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Anticonvulsant activity of various aryl, arylidene and aryloxyaryl semicarbazones

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Abstract – A number of aryl, arylidene and aryloxyaryl semicarbazones were evaluated as candidate anticonvulsants. In particular, insertion of an olefinic group between the carbimino carbon atom and an aryl ring (referred to as the proximal ring) led to series **6** in which there was retention in activity and **6b,c** were shown to be useful lead molecules. At the doses utilized, neurotoxicity was absent in these compounds when given orally to rats. Attachment of a 2-naphthyloxy group at the 4 position of the proximal ring gave **7** whose high activity in the rat oral maximal electroshock (MES) screen suggested that the binding site of the second aryl ring was capable of accommodating groups with molecular refractivity values of over 40. The greatest activity was displayed by a series of aryloxyaryl semicarbazones **8** which had oral activity in the MES screen substantially greater than phenytoin and with protection indices of over 100. A binding site hypothesis formulated as a result of the biodata generated was in accord with the information obtained by X-ray crystallography. © Elsevier, Paris

semicarbazone / anticonvulsant activity / binding site hypothesis / structure–activity relationship / X-ray crystallography

1. Introduction

Current drug therapy for epilepsy suffers from a number of disadvantages including the fact that the convulsions of approximately 25% of epileptics are inadequately controlled by medication [1]. Thus the evolution of novel antiepileptic agents is an urgent necessity. For the evaluation of compounds as candidate anticonvulsants, three screens are often employed initially. The maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens are claimed to detect compounds affording protection against generalized tonic-clonic and generalized absence seizures, respectively [2]. Detection of neurotoxicity (NT) may be made using the rotorod test in mice and by observation in rats [3].

A few years ago, the promising anticonvulsant activity of a number of aryl semicarbazones **1** in the MES and scPTZ tests after intraperitoneal (i.p.) administration to mice was described [4]. After oral administration to rats, potency in the MES screen was increased, NT was diminished while activity in the scPTZ test was virtually abolished [4]. Two molecular modifications of **1** that were undertaken which led to increased potency in the oral MES screen in rats were as follows. First, insertion of an olefinic linkage between the aryl ring and the carbimino carbon atom led to **2** with increased activity and an improved protection index (PI i.e. TD_{50}/ED_{50} where the TD_{50} and ED_{50} figures represent the doses to cause NT and afford protection in 50% of the animals, respectively) [4]. Second, the introduction of another aryl group, referred to as the distal ring, linked via an oxygen or sulphur atom to the aryl moiety in **1** (referred to as the proximal ring) led to the semicarbazones **3**, some of which had substantially improved potencies and PI values than **1** [5].

The aims of the present investigation were to prepare a variety of analogues of **1–3**; those compounds showing

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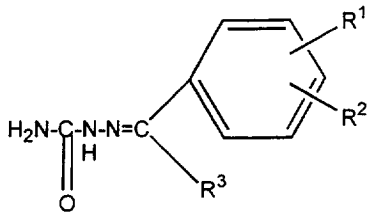
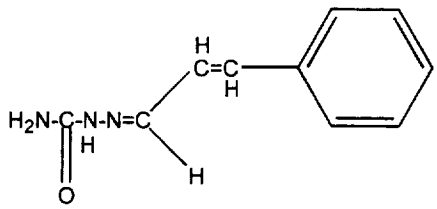


1

R^1=R^2=H, halogen, alkyl etc.

R^3=H, alkyl, aryl



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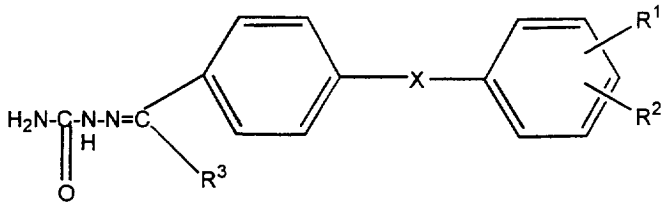
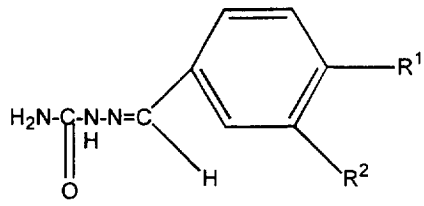


3

R^1,R^2=H, halogen, alkyl etc.

R^3=H,CH_3

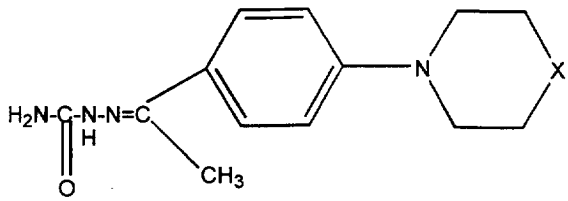
X=O,S



4

a: R^1=$O(CH_2)_3N(CH_3)_2$; R^2=H

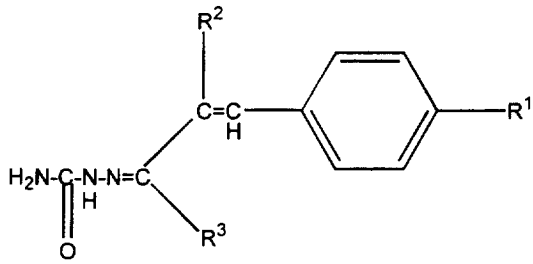
b: R^1=R^2=$OCH_2C_6H_5$



5

a: X=CH_2

b: X=O



6

a: R^1=Br; R^2=R^3=H

b: R^1=R^3=H; R^2=Br

c: R^1=OC_6H_4 4-F; R^2=R^3=H

d: R^1=R^2=H; R^3=C_6H_5

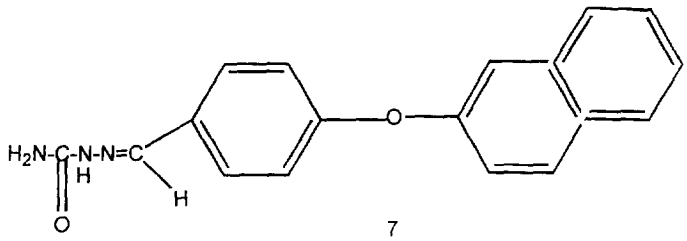
e: R^1=F; R^2=H; R^3=C_6H_5

f: R^1=R^2=H; R^3=C_6H_4 4-F

g: R^1=F; R^2=H; R^3=C_6H_4 4-F

h: R^1=R^2=H; R^3=$CH=CHC_6H_5$

Structures 1–6.



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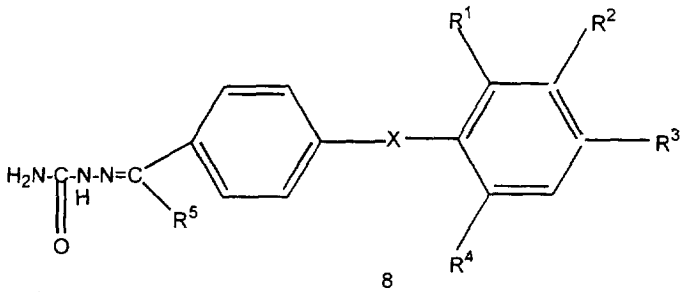


8

a: $R^1=CH_3$; $R^2=R^3=R^4=R^5=H$; X=O

b: $R^1=R^2=R^3=R^4=H$; $R^5=CH_3$; X=O

c: $R^1=R^2=R^3=R^4=H$; $R^5=CH_3$; X=S

d: $R^1=R^4=R^5=H$; $R^2=CH_3$; $R^3=F$; X=O

e: $R^1=CH_3$; $R^2=R^4=R^5=H$; $R^3=Cl$; X=O

f: $R^1=R^2=R^4=H$; $R^3=Br$; $R^5=CH_3$; X=S

g: $R^1=R^4=CH_3$; $R^2=R^3=R^5=H$; X=O

h: $R^1=R^2=R^4=H$; $R^3=R^5=CH_3$; X=O

i: $R^1=R^2=R^4=R^5=H$: $R^3=C(CH_3)_3$; X=O

j: $R^1=R^2=R^4=R^5=H$; $R^3=C(CH_3)_2CH_2CH_3$; X=O

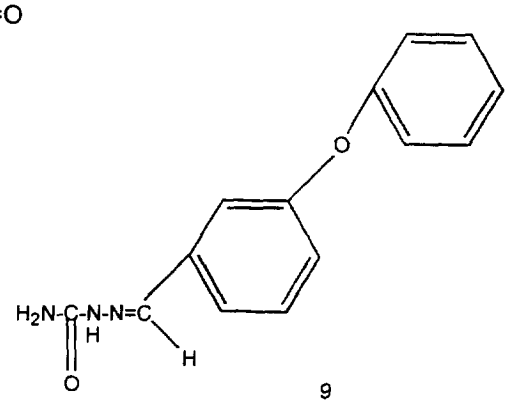
k: $R^1=R^2=R^4=R^5=H$; $R^3=CH(CH_3)C_2H_5$; X=O

l: $R^1=R^2=R^4=H$; $R^3=F$; $R^5=CH_3$; X=O

m: $R^1=R^2=R^4=H$; $R^3=Cl$; $R^5=CH_3$; X=O

n: $R^1=R^2=R^4=H$; $R^3=Br$; $R^5=CH_3$; X=O

o: $R^1=R^2=R^4=R^5=H$; $R^3=F$; X=O



9

Structures 7–9.

significant anticonvulsant activity would serve as prototypic molecules for subsequent molecular modification. In addition, the biodata generated may assist in refining the binding site hypothesis proposed for anticonvulsant semicarbazones [5, 6].

In order to achieve these objectives, the synthesis of **4–8** was planned for the following reasons. The increased activity of **3** in the MES screen compared to **1** may have been due, at least in part, to the presence of an electron rich atom attached at the para position of the aryl ring. Hence the preparation of **4** and **5** possessing one or two oxygen and/or nitrogen atoms was considered. Substitution in the aryl ring in series **1** by halogens led to a number of semicarbazones with low ED_{50} values in the rat oral MES screen accompanied by high PI values [7]. Hence the formation of **6a** was considered. The bioevaluation of the structural isomer **6b** may lead to an understanding of the importance of the size of the group at the olefinic double bond. Since the attachment of a 4-fluorophenoxy group to the aryl ring in series **1** led to compounds with marked increases in activity and PI values [5], the incorporation of this function to **2** leading to **6c** was considered to be of interest. In addition, this compound would have increased π bonding compared to **2** due to an additional aryl ring. Replacement of the proton on the carbimino carbon atom of **2** by phenyl (**6d**) or cinnamylidene (**6h**) would lead to increases in the size of the group at this position of the molecules. Such a modification could increase anticonvulsant activity due to additional van der Waals bonding or alternatively steric impedance to alignment at a binding site would occur leading to a reduction or abolition of bioactivity. Should **6d** display anticonvulsant activity, the presence of one or more fluoro atoms in the aryl rings (**6e–g**) may lead to marked increases in potency (see above).

The last cluster of compounds were based on series **3**. The anticonvulsant evaluation of **7** may shed some light on the size of the flat area on the binding site which interacts with the distal ring. Previous work revealed that the introduction of alkyl or halogen functions into the distal aryl ring led to compounds displaying high activity in the rat oral MES screen [5]. Since **8a–c** were promising lead molecules with ED_{50} figures of 5.65, 9.73 and 16.6 mg/kg in the rat oral MES screen [5], substitution in the distal aryl ring by halogens (**8d–f**) or methyl groups (**8d,e,g,h**) may lead to compounds with increased potencies. Previously the placement of a *t*-butyl group in the distal ring leading to **8i** was well tolerated at the binding site since ED_{50} figures in the mouse i.p. and rat oral MES screens of 8.87 and 1.68 mg/kg were observed [5]. Thus the quantitation in these tests of **8j**, which contains a *t*-pentyl group, was planned in order to ascertain the importance of the size and shape of the group at the 4 position of the distal ring on anticonvulsant activity.

Finally, after a review on the biological data had been generated, X-ray crystallography on selected molecules was planned in order to evaluate further the binding site hypotheses proposed for anticonvulsant semicarbazones [5, 6].

2. Chemistry

The semicarbazones **4a,b**, **5a,b**, **6b** were prepared by reaction of the appropriate aldehydes or ketones with semicarbazide. 4-Bromocinnamaldehyde was synthesized by the method outlined in *figure 1* except that 4-bromobenzaldehyde was used in place of 4-(4′-fluorophenoxy)benzaldehyde. *Figure 1* indicates the synthetic route to 4-(4′-fluorophenoxy)cinnamaldehyde. These two intermediates were reacted with semicarbazide to produce **6a,c**. A Claisen-Schmidt condensation between the appropriate aldehyde and 1-arylethanone led to the preparation of various chalcones which were converted into **6d–g**. Reaction of cinnamaldehyde with acetone led to 1,5-diphenyl-1,4-pentadien-3-one which on treatment with semicarbazide gave **6h**.

One of the initial attempts at the synthesis of an aryloxyaryl semicarbazone was made by treating 4-fluorobenzaldehyde semicarbazone with 4-chlorophenol in the presence of potassium carbonate and dimethylacetamide with a view to the preparation of 4-(4′-chlorophenoxy)benzaldehyde semicarbazone. The product isolated was identified by mass spectrometry, elemental analysis and independent synthesis as an azine whose structure, along with a proposed way in which it was prepared, is presented in *figure 2*. It is conceivable that the synchronous hydrolyses of the amidic and azomethine bonds would give rise to a hydrazone and an aldehyde which would interact yielding an azine. Subsequently the methodology employed involved the condensation of a 4-fluorophenyl aldehyde or ketone with the appropriate phenol or thiophenol followed by reaction with semicarbazide. This procedure was followed in the preparation of **7–9**.

3. Bioevaluations

The initial screening of **4a,b**, **5a,b**, **6a–h** has not been reported previously. It was undertaken using the mouse i.p. MES, scPTZ and NT screens and these data are summarized in *table I*. The activity of promising compounds was quantitated in both the mouse i.p. screen (*table II*) and/or the rat oral test (*table III*).

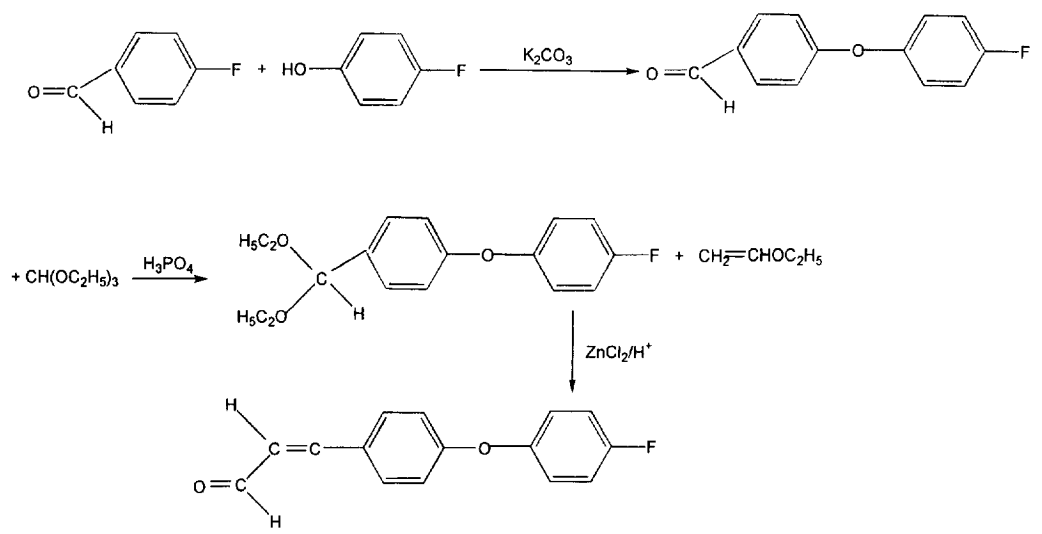


Figure 1. Schematic route to 4-(4′-fluorophenoxy)cinnamaldehyde.

4. Results and discussion

The evaluations of **4a,b**, **5a,b**, **6a–h** in the mouse i.p. MES, scPTZ and NT screens and for some of these compounds in the rat oral MES test are summarized in *table 1*. Compounds **4a,b**, **5a,b** possess oxygen or nitrogen atoms at the 4 positions of the proximal aryl ring. However, unlike many semicarbazones in series **3** these compounds were virtually devoid of protection in the MES and scPTZ screens suggesting the importance of the presence of the distal aryl group attached to a spacer atom between the two rings. While 4-benzyloxybenzaldehyde semicarbazone afforded protection in the mouse i.p. MES and scPTZ screens at 300 and 100 mg/kg, respectively [5], the introduction of a second benzyloxy group led to **4b** which was inactive. This observation suggests that the

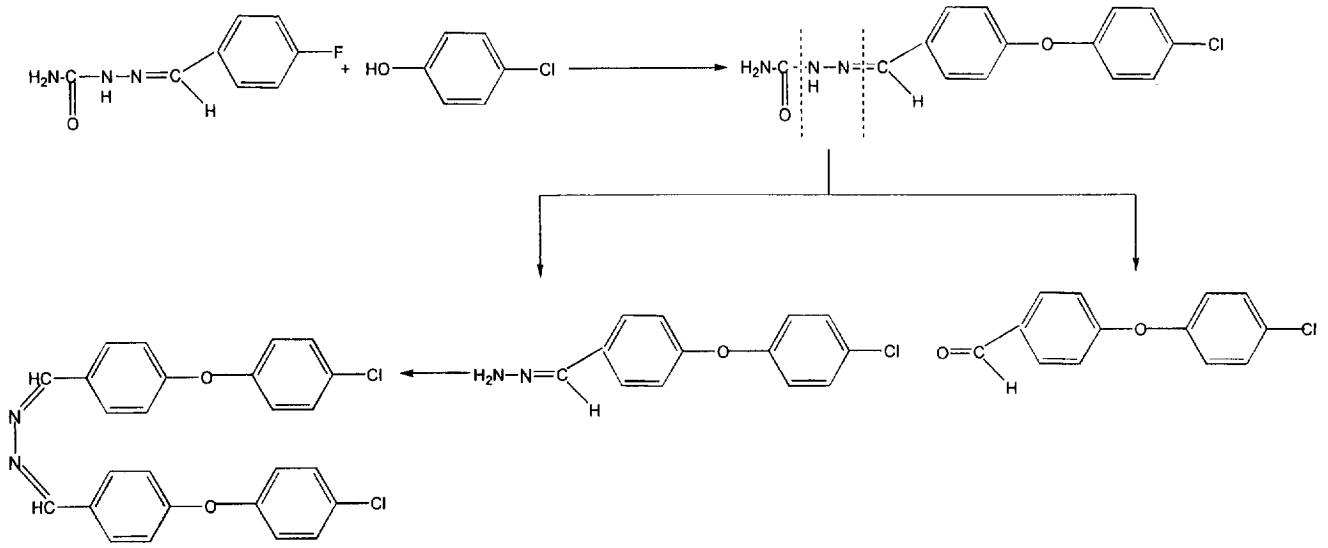


Figure 2. Formation of 4-(4′-chlorophenoxy)benzaldehyde azine.

Table I. Anticonvulsant evaluation of **4a,b**, **5a,b**, **6a–h** after intraperitoneal injection in mice and oral administration to rats.

Compound	Intraperitoneal injection in mice [a]						Oral administration to rats [b]					
	MES screen		scPTZ screen		NT screen		MES screen					
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	Dose (mg/kg)	0.25 h	0.5 h	1 h	2 h	4 h
4a	–	–	–	–	–	–	50	1	1	–	–	–
4b	–	–	–	–	–	–	×	×	×	×	×	×
5a	300	300	–	–	–	–	30	–	–	–	–	–
5b	–	–	–	–	–	–	×	×	×	×	×	×
6a	–	100	–	–	–	–	12.5	–	–	–	–	–
6b	100	–	30	–	300	–	50	4	4	4	3	3
6c	300	30	–	100	–	–	12.5	–	–	–	2	4
6d	300	300	–	–	–	–	×	×	×	×	×	×
6e	100	100	–	300	–	300	30	–	3	3	3	2
6f	–	100	–	300	–	300	30	–	3	1	4	1
6g	–	100	–	300	–	300	30	1	–	1	4	3
6h	–	–	–	–	–	–	×	×	×	×	×	×
Phenytoin	30	30	–	–	100	100	×	×	×	×	×	×

[a] Mice were injected with doses of 30, 100 and 300 mg/kg of the compounds in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and neurotoxicity (NT) screens and examined after 0.5 and 4 h. The figures are the doses affording protection or causing neurotoxicity in 50% or more of the animals. The designation – indicates the absence of activity or toxicity at the maximum dose administered (300 mg/kg) and × means that the compound was not screened.
[b] The figures in the table indicate the number of rats protected out of four 0.25, 0.5, 1, 2 and 4 h after administration. The designation – and × are the same as in [a].

additional bulky 3-benzyloxy group caused interference with alignment at a binding site (see *figure 3*).

Compounds **6a–h** were designed to examine the importance of the insertion of an olefinic group between the carbon atom attached to the semicarbazono group and an aryl ring. The semicarbazone **2** afforded protection in the mouse i.p. MES and scPTZ tests at 100 mg/kg and in the rat oral screen at 50 mg/kg. The data in *table I* for the mouse i.p. screens revealed that activity was increased (**6b,c**), retained (**6a,d,e–g**) or abolished (**6h**) relative to **2**. The presence of a 4-fluorophenoxy group (**6c**) but not a bromo atom (**6a**) led to greater potency than **2**. Increasing

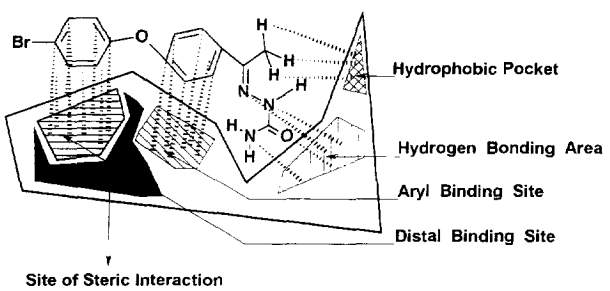


Figure 3. The proposed interaction of a representative compound **8n** at the binding site of anticonvulsant semicarbazones.

the size of the olefinic group in **6b** also raised activity compared to **2**. Replacement of the methine proton of **2** by a phenyl ring reduced activity (**6d**) but the insertion of a fluoro atom into the aryl rings led to **6e–g** with similar potency as **2**. If detoxification by hydroxylation at the 4 position of the aryl rings occurs, then this process will be blocked in one or both of the aryl rings in **6e–g** which may account for their greater potency than **6d**. Replacement of the methine proton of **2** by a styryl group led to **6h** which was devoid of anticonvulsant activity at the maximum doses employed. These results indicate that an olefinic group may be inserted between the carbimino carbon atom and an aryl ring with retention of anticonvulsant activity. In particular, **6b,c** are promising lead molecules. In this regard, quantification of the bioactivity of **6b** in the MES, scPTZ and NT screens was undertaken and the results are presented in *table II*. The ED_{50} values of **2** in the MES and scPTZ screens were 48.5 and 84.7 mg/kg, respectively with PI figures of 2.03 and 1.16, respectively [4]. Thus while anticonvulsant activity is the same for both compounds, the PI values of **6b** are approximately double that of **2**.

Compounds **6a–c,e–g** were examined for activity in the rat oral MES screen and these data are presented in *table I*. Initially doses of 50 mg/kg were employed but since complete protection using this dose was found early

Table II. Quantitative evaluation of selected compounds in the MES, scPTZ and NT screens after intraperitoneal injection in mice.

Compound	MES screen			scPTZ screen			NT 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	scPTZ
6b	0.25	48.6 (34.6-61.4)	5.98 (1.90)	0.25	94.1 (83.3-119)	8.53 (2.93)	1	204 (1.82-227)	12.1 (3.01)	4.20	2.17
7	2	26.8 (23.1-30.9)	10.5 (3.16)	2	> 500	–	4	> 500	–	> 18.7	–
8d	1	7.18 (5.65-9.20)	5.15 (1.57)	1	> 125	–	2	71.5 (55.4-99.1)	6.23 (1.85)	9.96	< 0.57
8e	2	6.97 (4.86-9.35)	4.07 (1.09)	2	> 100	–	2	46.05 (36.1-49.1)	32.5 (15.0)	6.60	< 0.46
8g	0.5	23.6 (17.1-29.7)	5.51 (1.79)	0.5	42.2 (33.2-52.8)	6.18 (1.88)	1	132 (106-163)	7.91 (2.07)	5.58	3.12
8h	1	5.62 (3.67-8.30)	3.68 (1.06)	1	19.2 (12.7-26.7)	3.95 (1.12)	1	57.9 (43.9-75.3)	6.43 (2.23)	10.3	3.02
8j	2	34.6 (28.3-49.0)	6.15 (1.93)	2	> 250	–	4	> 500	–	> 14.4	–
Phenytoin	1	6.32 (5.44-7.23)	11.2 (3.52)	1	> 50	–	0.5	41.2 (36.9-46.1)	14.4 (4.82)	6.52	< 0.82

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}.

in the bioevaluations, the dose was subsequently reduced. Hence a comparison of the potencies of different compounds was precluded. Nevertheless the general trends noted in the mouse i.p. screens were observed. Thus **6b,c** afforded complete protection against seizures confirming their potential utility as prototypic molecules and activity was noted with **6e–g** in the MES screen after oral administration. Compounds **6b,c** were examined in the scPTZ screen using doses of 50 and 25 mg/kg, respectively. The only activity noted was at the end of 0.5 (**6b**) and 0.5 and 2 h (**6c**) whereby 1/4 animals was protected. The semicarbazones **6a–c,e–g** were also evaluated for NT using the doses specified in *table I* except 25 mg/kg of **6c** was employed. No neurological deficit was noted.

In terms of interactions at the binding site, the aryl ring in **2,6a–g** may overlap both the proximal and distal binding sites. In the case of **6d–g**, it is of course conceivable that the aryl ring attached directly to the carbimino carbon atom may align with the aryl binding site and the β-arylethylenic group could be positioned at the hydrophobic pocket indicated in *figure 3*. However, the lack of activity of **6h** suggested that the hydrophobic pocket can accommodate an aryl ring but not a β-arylethylenic function.

The qualitative assessment of **7–9** after i.p. injection into mice as well as oral administration to rats have been described previously [5] and unreported quantitative data are portrayed in *tables II* and *III*. The results in *table II* revealed that all of the compounds afforded greater protection in the MES test than the scPTZ screen. A comparison of the data in *tables II* and *III* indicated that **7**, **8e,g,h,j** had lower ED_{50} figures in the MES test when given orally to rats than by intraperitoneal injection in mice. Previously a monocyclic distal aryl ring was incorporated into the design of the compounds in series **3**. An increase in the size of the distal ring led to **7**. An important feature of this compound was the absence of NT in the mouse i.p. screen at the maximum dose utilized namely 500 mg/kg. This observation is in contrast to all previous quantitative evaluations of the compounds in series **3** whereby toxicity was displayed at doses well below 500 mg/kg [5]. Compound **7** had 24% of the activity of phenytoin in the MES screen accompanied by a much higher PI value than the reference drug. The result in the rat oral MES screen was also encouraging insofar as a complete absence of NT over a 24 hour period was recorded. The potency and PI figures of **7** were more than five times that of phenytoin. One may note that **7** is a most promising prototypic molecule. Furthermore, the molecular refractivity (MR) values of the napththyl and phenyl groups are 41.59 and 25.36, respectively [8], and hence the size of the distal binding site is sufficiently large to accommodate an aryl group having a 64% greater size than a phenyl ring.

The incorporation of methyl groups with (**8d,e**) or without (**8g,h**) halogens into the distal aryl ring was undertaken. The data in *table II* revealed that the semicarbazones **8d,e,h** were equipotent with phenytoin in the MES screen. In addition, **8d,h** had higher PI values in this test than the established drug. Furthermore, **8h** has an

Table III. Quantitative evaluation of selected compounds in the MES and NT screens after oral administration to rats.

Compound	MES screen			NT screen			PI [a]
	t (h)	ED_{50} (mg/kg) (95% CI)	Slope (SE)	*t* (h)	TD_{50} (mg/kg) (95% CI)	Slope (SE)	
7	2	4.52 (3.00-6.12)	3.18 (0.85)	0.25-24 [b]	> 500	–	> 111
8e	4	1.12 (0.80-1.40)	6.21 (1.90)	2	144 (87.4-186)	6.00 (2.41)	128
8f	2	3.20 (2.01-5.08)	3.20 (0.94)	0.25-24 [b]	> 500	–	> 156
8g	1	6.13 (4.64-8.17)	5.20 (1.86)	2	> 101	–	> 16.5
8h	1	4.80 (3.15-7.24)	3.70 (1.05)	0.25-24 [b]	> 500	–	> 104
8j	4	1.52 (0.81-2.67)	2.06 (0.59)	0.25-24 [b]	> 500	–	> 329
Phenytoin	2	23.2 (21.4-25.4)	15.1 (4.28)	0.25-24 [b]	> 500	–	> 21.6

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}; [b] the compound was examined for NT at the end of 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h.

ED_{50} value of 19.2 mg/kg in the scPTZ screen whereas phenytoin showed no activity at doses up to and including 50 mg/kg. The activity in the rat oral MES screen for **8e,f–h** as revealed in *table III* indicated potencies 21, 7, 4 and 5 times that of phenytoin. The compounds did not display NT at the doses utilized with the exception of **8e**; nevertheless a PI value of 128 was achieved with this compound. One may conclude that in general the introduction of methyl and halogeno substituents into the distal ring leading to **8d–h** afforded compounds with comparable activity as phenytoin in the MES test when given intraperitoneally to mice. However, significantly greater potencies and PI values were obtained by oral administration to rats. Thus the distal binding site is capable of accommodating an aryl ring with at least two substituents on the distal ring.

The data in *table II* revealed that **8j** had 26% of the activity of **8i** in the MES screen but no NT was observed at 500 mg/kg in contrast to **8i** for which a TD_{50} figure of 106 mg/kg was recorded [5]. When administered orally to rats, the potency of **8j** was identical to **8i** and the absence of NT at the highest dose led to a PI figure of > 329 indicating its utility as a very promising lead molecule. The MR value of the *t*-pentyl substituent is 24.19 [9] and combined with a phenyl group (MR = 25.36) suggests that the distal binding site can accommodate groups even larger in size than the naphthyl function noted earlier.

The bioactivities noted suggested that the binding site for the semicarbazones affording protection in the MES screen may be represented as shown in *figure 3*. In order to examine this hypothesis further, X-ray crystallography of selected molecules was planned. The data in *tables II* and *III* revealed that the most promising compounds prepared in this study were the aryloxyaryl semicarbazones especially in view of their oral activity which is the preferred route of administration of antiepileptic drugs particularly for long-term therapy. Suitable crystals were obtained for **8k–n**. The semicarbazone **8k** has a large s-butyl substituent and **8l–n** have a methyl group on the carbimino carbon atom which may alter the alignment of the proximal and distal aryl rings compared to those analogues which have a proton at this function. The ORTEP diagrams of **8k–n** are portrayed in *figures 4-7*. For comparative purposes, two other compounds were chosen. First, the spatial orientation of **8o**, which is currently undergoing preclinical evaluation, may be compared to the analogs especially **8l**. Second, **9** was shown to be inactive in the rat oral MES screen using a dose of 50 mg/kg [5]. The X-ray crystallographic data of these six compounds was processed and the shapes of these molecules are presented in *figure 8*. In this diagram superimposition of four of the atoms in the semicarbazono group has taken place. The ED_{50} values in the rat oral MES screen of **8k–o** were 3.21, 3.37, 2.92, 1.52 and 1.59 mg/kg, respectively [5]. At the 95% confidence intervals, **8k,m** were equiactive with the other compounds while **8l** was equiactive with **8k,m** only and was less potent than **8n,o**.

The log *P* values of **8l,o** are 1.91 and 2.03, respectively which are similar to the figure obtained for the semicarbazone **9** namely 2.07 [5]. Hence it is unlikely that the

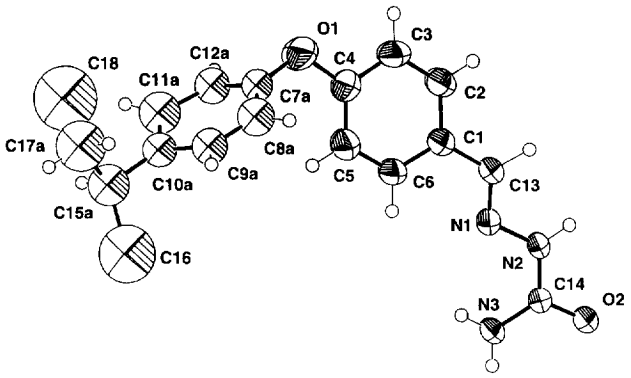


Figure 4. ORTEP diagram of **8k**.

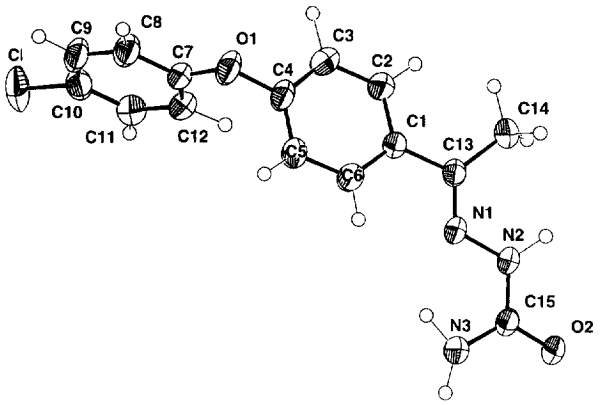


Figure 6. ORTEP diagram of **8m**.

difference in anticonvulsant activity between **8k–o** and **9** is due to variations in the extent/rate of transportation to a site of action i.e. the potency variation is a pharmacodynamic effect rather than a pharmacokinetic one. This being so, the alignment of the molecules at a binding site will profoundly affect bioactivity.

The following observations were made from the ORTEP diagrams of **8k–o**, **9** in *figure 8*. First, the proximal rings of **8l–n**, which have a methyl group attached to the carbimino carbon atom, are superimposable. Likewise **8k,o**, **9** have a proton at this juncture whose proximal rings overlap. The distal rings of **8l–n** also occupy a similar location while those of **8k,o** are found above. The average ED_{50} value in the rat oral MES screen for **8l–n** was 2.60 mg/kg while for **8k,o** the comparable figure was 2.40 mg/kg revealing the spatial tolerance of the proximal and distal binding sites. The width of the group at the 4 position of the distal ring of **8k** reinforces the view that the distal binding site is capable of accommodating bulky groups. Since the ED_{50} figure for benzaldehyde semicarbazone is 22.50 mg/kg in the rat oral MES screen [4], the lack of marked potency of **9** suggests its interaction at a site of steric interference and presumably prevents bonding at the binding sites.

An investigation was made to quantify the positions of the proximal and distal rings in relation to the ureido group. Such information will be useful in the design of further compounds not only with a view to increasing potency but for determining the spatial requirements for anticonvulsant activity in this novel series of anticonvulsants. *Figure 9* indicates the measurements made based on the X-ray crystallographic data. The terms d_p and d_d are the distances between the centres of the proximal and distal rings and the carbon atom of the ureido group. A

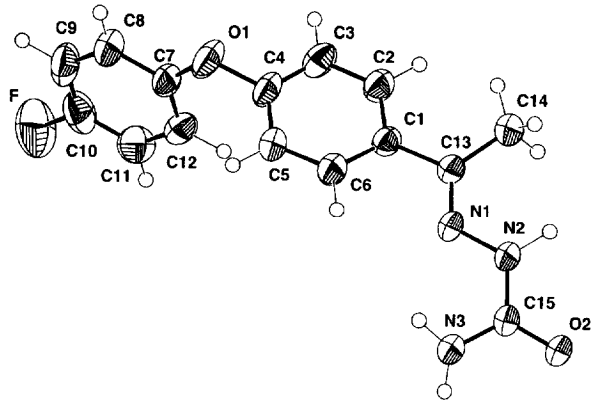


Figure 5. ORTEP diagram of **8l**.

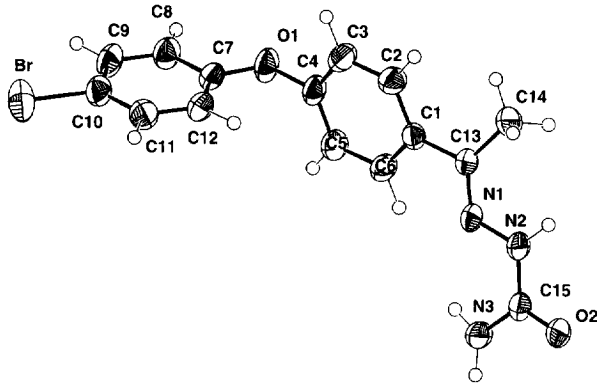


Figure 7. ORTEP diagram of **8n**.

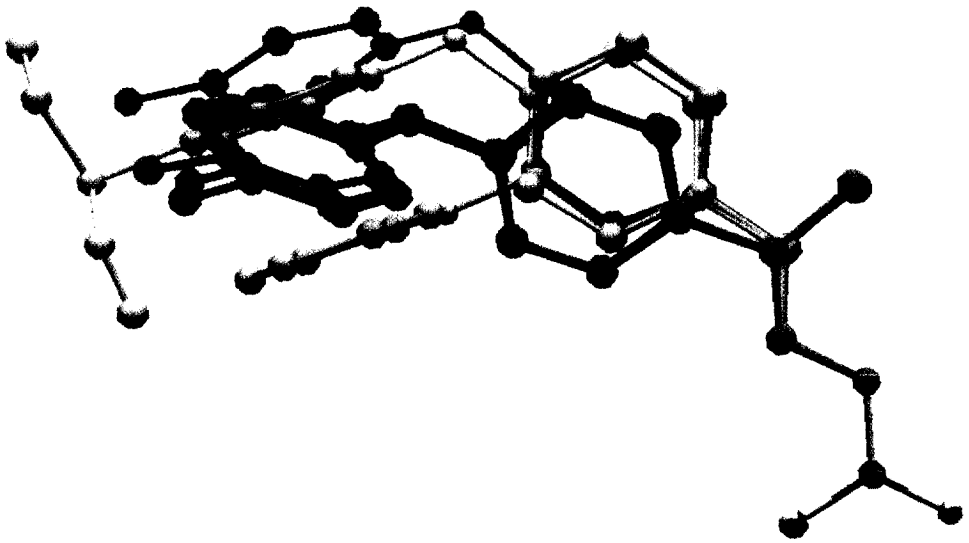


Figure 8. Orientation of **8k** (yellow), **8l** (red), **8m** (blue), **8n** (orange), **8o** (green) and **9** (grey) when the N3, C14(C15), O2, N2 atoms of each molecule were superimposed. For the sake of clarity, the hydrogen atoms were omitted. The data for **8o** and **9** were taken from [5] with the permission of the American Chemical Society. In the case of **8k**, which was a disordered racemic mixture, the diagram shows the dimensions occupied by the s-butyl group of both isomers.

vertical axis along the $C_{14}(C_{15})$–N_2 axis was constructed and the angles between this axis and the centres of the aryl rings were designated as θ_p and θ_d. In addition, the aryl rings can be located above or below the plane of the ureido group. The distances d'_p and d'_d and angles φ_p and φ_d reflect the locations of the aryl rings either above or below the plane of the ureido group. These measurements are illustrated in *figure 9* for a compound where both rings are above the plane of the ureido function and in *table IV* are displayed as positive values. Conversely negative figures reflect the locations of the aryl rings below the N_2–$C_{14}[C_{15}]$–(O_2)–N_3 plane.

The following observations were made regarding the proximal aryl rings of **8k–o**, **9**. The data in *table IV* revealed that there was only a 3% variation in the distance d_p for all six compounds. However the insertion of a methyl group on the carbimino carbon atom caused an increase in the θ_p angle by 59%; the average θ_p figure for **8k,o**, **9** being 33.18° while the corresponding value for **8l–n** was 52.74°. The proximal rings of **8k,o**, **9** were virtually coplanar with the ureido group. On the other hand for **8l–n**, which contain a methyl group on the carbimino function, the d'_p and φ_p figures revealed displacements of approximately 0.7 Å and 6.7°. The proximal binding site therefore is tolerant to some perturbations in the locations of the aryl ring.

In regard to the distal aryl rings of **8k–o**, **9**, the following conclusions were drawn. For the compounds displaying marked potency namely **8k–o**, the variation in the d_d values was less than 2%. However for **9**, there was an 18% reduction in the d_d distance compared to the average figure for the other five compounds. The semicarbazones **8k–o** had θ_d angles of between 41° and 59° which confirms the supposition expressed earlier that the distal binding site occupies an area larger than one aryl ring. The virtually inactive compound **9** had the highest θ_d angle. The d'_d and φ_d values revealed that the

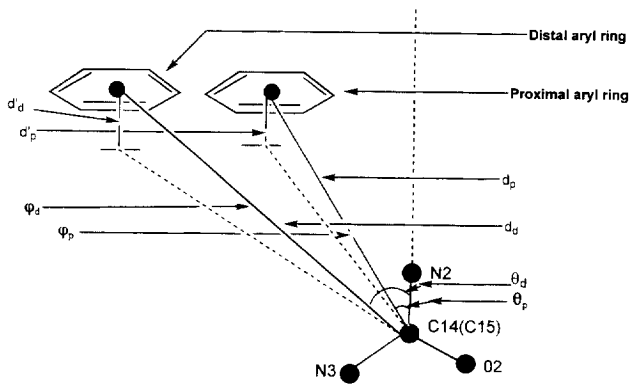


Figure 9. The positions of the proximal and distal aryl rings in relation to the ureido group of **8k–o**, **9** as determined by X-ray crystallography. Explanations of the various symbols are given in the text.

Table IV. Certain distances (Å) and angles (°) in **8k–o, 9** determined by X-ray crystallography. [a]

Compound	d_p	d_d	θ_p	θ_d	d'_p	d'_d	φ_p	φ_d
8k	5.89	9.98	34.42	54.56	0.02	0.77	0.15	4.41
8l	5.89	10.06	58.22	41.01	– 0.66	– 2.97	– 6.64	– 16.46
8m	5.88	10.01	58.84	41.32	– 0.69	– 3.12	– 6.69	– 17.34
8n	5.88	9.98	41.17	58.69	0.67	3.19	6.51	17.71
8o	6.07	10.13	32.65	50.59	– 0.21	0.71	– 2.02	4.02
9	6.06	8.53	32.46	64.21	– 0.16	2.33	1.55	15.27

[a] Explanations of the terms d_p, d_d, θ_p, θ_d, d'_p, d'_d, φ_p and φ_d are given in the text and illustrated in *figure 9*.

displacements of the distal rings were greater than in the case of the proximal rings in all cases. High activity was shown in compounds where the displacements were in the region of 0.7 to – 3.1 Å and 4 – 17°. The distal rings having the greatest displacement from the plane of the ureido group were **8l–n** which possess a methyl group on the carbimino function.

One may observe from these three dimensional measurements an appreciation of the different locations of the aryl rings which permit very high activities in the rat oral MES screen to be displayed by these compounds.

5. Conclusions

This study has identified a number of highly promising anticonvulsants from series **6–8** which serve as prototypic compounds for subsequent molecular modifications. The biodata generated led to the fine tuning of a binding site hypothesis which was in accord with the information garnered from X-ray crystallography.

6. Experimental protocols

6.1. Chemistry

Melting points are uncorrected. Elemental analyses (C,H,N) undertaken on **4a,b**, **5a,b**, **6a–h** and 4-(4′-chlorophenoxy) benzaldehyde azine prepared as per *figure 2* were within 0.4% of the calculated values. ^{1}H NMR spectroscopy was determined routinely using a Varian T-60 spectrometer. In addition, a Bruker AM 300FT instrument (300 MHz) was employed. Infrared spectra were determined as potassium bromide discs using a Beckman Acculab 4 machine. The mass spectrum was obtained using a VG 705Q machine. The accurate mass measurement was determined when the temperature of the solid probe was 200 C under electron impact conditions and an accelerating voltage of 6000 v. The source temperature was 200 °C. The instrument resolution was set to 7000 (140 ppm). The reference compound was perfluorokerosene and mass to charge ratios of 454.97284 and 466.97284 as reference markers were employed.

6.1.1. Synthesis of the semicarbazones ***4–9***

The aldehydes and ketones used in the synthesis of **4a,b**, **5a,b**, **6b** were obtained from the Aldrich Chemical Company, Inc., Milwaukee, WI, USA. 4-Bromocinnamaldehyde, required for the preparation of **6a**, was synthesized by a literature method [10]. 4-(4′-Fluorophenoxy)benzaldehyde was prepared by a reported procedure [5] and converted into 4-(4′-fluorophenoxy) cinnamaldehyde by a method which has been described previously [10]. The ketones used in the synthesis of **6d–h** were obtained by a literature method [11] and purified by recrystallization from ethanol. These intermediates were converted into the corresponding semicarbazones using a literature procedure [5] and purified by recrystallization from 95% ethanol. The melting points (°C) and yields (%) were as follows: **4a**: 182–184, 70; **4b**: 148–149, 60; **5a**: 234–235, 54; **5b**: 265–266, 46; **6a**: 240, 78; **6b**: 212–214, 60; **6c**: 205, 69; **6d**: 168, 60; **6e**: 202–204, 49; **6f**: 212–214, 40; **6g**: 218–220, 20; **6h**: 186–188, 40. The ^{1}H NMR spectrum (300 MHz) of a representative compound **6c** was as follows: δ (DMSO-d_6): 7.80 (s,1H, NH), 6.70–7.20 (m, 11H, carbimino, olefinic and aryl H), 6.20 (s, 2H, NH_2). The preparation of **7**, **8a–o**, **9** has been described previously [5].

6.1.2. Synthesis of 4-(4′-chlorophenoxy)benzaldehyde azine

A mixture of 4-fluorobenzaldehyde semicarbazone [7] (0.0144 mol), 4-chlorophenol (0.0204 mol), potassium carbonate (2.3 g) in dimethylacetamide (25 mL) was heated at 156 °C for 5 h under nitrogen. On cooling, water (100 mL) was added and the mixture was extracted with chloroform. The organic extract was washed with aqueous sodium hydroxide solution (4% w/v) and water and dried over anhydrous magnesium sulfate. The solvent was removed leaving a solid which was recrystallized from 95% ethanol to give 4-(4′-chlorophenoxy)benzaldehyde azine, m.p. 216 °C in 40% yield. Electron impact mass spectrum: calculated for $C_{26}H_{18}Cl_2N_2O_2$: 460.0742; found: 460.0745.

The title compound was also prepared by the following route. Hydrazine hydrate (0.01 mol) was added to a stirring solution of 4-(4′-chlorophenoxy)benzaldehyde [5] (0.02 mol) in methanol (25 mL) and the mixture was heated under reflux for 1 h. The resultant precipitate was collected, dried and recrystallized from 95% ethanol to give 4-(4′-chlorophenoxy)benzaldehyde azine, m.p. 205–207 °C in 60% yield. The infrared spectra of the products obtained from both reactions were identical.

6.1.3. X-ray crystallographic determinations on ***8k–n***

The compounds were crystallized from isopropanol-ethanol (**8k**), methanol-isopropanol (**8l,n**) and methanol-carbon tetrachlo-

ride (**8m**) by the vapour diffusion method. An Enraf-Nonius CAD-4 diffractometer with ω scan was used for data collection and the structures were solved by direct methods using XTAL 3.5 [12] and refined using SHELXL 97 [13]. Structure diagrams were drawn using ORTEP II [14] as implemented in XTAL 3.5 [12]. Atomic scattering factors and anomolous dispersion corrections were taken from the literature [15]. Unless indicated otherwise, all non-hydrogen atoms were found on the E-map and refined anisotropically. Hydrogen atoms were calculated and not refined.

The data for **8k** were as follows: $C_{18}H_{21}N_3O_2$, $M_r = 311.38$, colourless plates, $0.50 \times 0.20 \times 0.08$ mm, $a = 12.8815(24)$, $b = 7.9592(12)$, $c = 18.0640(21)$ Å, $\beta = 103.738(17)$, $V = 1799.1(5)$ Å^3, $Z = 4$, space group = $P2_1/a$, monoclinic, $D_x = 1.150$ g cm^{-3}, $\lambda(MoK\alpha) = 0.7093$ Å, $\mu = 0.7$ cm^{-1}, $F(000) = 664$, $T = 287$ K, $(\sin\theta)/\lambda_{max} = 0.5588$ Å^{-1}, $-14 \le h \le 13$, $0 \le k \le 8$, $0 \le l \le 20$. A total of 2761 reflections were measured of which 2620 were independent. Merging R is based on intensities 0.005 for 141 replicate reflections, 1578 observed reflections with $I > 2\sigma(I)$. The refinement on F^2, $R[F^2 > 2\sigma(F^2)] = 0.0723$, $wR(F^2)$ [all data] = 0.2584, $S = 1.055$. The refinement of the structure used 2620 observed reflections, parameters refined = 190, $w = 1/[\sigma^2(F_o)^2 + (0.1487P)^2 + 0.8790P]$ where $P = (F_o^2 + 2F_c^2)/3$; final $(\Delta/\sigma)_{max} = 0.000$. $\Delta\rho$ in the final difference map within + 0.53 and − 0.32 e Å^{-3}. The sample used was a racemic mixture of the two optical isomers formed by the asymmetric carbon atom (C15) of the s-butyl group. The two isomers occupy equivalent sites in the crystal packing, but the asymmetry of the s-butyl group results in disorder of the 4-s-butylphenoxy moiety. This disorder was described by using U_{iso} for all atoms of the 4-s-butylphenoxy unit. Two different 4-s-butylphenoxy groups with populations of 0.5 were found, offset by approximately 0.5 Å. The two sets of atoms were resolved except for the terminal carbons of the s-butyl group, where the population parameters were both 1.0, because the atoms from both isomers have coincident positions. Thus, C16 is a methyl when C18 is at the ethyl end of the butyl moiety and C18 is a methyl when C16 is at the ethyl end of the butyl moiety. H atoms were placed by geometry on all C and N atoms except C16 and C18.

The data for **8l** were as follows: $C_{15}H_{14}N_3O_2F{\bullet}CH_3OH$, $M_r = 319.33$, colourless blocks, $0.55 \times 0.5 \times 0.15$ mm, $a = 6.5348(5)$, $b = 8.6161(7)$, $c = 14.4411(9)$ Å, $\alpha = 104.174(7)$, $\beta = 96.694(6)$, $\gamma = 91.024(7)$, $V = 782.06(10)$ Å^{-3}, $Z = 2$, space group = $P\bar{1}$, triclinic, $D_x = 1.356$ g cm^{-3}, $\lambda(MoK\alpha) = 0.7093$ Å, $\mu = 1.0$ cm^{-1}, $F(000) = 336$, $T = 287$ K, $(\sin\theta)/\lambda_{max} = 0.5622$ Å^{-1}, $-7 \le h \le 7$, $0 \le k \le 9$, $-16 \le l \le 15$. A total of 2524 reflections were measured of which 2289 were independent. Merging R is based on intensities 0.007 for 235 replicate reflections, 1487 observed reflections with $I > 2\sigma(I)$. Refinement on F^2, $R[F^2 > 2\sigma(F^2)] = 0.0382$, $wR(F^2)$ [all data] = 0.1186, $S = 1.003$. The refinement of the structure used 2289 reflections. Parameters refined = 210, $w = 1/[\sigma^2(F_o^2) + (0.0534P)^2 + 0.11808P]$ where $P = (F_o^2 + 2F_c^2)/3$; final $(\Delta/\sigma)_{max} = 0.000$. $\Delta\rho$ in the final difference map within + 0.21 and − 0.20 e Å^{-3}.

The data for **8m** were as follows: $C_{15}H_{14}ClN_3O_2{\bullet}CH_3OH$, $M_r = 335.79$, colourless plates, $0.45 \times 0.30 \times 0.20$ mm, $a = 6.6250(6)$, $b = 8.5941(6)$, $c = 14.7183(10)$ Å, $\alpha = 77.691(6)$, $\beta = 82.854(6)$, $\gamma = 89.980(7)$, $V = 812.08(11)$ Å^3, $Z = 2$, space group = $P\bar{1}$, triclinic, $D_x = 1.373$ g cm^{-3}, $\lambda(MoK\alpha) = 0.7093$ Å, $\mu = 2.42$ cm^{-1}, $F(000) = 352$, $T = 287$ K, $(\sin\theta)/\lambda_{max} = 0.5940$ Å^{-1}, $-7 \le h \le 7$, $0 \le k \le 10$, $-16 \le l \le 17$. A total of 3113 reflections were measured of which 2842 were independent. Merging R is based on intensities 0.007 for 271 replicate reflections, 2205 observed reflections with $I > 2\sigma(I)$. Refinement on F^2, $R[F^2 > 2\sigma(F^2)] = 0.0371$, $wR(F^2)$ [all data] = 0.1119, $S = 1.035$. The refinement of the structure used 2842 reflections. Parameters refined = 207, $w = 1/[\sigma^2(Fo^2) + (0.0547P)^2 + 0.2888P]$ where $P = (F_o^2 + 2F_c^2)/3$; final $(\Delta/\sigma)_{max} = 0.000$. $\Delta\rho$ in the final difference map within + 0.24 and − 0.28 e Å^{-3}.

The data for **8n** were as follows: $C_{15}H_{14}N_3O_2Br{\bullet}CH_3OH$, $M_r = 380.24$, colourless plates, $0.45 \times 0.20 \times 0.15$ mm, $a = 6.6682(4)$, $b = 8.5537(4)$, $c = 15.0303(11)$ Å, $\alpha = 78.125(5)$, $\beta = 82.121(5)$, $\gamma = 89.807(5)$, $V = 830.76(9)$ Å^3, $Z = 2$, space group = $P\bar{1}$, triclinic, $D_x = 1.520$ g cm^{-3}, $\lambda(MoK\alpha) = 0.7093$ Å, $\mu = 24.6$ cm^{-1}, $F(000) = 388$, $T = 287$ K, $(\sin\theta)/\lambda_{max} = 0.5958$ Å^{-1}, $-7 \le h \le 7$, $0 \le k \le 10$, $-17 \le l \le 17$. A total of 3192 reflections were measured of which 2914 were independent. Merging R is based on intensities 0.010 for 278 replicate reflections, 2143 observed reflections with $I > 2\sigma(I)$. Refinement on F^2; $R[F^2 > 2\sigma(F^2)] = 0.0325$, $wR(F^2)$ [all data] = 0.0959, $S = 1.021$. The refinement of the structure used 2914 reflections. Parameters refined = 210, $w = 1/[\sigma^2(F_o^2) + (0.0531P)^2 + 0.3067P]$ where $P = (Fo^2 + 2F_c2)/3$; final $(\Delta/\sigma)_{max} = 0.000$. $\Delta\rho$ in the final difference map within + 0.26 and − 0.44 e Å^{-3}.

6.2. Screening

6.2.1. Anticonvulsant evaluation of compounds

The examination of the semicarbazones **4–7**, **8d–h,j** was undertaken by the Anticonvulsant Screening Project of the Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, USA, according to their protocols [3]. Compounds **7**, **8d,f–h,j** were evaluated using doses up to and including 202 (**8g**) or 250 (**7**, **8d,f,h,j**) mg/kg in the scPTZ screen after oral administration to rats. No anticonvulsant activity was observed except for **8h** where 2, 3 and 2 of 10 rats were protected at doses of 62.5, 125 and 250 mg/kg, respectively.

Acknowledgements

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