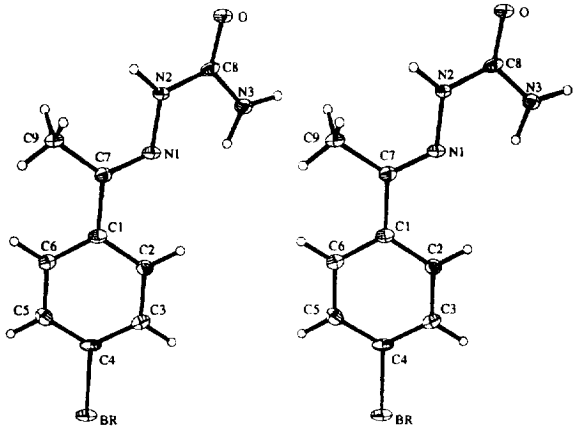

Fig 3. Ortep diagram of **3c**.

moieties joined together (**7**) or *via* a spacer group (**8**) may lead to compounds with marked anticonvulsant properties. The anticonvulsant activity in series **2** is probably influenced by the presence of an aryl ring which could form van der Waals bonds at a receptor; hence by increasing the flat area of the molecule improved activity may result. Such considerations led to the idea of preparing **9**. In order to note whether the electron donor atoms in these aryl semicarbazones contribute to anticonvulsant activity, semicarbazide hydrochloride **10** was included in the screening program.

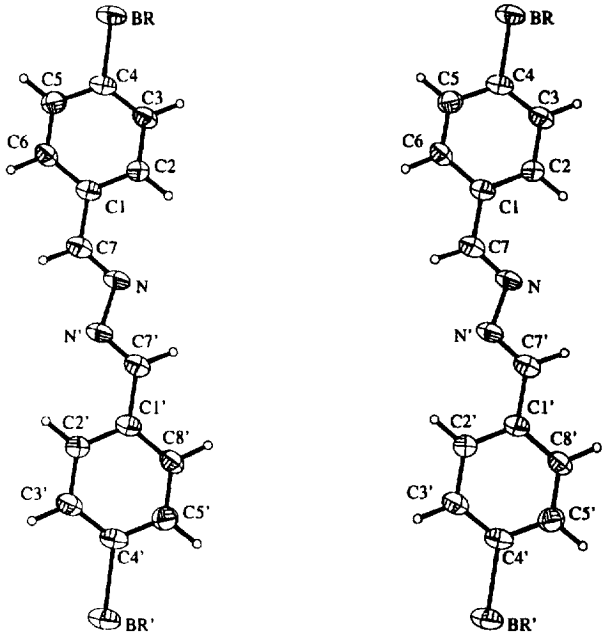

Fig 4. Ortep diagram of **7**.

Chemistry

Compounds **2–4** and **9** were prepared by reaction of the appropriate aryl aldehyde or aryl alkyl ketone with semicarbazide. Series **5** was synthesized from benzoylhydrazide and the corresponding aldehyde or ketone. Reaction of 4-bromobenzaldehyde with phenylhydrazine, hydrazine and carbohydrazide produced compounds **6–8** respectively.

The interplanar angles (θ) between the aryl rings and adjacent azomethine linkages of a number of compounds as determined by electronic absorption spectroscopy are listed in table II. The Ortep diagrams of the structures of **2a**, **3a**, **3c** and **7** determined by X-ray crystallography are presented in figures 1–4. The computational strategies employed in molecular modeling are described in the *Experimental protocols*. All the compounds in the series **2–5** and **9** for which ED_{50} values have been generated were used in the modeling. Each analog was described by 37 descriptors (listed in table III) including some new parameters (with an asterisk in table III) which combine steric, electronic and lipophilic information adding a third dimension to the QSAR analysis. A subset of descriptors which correlated with oral anticonvulsant activity (ED_{50}) in the MES test (table I) was selected from the full descriptor array using stepwise and backward-selective regression procedures at the 88% confidence level. At this level, eight descriptors were significant and they are listed in table IV ($r^2 = 0.89$).

Table III. Descriptors used in the molecular modeling studies of **2a–w**, **3a–l**, **4a**, **4b**, **5a–d** and **9**.

Geometric descriptors

1 Bond angle (°): C7=N1-N2
2 Dihedral angle (°): C7-N1-N2-C8
3 Sterimol parameter L
4 Sterimol parameter B_1
5 Sterimol parameter B_2
6 Sterimol parameter B_3
7 Sterimol parameter B_4
8 Total area (Å^2)
9 Total volume (Å^3)

Electronic descriptors

10 Atomic charge on atom C2
11 Atomic charge on atom C6
12 Atomic charge on atom C8
13 Atomic charge on atom N3
14 Molecular dipole *x*-component: μ_x
15 Molecular dipole *y*-component: μ_y
16 Molecular dipole *z*-component: μ_z
17 Molecular dipole moment
18 Energy of the HOMO (au)
19 Maximum MEP on the VDWS (au)
20 Minimum MEP on the VDWS (au)
21 Maximum MEP/minimum MEP on VDWS (au)

Graph theory descriptors

22 Randic index 1 ($x-1$)
23 Randic index 2 ($x-2$)
24 Randic index 3 ($x-3$)
25 Randic index 4 ($x-4$)
26 Kier–Hall index 1 ($xv-1$)
27 Kier–Hall index 2 ($xv-2$)
28 Kier–Hall index 3 ($xv-3$)
29 Kier–Hall index 4 ($xv-4$)
30 Zagreb index 1 (M1)
31 Zagreb index 2 (M2)
32 Platt index (F)

Physicochemical descriptors

33 Log *P* (Ghose parameters [10])
34 Log *P* (Kantola *et al* method [24])
35 Maximum MLP on the VDWS (au)
36 Minimum MLP on the VDWS (au)
37 Maximum MLP/minimum MLP on VDWS (au)

Pharmacology

Compounds **2–10** were administered to mice by the intraperitoneal route using doses of 30, 100 and 300 mg/kg and examined in the MES, scPTZ and neurotoxicity screens. Activity in the MES and scPTZ screens was displayed by 70 and 54% of the compounds respectively while neurotoxicity was found in 50% of the derivatives (details are given in the *Experimental protocols*). Thirteen representative compounds were evaluated quantitatively after intraperitoneal injection to mice and these results are summarized in table V. All of the semicarbazones **2–9** plus semicarbazide hydrochloride **10** were examined for activity in the MES screen and neurotoxicity after oral administration to rats; these data are presented in table I. Compounds **2–9** were virtually inactive in the scPTZ screen.

Table IV. Significant descriptors at the 88% confidence level.

Bond angle C7=N1-N2
Sterimol parameter L (O(C)...X(Ar))
Energy of the HOMO
Atomic charge on atom C2
z-component to the dipole moment (μ_z)
Randic index 3
Log *P* (calculated with Ghose parameters [10])
Maximum MLP/minimum MLP on the VDWS

Discussion

The initial anticonvulsant screening of all compounds was achieved by administering doses of 30, 100 and 300 mg/kg to mice by the intraperitoneal route. Slightly more compounds afforded protection in the MES screen than in the scPTZ test system. Quantitative data of representative compounds was obtained in order to develop structure–activity relationships, determined independently of whether there is preferential activity in the MES screen as suggested by the qualitative data, and finally to compare their potencies with those of clinically used drugs.

The data in table V revealed the following structure–activity relationships. First, the representative compounds in series **2** had the following order of potency in both the MES and scPTZ screens namely **2h > 2a–c > 2p**, **2s**. The six semicarbazones in series **3** had a potency order of **3i > 3a**, **3b > 3c**, **3d**, **3f** in the MES screen and **3i** is more potent than **3a–c** and equipotent

Table V. Evaluation of selected compounds in the MES, scPTZ and neurotoxicity screens after intraperitoneal injection in mice.

Compound	*MES screen*			*scPTZ screen*			*Neurotoxicity screen*			*PI*[a]	
	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)*	*MES screen*	*scPTZ screen*
2a[b]	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
2b[b]	1	74.84 (64.53–85.09)	8.90 (2.50)	0.25	135.1 (71.83–220.5)	2.26 (0.65)	2	252.2 (143.7–357.9)	3.46 (1.18)	3.37	1.87
2c[c]	1	71.99 (63.17–81.05)	13.50 (3.99)	1	106.61 (82.12–137.29)	4.87 (1.30)	1	427.26 (341.54–506.51)	6.58 (1.71)	5.93	4.01
2h	0.25	19.11 (15.82–23.58)	9.23 (2.89)	0.25	26.65 (14.23–36.42)	3.17 (1.11)	0.25	51.54 (40.26–72.34)	5.64 (1.70)	2.70	1.93
2p	2	103.37 (94.76–115.03)	14.51 (3.91)	2	352.60 (265.33–606.14)	3.51 (1.32)	0.25–24	> 500	–	> 4.84	> 1.42
2s	0.25	142.46 (110.82–190.02)	5.57 (1.76)	0.25	297.12 (193.52–434.57)	3.78 (1.34)	0.25	> 500	–	> 3.51	> 1.68
3a[b]	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
3b	0.25	41.41 (36.75–44.84)	19.9 (6.79)	0.25	76.88 (63.62–89.42)	11.59 (4.64)	0.50	77.32 (55.86–95.62)	5.98 (1.67)	1.87	1.01
3c	0.50	78.05 (62.04–86.07)	13.81 (5.01)	0.50	107.11 (71.57–148.56)	3.53 (1.00)	0.25	150.81 (111.50–210.64)	3.99 (1.28)	1.93	1.41
3d	0.50	95.98 (78.83–118.80)	8.05 (2.42)	0.50	69.31 (54.01–84.52)	5.61 (1.61)	0.25	221.71 (153.31–318.28)	3.99 (1.12)	2.31	3.20
3f	0.25	79.51 (68.44–90.69)	11.39 (3.57)	0.25	83.28 (46.08–157.08)	2.05 (0.59)	1	254.66 (165.56–338.90)	4.29 (1.31)	3.20	3.06
3i	0.25	24.96 (18.03–31.11)	6.44 (2.10)	0.25	44.19 (37.49–54.50)	7.45 (2.20)	0.5	101.21 (61.99–138.89)	3.82 (1.23)	4.05	2.29
5d	1	468.05 (350.11–692.19)	4.03 (1.22)	1	> 500	–	4	> 500	–	1.07	–
Phenytoin	2	6.48 (5.65–7.24)	12.4 (3.60)	2	> 50	–	0.5	42.8 (36.4–47.5)	10.2 (3.13)	6.60	–
Carbamazepine	0.25	9.85 (8.77–10.7)	20.8 (7.15)	0.25	> 50	–	0.25	47.8 (39.2–59.2)	7.98 (2.37)	4.85	–
Valproate	0.25	287 (237–359)	7.31 (2.48)	0.25	209 (176–249)	8.51 (2.69)	0.25	483 (412–571)	12.3 (4.01)	1.68	2.31

[a]PI, protection index, *ie* TD_{50}/ED_{50}. [b]Data taken from reference [1] and reproduced by permission of the copyright owner. [c]Data taken from reference [3] and reproduced by permission of the copyright owner.

with **3d, 3f** in the scPTZ test. Hence the compounds with the 4-fluoro substituent were the most active in both series of semicarbazones and in both screens. This observation may be of value in subsequent drug design. Second, the aryl substituents of **2a–c, 2h** and **3a–c, 3i** are similar. A comparison of the average ED_{50} figures of both groups of compounds revealed that greater overall potency was found in both screens by **3a–c, 3i**. In other words, in general the presence of a methyl group rather than a hydrogen atom adjacent to a carbimino group increased potency. Third, replacement of the terminal amino group of **3a** by a phenyl ring (**5d**) reduced activity more than 13- and sixfold in the MES and scPTZ screens, respectively. This primary amino group may therefore contribute significantly to anticonvulsant activity. In regard to the question of possible preferential activity in the MES screen, all of the compounds were active in both test

systems except for **5d** which displayed activity only in the MES screen. In the case of six of the compounds, namely **2c**, **2p**, **2s**, **3a**, **3b** and **3i**, greater activity was found in the MES screen whereas for the remaining semicarbazones, the confidence intervals on the data reveal equal protection in both screens. A comparison of the potency figures in table V with the established drugs phenytoin, carbamazepine and valproate revealed the following information. The compounds were similar to valproate insofar as most of them were active in both the MES and scPTZ screens. In the MES test, compound **5d** was equiactive with valproate while the remaining compounds were more potent than this useful drug but they were less potent than phenytoin and carbamazepine. The semicarbazones **2c**, **2h**, **3a–d**, **3f** and **3i** were more active than valproate in the scPTZ screen while, with the exception of **5d**, the remaining compounds were as potent as this drug.

In view of these encouraging results, the decision was made to administer all of the compounds **2–10** to rats by the oral route and to evaluate their activity in the MES, scPTZ and neurotoxicity screens. Since under clinical conditions, the oral route of administration of anticonvulsants is preferable, significant activity would be an important observation. The data in table I indicate the figures generated in the MES and neurotoxicity screens. In the scPTZ test, the compounds were either inactive or displayed marginal potencies when given up to and including the maximum doses. Hence a quite remarkable MES-selectivity was demonstrated by these compounds when given orally to rats.

In an attempt to find structure–activity relationships, the following analyses of the results were conducted. First, in order to examine whether the electronic, hydrophobic and steric properties of the aryl substituents of the compounds in series **2** and **3** influenced activity significantly in the oral MES screen, the following statistical evaluations were undertaken using the test for zero correlation [7]. Both linear and log–log plots were undertaken. In the case of series **2**, linear plots were made between the MES ED_{50} figure listed in table I and first the Hammett σ and/or Taft σ^* values, second the Hansch π figures, and third the molar refractivity (MR) constants of the aryl substituents in **2a–t**, **2v**, **1**, R^1 = 2-Cl, $R^2 = R^3$ = H and **1**, R^1 = 3-Cl, $R^2 = R^3$ = H. No significant correlation was noted ($p > 0.1$). Log–log plots between the MES ED_{50} figures and certain physicochemical constants of the nuclear substituents were undertaken, namely the σ and σ^* constants in **2b–p**, **1**, R^1 = 2-Cl, $R^2 = R^3$ = H, **1**, R^1 = 3-Cl, $R^2 = R^3$ = H ($p < 0.05$), the π values in **2a–t**, **2v**, **1**, R^1 = 2-Cl, $R^2 = R^3$ = H, **1**, R^1 = 3-Cl, $R^2 = R^3$ = H ($p > 0.1$) and the MR constants of **2a–t**, **2v**, **1**, R^1 = 2-Cl, $R^2 = R^3$ = H, **1**, R^1 = 3-Cl, $R^2 = R^3$ = H ($p > 0.1$). In series **3**, linear plots were made between the ED_{50} values of **3a–j**, **3l** and the σ and/or σ^* constants, π values and MR figures of the aryl substituents. No correlations were observed ($p > 0.1$). Log–log plots between the MES ED_{50} figures and the σ and/or σ^* constants in **3b–j** ($p < 0.05$), π values in **3b–i**, **3l** ($p > 0.1$) and MR constants in **3a–j**, **3l** ($p > 0.1$) were carried out. Thus in both series **2** and **3**, potency increases significantly as the σ and σ^* figures diminish and the correlation is of significance in the subsequent development of this series of compounds.

Second, in order to observe whether the torsional angles made by the aryl rings with the adjacent carbimino group influence bioactivity, the electronic absorption spectroscopy of some representative compounds was undertaken and these results are presented in table II. The interplanar angle θ was determined using equation [1] developed by Braude and coworkers [8, 9] in which ε and ε_0 refer to the molar absorptivities of the substituted and unsubstituted compounds, respectively.

$$\cos^2\theta = \frac{\varepsilon}{\varepsilon_0} \qquad [1]$$

The data in table II reveal that substitution in the *ortho* position of the aryl rings produces the highest θ values. Compounds with *meta* substituents also showed a lack of coplanarity of the aryl ring with the adjacent carbimino group, which may be due to a number of factors including a buttressing effect, *ie* the *meta* substituents force the adjacent hydrogen atom in the *ortho* position towards the carbimino group which impedes the coplanarity of the aryl ring with the adjacent unsaturated linkage. Linear and log–log plots were made using the test for zero correlation [7]. However in the case of log–log plots, compounds having θ values of zero were removed from the statistical analysis. Linear and log–log plots of the θ figures of compounds possessing *ortho* substituents (**1**, R^1 = 2-Cl, $R^2 = R^3$ = H, **2d**, **2f** and **2r**) as well as the unsubstituted compound (**2a**) against the MR values of the *ortho* substituents did not reveal any correlation ($p > 0.1$). A linear plot of the θ values of compounds with *meta* substituents (**1**, R^1 = 3-Cl, $R^2 = R^3$ = H, **2e**, **2g** and **2s**) as well as the unsubstituted compound (**2a**) revealed a correlation ($p < 0.1$), *ie* the θ value increased as the MR figure was elevated. However a log–log plot did not reveal any correlation ($p > 0.1$). With the exception of **2u**, for which an ED_{50} figure is unavailable, linear and log–log plots of the θ values of the compounds listed in table II against the MES ED_{50} data were undertaken; the figures for **1** (R^1 = 2-Cl, $R^2 = R^3$ = H) and **1** (R^1 = 3-Cl, $R^2 = R^3$ = H) are 38.78 and 24.38 mg/kg respectively [1]. While no correlation was noted in the linear plot made ($p > 0.1$), the

log θ figures were significantly correlated with the log ED_{50} values ($p < 0.1$), *ie* potency increased as the angle θ diminished.

Third, in order to evaluate the effect of replacing the hydrogen atom attached to the carbimino function (series **2**) by a methyl group (series **3**), a comparison of the MES ED_{50} figures of eight compounds possessing the same aryl substituents in both series was made. In six cases, the ED_{50} figures were the same while the values for **2d**, **3d**, **2e** and **3e** were different. The average ED_{50} value for the eight compounds in series **2** was 35.94 mg/kg while it was 49.20 mg/kg for the analogs in series **3**. A comparison of the potencies of **2a**, **3a** and **4a** revealed that **4a** was as active as **2a** but less potent than **3a**. The compounds **2c**, **3c** and **4b** were equiactive. One may tentatively conclude that a hydrogen atom attached to a carbimino group is marginally preferable to a methyl but not an ethyl group.

Fourth, replacement of the terminal amino group of **2a–c** and **3a** by an aryl ring led to the formation of **5a–d**. Thus the capacity of this amino group to form hydrogen bonds at a receptor was removed while an additional aryl ring in **5a–d** may permit increased van der Waals bonding to occur. While **2a** and **2b** were approximately 12 and 18 times more active than **5a** and **5b**, using the 95% confidence intervals, **5c** and **5d** were as active as the analogous semicarbazones **2c** and **3a**. In fact the potency of **5d** is noteworthy. Hence no conclusion can be drawn as to how replacement of an amino group by an aryl ring will affect potency.

Fifth, compound **2c** was selected as a lead molecule due to its excellent potency and high protection index [3]. In order to attempt to discern which portions of the molecule were important in conferring anticonvulsant activity, the following structural modifications were made. Replacement of the aminocarbonyl group of **2c** by an aryl ring gave **6** whereby potency was reduced 27-fold. Since the 4-bromophenylcarbimino group may make a significant contribution to anticonvulsant activity, the presence of two such functions joined together (**7**) or by a spacer group (**8**) were considered. At the maximum doses employed, neither **7** nor **8** displayed activity. The capacity for greater van der Waals bonding forces at a receptor in the case of **9** in contrast to **2a** did not lead to any increase in activity; in fact the compound was inactive at 500 mg/kg. While **10** has four electron-donor atoms, the absence of a hydrophobic group was probably responsible for its inactivity at the doses employed.

Finally, the potency of these compounds in the rat oral MES screen were compared with the three reference drugs phenytoin, carbamazepine and valproate. Compounds **2g**, **2h**, **2m–o**, **3i** and **5d** were more active than phenytoin and the following compounds were as active as this widely used drug: **2a–c**, **2e**, **2f**, **2j**, **2l**, **2p–s**, **3a–d**, **3f**, **3g**, **3j** and **4b**. The compounds described in this report were less active than carbamazepine. On the other hand, with the exception of **5a**, **5b** and **6–10**, all of the compounds for which ED_{50} figures were computed were more active than valproate. Hence aryl semicarbazones are a group of novel anticonvulsants.

X-ray crystallography was undertaken on four representative compounds for two reasons. First, to ascertain whether the shapes of molecules as revealed by this analytical technique could explain the differences in anticonvulsant potencies. Second, to compare certain bond distances and angles obtained by X-ray crystallography with those computed by molecular modeling techniques *vide infra*. The four compounds chosen were **2c**, **2d**, **3c** and **7**, which all contained the bromophenylcarbimino function; Ortep diagrams of these four molecules are presented in figures 1–4. The numbering sequence used in the case of the semicarbazones is indicated in figure 5.

Figure 2 reveals that compound **2d** exists in two crystallographically independent molecules which are designated without a prime (molecule A) and with a prime (molecule B). The *E* configuration of the carbimino group was noted in all of the compounds **2c**, **2d** (molecules A and B), **3c** and **7**. In the case of compounds **2c**, **2d** and **3c**, the semicarbazono group comprising the N1-N2-C8-O-N3 atoms was planar in these molecules. Hence the variation in the anticonvulsant activity in the MES screen after oral administration to rats is not due to differences in planarity of the semicarbazono group. On the other hand, these molecules were nonplanar due to the lack of coplanarity of the aryl ring with the adjacent carbimino (C=N) function, *ie* θ values greater than zero and the twisting of the molecule. Thus the θ values for **2c**, **2d** (molecule A), **2d** (molecule B) and **3c** were 8.9 (5)°, 5.6 (11)°, 4.2 (11)° and 15.5 (2)° respectively. The angles between the planes of the aryl rings (C1-C6) and the semicarbazono group for these molecules were 18.2 (2)°, 11.8 (3)°, 11.6 (3)° and 23.4 (2)° respectively. Compounds **2c** and **3c** are equiactive at the 95% confidence interval level and both are more potent than **2d**. Hence greater nonplanarity is

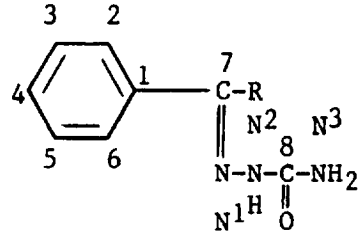

Fig 5. Numbering scheme of the semicarbazones used in X-ray crystallographic and molecular modeling studies.

displayed in the two most active compounds. Of interest is the observation that the smaller θ value of **2d** than **2c** noted by X-ray crystallography is in contrast to the data provided by ultraviolet-visible spectroscopy (table II). This discrepancy may be due to the fact that whereas the UV data were generated in solution, the X-ray determinations were made in the crystal state in which case such features as the packing of molecules in the unit cell could affect the orientation of different groups in the molecules. The planes of both aryl rings in the azine **7** are parallel to each other, but displaced, and although the group comprising the C7-N-N-C7 atoms is planar, it is at an angle of 5.8 (2)° to the two aryl rings. While the lack of anticonvulsant activity of **7** may be due to the absence of the semicarbazono group, the virtual planarity of the molecule may contribute to its inactivity.

Intermolecular hydrogen bonding was noted in the semicarbazones **2c**, **2d** and **3c** but not in the azine **7**. In the case of **2c**, the hydrogen bonds (symmetry position of the oxygen atoms in parentheses) were N2-HN2...0 ($1 - x$, $-1 - y$, $-z$) and N3-HN3B...0 ($1/2 - x$, $1/2 + y$, $-z$). Hydrogen bonding was noted in **2d** (molecule A) between the bonds N2-HN2...0 ($-x$, $-y$, $-z$) and N3-HN3B...0 ($2 - x$, $-y$, $-z$). For molecule B, bonds N2-HN2...0 ($3 - x$, $1 - y$, $-z$) and N3-HN3B...0 ($1 - x$, $-y$, $-z$) were observed. Finally in compound **3c**, a bond N2-HN2...0 ($1 - x$, $-1 - y$, $-z$) was detected. Thus the three compounds, **2c**, **2d** and **3c** possessing activity in the MES screen in the dose range 23–51 mg/kg each displayed intramolecular hydrogen bonding in contrast to the inactive azine **7** where the potential for such a phenomenon is absent.

In our previous paper we found that this family of semicarbazone anticonvulsants may be considered bifunctional [1], possessing a lipophilic moiety (substituted aromatic ring) and a hydrogen-bonding moiety (the semicarbazono terminal group, N1-N2-C8(O)-N3). This has been observed again in the present work. Statistical studies carried out over all of the compounds considered provided poor results due to the lack of structural communality within the entire family. However, when statistics were performed on those compounds with, at one end, a unique aromatic moiety and, at the other end, the semicarbazono group with an unsubstituted primary amino group (compounds **2a–w**, **3a–l**, **4a**, **b** and **9**), significant results were obtained. The eight statistically significant descriptors (table IV) suggest that the distance between the hydrogen bonding and lipophilic moieties is important for activity, as reflected by the Sterimol L parameter which is the longest distance found between two atoms in the molecule. In the case of this family of semicarbazones, the parameter L corresponds to the distance between a point in the van der Waals surface on the oxygen atom of the carbonyl group and a point in the surface of the most extreme atom in the aromatic moiety. This observation is in agreement with previous results [1], because it was found that the C4...O distance was a significant parameter. As was found previously [1], the geometry of the hydrogen bonding moiety (C7=N1-N2) is important for the anticonvulsant activity.

The energy of the HOMO (highest occupied molecular orbital) indicates the reactivity of the molecule. Thus, only those molecules with adequate HOMO energy would react satisfactorily in the active site of the receptor microenvironment. Activity is also influenced by the aromatic substitution pattern as shown by the charge on the C2 atom of the aromatic portion. In addition, the *z*-component of the molecular dipole moment also correlates with bioactivity. Since the semicarbazono moiety is responsible for the *z*-component, it can be postulated that the particular orientation of this moiety in the active site is important for the final drug–receptor interaction.

Two physicochemical descriptors were found to be significant for anticonvulsant activity namely the theoretical log *P* (calculated with the parameters of Ghose [10]) and the relation between the maximum and minimum of the molecular lipophilicity potential (MLP) calculated over the van der Waals surface (VDWS). Theoretical log *P* and MLP values reflect the ability of the drugs to cross biological barriers and membranes before effecting anticonvulsant bioactivity. Therefore, the molecular modeling calculations suggest that pharmacokinetic factors (which precede the interaction at the receptor site) play an important role in semicarbazone anticonvulsant activity. Moreover, the specific drug–receptor interaction seems to be equally important for the biological effect once the receptor environment has been attained. Specifically, the importance lies in the length and shape of the anticonvulsant because the Sterimol L parameter was a significant descriptor and the MLP over the VDWS, which represents the interrelation between molecular surface (shape) and lipophilicity, was found to be valuable in explaining the biological effect. Furthermore, a particular orientation in the hydrogen-bonding moiety (the semicarbazono portion) seems to be required for a desirable drug–receptor interaction, as shown by the significance of the *z*-component of the dipole moment.

This study also demonstrates the utility of some of the new descriptors (numbers 35, 36 and 37, table III) which allow for a three-dimensional representation of the drugs regarding both the shape and the interrelations between the surface and molecular properties. This is an essential consideration when evaluating the geometric, lipophilic and electronic properties of the drug–receptor interaction.

In conclusion, this study has revealed that a number of aryl semicarbazones afford protection in the MES screen when administered by the oral route to rats. The absence of neurotoxicity at the maximum dose utilized led to compounds displaying high protection indices. A number of physicochemical determinations and molecular modeling studies indicated a variety of structural features which are believed to contribute to anticonvulsant activity. From these data, ideas for future molecular modification leading to compounds with greater favorable pharmacological properties may be derived.

Experimental protocols

Chemistry

Elemental analyses (C, H, N) were undertaken on **2d–w**, **3b–l**, **4b**, **5a–d**, **6–10** by K Thoms, Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada and were within 0.4% of the calculated values except for **2i** (found: C: 47.58; calcd for $C_8H_7F_2N_3O$: C: 48.24) and **5a** (found: C: 74.50; calcd for $C_{14}H_{12}N_2O$: C: 74.98). In general, the melting points of compounds which have been described previously in the literature, were similar to the recorded values. Thin-layer chromatography was undertaken using silica-gel plates impregnated with a fluorescent indicator in order to determine whether the reaction product was homogeneous or not. Electronic absorption spectra were generated using a Perkin-Elmer Lambda 4B UV/vis spectrophotometer. A sample of semicarbazide hydrochloride **10** obtained from the Aldrich Chemical Company, Milwaukee, WI, USA was recrystallized from 95% ethanol prior to its bioevaluation.

Preparation of the semicarbazones 2–4

The preparation of compounds **2a**, **2b**, **3a** and **4a** has been described previously [1] and the synthesis of **2c** has recently been reported [3]. The general procedure for the preparation of **2c–w**, **3b–l** and **4b** was as follows. A solution of the aldehyde or ketone (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 (**2f**, **2h**, **2p–r**, **2t**, **2w**, **3b**, **3f–l** and **4b**) or 2.5 h (**2d**, **2e**, **2g**, **2i–o**, **2s**, **2u**, **2v** and **3c–e**). In order to dissolve the aldehyde and ketone in methanol, heating was applied in the synthesis of **2p** and **3h**. Deviations from this general procedure were made in some of the synthesis; the quantities of methanol used were 40 ml (**2g**, **2i**, **2k**, **2m**, **2n** and **2v**), 50 ml (**2j**), 20 ml (**2l**), 100 ml (**2o**) and 25 ml (**2s** and **2u**). The precipitates were removed by filtration, dried and recrystallized from absolute ethanol (**2d**, **2e**, **2l**, **2p**, **3c–e**, **3h** and **4b**), 2-propanol (**2f**, **2q**, **2r**, **2t**, **3f** and **3l**), methanol (**2g**, **2i**, **2j**, **2m–o**, **2s**, **2u** and **2v**), 1-propanol (**2h**), dimethylsulfoxide (**2k**), acetic acid (**2w**), 95% ethanol (**3b**, **3g**, **3i** and **3k**) or acetonitrile (**3j**). In the case of the following compounds, the melting points (°C) and yields were as follows: **2f**: 240.5–242, 68%; **2h**: 230.5–231.5, 79%; **2i**: 250–252, 87%; **2k**: 248–250, 82%; **2m**: 210.5–212, 66%; **2n**: 219–220, 83%; **2o**: 223–224, 90%; **2s**: 209–210, 89%; **2u**: 228–229, 76%; **2v**: 219–220, 81%; **2w**: 242–243, 27%; **3c**: 202–203, 79%; and **3j**: 251.5–253, 83%.

Preparation of the benzoylhydrazones 5

Compounds **5a**, **5b** and **5d** were prepared as follows. A mixture of the aldehyde or ketone (0.01 mol) and benzoyl hydrazide (0.01 mol) in methanol (20 ml) containing 2 or 3 drops of glacial acetic acid was stirred at room temperature for 20–24 h. The precipitate was collected and dried. Recrystallization of the reaction product from water/methanol gave **5a**, mp 204.5–206°C in 65% yield and **5b**, mp 171–173°C in 31% yield, while recrystallization from 2-propanol afforded **5d**, mp 151.5–153°C, in 68% yield.

Compound **5c**, mp 190–191°C, was prepared in 80% yield as follows. A mixture of 4-bromobenzaldehyde (0.01 mol), benzoyl hydrazide (0.01 mol), glacial acetic acid (0.01 mol) in dry ethanol (50 ml) was stirred at room temperature for 4 h. Pure **5c** precipitated from the reaction mixture. Refrigeration of the mother liquor at –20°C overnight gave rise to a precipitate which was recrystallized from absolute ethanol.

Preparation of 4-bromobenzaldehyde phenylhydrazone 6

A mixture of 4-bromobenzaldehyde (0.01 mol), phenylhydrazine (0.01 mol), acetic acid (0.002 mol) and sodium acetate (0.002 mol) in methanol (50 ml) was stirred at room temperature for 3 h. Water was added to increase the volume of the mixture to 100 ml and on cooling a precipitate formed, which was collected, dried and recrystallized from 95% ethanol to give **6**, mp 119–120°C, in 82% yield.

Preparation of the azine 7 and carbohydrazone 8

Hydrazine hydrate (0.01 mol) was added to a stirred solution of 4-bromobenzaldehyde (0.02 mol) in methanol (25 ml) and the mixture was heated under reflux for 1 h. The precipitate was collected, dried and recrystallized from absolute ethanol. The mother liquor was refrigerated overnight and the precipitate was collected, dried and recrystallized from absolute ethanol. This methodology led to the formation of **7**, mp 226–227°C in 87% yield.

Utilization of the same procedure except that carbohydrazide (0.01 mol) was used in place of hydrazine hydrate (0.01 mol) led to the formation of **8**, mp 229–230°C, in 85% yield.

Preparation of 9-anthraldehyde semicarbazone 9

9-Anthraldehyde (0.01 mol) was dissolved in hot ethanol (60 ml) and on cooling, the solution was added to a stirring mixture of semicarbazide hydrochloride (0.01 mol) and sodium acetate (0.01 mol) in water (20 ml). The mixture was stirred at room temperature for 4 h. The resultant precipitate was collected, washed with water, dried and recrystallization from absolute ethanol gave **9**, mp 289°C, in 76% yield.

Use of physicochemical constants

The Hammett σ values were taken from a published compilation [4, 11] and the Taft σ^* values were culled from the literature [12]. Both the π and MR figures were obtained from available data [13].

Determination of the θ values using electronic absorption spectroscopy

The compounds were weighed accurately and a stock solution was prepared in a mixture of methanol and acetonitrile (1:1). Four measurements were made at room temperature for each compound after dilution of the stock solution with the same solvent. The increases in molar absorptivity by the substituents compared with the unsubstituted compound were obtained by subtracting the ε value of **2a** from that of its 4-substituted analog. This figure was then subtracted from the ε values of compounds containing *ortho* and *meta* substituents prior to determining the θ figures.

X-ray crystallographic studies on 2c, 2d, 3c and 7

The compounds were crystallized from a mixture of diethyl ether and acetone (**2c**), diethyl ether and methanol (**2d**), benzene and methanol (**3c**) and cylclohexane and chloroform (**7**) by the vapor diffusion method. An Enraf-Nonius CAD-4

diffractometer with an $\omega/2\Theta$ scan was used for data collection and the structures were solved by direct methods using NRCVAX [14]. Structure diagrams were drawn using OrtepII [15]. Atomic scattering factors and anomalous dispersion corrections were taken from the literature [16].

The data for **2c** (4-bromobenzaldehyde semicarbazone) were as follows: $C_8H_8BrN_3O$, M_r = 242.07, monoclinic, $P2_1/a$, colorless rods, 0.48 x 0.10 x 0.08 mm, a = 11.9856(13), b = 4.4367(5), c = 17.3513(17) Å, β = 91.877(8)°, V = 922.19(17) Å^3, Z = 4, D_m = 1.741 Mg m^{-3}, D_x = 1.744 Mg m^{-3}, λ (MoK_α) = 0.71069 Å. Cell parameters obtained by least-squares fit of 25 reflections with 8.0 < Θ < 19.5°, μ = 4.37 mm^{-1}, F(000) = 479.0, T = 289K, Θ_{max} = 25.0°, $-14 \le h \le 14$, $0 \le k \le 5$, $0 \le l \le 20$. Three intensity standards monitored at intervals of 120 min showed no intensity variation. Merging R based on intensities 0.016 for 510 reflections. No absorption correction was applied. A total of 2128 reflections were measured, of which 1618 were independent. The refinement of the structure used 1136 observed reflections [$I > 2\ \sigma(I)$] and was accomplished using full-matrix least squares with anisotropic thermal factors for non-hydrogen atoms. The function minimized was $w(|F_o| - |F_c|)^2$ where $w = 1.0/(\sigma^2(F) + 0.00005F^2)$, 235 parameters. All hydrogen atoms which were placed in calculated positions on the corresponding C or N atoms (C-H = 1.00 Å and N-H = 0.90 Å) were not refined and the U of each hydrogen atom was set to the U_{eq} of the corresponding C or N atom plus 0.01. Refinement on F, $R(F)$ = 0.033, $wR(F)$ = 0.034, S = 2.11 and $(\Delta/\sigma)_{max}$ = 0.000. In the final difference map, $\Delta\rho_{max}$ = 0.39 e Å^{-3} and $\Delta\rho_{min}$ = –0.47 e Å^{-3}.

Compound **2d** (2-bromobenzaldehyde semicarbazone) crystallized in a monoclinic unit cell at room temperature. The crystal was rather small and diffracted too weakly to be collected at room temperature. On reducing the temperature to 123K, the two 90° angles in the room temperature monoclinic structure now deviated from 90°. The standard deviation on all three cell angles was 0.02°, including the γ angle of 89.53°. The deviation of the γ angle from 90° is believed to be real since past experience with the CAD-4 diffractometer has indicated right angles to be usually found to be within 0.10° of 90° even for crystals with poor peak shapes. The expected absences for $P2_1/a$ were all found. Use of the program NRCVAX CREDUC [14] revealed that the conversion of the triclinic space group into a monoclinic space group required a minimum delta of 0.478, which is unacceptably large. Merging of the triclinic data into a monoclinic space group led to the merging R (based on intensities) to be 0.146 which is much larger than the normal merging R figures of 0.01–0.03 found in the laboratory. If the true space group is monoclinic, then the two molecules in the $P\bar{1}$ solution would be highly correlated with one another, resulting in equivalent bond lengths being long in one molecule and short in the other. When the structure solution was refined in $P\bar{1}$ with two molecules in the asymmetric unit, the two molecules were found to have almost identical bond lengths and bond angles. The values for the bond lengths and bond angles in the two molecules are within one or two esd's of one another. The structure does solve and refine in $P2_1/a$, using merged data. The resultant R factor is approximately 0.2 lower than for the triclinic refinement. Thus from the evidence, in this structure there are both a pseudo-two-fold screw axis and a pseudo-glide plane, but the space group must be reported at $P\bar{1}$. The data for **2d** were as follows: $C_8H_8BrN_3O$, M_r = 242.07, triclinic, $P\bar{1}$, colorless plates, 0.60 x 0.15 x 0.05 mm, a = 3.8794(11), b = 13.885(3), c = 17.296(4) Å, α = 69.68(2)°, β = 89.87(2)°, γ = 89.53(2)°, V = 873.7(3) Å^3, Z = 4, D_m = 1.824 Mg m^{-3}, D_x = 1.840 Mg m^{-3}, λ(MoK_α) = 0.71069 Å. Cell parameters obtained by least-squares fit of 25 reflections with 15.0 < Θ < 19.0°, μ = 4.62 mm^{-1}, F(000) = 479.0, T = 123K, Θ_{max} = 25.0°, $-4 \le h \le 4$, $0 \le k \le 16$, $-18 \le l \le 20$. Three intensity standards monitored at intervals of 120 min showed no intensity variation. Merging R based on intensities 0.036 for 184 reflections. No absorption correction was applied. A total of 3222 reflections were measured, of which 3038 were independent. The refinement of the structure used 2299 observed reflections [$I > 2\ \sigma(I)$] and was accomplished using full-matrix least squares with anisotropic thermal factors for non-hydrogen atoms. The function minimized was $w(|F_o| - |F_c|)^2$ where $w = 1.0/(\sigma^2(F) + 0.0006F^2)$, 118 parameters. All hydrogen atoms, which were placed in calculated positions on the corresponding C or N atoms (C-H = 1.00 Å and N-H = 0.90 Å), were not refined and the U of each hydrogen atom was set to the U_{eq} of the corresponding C or N atom plus 0.01. Refinement on F, $R(F)$ = 0.054, $wR(F)$ = 0.068, S = 2.21 and $(\Delta/\sigma)_{max}$ = 0.000. In the final difference map, $\Delta\rho_{max}$ = 1.38 e Å^{-3} and $\Delta\rho_{min}$ = –1.92 e Å^{-3}.

The data for **3c** (4'-bromoacetophenone semicarbazone) were as follows: $C_9H_{10}BrN_3O$, M_r = 256.10, monoclinic, $P2_1/a$, colorless prisms, 0.20 x 0.10 x 0.08 mm, a = 15.199(3), b = 4.0348(9), c = 17.195(3) Å, β = 113.51(2)°, V = 966.7(4) Å^3, Z = 4, D_m = 1.713 Mg m^{-3}, D_x = 1.760 Mg m^{-3}, λ(MoK_α) = 0.71069 Å. Cell parameters obtained by least-squares fit of 25 reflections with 16.0 < Θ < 20.0°, μ = 4.18 mm^{-1}, F(000) = 511.0, T = 123K, Θ_{max} = 25.0°, $-18 \le h \le 16$, $0 \le k \le 4$, $0 \le l \le 20$. Three intensity standards monitored at intervals of 120 min showed no intensity variation. Merging R based on intensities 0.009 for 181 reflections. No absorption correction was applied. A total of 1845 reflections were measured, of which 1664 were independent. The refinement of the structure used 1423 observed reflections [$I > 2\ \sigma(I)$] and was accomplished using full-matrix least squares with anisotropic thermal factors for non-hydrogen atoms. The function minimized was $w(|F_o| - |F_c|)^2$ where $w = 1.0/(\sigma^2(F) + 0.0001F^2)$, 127 parameters. All hydrogen atoms which were placed in calculated positions on the corresponding C or N atoms (C-H = 1.00 Å and N-H = 0.90 Å) were not refined and the U of each hydrogen atom was set to the U_{eq} of the corresponding C or N atom plus 0.01. Refinement on F, $R(F)$ = 0.032, $wR(F)$ = 0.038, S = 2.00 and $(\Delta/\sigma)_{max}$ = 0.000. In the final difference map, $\Delta\rho_{max}$ = 0.77 e Å^{-3} and $\Delta\rho_{min}$ = –0.98 e Å^{-3}.

The data for **7** (4-bromobenzaldehyde azine) were as follows: $C_{14}H_{10}Br_2N_2$, M_r = 366.06, monoclinic, $P2_1/c$, brown prisms, 0.25 x 0.17 x 0.10 mm, a = 7.0301(5), b = 4.0264(6), c = 23.2730(18) Å, β = 92.166(7)°, V = 658.29(12) Å^3, Z = 2, D_m = 1.842 Mg m^{-3}, D_x = 1.847 Mg m^{-3}, λ(MoK_α) = 0.71069 Å. Cell parameters obtained by least-squares fit of 25 reflections with 13.0 < Θ < 22.0°, μ = 6.08 mm^{-1}, F(000) = 354.92, T = 287K, Θ_{max} = 25.0°, $-8 \le h \le 8$, $0 \le k \le 4$, $0 \le l \le 27$. Three intensity standards monitored at intervals of 120 min showed no intensity variation. Merging R based on intensities 0.015 for 382 reflections. No absorption correction was applied. A total of 1525 reflections were measured, of which 1143 were independent. The refinement of the structure used 926 observed reflections [$I > 2\ \sigma(I)$] and was accomplished using full-matrix least squares with anisotropic thermal factors for non-hydrogen atoms. The function minimized was $w(|F_o| - |F_c|)^2$ where $w = 1.0/(\sigma^2(F) + 0.0003F^2)$, 82 parameters. All hydrogen atoms which were placed in calculated positions on the corresponding C atoms (C-H = 1.00 Å) were not refined and the U of each hydrogen atom was set to the U_{eq} of the corresponding C atom plus 0.01. Refinement on F, $R(F)$ = 0.052, $wR(F)$ = 0.063, S = 2.62 and $(\Delta/\sigma)_{max}$ = 0.000. In the final difference map, $\Delta\rho_{max}$ = 0.98 e Å^{-3} and $\Delta\rho_{min}$ = –1.56 e Å^{-3}.

Molecular modeling

All the structures were built and optimized using the Biograf 3.0 package and the Dreiding force-field [17]. Calculations at the molecular orbital semiempirical level were carried out using the AM1 Hamiltonian [18] as implemented in the Mopac 6.0 program [19]. For statistical calculations, the SAS package of statistical programs, version 6.06, was used [20]. Molecular mechanics and semiempirical molecular orbital 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.

All the semicarbazones studied exhibit a long flexible chain, which have a high degree of rotational freedom. The conformational space of all these compounds was scanned by carrying out quenched high-temperature molecular dynamics. In all cases the molecules were heated to 1500 K, increasing the temperature 12K every 0.1 ps. This heating step was followed by 10 ps of equilibration and 78 ps of stimulation, during which 300 structures were stored at equal intervals. These structures were cooled and minimized during 200 cycles using the Dreiding force field. All those conformers within a range of 5 kcal•mol^{-1} from the global minimum were considered and only those with different structural configuration were evaluated with semiempirical optimization using the AM1 method. The precision for each calculation was incremented by a factor of 100 and the structures were optimized to a gradient norm smaller than 1.0 kcal•Å^{-1}. All conformers were characterized to be real minima by calculating their frequencies. These new sets of conformers were compared structurally to the X-ray structures of compounds **2c** and **3c**. Consequently, the bioactive structure was selected to be that conformer, minimum in energy, with similar geometric disposition to those crystallographic structures. Compounds containing a primary amino terminal group (**2a–w**, **3a–l**, **4a**, **b** and **9**) were used for the structure–activity relationship studies.

The parameters describing these compounds can be classified in four different groups: (i) geometric descriptors represent three dimensional properties and reflect aspects of molecular size and shape (*eg*, bond and torsional angles, total areas, total volumes, Sterimol parameters); (ii) electronic descriptors represent the electron distribution through the molecular framework which is important in conceptualizing the drug–receptor interaction (*eg*, atomic charge densities, molecular dipole components, relation between the maximum and minimum molecular electrostatic potential (MEP) calculated over the VDWS [21]); (iii) graph theory descriptors encode aspects of molecular composition and connectivity (*eg*, Randic indices [22], Kier–Hall indices [23]); and (iv) physicochemical parameters describe molecular lipophilicity (*eg*, theoretical log P [10, 24], relation between the maximum and minimum MLP calculated over the VDWS [25]). The geometric and electronic descriptors were obtained from the AM1 calculations. The maxima and minima MEP values over the VDWSs were obtained by single point *ab initio* calculations (minimal basis set STO-3G) on an isotropic distribution of points over the VDWS [26] of the AM1 optimized structures [21]. The graph theory descriptors were determined from graph theory calculations. The theoretical log *P* values were calculated by the additive approach using the parameters of Ghose [10] and by the method of Kantola *et al* [24] based on the AM1 charges obtained for each molecule. The maximum and minimum MLP values over VDWSs were obtained by using the potential equation of Audry [25] with the atomic lipophilic parameters of Ghose [10] on an isotropic distribution of points over the VDWS [26] of the AM1 optimized structures. Descriptors are listed in table III.

Each compound was described by 37 descriptors. Multivariable regression statistical analyses were performed to establish a relationship between the molecular structure and the biological activity and to find the minimal number of descriptors needed for identifying optimal bioactivity.

Screening. Anticonvulsant evaluation of compounds 2–10

The examination of the compounds **2–10** was carried out under the auspices of the Anticonvulsant Screening Project of the Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, USA according to their protocols [27, 28]. Initial anticonvulsant evaluation of the compounds was achieved by injection of doses of 30, 100 and 300 mg/kg into mice by the intraperitoneal route. Activity was displayed in the MES screen by the following compounds namely **2a–d**, **2f–h**, **2j**, **2l–t**, **3a–d**, **3f–l**, **4a**, **4b**, **5d** and **8** while **2a–d**, **2g**, **2h**, **2j**, **2m–r**, **2t**, **3a–d**, **3f**, **3g**, **3i–k**, **4b** and **8** were active in the scPTZ test. Neurotoxicity was displayed by **2a**, **2b**, **2g**, **2h**, **2j**, **2k**, **2m–o**, **2r**, **3a–d**, **3f**, **3h**, **3i**, **3k**, **4a**, **4b**, **5b**, **6** and **10**. The data for examining **2–10** in the MES and neurotoxicity screens after oral dosing in rats are given in table I. In addition, compounds **2–9** were evaluated in the scPTZ test at doses up to and including 120 mg/kg (**2v**), 300 mg/kg (**3a**, **3k**) and 500 mg/kg (**2d–f**, **2p**, **3c**, **3e**, **4b**, **5c** and **6–9**); the maximum dose in all other cases was 250 mg/kg. The only protection noted was in the following cases (number of animals protected/total number of rats at dose of compound in mg/kg): **2d** (1/4 at 500), **2f** (2/8 at 125; and 1/8 at 250 and 500), **2h** (1/8 at 250), **2j** (1/8 at 125), **2o** (1/2 at 31.3), **2p** (1/8 at 250), **2u** (1/4 at 120), **3c** (1/4 at 500) and **4b** (1/4 at 500).

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