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Original article

Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds

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Abstract – Various acetylhydrazones, oxamoylhydrazones and semicarbazones were prepared as candidate anticonvulsants with a view to examining the viability of a putative binding site hypothesis. Atomic charge calculations were undertaken to determine the hydrogen bonding capacities of various molecules. The biological results obtained revealed that in general the acetylhydrazones and semicarbazones afforded good protection against convulsions while the oxamoylhydrazones were significantly less active. These data suggest that terminal electron-donating groups enhanced the hydrogen bonding capabilities and anticonvulsant properties of these molecules. © 2000 Éditions scientifiques et médicales Elsevier SAS

semicarbazones / acetylhydrazones / oxamoylhydrazones / anticonvulsant / atomic charges

1. Introduction

Today there is a need for new antiepileptic drugs since approximately one quarter of epileptic patients find that drug therapy inadequately controls their convulsions [1]. In addition, many currently used antiepileptic drugs cause significant side effects which may limit their maximal usefulness [2]. In the course of investigations aimed at developing structurally novel anticonvulsants, a number of aryl semicarbazones, including series **1**, were found to display significant activity [3, 4]. These compounds were believed to interact at two locations on a putative binding site designated a hydrogen bonding area and an aryl binding site [5]. However, since the aryl group can be replaced by other hydrophobic moieties with retention of anticonvulsant activity [6], the portion of the binding site with which the aryl group of series **1** and related compounds interacts will be referred to as a hydrophobic bonding area rather than an aryl binding site. The principal objective of the present investigation was the preparation of a number of analogues of **1** with a view to further evaluating the binding site hypothesis (*figure 1*).

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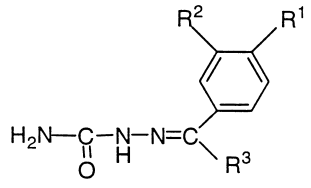
Initially, the nature of the hydrogen bonding area was investigated. In the absence of an identified binding site the assumption was made that it was a peptide chain whereby the repeating amidic functions allow hydrogen bond formation (*figure 2*). If this supposition is correct, then changing the nature of the group X should affect the capacity of the compounds to form hydrogen bonds at the binding site, which will be reflected by alterations in anticonvulsant activity. The maximum electronic effect caused by varying X would be predicted to be on atoms 6 and 7, while smaller changes in the electron densities would be predicted to be on atoms 1–5 due to tautomerism of the 5 hydrogen atom and the subsequent rearrangement of single and double bonds [7]. Thus two series of compounds were proposed whereby substituent X was changed from an amino group in **1a–f** to a methyl function in the acetylhydrazones **2a–f** and an aminocarbonyl moiety leading to the oxamoylhydrazones **3a–f**. The terminal amino, methyl and aminocarbonyl groups found in series **1**, **2** and **3**, respectively, would be expected to exercise different electronic effects on atoms 1–7, which in turn should affect hydrogen bonding capabilities with the putative binding site and hence anticonvulsant activity.



1

a: $R^1 = R^2 = R^3 = H$

b: $R^1 = Cl$; $R^2 = R^3 = H$

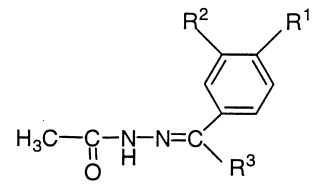
c: $R^1 = R^2 = Cl$; $R^3 = H$

d: $R^1 = CH_3$; $R^2 = R^3 = H$

e: $R^1 = OCH_3$; $R^2 = R^3 = H$

f: $R^1 = Br$; $R^2 = R^3 = H$

g: $R^1 = R^2 = H$; $R^3 = CH_3$



2

a: $R^1 = R^2 = R^3 = H$

b: $R^1 = Cl$; $R^2 = R^3 = H$

c: $R^1 = R^2 = Cl$; $R^3 = H$

d: $R^1 = CH_3$; $R^2 = R^3 = H$

e: $R^1 = OCH_3$; $R^2 = R^3 = H$

f: $R^1 = Br$; $R^2 = R^3 = H$

g: $R^1 = R^2 = H$; $R^3 = CH_3$

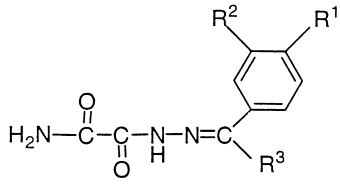
h: $R^1 = Cl$; $R^2 = H$; $R^3 = CH_3$

i: $R^1 = R^2 = Cl$; $R^3 = CH_3$

j: $R^1 = CH_3$; $R^2 = H$; $R^3 = CH_3$

k: $R^1 = OCH_3$; $R^2 = H$; $R^3 = CH_3$

l: R^1 =Br; $R^2 = H$; $R^3 = CH_3$



3

a: $R^1 = R^2 = R^3 = H$

b: $R^1 = Cl$; $R^2 = R^3 = H$

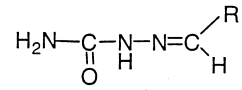
c: $R^1 = R^2 = Cl$; $R^3 = H$

d: $R^1 = CH_3$; $R^2 = R^3 = H$

e: $R^1 = OCH_3$; $R^2 = R^3 = H$

f: $R^1 = Br$; $R^2 = R^3 = H$

g: $R^1 = R^2 = H$; $R^3 = CH_3$



4

a: $R = (CH_2)_7CH_3$

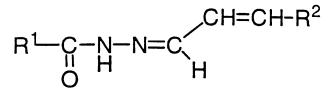
b: $R = CH{=}CHC_6H_5$

c: $R = CH{=}CH(CH_2)_7CH_3$

d: $R = CH{=}CH(CH_2)_4CH_3$

e: $R = CH{=}CH(CH_2)_5CH_3$

f: $R = (CH{=}CH)_2C_2H_5$



5

a: $R^1 = CH_3$; $R^2 = C_6H_5$

b: $R^1 = CH_3$; $R^2 = (CH_2)_5CH_3$

c: $R^1 = H_2NCO$; $R^2 = C_6H_5$

d: $R^1 = H_2NCO$; $R^2 = (CH_2)_5CH_3$

Figure 1. Structures of **1**–**5**.

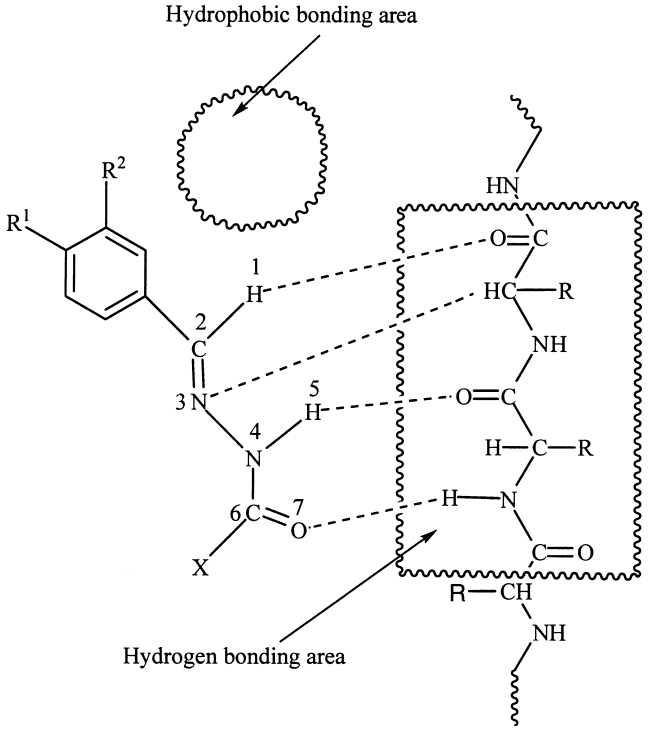


Figure 2. Proposed interactions of compounds **1–5** at a binding site.

A previous study revealed that replacement of the methine proton of **1a** by a methyl group led to **1g** with an approximately 2-fold increase in anticonvulsant activity when the compounds were administered by the intraperitoneal route to mice [3]. In order to explore whether this observation was indicative of a general trend, which may therefore afford some insight into the existence of an additional hydrophobic binding site, the synthesis and bioevaluation of **2g–l** were suggested whose bioactivity could be compared to **2a–f**. In addition **3g** was also considered for comparison with **3a**.

In order to verify that changes in the nature of substituent X would alter the polarity of the group interacting at the hydrogen bonding area, the decision was made to determine the atomic charges of the atoms 1–7 noted in *figure 2*.

The second phase of the study was directed to examining some of the structural requirements at the hydrophobic bonding area. In this regard, the preparation of series **4** was suggested. Replacement of the phenyl ring of **1a** by a hydrophobic n-octyl group leading to **4a** would retain hydrophobic bonding while creating spatial property differences between the phenyl and n-octyl groups. Since the substitution of the phenyl ring of **1a** by a β-phenylvinyl group producing **4b** led to increased anticonvulsant activity [3], a comparison of the activity of **4b** with **4c** was considered in order to provide further information of the properties of the groups that could be accommodated at the hydrophobic bonding area. The remaining analogues **4d–f** were designed in order to discern the influence on anticonvulsant activity of the length and increasing unsaturation of the n-alkyl chains.

Compounds **4b** and **4e** had promising anticonvulsant activities vide infra. In order to explore further the generality of the effect on anticonvulsant properties of replacing the amino group of the semicarbazones by methyl and aminocarbonyl functions, the preparation and bioevaluation of **5a–d** was suggested.

In summary therefore, the principal aim of the present investigation was the synthesis of the compounds in series **2–5** for anticonvulsant evaluation with a view to obtain-

Table I. Atomic charges on different atoms of various compounds in series **1–5**.

Compound	X	Atoms[a]						
		1	2	3	4	5	6	7
1a	NH_2	–0.028	0.051	–0.045	–0.146	0.095	0.448	–0.378
2a	CH_3	–0.028	0.052	–0.050	–0.132	0.097	0.351	–0.317
3a	H_2NCO	–0.026	0.059	–0.056	–0.119	0.105	0.291	–0.303
1g	NH_2	–	0.077	–0.066	–0.149	0.094	0.448	–0.379
2g	CH_3	–	0.077	–0.071	–0.134	0.095	0.351	–0.319
3g	H_2NCO	–	0.082	–0.077	–0.124	0.113	0.291	–0.297
4b	H_2N	–0.019	0.057	–0.047	–0.146	0.094	0.448	–0.378
5a	CH_3	–0.020	0.057	–0.053	–0.132	0.096	0.351	–0.317
5c	H_2NCO	–0.014	0.070	–0.083	–0.128	0.105	0.287	–0.275
4e	H_2N	–0.019	0.058	–0.050	–0.148	0.093	0.448	–0.379
5b	CH_3	–0.021	0.062	–0.061	–0.134	0.093	0.351	–0.320
5d	H_2NCO	–0.013	0.072	–0.085	–0.130	0.104	0.287	–0.277

[a]The atoms corresponding to the numbers are shown in *figure 2*.

Table II. Evaluation of compounds **1–5** in the mouse intraperitoneal MES, scPTZ and NT screens[a].

Compound	MES screen		scPTZ screen		NT screen	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1a	100	–	300	–	300	–
1b	100	100	300	–	–	300
1c	–	300	–	–	–	–
1d	100	–	300	–	–	–
1e	–	–	–	–	–	–
1f	100	300	300	–	–	–
1g	100	300	100	–	100	–
2a	100	300	300	–	300	300
2b	30	100	100	300	100	300
2c	100	100	300	–	–	300
2d	300	–	300	300	300	300
2e	300	300	300	–	300	300
2f	100	–	100	300	300	300
2g	300	–	–	–	300	300
2h	100	–	–	–	300	300
2i	–	–	–	–	–	100
2j	–	–	–	–	–	300
2k	–	–	–	–	300	300
2l	300	–	300	–	300	100
3a	300	300	–	–	–	–
3b	–	–	–	–	–	–
3c	–	300	–	–	–	–
3d	–	–	–	–	–	–
3e	–	300	–	–	300	–
3f	300	–	–	–	–	–
3g	300	–	–	–	300	–
4a	300	–	–	–	300	–
4b	100	300	100	300	300	–
4c	100	300	100	300	300	300
4d	100	–	100	–	100	300
4e	100	300	–	–	–	–
4f	100	–	300	–	300	–
5a	100	300	–	–	300	300
5b	100	300	300	–	300	300
5c	–	–	–	–	300	–
5d	–	300	–	–	–	–
Phenytoin	30	30	–	–	100	100
Carbamazepine	30	100	100	300	100	300
Valproic acid	–	–	300	–	–	–

[a]Doses of 30, 100 and 300 mg/kg of the compound were administered and the protection and neurotoxicity measured after 0.5 and 4 h. The figures indicate the minimal dose required to cause protection or neurotoxicity in 50% or more of the animals. – indicates the absence of anticonvulsant activity or neurotoxicity.

ing a greater understanding of the nature of the binding site with which these compounds are believed to interact.

2. Chemistry

Reaction of acetic acid hydrazide with various aldehydes or ketones led to the formation of **2a–l**, while the oxamoylhydrazones **3a–g** were synthesized from the appropriate aryl aldehydes or ketones and oxamic acid hydrazide. The semicarbazones **4a–f** were prepared from semicarbazide and various saturated and unsaturated aliphatic aldehydes. Condensation of cinnamaldehyde and 1-nonenal with both acetic and oxamic acid hydrazides led to **5a** and **5b**, and **5c** and **5d**, respectively. The charge densities of a number of atoms in selected compounds were calculated (*table I*).

Table III. Comparison of the protection scores with various groups of compounds in the mouse intraperitoneal MES screen.

Compounds	Protection score[a]		
	0.5 h	4 h	total
1a–f	12	5	17
2a–f	21	8	29
3a–f	2	3	5
1a, 2a, 3a	7	2	9
1g, 2g, 3g	5	1	6
2a–f	21	8	29
2g–l	5	0	5

[a]The figures in the table are the total scores for each cluster of compounds (see Results and discussion section regarding the calculation of the PS values).

3. Pharmacology

All of the compounds were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and neurotoxicity (NT) screens using doses of 30, 100 and 300 mg/kg at two different time intervals. These data are presented in *table II*. Comparisons of the activities of various groups of compounds are summarized in *table III*. Approximately half of the compounds were also evaluated orally in rats for activity in the MES test at several time points (*table IV*).

4. Results and discussion

In order to study the contribution of the electronic effects of the amino, methyl and aminomethyl groups at the putative binding site, the atomic charges on the seven atoms indicated in *figure 2* were computed for four groups of compounds, each group consisting of three analogues. These data are presented in *table I*. The bonding that can occur between the ligands and a peptide chain is considered to be hydrogen bonding. In the case of the N3···HCR bond, the proton of the peptide chain is considered to be acidic due to the electron-withdrawing

Table IV. Evaluation of various compounds for activity in the rat oral MES screen[a].

Compound	Time of test (h)				
	0.25	0.5	1	2	4
1a	4	4	4	4	–
1b	–	2	4	4	1
1c	–	–	4	3	4
1d	3	–	–	–	–
1e	–	–	–	–	–
1f	2	4	4	4	3
1g	4	4	4	1	–
2a	–	1	–	–	–
2c	–	–	2	2	1
2d	1	1	2	1	–
2f	1	1	1	2	–
2g	1	–	–	–	–
3g	2	1	–	–	–
4b	4	4	4	4	1
4c	–	3	3	4	3
4e	4	4	3	2	1
4f	1	3	2	–	1
5a	1	1	–	1	–
5b	2	1	2	1	–
5c	–	–	–	2	–
Phenytoin	1	4	3	3	3

[a]The doses administered were 50 mg/kg (**1a**–**g** and **4b**), 25 mg/kg (**2f**) and 30 mg/kg in the remaining cases, including phenytoin. Four rats were used for each compound and the figures in the table indicate the number of rats protected. – indicates an absence of anticonvulsant activity.

influence of the adjacent carbonyl group; however, it is possible that it is a dispersion force rather than a hydrogen bond. Nevertheless, from the standpoint of the arguments developed in this study, the term hydrogen bonding will be used in all four ligand–peptide interactions as illustrated in *figure 2*. The magnitude of the charges on the atoms which could be involved in hydrogen bonding is in the order of 7 > 5 > 3 > 1. In particular the large negative charge on the 7 oxygen atom would be predicted to exert an enormous effect on hydrogen bonding at a binding site. In each of the 4 groups of compounds, the negative electronic charge of the 7 oxygen atom was in the order of amino > methyl > aminocarbonyl. The average percentage reductions in the atomic charges on the 7 oxygen atom of the acetyl and oxamoyl hydrazones compared to the corresponding semicarbazones were 16 and 24, respectively.

The second portion of these molecules, where hydrogen bonding could occur, is at position 5. In this case, hydrogen bonding will be facilitated as the positive charge on the 5 hydrogen atom increases. With the exception of **4e** and **5b** which have the same electron densities, the magnitude of the positive charges on the 5 hydrogen atoms reflects the electronic properties of group X, e.g., the greatest charges are on the 5 hydrogen atoms found in the oxamoylhydrazones in which X is the most electron-attracting group. The two remaining atoms where hydrogen bonding could occur, namely at positions 1 and 3, had small atomic charges. It is likely therefore that they would make only minor contributions to the binding of these molecules to cellular macromolecules. In summary therefore, the data provided by the charge density calculations support the hypothesis outlined previously that variation in the nature of group X will markedly affect the capacity for hydrogen bonding at a binding site.

Candidate anticonvulsants are often evaluated initially in the MES and scPTZ screens [8]. Compounds affording protection in the MES test may prove to be useful in treating generalized tonic–clonic and complex partial seizures, while activity in the scPTZ screen is claimed to denote agents of value in treating absence seizures. Neurotoxicity in mice may be measured by the rotorod procedure [9], while minimal motor impairment in rats is detected by overt evidence of ataxia and abnormal gait and stance. These procedures were undertaken in the present investigation.

The results of intraperitoneal screening in mice are summarized in *table II*. Two general trends may be discerned. First, the data for the MES and scPTZ tests revealed that 74 and 92%, respectively, of the compounds **2–5** had greater activity at the end of 0.5 h than after 4 h. Thus, in general, these compounds are short acting anticonvulsants. Second, protection was afforded by 79 and 41% of the compounds **2–5** in the MES and scPTZ screens, respectively. In addition, 55% of the compounds had greater activity in the MES test rather than the scPTZ screen, while for the remaining cases equal activity was demonstrated. Furthermore, as will be presented later, in the rat oral MES screen **1b**, **1g**, **2f** and **4b** afforded protection, but these compounds were inactive in the scPTZ test using the same doses. Thus the majority of the compounds displayed preferential or exclusive MES-selectivity and hence further discussion in regard to the binding site hypothesis and structure–activity relationships will be devoted to the data provided from MES screens only.

In order to compare the activities of various clusters of candidate anticonvulsants in the mouse intraperitoneal MES screen, compounds which were active at 30, 100 and 300 mg/kg were assigned scores of 10, 3 and 1, respectively. In this way, the protection score (PS) values for different groups of compounds were obtained and are presented in *table III*. A comparison of the figures for **1a–f**, **2a–f** and **3a–f** revealed that the order of anticon-

vulsant activity was the acetylhydrazones > semicarbazones > oxamoylhydrazones. While the greater activity of the acetylhydrazones than the semicarbazones was unanticipated, the results indicate that the oxamoylhydrazones, having the smallest negative atomic charges on the 7 oxygen atom, possessed the lowest anticonvulsant activity. It is conceivable that the terminal methyl group in **2a–f** forms van der Waals bonding with a corresponding area of the binding site; such interactions would not occur when X is either an amino or aminocarbonyl group.

A comparison of the PS values for **1a**, **2a** and **3a** with **1g**, **2g** and **3g** revealed that greater anticonvulsant activity was displayed by the nor analogues **1a**, **2a** and **3a**. In order to evaluate the generality of this observation, the PS figures for **2a–f** and **2g–l** were contrasted. The data in *table III* reveal that the acetylhydrazones having a methine proton were significantly more active than the homo analogues. Thus a site of steric repulsion may be present on the binding site hindering alignment of the molecules and hence lowering anticonvulsant activity.

Replacement of the phenyl ring of **1a** (PS = 3) by an n-octyl group led to **4a** (PS = 1) with reduced activity. However the preparation of analogues in which an olefinic double bond was attached to the carbimino (C=N) group led to increases (**4b**, **4c** and **4e**; PS = 4) or retention (**4d** and **4f**; PS = 3) of activity. Other structural features incorporated into the R group of series **4** namely an aryl ring (**4b**), alkyl chains of varying lengths (**4c–e**) and an additional olefinic double bond (**4f**) seemed to be relatively unimportant contributors to bioactivity.

The biodata generated in the mouse intraperitoneal MES screen for series **5** confirmed two generalizations noted earlier. First, as observed in the case of **4b** and **4e**, the hydrophobic bonding area can accommodate both the β-phenylvinyl and 1-octenyl groups present in **5a** and **5b**; both compounds have a PS value of 4. Second, while the acetylhydrazones **5a** and **5b** have the same PS value of 4 as the corresponding semicarbazones **4b** and **4e**, the oxamoylhydrazones **5c** (PS = 0) and **5d** (PS = 1) are much less potent. These data support the conclusion drawn earlier that in order for significant anticonvulsant activity to be displayed, group X in *figure 2* may be an amino or methyl but not an aminocarbonyl function.

In order to confirm the utility of these alkylidene semicarbazones, quantification of a representative compound **4c** was undertaken. The ED_{50} figure in the MES screen was 21.3 mg/kg and a protection index (PI: i.e., TD_{50} in the rotorod screen/ED_{50} in the anticonvulsant test) was 12.4. These figures compare favourably with **1a** for which the ED_{50} and PI values were 69.7 and 2.93, respectively [3]. In addition, while **4c** has approximately one-third of the potency of phenytoin (ED_{50} in the MES screen = 6.32; PI = 6.52), the PI value of **4c** is virtually double that of the established drug. Thus **4c** and related compounds serve as useful molecules for further development.

In the case of those series in which there was a variation in the aryl substituent patterns, the groups in **1a–e**, **2a–e** and **2g–k**, and **3a–e** were chosen for a Topliss analysis [10]. The 4-bromo substituent in **2f** and **2l**, and **3f** was used owing to the promising anticonvulsant activity of **1f** [11]. In the light of the results obtained, a Topliss analysis did not reveal any parameter dependencies nor did the 4-bromo derivatives **2f** and **2l**, and **3f** demonstrate markedly superior activity than their analogues.

A number of compounds were examined for activity in the rat oral MES screen and the results are summarized in *table IV*. The initial screening using **1a–g** and **4b** employed doses of 50 mg/kg. However, since protection in all animals was often obtained, the doses of the analogues were generally reduced to 30 mg/kg in order to detect the most active compounds. The ED_{50} figures in the rat oral MES screen for **1a**, **1c**, **1f** and **1g** were 20–23 mg/kg [3, 4] and the related acetylhydrazones possessed comparable (**2c** and **2f**) or reduced (**2a** and **2g**) activity when 30 mg/kg of the compound was administered. In general therefore, the semicarbazones afforded greater protection than the corresponding acetylhydrazones in the rat oral MES screen. This trend was also observed by noting that the semicarbazones **4b** and **4e** were more active than the analogous acetylhydrazones **5a** and **5b**. These observations support the hypothesis outlined previously that the greater electron-donating capabilities of the amino group compared to the acetyl function leading to greater hydrogen bond formation favours bioactivity. The promising results obtained in the mouse intraperitoneal MES screen for the semicarbazones which contained olefinic linkages were also noted when administered orally to rats. Thus protection in the MES screen was 100% (**4b**, **4c** and **4e**) or 75% (**4f**) affording further evidence towards the profitability of developing this series of compounds. At the doses utilized in the MES screen, none of the compounds displayed neurotoxicity and **1b**, **1g**, **2f** and **4b** gave no protection in the scPTZ test.

5. Conclusions

The synthesis of a number of acetylhydrazones, oxamoylhydrazones and semicarbazones of aryl aldehydes and related compounds as candidate anticonvulsants is reported in this study. Measurements of the atomic charges on certain atoms of representative compounds revealed changes in electronic properties caused by varying the terminal group from amino to methyl and aminocarbonyl

functions. Evaluation in the mouse intraperitoneal MES and scPTZ screens showed that the compounds were rapidly acting and afforded greater protection in the MES screen. The biodata generated suggested that a site of steric repulsion close to atom 1 (*figure 2*) is possible. Confirmation that the hydrophobic bonding area could accommodate groups other than aryl rings was noted and in particular 1-nonenal semicarbazone **4c** is clearly a useful lead compound. Most of the semicarbazones in series **1** and **4** afforded complete protection in the rat oral MES screen and were bereft of NT at the doses employed. In general the results obtained revealed that the acetylhydrazones and semicarbazones afforded greater protection in the MES screens than the corresponding oxamoylhydrazones which may have been due to the higher negative charges on the 7 oxygen atom with the more active compounds.

6. Experimental protocols

6.1. Chemistry

Melting points are uncorrected and are quoted in degrees Centigrade. Elemental analyses (C, H, N) were undertaken on **2a–e** and **2g–l**, **3a–g**, **4a** and **4c–f**, and **5a–d** by Mr K. Thoms, Department of Chemistry, University of Saskatchewan and were within 0.4% of the calculated values. All compounds were shown to be homogenous by TLC using silica gel 60 F_{254} precoated plastic sheets and a solvent system of chloroform:methanol (7:3). ^{1}H-NMR spectroscopy utilized a Bruker AMX 500 FT (500 MHz) instrument.

6.1.1. Synthesis of compounds **2a–l**, *and* **5a** *and* **5b**

The acetylhydrazone **2f** was prepared by the method described previously [12]. The remaining analogues were synthesized as follows. A mixture of the appropriate aldehyde or ketone (0.01 mol) and acetic acid hydrazide (0.01 mol) in ethanol (25 mL) was stirred at room temperature for 3 h (**2a–e,** and **5a** and **5b**), 24 h (**2i** and **2l**), 48 h (**2g**, **2h** and **2j**) or 72 h (**2k**). The mixture was concentrated in vacuo and the precipitated compound was collected by filtration and recrystallized from ethanol (**2a–c**, **2l** and **5a**), ethyl acetate (**2d**, **2e** and **2i**), ethanol (70% v/v, **2g**, **2h**, **2j** and **2k**) or hexane (**5b**) to yield the acetylhydrazones. The m.p.'s and yields are: **2a**: 133–135° (lit. [13] 136°), 42%; **2b**: 136–138°, 84%; **2c**: 198–200°, 76%; **2d**: 128–130° (lit. [14] 132°), 27%; **2e**: 129–131° (lit. [15] 139°), 62%; **2g**: 120–122° (lit. [16] 131–132°), 71%; **2h**: 163–165°, 83%; **2i**: 153–155°, 75%; **2j**: 160–162°, 69%; **2k**: 150–152°, 92%; **2l**: 176–178°, 75%; **5a**: 164–166° (lit. [15] 171°), 81% and **5b**: 69–71°, 74%. The ^{1}H-NMR spectrum of a representative compound **2d** was as follows: δ ($CDCl_3$): 2.36 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.18 (d, 2H, 3,5 aryl H), 7.55 (d, 2H, 2,6 aryl H), 7.80 (s, 1H, CH=N), 10.11 (s, 1H, NH) ppm.

6.1.2. Synthesis of compounds **3a–g**, *and* **5c** *and* **5d**

A mixture of the appropriate aldehyde and ketone (0.01 mol) and oxamic acid hydrazide (0.01 mol) in ethanol (70% v/v, 100 mL) was heated under reflux for 3 h. On cooling, the precipitate was collected, washed thoroughly with ethanol (70% v/v) and dried to yield the pure oxamoylhydrazones. The m.p.'s and yields are: **3a**: 286–288° (lit. [17] 215–216°), 92%; **3b**: 312–314°, 93%; **3c**: 286–290°, 91%; **3d**: 294–296°; 85%; **3e**: 290–292°, 78%; **3f**: > 300° (lit. [17] > 300°), 96%; **3g**: 211–213°, 77%; **5c**: 304–306°, 92% and **5d**: 245° (dec), 90%. The ^{1}H-NMR spectrum of a representative compound **3d** was as follows: δ (DMSO-d_6): 2.33 (s, 3H, CH_3), 7.26 (d, 2H, 3,5 aryl H), 7.57 (d, 2H, 2,6 aryl H), 7.93 (s, 1H, CH=N), 8.27 (s, 1H, $CONH_2$), 8.51 (s, 1H, $CONH_2$), 12.02 (s, 1H, CONHN) ppm.

6.1.3. Synthesis of compounds **4a–f**

Compound **4b** has been prepared previously [3]. The semicarbazones **4a** and **4c–f** were synthesized as follows. A solution of the appropriate aldehyde (0.01 mol) in ethanol (95% v/v, 25 mL) was added to a solution of semicarbazide hydrochloride (0.01 mol) and sodium acetate (0.013 mol) in water (10 mL). The mixture was stirred at room temperature for 1 h, cooled (4 °C) and the precipitates were collected and recrystallized from ethanol (80% v/v, **4a**) or ethanol (**4c–f**) to give the semicarbazones. The m.p.'s and yields are: **4a**: 93–95° (lit. [18] 100°), 92%; **4c**: 160–161° (lit. [19] 161.5–162.5°), 92%; **4d**: 166–168° (lit. [20] 163°), 72%; **4e**: 162–164° (lit. [19] 161–162°), 96% and **4f**: 193–195° (lit. [21] 211–213°), 93%. The ^{1}H-NMR spectrum of a representative compound **4c** was as follows: δ (DMSO-d_6): 0.84 (t, 3H, CH_3), 1.24 [def s, 10H, $(CH_2)_5$], 1.26 [def t, 2H, CH_3 $(CH_2)_5C\underline{H}_2$], 2.12 (q, 2H, $C\underline{H}_2CH$=CH), 5.95–6.07 (m, 2H, CH=CH), 6.18 (s, 2H, NH_2), 7.47 (d, 1H, CH=N), 9.92 (s, 1H, $N\underline{H}CONH_2$) ppm.

6.1.4. Charge density calculations

The structures of the compounds **1a** and **1g**, **2a** and **2g**, **3a** and **3g**, **4b** and **4e** and **5a–d** were built and minimized using the HyperChem molecular modelling programme [22]. Initially the molecules were optimized using the MM^+ molecular mechanics module with the Polak-Ribiere algorithm (conjugant gradient). Molecules were deemed to be minimized when there was a minimum energy difference between cycles of less than

0.001 kcal/mol. Non-bonded electrostatic interactions were calculated using bond dipole interactions. The minimum energy conformers from the MM^+ calculations were obtained and the CNDO semiempirical force field was used to calculate their wave functions from which the charges listed in *table I* were generated.

6.2. Anticonvulsant evaluations

The screening of the compounds for anticonvulsant activity and neurotoxicity was undertaken by the National Institute of Neurological Disorders and Stroke, Bethesda, USA according to their protocols [8].

Side effects were noted in the mouse intraperitoneal NT screen in the case of the following compounds (dose of compound in mg/kg and time of observation in h in parentheses): anaesthesia: **1g** (300, 0.5), loss of righting reflex: **2b** (300, 0.5), **4d** (300, 0.5), death: **2g** (300, 0.5 and 4), **2j** (300, 4), **4a** (300, 0.5), **4d** (300, 0.5 and 4), unable to grasp rotorod: **2a** (300, 0.5), **2b** (300, 4), **2c** (300, 4), **2d** (300, 0.5 and 4), **2f** (300, 0.5 and 4), **2h** (300, 4), **2i** (300, 4), **2k** (300, 4), **2l** (300, 4), **4a** (300, 0.5), **5a** (300, 4), **5b** (300, 0.5). The neurological disorders noted in the scPTZ test were as follows: tonic extension: **2a** (100, 4), **2h** (30, 0.5), **2j** (30 and 300, 0.5), **3f** (100, 0.5), **5b** (30, 0.5), continuous seizure activity: **2a** (300, 4), **3f** (100, 0.5), **5a** (30, 100 and 300, 0.5), **5b** (300, 4), death following tonic extension: **2i** (300, 4), death following clonic seizure: **2l** (100 and 300, 4). In the rat oral scPTZ screen, death following continuous seizure activity was noted 0.25, 0.5 and 2 h after administration of 25 mg/kg of **2f**. In the case of phenytoin, NT was determined using a dose of 1 000 mg/kg at the following times (number of rats displaying neurotoxicity out of eight) ie. 0.25 (0), 0.5 (0), 1 (0), 2 (0), 4 (0), 6 (2), 8 (2) and 24 (1) h.

Quantitation of **4c** in the mouse i.p. MES, scPTZ and NT screens was undertaken at the time of peak effect, namely 1 h. The ED_{50} and TD_{50} figures (95% confidence intervals) in the MES, scPTZ and NT screens for **4c** were 21.26 (14.44–30.61), 53.88 (34.51–74.05) and 263.85 (140.96–628.86) mg/kg, respectively. The related figures for phenytoin were 6.32 (5.44–7.23), > 50 and 41.2 (36.9–46.1) mg/kg, respectively, determined 1, 1 and 0.5 h, respectively, after administration of the drug.

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