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Laboratory note

Evaluation of the thiosemicarbazones of some aryl alkyl ketones and related compounds for anticonvulsant activities

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Summary — The thiosemicarbazone of acetophenone (**1a**) had been shown previously to afford protection against experimentally-induced seizures. This report describes the systematic chemical modification of **1a** and the activities of these analogues in the maximal electroshock seizure (MES) test, subcutaneous maximal pentylenetetrazole seizure (scPTZ) test and neurotoxicity screen in mice when administered by the intraperitoneal route. Most of the compounds were active and representative compounds examined for oral activity in rats revealed that in most cases protection against seizures induced in the MES screen but not the scPTZ test was achieved at a dose of 50 mg/kg. Some correlations between chemical structures and anticonvulsant properties were found.

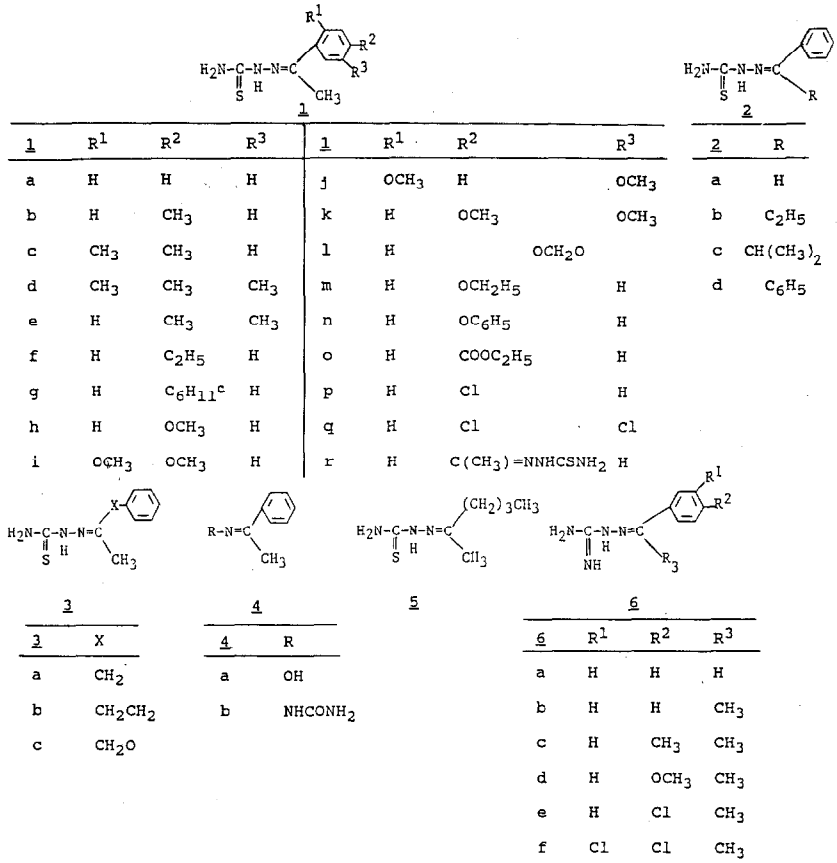
Résumé — Evaluation de thiosemicarbazones de quelques arylalkylcétones et composés apparentés pour leurs activités anticonvulsivantes. *On a déjà démontré que l'acétophénone thiosemicarbazone (**1a**) protège contre les crises déclenchées expérimentalement. Cet article décrit la modification systématique de la molécule **1a** et l'activité observée dans les tests d'électrochoc maximal (ECM) et d'injection sous-cutanée de pentétrazole (ScPTZ). La neurotoxicité est évaluée lorsque l'on administre ces analogues par une SP à des souris. La plupart de ces composés sont actifs. Administrés oralement à des rats, à une dose de 50 mg·kg^{-1}, les composés les plus remarquables protègent contre les crises déclenchées par l'ECM mais non contre le PTZ. Des corrélations entre les propriétés anticonvulsivantes et la structure chimique des analogues de **1a** sont présentées.*

thiosemicarbazones of ketones / anticonvulsant activities / structure–activity relationships

The initial evaluation of many candidate anticonvulsant agents is performed using the maximal electroshock seizure (MES) and the subcutaneous pentylenetetrazole threshold (scPTZ) screens which are claimed to detect compounds of value in treating grand mal and petit mal respectively [1]. During the last few years, a number of thiosemicarbazones of arylidene and alkyl ketones were shown to have anticonvulsant properties [2, 3] and of particular interest was **1a** with activity in the MES screen when administered by both the intraperitoneal and oral routes [3]. The objective of the present study was to modify **1a** in an attempt to discern correlations between chemical structures and anticonvulsant activities. Thus, various substituents were planned to be introduced into the aryl ring of **1a** leading to compounds **1b–r** and replacement of the alkyl methyl group of **1a** by another atom or groups (series **2**) was considered. In addition, modifications of **1a** were envisaged in which spacer groups would be introduced between the substituted carbimino (C=N) function and the phenyl ring (series **3**) and replacement of the thioureido function by other electron rich groups (series **4**) would take place. In addition, since the π values of the phenyl and *n*-butyl groups are similar, namely 1.96 and 2.13 respectively [4], the synthesis of compound **5** was suggested in order to see if activity was retained in this compound. In series **6** the sulphur atom of the thiosemicarbazono group has been replaced by an imino function.

Chemistry

The compounds were prepared by reacting the appropriate aldehyde or ketone with thiosemicarbazide (series **1–3**, **5**), hydroxylamine or semicarbazide (series **4**) or aminoguanidine (series **6**) (scheme 1). Since compounds containing a carbimino group are capable of existing as *E* and *Z* isomers in solution, a representative compound **1j** was dissolved in deutero-



1	R^1	R^2	R^3
a	H	H	H
b	H	CH_3	H
c	CH_3	CH_3	H
d	CH_3	CH_3	CH_3
e	H	CH_3	CH_3
f	H	C_2H_5	H
g	H	C_6H_{11}[c]	H
h	H	OCH_3	H
i	OCH_3	OCH_3	H
j	OCH_3	H	OCH_3
k	H	OCH_3	OCH_3
l	H	OCH_2O	
m	H	OCH_2H_5	H
n	H	OC_6H_5	H
o	H	$COOC_2H_5$	H
p	H	Cl	H
q	H	Cl	Cl
r	H	$C(CH_3)=NNHCSNH_2$	H

2	R
a	H
b	C_2H_5
c	$CH(CH_3)_2$
d	C_6H_5

3	X
a	CH_2
b	CH_2CH_2
c	CH_2O

4	R
a	OH
b	$NHCONH_2$

6	R^1	R^2	R^3
a	H	H	H
b	H	H	CH_3
c	H	CH_3	CH_3
d	H	OCH_3	CH_3
e	H	Cl	CH_3
f	Cl	Cl	CH_3

Scheme 1.

chloroform and examined by high resolution ^1H NMR spectroscopy. On dissolution and also after 0.5 h, the spectra revealed the presence of two methoxy and one methyl signals of one isomer. However, spectra recorded after 5, 10 and 23 h showed the presence of two additional methoxy and one extra methyl absorptions due to a second isomer which was present to the extent of 21% approximately. It is likely that the major isomer has the *E* configuration for the following reasons. First, Fisher–Taylor–Hirschfelder models reveal that the phenyl ring can be coplanar with the carbimino group only in the case of the *E* isomer. Second, the methyl signal of the major isomer was at slightly higher field than in the case of the minor isomer which may be due to the greater shielding of the methyl group by the thiosemicarbazono function in the *E* isomer.

Pharmacology

The results of evaluating series **1–5** after intraperitoneal injection to mice in the MES, scPTZ and neurotoxicity screens are presented in table I; series **6** are not included due to the virtual absence of anticonvulsant activity in this group of derivatives. A number of compounds were evaluated for anti-convulsant activity when administered orally to rats and some of this data is summarized in table III.

Results and discussion

Previous work from these laboratories with arylidene alkyl ketone thiosemicarbazones had shown that X-ray crystallographic analysis of 4-(4-methylphenyl)-3-buten-2-one thiosemicarbazone had the *E* configuration with regard to the stereochemistry of the carbimino group [3]. On dissolution, the ^1H NMR spectrum of this derivative revealed the presence of two geometrical isomers in solution and the major one was assumed to have the stereochemistry established by X-ray crystallography. An isomeric equilibrium was established rapidly with the *E* isomer predominating. In addition, a solution of a related analog lacking the aryl methyl group gave rise to an equilibrium mixture

Table I. Evaluation of compounds **1–5** in the maximal electroshock seizure (MES), subcutaneous pentylenetetrazole seizure (scPTZ) and neurotoxicity screens after intraperitoneal injection in mice. The figures indicate the minimum doses at which activity or toxicity was noted in half or more of the animals. The letters I and NT refer to the compound being inactive and nontoxic respectively at the maximum dose of 300 mg/kg unless otherwise indicated. The data for compounds **1a**, **b** in the MES, scPTZ and neurotoxicity screens, except for the neurotoxicity test for **1b** at the end of 0.5 h, is taken from reference [3] and is reproduced by permission.

Compd	*MES screen (h)*		*scPTZ screen (h)*		*Neurotoxicity (h)*	
	0.5	*4*	*0.5*	*4*	*0.5*	*4*
1a[a]	100	I	300	I	100	100
1b	I	I	30	30	300	100
1c	I	I	300	I	NT	NT
1d	300	I	100	I	NT	300
1e	I	I	I	I	NT	100
1f[a]	100	I	300	I	300	30
1g	300	I	300	30	NT	30
1h	300	I	I	I	NT	NT
1i	300	I	I	I	NT	NT
1j	I	I	I	I	NT	NT
1k	I	300	I	300	NT	300
1l	300	I	300	I	NT	300
1m	100	I	300	I	NT	100
1n	300	300	300	300	NT	30
1o	I	300	I	300	NT	NT
1p	100	I	100	I	300	30
1q	300	300	I	I	NT	30
1r	I	I	I	I	NT	NT
2a[a]	30	100	30	100	30	100
2b[a]	30	100	100	I	100	100
2c[a]	100	100	I	I	100	NT
2d[a]	100	100	100	I	NT	100
3a	100	300	100	300	300	100
3b	100	100	100	I	100	100
3c	100	300	300	I	300	300
4a	100	I	300	I	300	NT
4b	100	300	100	I	100	NT
5[a]	100	I	I	I	300	100

[a]Animals receiving a dose of 300 mg/kg died before the 4 h test. Thus data after 4 h refers to testing at doses of 30 and 100 mg/kg only.

of isomers in which the *E* conformer predominated and in both cases even after 13 h no decomposition to other products occurred [3]. A similar observation was made in the present study whereby the ^{1}H NMR spectra of a solution of a representative compound **1j** showed that an equilibrium mixture of geometrical isomers occurred, the major isomer was considered to have the *E* configuration and no breakdown products were detected after 23 h in solution. While extrapolation of such data to *in vivo* conditions must be tentative, the NMR evidence suggests that administration of the thiosemicarbazones of aryl methyl ketones described herein to rodents may give rise to a mixture of isomers with the *E* conformer predominating and the compounds do not give rise to other products such as the corresponding ketones. Hence it is conceivable that the thiosemicarbazones *per se* are responsible for any bioactivity observed.

Approximately 90% of the compounds listed in table I showed activity in the initial MES and/or scPTZ screens at doses of 300 mg/kg or less. It is important to attempt to find the structural requirements which produce activity in these initial screens since inactive compounds, even if demonstrating anticonvulsant properties when given by other routes of administration, will be undetected in these preliminary screens. Thus, an inquiry into the discernment of any correlations between chemical structures and anticonvulsant activities was made as follows. In the case of series **1**, physicochemical constants reflecting the steric (MR), hydrophobic (π) and electronic (σ, σ^*) properties of the aryl substituents are available except for **1l**, **r**. Hence the remaining compounds in series **1** were divided into 3 groups, namely those derivatives which were active or neurotoxic at 100 mg/kg or less (group A), the thiosemicarbazones which were active or toxic at 300 mg/kg but not 100 mg/kg (group B) and group C contained the compounds which were inactive or nontoxic at the maximum dose administered namely 300 mg/kg. For example in the MES screen, group A consisted of **1a**, **f**, **m**, **p**. Table II indicates the data generated which reveals that at the 95% confidence limit ($P \leq 0.05$), only the MR values in the MES screen of groups A, B and C vary *ie* the values for A and B are different although the value of C (11.00) is not statistically distinguishable from either 7.46 or 17.53. In all other cases P values > 0.05 were found. In addition, when the MR, π and σ, σ^* values in group A were compared in the MES, scPTZ and neurotoxicity screens, *eg* 7.46, 13.83 and 12.58 for the MR values, no statistical difference among the figures was noted. A similar situation prevailed when groups B and C were examined in all 3 screens.

In series **2**, the most active compounds were **2a**, **b**. The screening results of series **3–5** indicated that activity was retained firstly when the aryl ring was separated by spacer groups consisting of one (**3a**) or two (**3b**, **c**) atoms from the carbon atom bearing the methyl and thiosemicarbazono groups, secondly the thiosemicarbazono function may be replaced by a hydroxyimino (**4a**) or semicarbazono (**4b**) groups and thirdly when the aryl ring is replaced by a *n*-butyl group (**5**). Compounds in series **6** were lethal to all animals given a dose of 100 mg/kg except for **6d** in which case two out of 4 animals were alive at the end of four hours. No activity nor fatalities were noted for

Table II. The average values of the molar refractivity (MR), Hansch hydrophobicity (π) and electronic (σ, σ^*) constants of the aryl substituents of various compounds in series 1. See text for a classification of the compounds in series **1**. The *P* values were obtained using the analysis of variance procedure. Means with different superscripts are significantly ($P \leq 0.05$) different from one another (Student-Newman-Keuls multiple comparison procedure).

Physicochemical parameters	MES screen				scPTZ screen				Neurotoxicity			
	A(n=4)	B(n=8)	C(n=4)	p	A(n=4)	B(n=7)	C(n=5)	p	A(n=9)	B(n=2)	C(n=5)	p
MR	7.46^{α}	17.53^{β}	$11.00^{\alpha,\beta}$	0.0349	13.83	13.71	12.54	0.9564	12.58	16.35	13.62	0.8108
π	0.53	1.01	0.69	0.6130	1.37	0.72	0.49	0.2641	1.09	0.82	0.31	0.2297
$\Sigma\sigma,\sigma^*$	0.94	1.03	1.10	0.9401	0.78	0.83	1.50	0.0748	0.93	0.55	1.38	0.1783

Table III. Evaluation of selected compounds for oral activity in the maximal electroshock seizure (MES) screen in rats using a dose of 50 mg/kg.

Compd	*Number of rats out of 4 protected against seizures at different times (h)*				
	0.25	0.5	1	2	4
1a[a]	0	2	3	1	1
1d	0	0	0	0	0
1f	0	0	2	1	0
1h	0	0	0	0	0
1m	0	1	1	0	0
1p	4	3	3	3	1
1q	0	1	1	0	0
2a	3	4	4	4	3
2b	3	1	2	1	3
2c	3	3	4	2	2
2d	0	0	0	0	1
3a	0	0	0	0	0
3b	3	2	3	0	0
3c	1	4	0	0	0
4a	0	2	1	2	0
4b	4	4	4	1	0
5	1	2	2	0	0
6b	1	0	2	3	2

[a]Dose of 20 mg/kg used.

mice receiving 30 mg/kg of **6a**, **c**, **d**, **f**. In the case of **6b**, at the end of 4 h, 3 out of the 4 animals receiving 30 mg/kg were dead while the remaining mouse was protected against MES convulsions and demonstrated neurological deficit. Mice given 30 mg/kg of **6e** died between 1/2 and 4 h of respiratory depression. In the neurotoxicity screen, of the 6 compounds which did not show any neurological deficit at 300 mg/kg, 4 derivatives namely **1c**, **h**, **i**, **o** had anticonvulsant properties.

A valuable property of a candidate anticonvulsant agent is its ability to inhibit convulsions when given by the oral route. Table III indicates the evaluation of 18 compounds in the oral MES screen. With the exception of **1a**, the same dose of compound namely 50 mg/kg was administered in order to obtain an approximate evaluation of relative potency. At this dose with the exception of **1a** none of the compounds listed in table III showed neurotoxicity. Compounds **1c**, **d**, **g**, **p**, **2a**, **b**, **d**, **3a–c**, **4b** were inactive in the scPTZ screen when given orally to rats at a dose of 50 mg/kg except **3b** affording protection in 1 of 4 animals after 1/4 h. While it is possible that elevation of the dose might produce compounds with oral activity in the scPTZ screen, the results generated suggest that these derivatives display selective oral activity in the MES test.

Of the 7 compounds examined in series **1**, the greatest oral activity was displayed by **1a**, **p**. The ED_{50} values for **2a**, **1a**, **2b**, **c** were 12.84, 16.89 [3], 34.18 and 39.01 mg/kg respectively and correlation coefficients (*r*) found from the plots of these ED_{50} figures *versus* MR, field ($\mathcal{F}$) and resonance ($\mathcal{R}$) constants were 0.97, 0.80 and 0.47 respectively. Thus steric and field effects play an important role in determining anticonvulsant activity in series **2**. In series **3**, the activity of **3b**, **c** in contrast to **3a** suggests that spacer groups between the phenyl ring and the carbon atom bearing the methyl and thiosemicarbazono groups should contain two and not one atoms. Both **4a**, **b** are active by the oral route and the complete protection from one-quarter to one hour by **4b** indicates that isosteric replacement of sulphur by oxygen

in the thiosemicarbazones may be a profitable avenue to pursue. Compound **5** is clearly less active than **1a**. The tolerance by the rats of a dose of 50 mg/kg of **6b** by the oral route plus the activity during the 4 h period of observation was noteworthy.

From this study the following conclusions may be drawn pertaining to the anticonvulsant activities of the compounds described in this report. Firstly, evaluation of the compounds in series **1–5** after intraperitoneal injection revealed that 89% of the compounds were active in the MES and/or scPTZ screens. On oral administration, however, significant activity was seen in the MES test system only. Secondly, compounds **1a**, **p**, **2a–c**, **3c**, **4b** gave complete protection against seizures in the MES screen when the derivatives were administered by the oral route at a dose of 50 mg/kg to rats.

Experimental protocols

Chemistry

Elemental analyses (C, H, N) were undertaken by K Thoms, Department of Chemistry, University of Saskatchewan on **1c–g**, **i–o**, **r**, **2c**, **3a–c**, **4a**, **b**, **5**, **6a–f** and are within 0.4% of the calculated values except for the carbon analysis of **1r** (calcd for $C_{12}H_{16}N_6S_2$: C, 46.73. Found: 47.32). Melting points, which are uncorrected, were generally in accord with literature values for previously described compounds, namely **1c–f**, **h**, **l–n**, **p**, **q**, **2a–d**, **3a**, **c**, **4a**, **b**, **6a**, **b**: where mp's were at variance with those reported earlier, elemental analysis of the product agreed with the calculated figures.

*Synthesis of **1c–g, i–o, r, 3a–c, 5***

The general procedure was as follows. A mixture of thiosemicarbazide (0.01 mol) was added slowly to a stirred solution of the appropriate ketone (0.01 mol) in ethanol (20 ml) and 37% w/v aqueous hydrochloric acid (2 ml). The mixture was heated under reflux for 72 h and on cooling the precipitate was collected, dried and recrystallized from ethanol (95% or absolute). Exceptions to this general method were as follows. In the following cases, the reactants were stirred at room temperature for 18 (**1c**), 60 (**1d**), 0.25 (**1e**), 24 (**1j**), 60 (**3b**) and 120 (**3c**) h. On occasions the products were prepared by heating the reactants under reflux for 9 (**1d**), 48 (**1l**), 22 (**3b**) and 48 (**3c**) h. Some of the compounds were recrystallized from the following solvents namely water-ethanol (**1f**, **i**), acetone-ethanol (**1k**) and methanol (**1o**). Compound **1r** was prepared by this general route except that the quantity of 1,4-diacetylbenzene was 0.005 mol, the mixture was heated under reflux for 96 h and the crude product was recrystallized from chloroform-dimethylsulphoxide. The mp's (°C) and yields (%) for these compounds were as follows: **1c**: 167.5, 37; **1d**: 160.5, 54; **1e**: 190.0, 63; **1f**: 137.8, 52; **1g**: 166.0, 69; **1i**: 157.1, 32; **1j**: 137.2, 48; **1k**: 227 (dec), 48; **1l**: 186.0, 39; **1m**: 159.7, 34; **1n**: 170.2, 49; **1o**: 215.8, 53; **1r**: 200–202 (dec), 43; **3a**: 145.8, 41; **3b**: 100.8–102.6, 50; **3c**: 135.0, 32; **5**: 52.5–53.0, 28.

*Synthesis of **1h, q, 2a***

Compounds **1h**, **q**, **2a** were prepared by heating under reflux a solution of thiosemicarbazide (0.01 mol) and the ketone or aldehyde (0.01 mol) in ethanol (40 ml) for 24 h. On cooling, the product was collected and recrystallized from methanol. The mp's (°C) and yields (%) of these thiosemicarbazones were as follows: **1h**: 178.5, 65; **1q**: 197.4, 59; **2a**: 153.5–154.5, 60.

*Synthesis of **1p, 2b–d***

The thiosemicarbazones **1p**, **2b–d** were synthesized as follows. Thiosemicarbazide (0.01 mol) was added to a solution of glacial acetic acid (2 ml) in water (30 ml) and the mixture was warmed on a steam bath to give a clear solution. On cooling the appropriate ketone (0.01 mol) in ethanol (25 ml) was added and the solution heated under reflux for 0.25 (**1p**), 0.25 (**2b**), 24 (**2c**) and 4 (**2d**) h. The precipitates were collected and recrystallized from methanol. The mp's (°C) and yields (%) of these compounds were as follows: **1p**: 178.7–179.5, 81; **2b**: 111.7–112.8, 70; **2c**: 119.1–120.3, 40; **2d**: 165.3–166.5, 32.

*Synthesis of **4a, b***

Compound **4a** was prepared as follows. A mixture of acetophenone (1.0 g), hydroxylamine hydrochloride (2.0 g), sodium acetate (4.0 g) in water (10 ml) was warmed on a water bath at 35–40°C and sufficient ethanol was added dropwise to obtain a clear solution. On cooling, the reaction mixture was basified with aqueous sodium hydroxide solution and extracted with ether. A mixture of ethanol (≈ 15 ml) and water (≈ 5 ml) was added to the ethereal extract and refrigerated. The crystals formed were collected and recrystallized from water to give **4a**, mp 59–60°C in 74% yield.

The semicarbazone **4b**, mp 201.6°C, was prepared in 66% yield by the same procedure as described in **1c** and related compounds except that semicarbazide was used in place of thiosemicarbazide, the mixture was heated under reflux for 96 h and the product was recrystallized from water-ethanol.

*Synthesis of **6***

Compound **6a** was prepared in 52% yield by a literature method [5] except that aminoguanidine nitrate was used in place of aminoguanidine bicarbonate. The crude product was recrystallized from water, mp 179–180°C. The general method for preparing **6b–f** was by heating under reflux a mixture of aminoguanidine bicarbonate (0.01 mol), the appropriate ketone (0.01 mol), ethanol (95%, 20 ml) HCl (37% w/v, 1 ml) and water (1 ml) for 1 (**6b–d**), 2.5 (**6e**) or 1.25 (**6f**) h. The solvent was removed and the reaction product was treated with excess of aqueous sodium hydroxide solution. The crude product was collected and recrystallized from ethanol (**6b–c**), water-ethanol (**6d**), benzene (**6e**) or isopropanol (**6f**). The mp's (°C) and yields (%) of **6b–f** were as follows: **6b**: 182–184,22; **6c**: 184.5–185.5, 11; **6d**: 214 (dec), 57; **6e**: 183–184.5, 19; **6f**: 172.5–174,26.

^1H NMR spectroscopy

High resolution ^1H NMR spectra were recorded using a Bruker AM 300 FT NMR instrument equipped with an Aspect 300 computer. A solution of **1j** in deuterochloroform (10 μM in 1 ml) was examined at room temperature. The methyl signals of the major and minor isomers appeared at 2.24 and 2.26 ppm respectively. The methoxy absorptions of the major isomer were at 3.80 (1141.58 Hz) and 3.79 ppm and for the minor component, the two methoxy signals appeared at 3.81 and 3.80 (1139.41 Hz) ppm. The percentages of the minor isomer as obtained by integration of the methyl signals after 5, 10 and 23 h were 19, 17 and 26 respectively while integration of the methoxy signals revealed the presence of 20, 18 and 25% of the minor isomer after 5, 10 and 23 h respectively.

Use of physicochemical constants

The MR values for aromatic (series **1**) and aliphatic (series **2**) substituents were taken from the literature [6] and the π values were obtained from published data [7]. The σ and σ^* constants were obtained from a reference source [8] and the $\mathcal{F}$ and $\mathcal{R}$ values were taken from the literature [9].

Pharmacology

*Evaluation of **1–6** for anticonvulsant activity and neurotoxicity*

The screens for anticonvulsant activity and neurotoxicity were undertaken by the Antiepileptic Drug Development Program, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland, USA according to their protocols [10, 11]. The initial experiments in which compounds were injected by the ip route to mice were undertaken be administering the compounds in aqueous polyethyleneglycol 400 (30%) to male Carworth number 1 mice. The ED_{50} values (TD_{50} figures *ie* dose of drug causing neurotoxicity in 50% of the animals) of 3 reference compounds active against grand mal epilepsy and in the MES screen in mice are as follows: phenobarbitone 21.8 (69.0), phenytoin 9.50 (65.5) and primidone 11.4 (680) mg/kg (1). The ED_{50} figures (TD_{50} values) of 3 reference compounds active versus petit mal seizures and in the scPTZ screen are as follows: trimethadione 300 (819), methsuximide 68.3 (188) and clonazepam 0.009 (0.18) mg/kg (1).

In addition to the data presented in table I, the following observations were made during this bioevaluation process. In the MES screen, delayed tonic extension was noted with **1p** at doses of 100 and 300 mg/kg at the end of 4 h. In the scPTZ screen, the phenomena noted in the case of certain compounds (dose in mg/kg, time of observations in h after administration of compound) were as follows: the animals died during the test without having a seizure: **1a** (100,4), **1f** (100,4), **1g** (100,4) and **5** (300, 0.5); continuous jerking movement: **1n** (300,4); continuous jerking movement followed by death: **1b** (300, 0.5 and 4); continuous tremors followed by tonic extension: **1f** (30,4) and **1h** (300,4); continuous tremors: **1q** (30,4); continuous tremors followed by tonic extension and death: **1q** (100,4); clonic seizure followed by continuous seizure activity: **1q** (300,4); **2d** (300, 0.5) and **3b** (100,4); tonic extension: **1r** (300, 0.5) and continuous seizure activity **2c** (300, 0.5).

A number of compounds were examined for anticonvulsant activity after oral administration to male Sprague-Dawley rats. The data for the MES screen is presented in table III. The following compounds were examined in the scPTZ screen namely **1c, d, g, p, 2a, b, d, 3a–c** and **4b** using a dose of 50 mg/kg. Observations were made at the end of 0.25, 0.5, 1, 2 and 4 h except for **3c** which was examined at the end of 0.25, 0.5, 1 and 2 h only. All compounds were inactive in the scPTZ screen except **3b** where 1/4 animals were protected at the end of 0.25 h. A number of the animals died following seizures after receiving **1c** and some of the rats had continuous seizure activity and/or death after administration of **2b** while 2/4 animals died 0.25 h after receiving **3c**. The thiosemicarbazone **1a** was evaluated differently in the oral scPTZ screen whereby doses of 10, 30 and 100 mg/kg were administered using one animal per dose and the rats were examined at the end of 0.5 and 4 h only. No protection was observed. Neurotoxicity was noted in 0/8, 1/8, 5/8, 3/8, 1/8, 1/8, 0/8 and 0/8 rats at a dose of 100 mg/kg of **1a** at the end of 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h respectively.

Quantification of the oral activity for **2a–c** was undertaken as follows. The compounds in aqueous methylcellulose (0.5% w/v) were administered to Sprague-Dawley rats. Eight animals per dose were used in the MES test and observations were made at the end of 2 (**2a**), 0.5 (**2b**) and 1 (**2c**) h. Five doses of each compound were made except for **2b** in which case, 4 doses were employed. The ED_{50} values (95% confidence intervals) were as follows: **2a**: 12.84 (8.98–16.64), **2b**: 34.18 (22.54–47.27) and **2c**: 39.01 (22.97–58.54) mg/kg. In the scPTZ test, two animals per dose were used and **2a–c** were inactive at the maximum doses employed namely 400, 500 and 250 mg/kg respectively at the end of 2 (**2a**), 0.5 (**2b**) and 1 (**2c**) h. These compounds were examined for neurotoxicity using two animals per dose and 4 doses of compound were employed except 8 animals per dose were used in the case of **2c**. Compounds **2a, b** were evaluated over the 0.25–24 and 0.25–6 h time periods respectively and the TD_{50} values were > 200 and > 250 mg/kg for **2a, b**. The TD_{50} value for **2c** determined at the end of 2 h was 67.93 (34.22–105.80) mg/kg. The ED_{50} (TD_{50}) values for 3 reference substances in the MES screen when given orally to rats are as follows: phenobarbitone 9.1 (61.1), phenytoin 29.8 (> 3000) and primidone 6.2 (233.9) mg/kg (11). The ED_{50} (TD_{50}) figures for 3 drugs active orally in the scPTZ screen are as follows: trimethadione 233.7 (1326.9), methsuximide 25.3 (59.8) and clonazepam 0.06 (71.6) mg/kg (11).

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References

1 Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) *Epilepsia* 19, 409–428
2 Dimmock JR, Smith DC, Brenner JM, Jonnalagadda SS, Sardessai MS, Wood JD, Bigam GE (1986) *Eur J Med Chem* 21, 187–192
3 Dimmock JR, Jonnalagadda SS, Hussein S, Tewari S, Quail JW, Reid RS, Delbaere LTJ, Prasad L (1991) *Eur J Med Chem* 25, 581–588
4 Hansch C, Leo AJ (1979) *In: Substituent Constants for Correlation Analysis in Chemistry and Biology,* John Wiley and Sons, New York, 116, 129
5 Finnegan WG, Henry RA, Smith GBL (1952) *J Am Chem Soc* 74, 2981–2983
6 Hansch C, Leo AJ (1979) *In: Substituent Constants for Correlation Analysis in Chemistry and Biology,* John Wiley and Sons, New York, 49–53
7 Hansch C, Leo AJ (1979) *In: Substituent Constants for Correlation Analysis in Chemistry and Biology,* John Wiley and Sons, New York, 49–51
8 Perrin DD, Dempsey B, Serjeant EP (1981) *In: pK_a Prediction for Organic Acids and Bases,* Chapman and Hall, London, 109–114, 116, 119, 120
9 Hansch C, Leo A, Unger SH, Kim KH, Nikartani D, Lien EJ (1973) *J Med Chem* 16, 1207–1216
10 Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institutes of Health, DHEW Publication No (NIH) 78–1093 (1978)
11 Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B, White BG (1984) *Cleve Clin Q* 51, 293–305