Eur J Med Chem (1995) 30, 303–314
© Elsevier, Paris

Evaluation of the semicarbazones, thiosemicarbazones and bis-carbohydrazones of some aryl alicyclic ketones for anticonvulsant and other biological properties

JR Dimmock[1], SN Pandeya[1], JW Quail[2], U Pugazhenthi[2], TM Allen[3], GY Kao[3], J Balzarini[4], E DeClercq[4]

[1]*College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon;*
[2]*Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0;*
[3]*Department of Pharmacology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada;*
[4]*The Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium*

(Received 18 August 1994; accepted 29 November 1994)

Summary — A number of aryl alicyclic ketones were converted to their corresponding semicarbazones, thiosemicarbazones and bis-carbohydrazones. Anticonvulsant activity was displayed by most of the compounds in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens when given intraperitoneally to mice. However, on oral administration to rats, a marked selective activity in the MES screen only was noted. X-ray crystallography on five semicarbazones was undertaken in order to find correlations between the shapes of these molecules and anticonvulsant properties. The thiosemicarbazones displayed greater cytotoxicity to P388D1 and L1210 cells than the semicarbazones while a number of human tumors and different viruses were, in general, insensitive to representative compounds.

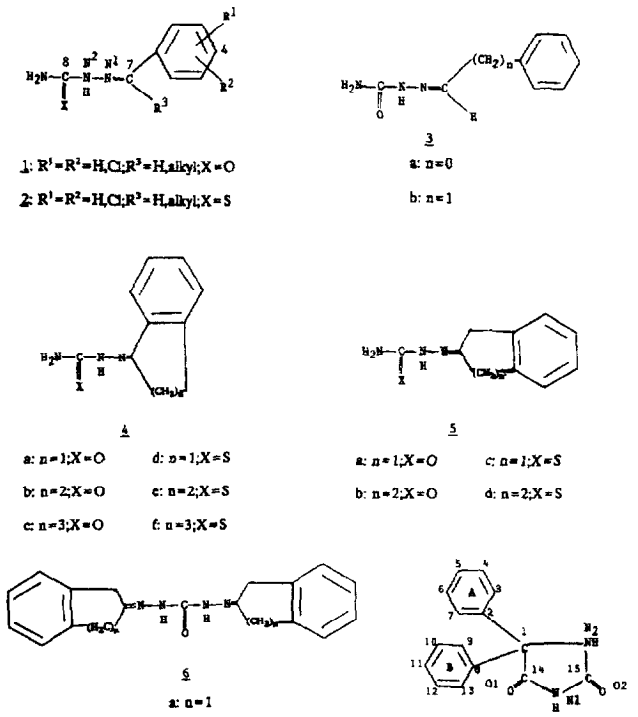
semicarbazone / thiosemicarbazone / anticonvulsant agent / X-ray crystallography / cytotoxic agent

Introduction

Two major pharmacological screening tests used to evaluate compounds for anticonvulsant activities are the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens [1]. These tests are claimed to detect compounds possessing activity against generalized tonic-clonic *(grand mal)* and generalized absence *(petit mal)* seizures, respectively [1]. The structural requirements for activity in the MES screen have been stated to be the presence of a large hydrophobic group which is in close proximity to at least two electron-donor atoms [2]. For activity in the scPTZ screen, a smaller, less hydrophobic group than is required for activity in the MES screen should be present near to a minimum of two electron-donor atoms [2].

Based on these considerations, a number of aryl semicarbazones **1** have been prepared, which contained a hydrophobic moiety, namely an aryl ring, as well as four electron-donor atoms in the semicarbazono group [3]. Most of these compounds displayed activity in both the MES and scPTZ screens when given by intraperitoneal injection to mice, although neurotoxicity was noted in a number of cases. Hence quantification of selected compounds revealed that most semicarbazones had a low protection index (PI *viz* TD_{50}/ED_{50}, where TD_{50} and ED_{50} refer to the doses of compounds which cause neurotoxicity and afford protection in 50% of the animals, respectively). However, when given orally to rats, the semicarbazones in general displayed significant anticonvulsant activity in the MES screen accompanied by high PIs, but afforded virtually no protection in thc scPTZ test. In addition, some related thiosemicarbazones **2** also displayed anticonvulsant properties, but on occasions greater neurotoxicity was revealed with these compounds than with the corresponding semicarbazones.

After oral administration to rats, **3a** was shown to be equipotent with mephenytoin in the MES screen [3]. However, it displayed lower neurotoxicity than this established drug and the PI of **3a** is 2.4 times that of mephenytoin. While **3b** is only half as potent as **3a**, the neurotoxicity of this compound is lower and thus a higher PI was obtained in the MES screen after oral administration to rats for **3b**. Hence these two compounds were taken as the lead molecules for



Structures **1**–**7**.

molecular modification with a view to discerning the structural features contributing to anticonvulsant activity, particularly when the compounds are administered by the oral route.

The specific objectives of the present study were as follows. First, the synthesis of a selected number of analogues of **3a** and **3b** was planned in which the orientations of the phenyl ring relative to the semicarbazono group would be restricted. This may be accomplished by the attachment of the aryl ring to an alicyclic ketone and then forming the corresponding semicarbazone. Such considerations led to the decision to prepare **4a–c**, **5a** and **5b**. Evaluation of these compounds may reveal whether the frozen conformations have higher anticonvulsant activities than the more flexible analogs **3a** and **3b**. Second, in order to compare the shapes of some of these conformationally restricted molecules with **3a** and **3b**, X-ray crystallography on representative compounds was considered. Third, the preparation of the thiosemicarbazones **4d–f**, **5c** and **5d** was planned in order to compare their bioactivities with their isosteres **4a–c**, **5a** and **5b**. Fourth, the preparation of **6a** and **6b** was suggested in which the two potentially pharmacophoric aryl rings are now in close proximity to five electron-donor atoms. These molecules may be regarded as being constructed from two molecules of **3b** which share an aminocarbonyl function.

Evaluation of the bioactivities of these compounds was proposed as follows. The decision was made to examine the series **4–6** in the MES, scPTZ and neurotoxicity screens after intraperitoneal injection into mice. Promising compounds would be administered to rats by the oral route. In addition, since a number of semicarbazones and thiosemicarbazones have demonstrated cytotoxic and anticancer properties [4], their *in vitro* evaluation against murine leukemia cells and also *versus* a number of human tumors was considered. Finally, various thiosemicarbazones such as benzaldehyde thiosemicarbazone have antiviral activities [5] and the examination of a number of these compounds for such properties was therefore warranted.

Chemistry

Reaction of semicarbazide with 1-indanone, 1-tetralone, 1-benzosuberone, 2-indanone and 2-tetralone led to the formation of **4a–c**, **5a** and **5b**, respectively. Condensation of these ketones with thiosemicarbazide produced the corresponding thiosemicarbazones **4d–f**, **5c** and **5d**. Both 2-indanone and 2-tetralone were reacted with carbohydrazide leading to the formation of **6a** and **6b**, respectively. The yields of compounds **4–6** were between 60 and 75%. The structures of **4a–c**, **5a** and **5b** were determined by X-ray crystallography and Ortep diagrams of these compounds along with that of **3a** are portrayed in figures 1–6.

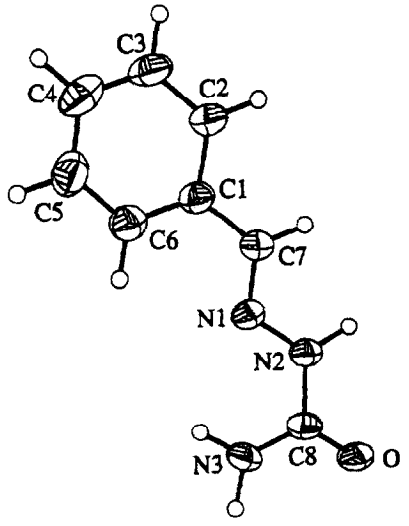


Fig 1. Ortep drawing of **3a**. Reprinted with permission from reference [3]. Copyright (1993) American Chemical Society.

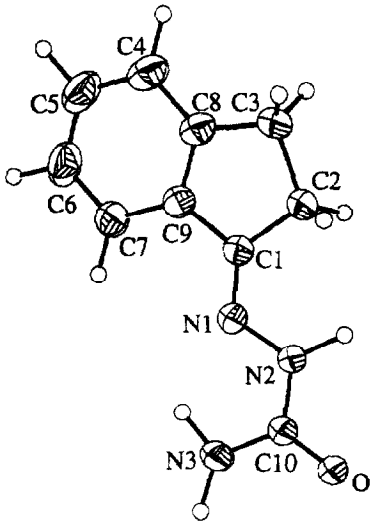


Fig 2. Ortep drawing of **4a**.

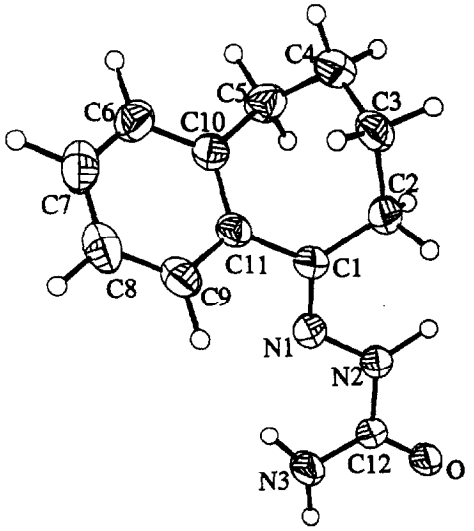


Fig 4. Ortep drawing of **4c**.

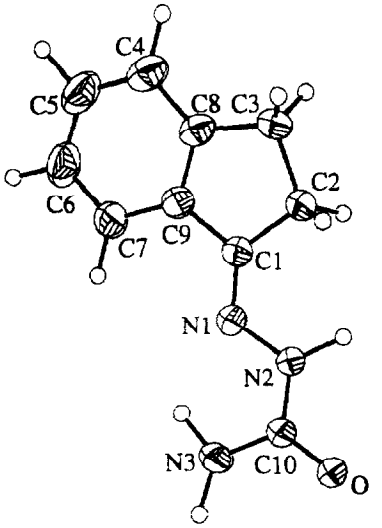


Fig 3. Ortep drawing of **4b**.

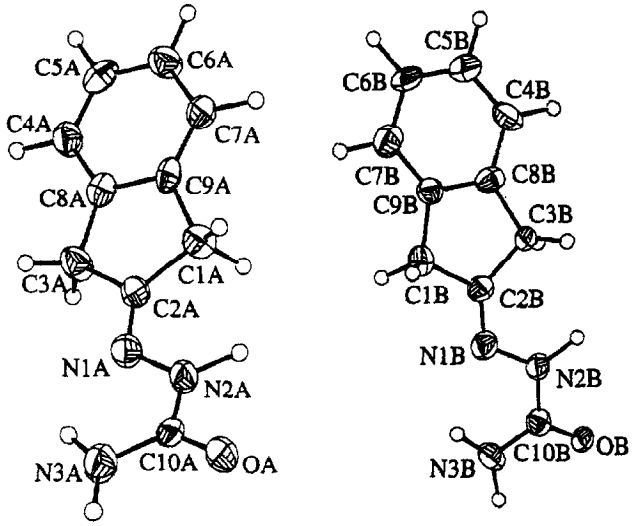


Fig 5. Ortep drawing of **5a** (structures A and B).

Bioevaluation

The compounds in series **4–6** were initially examined in the MES, scPTZ and neurotoxicity screens using the intraperitoneal route in mice. These data are presented in table I. Subsequently, these derivatives were administered by the oral route to rats in the MES and neurotoxicity tests and over half of the compounds were evaluated for protective capabilities in the scPTZ screen. Quantitation of the oral activity of two thirds of the compounds was accomplished. The results of these evaluations in the rat after oral administration are given in table II.

In addition, the derivatives in series **4–6** were examined for *in vitro* cytotoxicity towards murine P388 lymphocytic leukemia cells and a number of compounds were assessed against murine L1210 lymphoid leukemia cells and human T4 lymphocytes and a variety of human tumors (see table III). The antiviral activity and cytotoxicity of compounds **4a**,

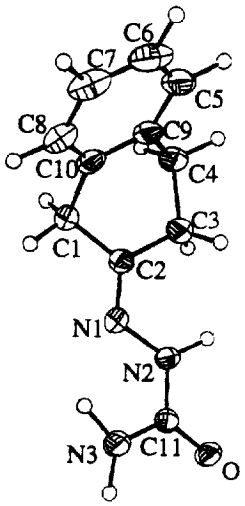


Fig 6. Ortep drawing of **5b**.

4c, **4d**, **4f**, **5a**, **5c** and **6b** were undertaken, using (i) herpes simplex-1 (KOS), herpes simplex-1 (TK- B2006), herpes simplex-1 (TK- VMW1837), herpes simplex-2 (G), vaccinia and vesicular stomatitis virus in E_6SM cell cultures; (ii) vesicular stomatitis, Coxsackie and polio-1 virus in HeLa cell cultures; (iii) parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4 and Semliki forest virus in Vero cell cultures; and (iv) human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in human T-lymphocytes (CEM). Activity was displayed by **4a**, **4d** and **5a** against herpes simplex-1 (TK-1 VMW 1837) in which cases the IC_{50} values were 793, 195 and 1057 μM respectively, and **4d** was also active against vaccinia and herpes simplex-1 (TK- B2006) displaying IC_{50} figures against these two organisms of 97 and 731 μM, respectively. The concentrations required to cause a microscopically detectable alteration of normal cell morphology of E_6SM cells by **4a**, **4d** and **5a** were 2114, ≥ 974 and ≥ 2114 μM, respectively. In all other instances no inhibition of virus-induced cytopathogenicity was noted with any of the compounds at the highest concentrations utilized.

Discussion

The data in table I reveal that all of the compounds in series **4** and **5** afforded protection in the MES screen while 70% of these derivatives were active in the scPTZ test. Hence the compounds display some MES selectivity. In general, anticonvulsant activity was noted at the end of 30 min rather than 4 h, *ie* the onset of action was rapid. It is of interest to note that of the five semicarbazones **4a–c**, **5a** and **5b**, only **5a** and **5b** demonstrated neurotoxicity while all of the thiosemicarbazones **4d–f**, **5c** and **5d** caused neurological deficit. Neither anticonvulsant nor neurotoxic properties were displayed by **6a** and **6b**.

All of the compounds in series **4–6** were administered to rats by the oral route and evaluated in the MES screen. The data in table II reveal that, with the

Table I. Minimum doses of compounds **4–6** to produce activity in the majority of animals in the MES, scPTZ and neurotoxicity screens after intraperitoneal injection into mice.

Compound	*MES screen*[a]		*scPTZ screen*[a]		*Neurotoxicity screen*[a]	
	0.5 h	*4 h*	*0.5 h*	*4 h*	*0.5 h*	*4 h*
4a	100	–	100	–	–	–
4b	300	–	–	–	–	–
4c	300	300	–	–	–	–
4d	30	–	100	–	100	300
4e	–	300	–	–	–	300
4f	100	–	100	–	–	300
5a	100	300	100	–	300	300
5b	100	–	100	–	300	–
5c	100	–	100	–[b]	100	30
5d	30	300	100	100	100	100
6a	–	–	–	–	–	–
6b	–	–	–	–	–	–

[a]The doses used were 30, 100 and 300 mg/kg in all screens and the mice were examined 0.5 and 4 h after injection. The dashes – indicate an absence of activity or toxicity. [b]Animals receiving doses of 100 and 300 mg/kg were not observed at the end of 4 h.

Table II. Evaluation of compounds **4–6** in the MES and neurotoxicity screens after oral administration to rats.

Compound	*Maximum number of rats protected (out of 4) in the MES screen*[a]		*Quantification studies*[b]						*PI*[c]
			MES screen			*Neurotoxicity screen*			
	Time (h)	*Protection*	*Time (h)*	*ED_{50} (mg/kg) (95% CI)*	*Slope (SE)*	*Time (h)*	*TD_{50} (mg/kg) (95% CI)*	*Slope (SE)*	
4a	0.5	2	0.5	26.96 (18.55–34.89)	4.95 (1.48)	0.25–24	> 126	–	> 4.67
4b	1	3	1	56.12 (34.48–86.40)	2.83 (0.73)	0.25–24	> 500	–	> 8.91
4c	0.5	4	0.25	37.34 (23.59–52.24)	3.27 (0.85)	0.25–24	> 450	–	> 12.1
4d	1	3	1	20.10 (13.04–27.93)	2.96 (0.79)	24	121.31 (92.26–144.85)	9.15 (3.02)	6.04
4e	0.5, 2	1	0.50	107.58 (71.66–143.27)	4.39 (1.35)	24	94.90 (76.08–116.10)	6.57 (1.70)	0.88
4f	0.25, 1	3	–	–	–	–	–	–	–
5a	1	2	1	41.36 (31.04–56.89)	3.75 (1.08)	0.25–24	> 82	–	> 1.98
5b	0.25, 0.5	4	0.25	14.35 (9.00–20.63)	2.76 (0.69)	1	569.73 (443.51–951.34)	4.35 (1.35)	39.7
5c	1, 2	2	–	–	–	–	–	–	–
5d	1	4	0.50	27.70 (20.42–35.83)	5.37 (1.63)	24	69.28 (47.15–94.68)	4.57 (1.27)	2.50
6a	2	1	–	–	–	–	–	–	–
6b	0.25–4	0	–	–	–	–	–	–	–
Phenytoin	–	–	2	23.2 (21.4–25.4)	15.1 (4.28)	0.25–24	> 500	–	> 21.6
Carbamazepine	–	–	1	3.57 (2.41–4.72)	3.84 (1.15)	1	361 (319–402)	11.4 (2.96)	101
Valproate	–	–	0.5	395 (332–441)	8.13 (2.76)	0.5	859 (719–1148)	6.57 (2.17)	2.17

[a]Doses of 50 mg/kg were used except for **4a**, **4d**, **5a** and **5c** in which cases 25 mg/kg were employed. The rats were observed 0.25, 0.5, 1, 2 and 4 h after the compound was administered. The data for the reference drugs are unavailable. [b]The dashes – indicate that the compound was not evaluated (**4f**, **5c**, **6a** and **6b**) or the slopes (SE) were not calculated. [c]PI indicates the protection index *ie* TD_{50}/ED_{50}.

Table III. Cytotoxic evaluations using P388D1 and L1210 leukemia cells, human T-lymphocytes and human tumors.

Compound	*P388D1 cells IC_{50} (μM)*[a]	*L1210 cells and human T-lymphocytes cells (Molt-4/C8 and CEM) IC_{50} (μM)*[a,b]					*Human tumor cell lines $log_{10} GI_{50}$ (M) MG MID figures*[c]
		L1210	*Molt 4/C8*	*TI*	*CEM*	*TI*	
4a	> 50	> 1057	> 1057	–	> 1057	–	–
4b	> 50	–	–	–	–	–	–
4c	> 50	206.7 ± 32.2	111.8 ± 11.5	0.54	685.8 ± 299.2	3.32	> – 5.00
4d	14.3	75.5 ± 5.35	204.6 ± 36.5	2.71	82.8 ± 12.67	1.10	–
4e	6.2	–	–	–	–	–	–
4f	1.6	19.8 ± 4.67	13.46 ± 0.6	0.68	44.57 ± 25.72	2.25	– 5.01
5a	> 50	502.1 ± 41.22	397.44 ± 58.66	0.79	344.6 ± 108.3	0.69	> – 4.00
5b	37	–	–	–	–	–	–
5c	8.2	93.54 ± 3.9	107.18 ± 45.8	1.15	95.48 ± 19	1.02	– 4.01
5d	11.3	–	–	–	–	–	– 4.06
6a	> 50	–	–	–	–	–	– 4.10
6b	2.3	42.15 ± 1.73	45.61 ± 7.5	1.08	41.86 ± 5.77	0.99	> – 5.00
Melphalan	0.2	200	176 ± 28	0.88	227 ± 63	1.13	– 4.58
Ara C[d]	–	0.535 ± 0.08	0.493 ± 0.08	0.92	0.617 ± 0.08	1.15	–
Methotrexate	–	0.22 ± 0.022	0.374 ± 0.066	1.70	0.264 ± 0.022	1.20	–

[a]The IC_{50} figures refer to the concentrations of compounds required to inhibit the growth of the cells by 50%. [b]The letters TI refer to therapeutic index; *viz*, IC_{50} for human lymphocytes/IC_{50} for murine leukemia L1210 cells. [c]The log_{10} GI_{50} MG MID data indicate the concentration of compounds necessary to inhibit the growth of 54 (± 6) human tumors by 50% (see *Discussion* for further details). [d]1-β-(D-Arabinofuranosyl)cytosine.

exception of **6b**, all of the compounds afforded some protection in this test. In general, the compounds had a rapid onset of action as noted when they were given intraperitoneally to mice. At the same doses as used in the MES screen, compounds **4a**, **d**, **f** and **5a–d** were inactive or showed only marginal anticonvulsant properties in the scPTZ test (not shown). Hence, the compounds displayed a marked MES-selectivity when given orally to rats. At the maximum doses administered, no neurotoxicity was noted with any of the 12 compounds. Clearly, both the mouse and rat data revealed that the incorporation of two potentially pharmacophoric groups into the molecules **6a** and **6b** does not lead to compounds with promising anticonvulsant activity.

In view of these encouraging results for the compounds in series **4** and **5**, the decision was made to quantify the activity of the five semicarbazones (**4a–c**, **5a** and **5b**) and three thiosemicarbazones (**4d**, **4e** and **5d**) in the MES screen. These results are presented in table II, which also includes an evaluation of neurotoxicity.

One of the major aims in this investigation was a comparison of the anticonvulsant activity of the acyclic lead molecules **3a** and **3b** with the cyclic analogues **4a–c**, **5a** and **5b**. The ED_{50} (mg/kg) and TD_{50} (mg/kg) figures along with the PI values of **3a** are 22.50, 254.3 and 11.30 respectively, while for **3b** the corresponding data are 42.78, > 500 and > 11.69 [3]. The anticonvulsant activities of **4a**, **4b**

and **4c** are 0.83, 0.40 and 0.60 that of the related acyclic analog **3a**. Hence, in terms of potency in the MES screen, the formation of comparatively rigid structures is not beneficial. On the other hand, the potencies of **5a** and **5b** were 1.03 and 2.98 times that of the acyclic analogue **3b**. Thus increased rigidity leading to retention of potency (**5a**) or an approximately threefold increase in activity (**5b**) may therefore be a useful molecular modification in the development of related anticonvulsants. This conclusion was strengthened by the significant potency of **5b** which had greater activity than both phenytoin and valproate with a PI of approximately 40. It is of interest to note that the four other semicarbazones **4a–c** and **5b** all had much higher activity in the MES screen than valproate, and **4a** and **4c** were equiactive with phenytoin.

A comparison of the activities in the MES screen and neurotoxicities of the semicarbazones **4a**, **4b** and **5b** with the isosteres **4d**, **4e** and **5d** was made. Compounds **4a** and **4d** were equipotent and had similar PI values. On the other hand, the ED_{50} values of the semicarbazones **4b** and **5b** were approximately half of the corresponding thiosemicarbazone figures. In addition, the PI of **4b** and **5b** were substantially greater than **4e** and **5d** and hence future developments should utilize the semicarbazono pharmacophore.

The questions of whether attachment of the aryl ring directly to the carbimino group or using a one carbon spacer group would lead to increased anticonvulsant activity and also whether an optimal size of the alicyclic ring could be discerned were both unresolved. Thus in the first case, while **4a** has 1.53 times the potency of **5a**, the semicarbazone **5b** and the thiosemicarbazone **5d** were 3.91 and 3.88 times as potent as the analogues **4b** and **4e** respectively. Secondly, although **4a**, which contains a five-membered alicyclic ring, is 2.08 times as active as the homo analogue **4b**, the semicarbazone **5b** has 2.88 times the potency of **5a**. In addition, increasing the size of **4a** led to compounds with higher (**4b**) and lower (**4c**) ED_{50} values.

The Ortep diagrams of **3a**, **4a–c**, **5a** and **5b** are displayed in figures 1–6. In the case of **5a** two crystallographically independent molecules designated A and B were noted. In addition, the X-ray structure of the MES-active anticonvulsant phenytoin **7** has been reported [6] and hence a comparison of its structure with the semicarbazones was considered of interest. In an attempt to discern correlations between the shapes of these molecules and anticonvulsant potencies, the hypothesis was made that the alignments of both the terminal four atoms of the semicarbazono group and the aryl ring at a receptor site were critical factors influencing bioactivity considerably. Thus the following measurements were made.

First, the stereochemistry of the semicarbazono group was examined as well as its relationship with the aryl ring. This was accomplished by considering the planes 1–3 which are indicated in figure 7. The angles made between these planes, namely θ_A, θ_B and θ_C, are presented in table IV. The θ_A angles indicate the deviation from coplanarity of the atoms in plane 1 with the adjacent plane 2. The semicarbazono group in **3a**, **4b** and **4c** was nearly planar (θ_A = 5.1° on average) and hence similar to phenytoin whereas the terminal aminocarbonyl group in **4a** and **5a** deviated markedly from coplanarity with plane 2 (average θ_A value = 14.2°). The θ_A value for **5b** was intermediate between the other two groups of compounds. When the N-O atoms of the terminal aminocarbonyl group of the compounds **3a**, **4a–c**, **5a**, **5b** and **7** were superimposed, the angle θ_B between these atoms and the aryl ring varied considerably. Similarly the angles made between the aryl ring and plane 2 (θ_C) also differ markedly in this group of compounds. It is of interest to note that among the semicarbazones listed in table IV the most potent anticonvulsant **5b** has the largest θ_B and θ_C angles and was thus similar to phenytoin while the least active analogue **4b** had very small θ_B and θ_C angles. Using the test for zero correlation [7], linear plots between each of the θ_B and θ_C angles with the MES ED_{50} figures obtained after oral administration to rats of compounds **3a**, **4a–c**, **5a**, **5b** and **7** revealed a correlation at the 85% but not 90% confidence levels. No correlation was observed between the MES figures and θ_A ($p > 0.15$). Second, the length of organic molecules may affect bioactivity [8] and hence the distances between the oxygen atoms and the protons at the maximum distance from the oxygen atoms were determined. This information is presented in table V. Linear plots of these distances against the oral rat MES ED_{50} figures did not reveal any correlation ($p > 0.15$).

Third, figures 1–6 indicate that the positions of the aryl rings with reference to the terminal portion of the semicarbazono group vary. Hence the aryl rings in

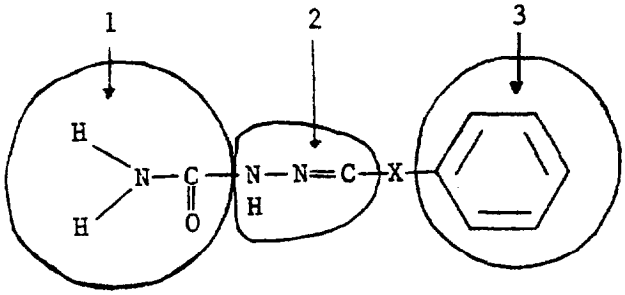


Fig 7. Planes 1–3 used in evaluating the X-ray data of compounds **3a**, **4a–c**, **5a**, **5b** and **7**.

Table IV. Angles between different planes in compounds **3a**, **4a–c**, **5a**, **5b** and **7** obtained by X-ray data.

Compound	*Atoms*			*Angles (esd) between planes*		
	Plane 1	*Plane 2*	*Plane 3*	*1 and 2 (θ_A)*	*1 and 3 (θ_B)*	*2 and 3 (θ_C)*
3a	N3-C8-O	N2-N1-C7-C1	C1 to C6	4.8 (3)	2.4 (1)	4.8 (2)
4a	N3-C10-O	N2-N1-C1	C4 to C9	14.3 (4)	19.5 (3)	5.5 (1)
4b	N3-C11-O	N2-N1-C1	C5 to C10	4.9 (3)	2.6 (2)	3.9 (2)
4c	N3-C12-O	N2-N1-C1	C6 to C11	5.6 (2)	42.7 (2)	46.8 (2)
5a (A)	N3A-C10A-O1A	N2A-N1A-C2A	C4A to C9A	13.9 (12)	24.0 (10)	10.2 (6)
5a (B)	N3B-C10B-O1B	N2B-N1B-C2B	C4B to C9B	14.5 (12)	21.2 (11)	7.0 (5)
5b	N3-C11-O	N2-N1-C2	C5 to C10	9.4 (2)	57.5 (2)	56.5 (1)
7	N2-C15-O2	N1-C14-C1	C2 to C7 (plane 3A)	4.0 (1)	64.6 (11), (θ_{B1})[a]	66.5 (6), (θ_{C1})[a]
			C8 to C13 (plane 3B)		68.4 (10), (θ_{B2})[a]	64.7 (10), (θ_{C2})[a]

[a]The angles θ_{B1} and θ_{C1} were obtained using plane 3A and θ_{B2} and θ_{C2} are the angles made using plane 3B.

these compounds would assume different positions on the aryl binding site if the aminocarbonylamino group at the end of the molecules occupied the same location on the hydrogen bonding area (fig 8).

In order to construct a receptor map indicating the locations of the centers of the aryl rings in relation to a common axis, the distances between the carbonyl carbon atom and the centers of the aryl ring were obtained. In addition, the bond angle θ was measured, which was the angle between the centers of the aryl

Table V. Distances between the aryl hydrogen atom at the maximum distance from the oxygen atom and the oxygen atom in compounds **3a**, **4a–c**, **5a**, **5b** and **7**.

Compound[a]	*Distance (Å)*
3a	9.527
4a	9.619
4b	9.411
4c	9.414
5a (A)	10.058
5a (B)	10.104
5b	9.618
7	7.808, 7.599

[a]The following hydrogen atoms were used in these calculations: H4 (**3a**), H5 (**4a**), H6 (**4b**), H7 (**4c**), H5A (**5a**-A), H6B (**5a**-B) and H6 (**5b**). For compound **7**, the distances measured were between the O2 oxygen atom and the H5 and H11 protons, respectively.

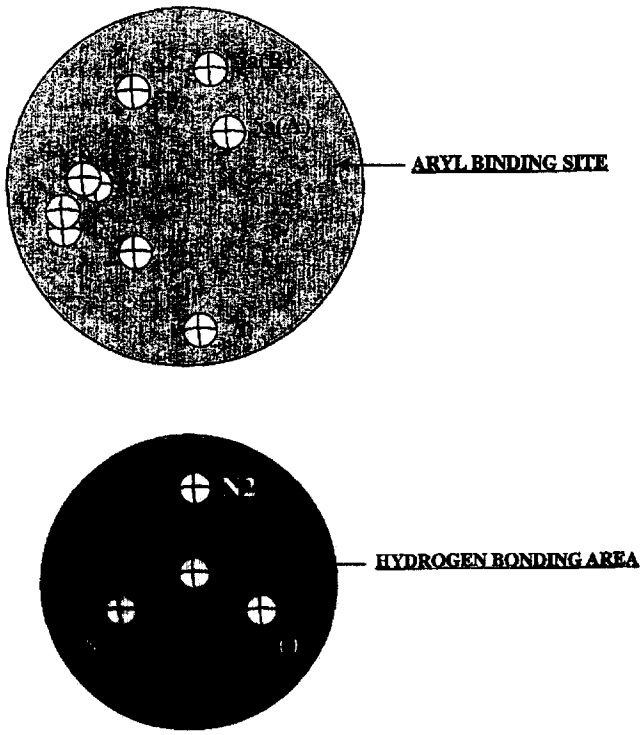


Fig 8. Projections of the centers of the aryl rings of **3a**, **4a–c**, **5a**, **5b** and **7** in the plane of the N3-C-O-N2 atoms.

ring, the carbonyl carbon atom and the N2 atom. Furthermore, when the four atoms N3-C-O-N2 were in the same plane, the centers of the aryl rings were either above (+) or below (–) this plane. The angle ψ is a measure of the deviation of the aryl ring from coplanarity with the common plane. The measurements of θ and ψ for one of the compounds is illustrated in figure 9 and the data are presented in table VI.

The data in figure 8 reveal that the aromatic rings of compounds **3a**, **4a–c**, **5a**, **5b** and **7** may be viewed as being located on two main areas of the putative aryl binding site. On the one hand, the semicarbazones **3a**, **4a–c** and **7** are found which have an average ED_{50} figure in the rat oral MES screen of 33.2 mg/kg while the comparable figure for **5a** and **5b** was 27.9 mg/kg. No correlation [7] was noted between the potencies of these compounds in the rat oral MES screen and either the aryl-C distances or the θ and ψ angles ($p > 0.1$). From these observations one may conclude that the receptor accommodates an aryl ring over a wide area and other considerations must influence potencies. Hence future work will be directed to preparing compounds whereby the aryl-C distances and θ values in particular will fall outside the area indicated in figure 8. In this manner, the goal of delineating the nature of the binding site at which aryl semicarbazones interact may be realized.

The P388 and L1210 leukemia screens have been widely used to detect useful anticancer drugs [9] and the evaluation of a number of compounds in these tests is presented in table III. The P388 data revealed that the five thiosemicarbazones (**4d–f**, **5c** and **5d**) are significantly more cytotoxic than the corresponding semicarbazones (**4a–c**, **5a** and **5b**). In fact, among the

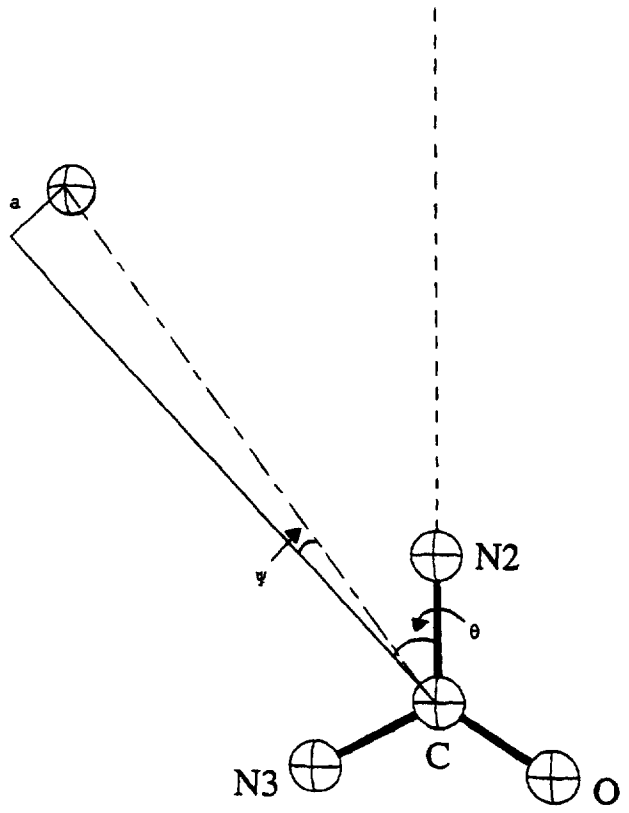


Fig 9. Measurements of the location of the center of the aryl ring from the carbonyl carbon atom for a representative compound. The letter 'a' indicates the displacement of the center of the aryl ring from the plane comprising the atoms N3-C-O-N2.

Table VI. Measurements of the projections of the centers of the aryl rings of compounds **3a**, **4a–c**, **5a**, **5b** and **7** in the plane of the N3-C-O-N2 atoms.

Compound	*Aryl-C distance (Å)*[a]	*Angle θ (°)*[b]	*Angle ψ (°)*[c]
3a	6.1	33.8	1.2
4a	6.1	36.0	8.1
4b	6.0	40.9	3.8
4c	5.9	41.5	–2.5
5a (A)	6.8	19.7	14.7
5a (B)	6.8	17.5	–12.6
5b	6.7	30.7	18.5
7[d]	4.7	41.0	25.7
7[e]	4.6	40.7	–29.2

[a]The aryl-C distance is calculated between the centers of the aryl rings and the carbonyl carbon atom. [b]The θ values are the angles made between the centers of the aryl ring, the carbonyl carbon and N2 atoms. [c]The angle ψ is a measurement of the deviation of the centers of the aryl rings from coplanarity with the N3-C-O-N2 plane. [d]These measurements refer to aryl ring A. [e]These figures are obtained using aryl ring B.

semicarbazones only **5b** had an IC_{50} value of less than 50 μM. In the series **4d–f**, **5a** and **5b**, activity rose as the lipophilicity of the molecule increased. In order to evaluate the generality of these observations, selected compounds were also examined in the L1210 screen. The thiosemicarbazones **4d**, **4f** and **5c** were all more cytotoxic than the corresponding semicarbazones **4a**, **4c** and **5a**. Potency was elevated as the size of the alicyclic group increased, *viz* **4c** > **4a** and **4f** > **4d**. Both the P388 and L1210 bioevaluations revealed that **4f** and **6b** had the lowest IC_{50} figures, *ie* these compounds were the most cytotoxic.

To verify whether any chemoselective toxicity would be displayed towards the L1210 cells in contrast to human T-lymphocytes, **4a**, **4c**, **4d**, **4f**, **5a**, **5c** and **6b** were examined for cytotoxicity against Molt4/C8 and CEM cells. The results are given in table III. Employment of Molt4/C8 lymphocytes revealed that **4d** displayed significant selective toxicity to the L1210 cells while use of CEM lymphocytes indicated that **4c** and **4f** definitely had preferential activity towards L1210 cells.

Seven compounds were examined against a variety of human tumors from nine specific diseases, namely leukemia, melanoma, non-small cell lung, colon, central nervous system, ovarian, renal, prostate and breast cancers. The molar quantities required to reduce the growth of the cells by 50% relative to the untreated cells are referred to as the GI_{50} figures. Most of the cell lines were insensitive to the compounds at the maximum concentrations used namely $\log_{10}$–4.00 M (**5a**, **5c**, **5d** and **6a**) or $\log_{10}$–5.00 M (**4c**, **4f** and **6b**) and these figures were included in calculating the average cytotoxicity data for all tumors. Hence the term MG MID (meangraph midpoint) rather than mean value is used in table III. A $\log_{10}$ GI_{50} figure was not obtained for any cell line in the case of compounds **4c**, **5a** and **6b**. For the remaining derivatives, $\log_{10}$ GI_{50} values were obtained against the following number of cell lines, *viz* **4f**: 1/48; **5c**: 1/60; **5d**: 9/55; and **6a**: 4/57. Hence, **5d** showed the widest spectrum of activity, but, in general, the compounds showed little or no activity towards human tumor cell lines.

Compounds **4a**, **4c**, **4d**, **4f**, **5a**, **5c** and **6b** were examined for their displayed antiviral activity. In order for a compound to be considered a useful lead as an antiviral agent, it should be active at a concentration at least ten-fold lower than its toxic concentration to normal cells. This criterion was only achieved by **4d** against vaccinia virus, and hence in general these compounds have cytostatic activities rather than antiviral properties.

In conclusion, this study has revealed that a number of semicarbazones and thiosemicarbazones derived from aryl alicyclic ketones have anticonvulsant properties. Greater PI values were obtained with the semicarbazones, and, in particular, the potency and PI of 2-tetralone semicarbazone (**5b**) are noteworthy. The thiosemicarbazones displayed greater cytotoxicity than the semicarbazones towards two murine leukemic cell lines while representative compounds were mainly inactive towards a variety of human tumors and viruses.

Experimental protocols

Chemistry

Melting points are uncorrected. Elemental analyses (C, H, N) were undertaken on the compounds in series **4–6** and are within ±0.4% of the calculated values except for **6a** (calcd for $C_{19}H_{18}N_4O$: N; 17.59%; found: N; 16.95%). The purity of the compounds was confirmed by thin-layer chromatography using silica-gel plastic-backed sheets and a solvent system benzene/methanol (8:2).

*Synthesis of the semicarbazones **4a–c**, **5a** and **5b***

A mixture of semicarbazide hydrochloride (0.01 mol), sodium acetate (0.01 mol) and water (10 ml) was added slowly to a stirred solution of the ketone (0.01 mol) in ethanol (95%, 30 ml). The reaction mixture was stirred at room temperature for 4 h (**4a** and **5a**) or 24 h (**4b**, **4c** and **5b**). The precipitate was collected, washed with ether and water and dried. Recrystallization from water/acetic acid (**4a**) or ethanol (95%, **4b**, **4c**, **5a** and **5b**) gave semicarbazones with melting points (°C) as follows: **4a**: 240; **4b**: 224; **4c**: 220; **5a**: 218 (dec); and **5b**: 200.

*Syntheses of the thiosemicarbazones **4d–f**, **5c** and **5d***

A solution of thiosemicarbazide (0.02 mol) in ethanol (95%, 10 ml) was added slowly to a stirring solution of the appropriate ketone (0.02 mol) in ethanol (95%, 30 ml) and acetic acid (4 ml). The reaction mixture was stirred at room temperature for 24 h (**4d** and **5c**) or heated under reflux for 24 h (**4e**, **4f** and **5d**). On cooling, the precipitate was collected, washed with ether and water and dried. Recrystallization from ethanol (95%) provided the following thiosemicarbazones with melting points (°C) as follows: **4d**: 188; **4e**: 210; **4f**: 160; **5c**: 204 (dec); and **5d**: 160.

*Synthesis of the bis-carbohydrazones **6a** and **6b***

A solution of carbohydrazide (0.01 mol) in ethanol (95%, 30 ml) was added to a solution of 2-indanone (0.02 mol) in ethanol (95%, 25 ml). The mixture was stirred at room temperature for 2 h and the precipitate was collected and recrystallized initially from ethanol (95%) and finally benzene to give **6a**, mp 210°C (dec).

A solution of carbohydrazide (0.01 mol) in methanol (20 ml) was added to a solution of 2-tetralone (0.02 mol) in methanol (25 ml) and the resultant mixture was stirred at room temperature for 20 min. The solid was collected and recrystallization from ethanol (95%) gave **6b**, mp 164°C (dec).

*X-ray crystallographic studies of **4a–c**, **5a** and **5b***

The compounds were recrystallized from isopropanol/ethanol (**4a**), isopropanol/methanol (**4b**), methanol by slow evaporation (**4c**) or diethylether/ethanol by vapor diffusion (**5a** and **5b**). An Enraf-Nonius CAD-4 diffractometer with $\omega/2\theta$ scan was used for data collection and the structures were solved by direct

methods using NRCVAX [10]. Atomic scattering factors were taken from the literature [11]. All non-hydrogen atoms were found on the E map and refined anisotropically. Hydrogen atoms were calculated and not refined.

The data for **4a** were as follows: $C_{10}H_{11}N_3O$, M_r = 189.21, colorless rectangular prisms, 0.15 x 0.125 x 0.125 mm, a = 7.0517(7), b = 7.2903(7), c = 9.6891(10) Å, α = 96.49(1), β = 101.42(1), γ = 102.78(1)°, V = 469.70(5) Å^3, Z = 2, space group = P1, triclinic, D_m = 1.350, D_x = 1.338 gcm^{-3}, λ(MoK_α) = 0.7093 Å, μ = 0.08 mm^{-1}, F(000) = 200, T = 287K, $(\sin\theta)/\lambda_{max}$ = 0.6180 Å^{-1}, $-8 \le h \le 8$, $0 \le k \le 8$, $-11 \le l \le 11$. Merging R is based on intensities 0.007 for 343 replicate reflections. $R(F)$ = 0.050, R_w = 0.056 and S = 1.91. A total of 2179 reflections were measured of which 1836 were independent. The refinement of the structure used 1177 observed reflections [$I > 2.5\sigma(I)$]. Parameters refined = 127, [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.000. $\Delta\rho$ in the final difference map was within +0.19 and –0.35 e Å^{-3}.

The data for **4b** were as follows: $C_{11}H_{13}N_3O$, M_r = 203.24, colorless rods 0.50 x 0.125 x 0.075 mm, a = 6.3183(17), b = 21.323(4), c = 7.2944(13) Å, β = 97.608(20)°, V = 974.1(4) Å^3, Z = 4, space group = P2$_1$/a, monoclinic, D_m = 1.362, D_x = 1.359 gcm^{-3}, λ(MoK_α) = 0.7093 Å, μ = 0.084 mm^{-1}, F(000) = 432, T = 123K, $(\sin\theta)/\lambda_{max}$ = 0.5958 Å^{-1}, $-7 \le h \le 7$, $0 \le k \le 25$, $-1 \le l \le 8$. Merging R is based on intensities 0.021 for 430 replicate reflections. $R(F)$ = 0.046, R_w = 0.051 and S = 1.81. A total of 2142 reflections were measured of which 1712 were independent. The refinement of the structure used 1086 observed reflections [$I > 2.5\sigma(I)$]. Parameters refined = 136, [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.000. $\Delta\rho$ in the final difference map was within +0.20 and –0.28 e Å^{-3}.

The data for **4c** were as follows: $C_{12}H_{15}N_3O$, M_r = 217.27, colorless thick plates 0.50 x 0.45 x 0.10 mm, a = 9.6452(10), b = 7.4773(7), c = 30.8784(16) Å, β = 97.67(9), V = 2207.0(3) Å^3, Z = 8, space group = A2/n, monoclinic, D_m = 1.312, D_x = 1.308 gcm^{-3}, λ(MoK_α) = 0.7093 Å, μ = 0.08 mm^{-1}, F(000) = 928, T = 287K, $(\sin\theta)/\lambda_{max}$ = 0.6180 Å^{-1}, $0 \le h \le 11$, $-8 \le k \le 8$, $-36 \le l \le 36$. Merging R is based on intensities 0.034 for 702 replicate reflections. $R(F)$ = 0.058, R_w = 0.073 and S = 2.73. A total of 4605 reflections were measured of which 3903 were independent. The refinement of the structure used 2851 observed reflections [$I > 2.5\sigma(I)$]. Parameters refined = 145, [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.001. $\Delta\rho$ in the final difference map was within +0.22 and –0.35 e Å^{-3}.

The data for **5a** were as follows: $C_{10}H_{11}N_3O$, M_r = 189.22, colorless plates 0.30 x 0.10 x 0.05 mm, a = 7.4783(7), b = 14.4373(14), c = 17.0392(17) Å, V = 1839.7(3) Å^3, Z = 8, space group = Pbn2$_1$, orthorhombic, D_m = 1.372, D_x = 1.366 gcm^{-3}, λ(MoK_α) = 0.7093 Å, μ = 0.09 mm^{-1}, F(000) = 800, T = 287K, $(\sin\theta)/\lambda_{max}$ = 0.6180 Å^{-1}, $0 \le h \le 9$, $0 \le k \le 17$, $0 \le l \le 20$. Merging R is based on intensities 0.012 for 128 replicate reflections. $R(F)$ = 0.047, R_w = 0.048, S = 1.52. A total of 1986 reflections were measured of which 1858 were independent. The refinement of the structure used 1029 observed reflections [$I > 2.5\sigma(I)$]. Parameters refined = 252, [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.000. $\Delta\rho$ in the final difference map was within +0.20 and –0.21 e Å^{-3}.

The data for **5b** were as follows: $C_{11}H_{13}N_3O$: M_r = 203.24, colorless rectangular prisms, 0.43 x 0.35 x 0.15 mm, a = 11.2810(11), b = 6.8040(7), c = 13.7833(14) Å, β = 108.850(11)°, V = 1001.21(17) Å^3, Z = 4, space group = P2$_1$/c, monoclinic, D_m = 1.374, D_x = 1.348 gcm^{-3}, λ(MoK_α) = 0.7093 Å, μ = 0.08 mm^{-1}, F(000) = 432.17, T = 287K, $(\sin\theta)/\lambda_{max}$ = 0.6180 Å^{-1}, $-13 \le h \le 13$, $0 \le k \le 8$, $0 \le l \le 16$. Merging R is based on intensities 0.023 for 1752 replicate reflections. $R(F)$ = 0.043, R_w = 0.061, S = 2.50. A total of 3710 reflections were measured of which 1958 were independent. The refinement of the structure used 1530 observed reflections [$I > 2.5\sigma(I)$]. Parameters refined = 136, [$w = 1/\sigma^2(F)$]; final $(\Delta/\sigma)_{max}$ = 0.000. $\Delta\rho$ in the final difference map was within +0.24 and –0.32 e Å^{-3}.

Bioevaluation

Anticonvulsant screening

The data presented in tables I and II were provided by the Anticonvulsant Drug Development Program, Division of Convulsive, Developmental, and Neuromuscular Disorders, NIH, USA using their protocols [12]. Oral administration of **4a**, **4d**, **5a** and **5c** (25 mg/kg) and **4f**, **5b** and **5d** (50 mg/kg) to rats revealed the following protection in the scPTZ screen: **4a**: 0/4; **4d**: 0/4; **4f**: 2/4 (0.25 h); **5a**: 1/4 (2 h); **5b**: 1/4 (0.5, 1 h); **5c**: 0/4; and **5d**: 1/4 (0.25 h).

Cytotoxicity evaluation

The bioactivity of the compounds in series **4–6** using P388D1 cells was obtained using a literature procedure [13] while the evaluation using the L1210 screen was undertaken by the previously described protocol [14]. The assay using approximately 54 human tumors was performed by the National Cancer Institute, USA, using their protocols [15].

Antiviral screening

The methodology for evaluating **4a**, **4c**, **4d**, **4f**, **5a**, **5c** and **6b** for antiviral activity has been described previously [16, 17].

Acknowledgments

The authors record their appreciation to the following organizations and individuals who supported and assisted in this investigation. Nordic Merrell Dow Research Canada provided a grant to JRD. Banaras Hindu University, India, provided a leave of absence to SNP to study at the University of Saskatchewan; this sabbatical was financed by the Canadian Commonwealth Scholarship and Fellowship Plan. The Natural Sciences and Engineering Council of Canada provided an operating grant to JWQ and money for an X-ray diffractometer. The National Cancer Institute of Canada provided a grant to TMA. The AIDS Basic Research Programme of the European Community and the Belgian FGWO (fonds voor Geneeskundig Wetenschappelijk Onderzoek) provided funds for EDC. The excellent technical assistance of A Absillis, F De Meyer, L Van Berckelaer and A Van Lierde working in the laboratoy of EDC is gratefully acknowledged. In particular, the extraordinary assistance of JP Stables and members of the Anticonvulsant Drug Development Program, USA, is recorded. The National Cancer Institute, USA, examined a number of the compounds for activity towards a variety of human tumors.

References

1 Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) *Epilepsia* 19, 409–428
2 Jones GL, Woodbury DM (1982) *Drug Dev Res* 2, 333–355
3 Dimmock JR, Sidhu KK, Thayer RS *et al* (1993) *J Med Chem* 36, 2243–2252
4 Pandeya SN, Dimmock JR (1993) *Pharmazie* 48, 659–666
5 Sidwell RW, Witkowski JT (1979) *Burger's Medicinal Chemistry, 4th Edition Part II* (Wolff ME, ed) John Wiley and Sons, New York, USA, 553–554

6 Camerman A, Camerman N (1971) *Acta Cryst* B27, 2205–2211
7 Bolton S (1984) *Pharmaceutical Statistics*. Marcel Dekker, NY, USA, 207–209
8 Hansch C, Leo A (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*. John Wiley and Sons, New York, USA, 11
9 Hakola MT, Rustum YM (1979) *Methods in Cancer Research, Volume XVI* (DeVita Jr VT, Busch H, eds) Academic Press, NY, USA, 247–287
10 Gabe EJ, LePage Y, Charland JP, Lee FL, White PS (1989) *J Appl Crystallogr* 22, 384–387
11 *International Tables for X-ray crystallography, Volume IV* (1974) Kynoch, Birmingham, UK
12 Porter RJ, Cereghino JJ, Gladding GD *et al* (1984) *Cleveland Clin Q* 51, 293–305
13 Phillips OA, Nelson LA, Knaus EE, Allen TM, Fathi-Afshar R (1989) *Drug Design Delivery* 4, 121–127
14 Balzarini J, De Clercq E, Mertes MP, Shugar D, Torrence PF (1982) *Biochem Pharmacol* 31, 3673–3682
15 Grever M, Schepartz SA, Chabner BA (1992) *Seminars in Oncology* 19, 622–638
16 De Clercq E (1985) *Antimicrob Agents Chemother* 28, 84–89
17 Balzarini J, Karlsson A, De Clercq E (1993) *Mol Pharmacol* 44, 694–701