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Anticonvulsants Containing the N-(3-Aryl-2-propenoyl) amido Pharmacophore

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A series of 1-(3-aryl-2-propenoyl)-4-oxopiperidines (1) as well as some related semicarbazones (2) and thiosemicarbazones (3) were prepared in order to determine whether the relative locations of aryl rings and amidic groups would lead to novel anticonvulsant agents. Initially the compounds were administered intraperitoneally to mice and examined in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and neurotoxicity (NT) screens. The biodata revealed that anticonvulsant properties were displayed by most of the compounds in series (1), in half of the semicarbazones (2) while protection was absent by members of series (3). Molecular modeling was utilized in order to compare the positions of a phenyl ring in relation to amidic groups in representative compounds in series (1–3) with previously reported anticonvulsant agents. Molecular simplification of 4-oxo-1-(3-phenyl-2-propenoyl)piperidine (1a) led to 1-(3-phenyl-2-propenoyl)piperidine (7) and N,N-diethylcinnamamide (8) with retention of anticonvulsant properties. Both (1a) and (8) afforded protection in the hippocampal kindling screen in rats. When administered orally to rats, (1a) and (8) demonstrated activity in the MES screen and in the case of (8), a huge protection index was observed revealing it to be an important lead compound. The IC_{50} values of all of the compounds towards murine P388 cells were in excess of 50 μM while several compounds displayed cytotoxicity towards *Mycobacterium tuberculosis*.

Keywords: Anticonvulsants; N-acyl-4-piperidones; Molecular modeling; Semicarbazones; Thiosemicarbazones

INTRODUCTION

The objective of the present study was the preparation and bioevaluation of a small series of prototypic molecules designed as candidate anticonvulsant agents. The initial series of compounds **1–3** possess one potential pharmacophoric group comprising an aryl ring and an amidic function which are displayed in a number of antiepileptic drugs.[1] In the case of series **2** and **3**, a semicarbazono or thiosemicarbazono group was added in order to simulate the aryl ring and semicarbazono or thiosemicarbazono substituents which are considered to contribute significantly to the activity of the anticonvulsant aryloxyaryl semicarbazones and thiosemicarbazones.[2] The structures of the compounds in series **1–3** are presented in Figure 1.

A number of major groups of heterocyclic antiepileptic drugs have the following structural features, namely (1) an aryl group attached to a carbon atom of a heterocyclic ring, (2) a dicarboximide (CONHCO) or substituted dicarboximide (CONRCO) portion of the heterocyclic ring, and, (3) the groups which make a major contribution to bioactivity are fixed in relation to each other.[1] The compounds in series **1** incorporated some of these three structural characteristics. Hence an aryl ring was present in series **1**. However since the dicarboximide group has been associated with toxicity,[3] the related amidic function (CONH) was employed instead. The rigidity of the molecules in this study was achieved in two ways, namely through the placement of an olefinic spacer group between the aryl ring and amidic group as well as the incorporation of the nitrogen atom of the amide into a piperidine ring. A 4-oxo group was placed on the piperidine ring in series **1** to increase the possibility of hydrogen bonding at a binding site.

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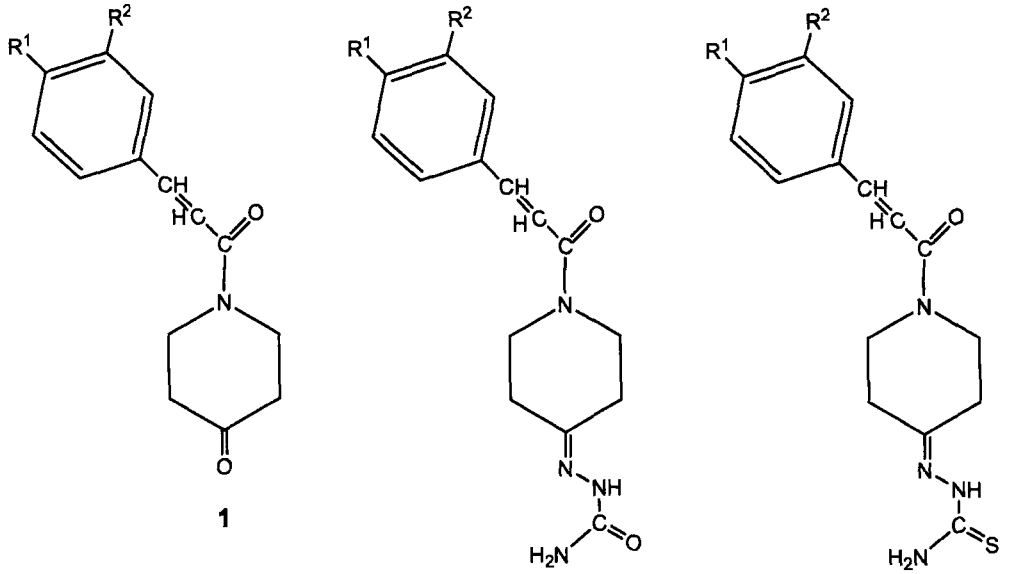
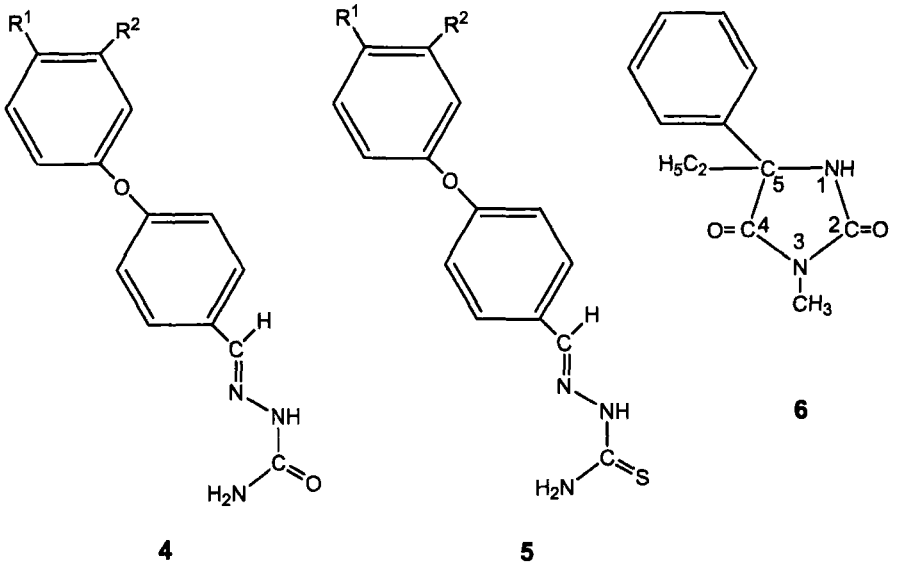


1 2 3

FIGURE 1 The structures of the compounds in series **1–3**. The nature of the R^1 and R^2 groups was as follows: **a**: R^1=R^2 = H; **b**: R^1=R^2=Cl; **c**: R^1=Cl, R^2=H; **d**: R^1=F, R^2=H; **e**: R^1=CH_3, R^2=H; **f**: R^1=OCH_3, R^2=H.

Recently the discovery of the anticonvulsant properties of various aryloxyaryl semicarbazones **4** and thiosemicarbazones **5** has been disclosed. The structures of these compounds as well as a reference drug mephenytoin **6** are presented in Figure 2. In the studies using these compounds, a triine interaction between the two aryl rings and either the semicarbazono or thiocarbazono groups of portions of **4** and **5** with a receptor was postulated. In the present investigation, the aryl ring of **2** and **3** and the terminal aromatic ring in **4** and **5** were considered to be approximately equidistant from the semicarbazono or thiosemicarbazono groups. Hence the aspiration was made that a three point drug-receptor interaction would occur with the compounds in series **2** and **3**. In other words, the aryl ring, amidic group and semicarbazono or thiosemicarbazono functions of **2** and **3** would form bonds with three complementary areas on a binding site.

In order to compare the shapes of the compounds in series **1–3** with established antiepileptic and anticonvulsant molecules, the decision was made to determine a number of interatomic distances as well as bond and torsion angles of the compounds prepared in this study with reference substances using molecular modeling.



4 5 6

FIGURE 2 The structures of series **4** and **5** and mephenytoin **6**.

A final aspect of this investigation was as follows. The compounds prepared in this study contain partial structures which are found in both cytotoxic and antitubercular agents. For example, the β-aryl-α,β-unsaturated keto group is present in a variety of compounds which display antineoplastic properties[4,5] while a number of thiosemicarbazones are antimycobacterials.[6] The decision was made therefore to evaluate these molecules towards both murine P388 lymphocytic leukemia cells and *Mycobacterium tuberculosis in vitro*.

MATERIALS AND METHODS

Chemistry

Melting points were uncorrected and are given in degrees Celsius. Yields of compounds are expressed as percentages. Elemental analyses (C, H, N) were undertaken on **1a–f**, **2a–f**, **3a–d**, **7** and **8** by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan and were within 0.4% of the calculated values. ^{1}H NMR spectra were determined routinely which confirmed the structures proposed. TLC used silica gel sheets.

Syntheses of the 1-(3-Aryl-2-propenoyl)-4-piperidones 1a–f

3-Phenyl-2-propenoyl chloride was obtained commercially while the acid chlorides required in the syntheses of **1b–f** were prepared as follows. The 3-aryl-2-propenoic acids were synthesized using a literature procedure[7] and the crude products were purified by recrystallization from water–ethanol until homogeneous by TLC using a solvent system of chloroform: methanol (8:2). The acids were converted to the corresponding acid chlorides using a previously reported procedure[8] and were used without further purification.

The appropriate propenoyl chloride (0.01 M) and sodium carbonate (0.02 M) were added to a solution of 4-piperidone hydrochloride monohydrate (0.01 M) in chloroform (50 ml). The mixture was heated under reflux with stirring for 8–10 h and on cooling, the solution was filtered. Evaporation of the solvent gave a residue which was washed successively with water, dilute hydrochloric acid and water and then dried. The resultant product was recrystallized from petroleum spirit bp 100–120° (**1a,e,f**) or 95% ethanol (**1b–d**) to give the following compounds (melting points and percentage yields in parentheses); **1a** [109–110°, lit.[9] 107–109°, 65], **1b** (151°, 70), **1c** (208–210°, 80), **1d** (172–173°, 70), **1e** (96–97°, 45), **1f** (157–158°, 30). The ^{1}H NMR spectrum (500 MHz) of a representative compound **1a** was as follows: δ ($CDCl_3$): 2.55–2.57 (t, 4H, 2 × CH_2CO), 3.99 (br s, 4H, 2 × CH_2N), 6.93–6.97 (d, 1H, =CH, J = 15.4 Hz), 7.39 (m, 3H, aryl H), 7.55–7.56 (m, 2H, aryl H), 7.74–7.77 (d, 1H, =CH, J = 15.4 Hz).

Synthesis of the Semicarbazones 2a–f

The N-acyl-4-piperidones **1a–f** were converted into the corresponding semicarbazones by a literature procedure[2] and purified by recrystallization from 95% ethanol to give the following compounds (melting points and yields in parentheses); **2a** (189–191°, 55), **2b** (210–212°, 58), **2c** (201–203°, 80), **2d** (202–204°, 62), **2e** (196–199°, 50), **2f** (185–188°, 40). The ^{1}H NMR spectrum (500 MHz) of a representative compound **2a** was as follows: δ ($CDCl_3$): 2.55 (br s, 4H, 2 × CH_2—C=N), 3.83–3.88 (br d, 4H, 2 × CH_2N), 5.06 (br s, 1H, NHCO, D_2O exchangeable), 6.14 (br s, 1H, NHCO, D_2O exchangeable), 6.90 (t, 1H, =CH), 7.36–7.40 (m, 3H, aryl H), 7.54–7.55 (m, 2H, aryl H), 7.70–7.73 (d, 1H, =CH, J = 15.3 Hz), 8.91 (s, 0.5H, NH, D_2O exchangeable), 9.19 (s, 0.5H, NH, D_2O exchangeable).

Synthesis of the Thiosemicarbazones 3a–d

A solution of thiosemicarbazide (0.01 M) in absolute alcohol (20 ml) was prepared by gentle heating and then added dropwise to a solution of the appropriate N-aryl-4-piperidone (0.01 M) in absolute alcohol (20 ml). The mixture was stirred at room temperature for 2–4 h and the product collected by filtration. Recrystallization from absolute alcohol afforded the following thiosemicarbazones (melting points and percentage yields in parentheses); **3a** (168–170°, 43), **3b** (215–217°, 75), **3c** (204–206°, 80), **3d** (205–207°, 85). The ^{1}H NMR spectrum (500 MHz) of a representative compound **3a** was as follows: δ ($CDCl_3$): 2.53 (t, 2H, CH_2C=N), 2.59 (br s, 2H, CH_2C=N), 3.75 (br s, 4H, 2 × CH_2N), 6.29 (s, 1H, NHCO, D_2O exchangeable), 6.88–6.91 (d, 1H, =CH, J = 15.3 Hz), 7.24 (s, 1H, NHCO, D_2O exchangeable), 7.40 (br s, 3H, aryl H), 7.55 (br s, 2H, aryl H), 7.73–7.76 (d, 1H, =CH, J = 15.4 Hz), 8.64 (s, 1H, NH, D_2O exchangeable).

Synthesis of the Amide 7

1-(3-Phenyl-2-propenoyl)piperidine **7** was prepared from piperidine and 3-phenyl-2-propenoyl chloride using the same procedure as employed in the synthesis of **1a–f**. The product was recrystallized from 95% ethanol to give **7**, mp 118–119° (lit.[10] 118–120°) in 80% yield.

Synthesis of 8

A solution of *E*-cinnamoyl chloride (0.02 M) in diethyl ether (75 ml) was added dropwise to a solution of

diethylamine (0.03 M) in diethyl ether (75 ml) which was cooled using an ice bath over a period of 0.5 h. The reaction mixture was then stirred at room temperature for 1.5 h. Water (100 ml) was added and the ethereal layer was separated and washed repeatedly with hydrochloric acid (10% w/v) and subsequently with sodium bicarbonate solution (10% w/v). After drying over anhydrous magnesium sulphate, the organic solvent was removed *in vacuo* to produce an oil which solidified. Recrystallization from 50% aqueous ethanol led to the isolation of **8**, mp 71–72° (lit.[11] 68–69°) in 95% yield.

Molecular Modeling

Models of **1a**,**2a**,**3a**,**4a**,**5a** and **6** were built using the MacroModel 8.0 programme[12] which was followed by a Monte Carlo search for the conformations having the lowest energies; this latter process used an Amber force field of 1000 initial conformations. The data used were obtained on the lowest energy conformations.

Statistical Analyses

The sigma, pi and molar refractivity (MR) values of both of the R^1 and R^2 groups of **3a–d** were taken from the literature.[13] The linear and semi-logarithmic plots between each of these values and the antimycobacterial potency ratios were generated using a commercial software package.[14]

Bioevaluations

Evaluation of Compounds for Anticonvulsant Activity and Toxicity

The anticonvulsant and toxicity experimentation was undertaken by the National Institutes of Neurological Disorders and Stroke, USA under the Anticonvulsant Screening Program. All animals were handled, fed and housed using the procedures outlined in the National Research Council Publication, "Guide for the Care and Use of Laboratory Animals". The mice and rats were euthanized consistent with the policies of the Institute of Laboratory Resources which describe the humane care of laboratory animals. The anticonvulsant and toxicity screens were undertaken by literature procedures.[15] All of the compounds in series **1–3**, **7** and **8** were evaluated initially in mice. In most cases, doses of 30, 100 and 300 mg/kg were injected intraperitoneally and examined after 0.5 and 4 h in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and neurotoxicity (NT) screens. In general, the following numbers of animals were employed in each determination. One mouse was used in the MES screen except for the 100 mg/kg dose in which case three animals were utilized. One mouse was used in the scPTZ test. In the case of the toxicity screen, two animals which received 30 and 300 mg/kg of the compounds were observed after 4 h. After administration of 30, 100 and 300 mg/kg of the compounds, four mice were observed after 0.5, 4 and 0.5 h, respectively. Eight animals were employed when 100 mg/kg of the compounds were injected and the mice were observed after 0.5 h. The results are summarized in Table I. Other observations made from these three initial tests were as follows. In the scPTZ screen, after 4 h **2a** caused tonic extension (100 mg/kg dose) and death following a clonic seizure was noted when 300 mg/kg of **3a** was administered. After 0.5 h, the following observations were made in the NT test (dose in mg/kg in parentheses), namely inability to grasp the rotorod: **1b** (100), **1d** (300), **1e** (100), anesthesia: **1b** (300), **8** (300), and loss of righting reflex: **1f** (300). After 0.5 h, death was caused by the following compounds (number of animals dead/number of animals treated, dose in mg/kg), namely **1a** (2/4, 300), **1b** (1/4, 300), **1e** (6/8, 100; 4/4, 300), **1f** (2/4, 300, **2b** (1/4, 300), **2c** (1/4, 300), and **8** (1/4, 300). Four hours after administration of 300 mg/kg of **3a**, clonic seizures and wild running were noted.

Quantitation of the potencies of **8** in the MES, scPTZ and NT screens were made 0.25 h after intraperitoneal injection in mice. The 95% confidence intervals are indicated in parentheses. The ED_{50} figures of **8** in the MES and scPTZ screens were 52.0

TABLE I Doses of **1a–f**, **2a–f**, **3a–d**, **7** and **8** which elicit anticonvulsant and neurotoxic properties in mice[a]

	MES screen		scPTZ screen		NT screen	
Compound	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1a	100	300	30	b	300	–
1b	100	100	100	100	100	100
1c	–	–	300	300	300	300
1d	300	300	100	300	100	100
1e	c	c	b	b	100	b
1f	300	b	300	b	300	b
2a	300	300	300	–	300	300
2b	–	–	–	b	300	300
2c	–	100	30	b	300	300
2d	–	–	–	300	100	100
2e	–	–	–	–	–	–
2f	–	–	–	–	300	–
3a	–	–	–	–	100	100
3b	–	–	–	–	–	–
3c	–	–	–	–	–	300
3d	–	–	–	–	–	–
7	300	–	300	–	100	–
8	100	300	100	b	300	–

[a] In most cases, doses of 30, 100 and 300 mg/kg of each compound were injected intraperitoneally into mice. The figures in the table indicate the lowest doses at which biological responses occurred in 50% or more of the animals. The number of animals used in the tests is stated in the Experimental section. The designation – indicates a lack of bioactivity.
[b] Evaluated at doses of 30 and 100 mg/kg only. No bioactivity was noted.
[c] Compound was only evaluated using a dose of 30 mg/kg. No activity was observed.

(48.4–57.3) and 69.4 (56.3–80.4) mg/kg, respectively, while the TD_{50} value (this is the dose of the compound causing toxic symptoms to appear in 50% of the treated animals) was 114.4 (73.0–170.2) mg/kg. The data for mephenytoin in the MES and scPTZ screens determined 0.5 h after intraperitoneal injection in mice were 60.5 (49.5–70.3) and 30.5 (19.6–39.5) mg/kg, respectively, while the TD_{50} figure, generated 0.25 h after administration, was 154 (133–179) mg/kg.[16]

The N-acylpiperidones **1a**, **b**, **f**, **2c** and **8** were administered orally to rats and examined at the end of 0.25, 0.5, 1, 2 and 4 h in different screens. In the case of the MES test, doses of 30 mg/kg of each compound were used and the number of animals (out of 4) which were protected after 0.25, 0.5, 1, 2 and 4 h were as follows: **1a**: 1, 0, 2, 1, 4; **1b**: 2, 1, 0, 0, 2; **2c**: 0, 1, 2, 0, 0 and **8**: 1, 4, 2, 3, 0 while **1f** afforded no protection. Compounds **1b**, **2c** and **8** were evaluated in the scPTZ test using a dose of 50 mg/kg and displayed no anticonvulsant activity. Using doses of 30 (**1a**, **1f**, **2c**) and 50 (**1b**, **2c**, **8**) mg/kg, no neurological deficit was noted by these compounds within the 0.25–4 h time frame.

Quantitation of **1a** and **8** in the MES, scPTZ and NT screens after oral administration to rats was undertaken (95% confidence intervals in parentheses). After 2 h, the ED_{50} value of **1a** in the MES screen was 20.7 (10.8–37.1) mg/kg while at the same time interval, no protection was afforded in the scPTZ screen using doses up to and including 250 mg/kg. The toxicity of **1a** was evaluated after 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h using doses of 125 and 250 mg/kg. No neurotoxicity was noted using the 125 mg/kg dose. However administration of 250 mg/kg of **1a** revealed that 2/2 rats displayed neurological deficit during the 0.25–4 h time frame after which no neurotoxicity was noted. The ED_{50} value of **8** in the MES screen was 25.3 (17.8–35.4) mg/kg when determined 1 h after administration. At the same time interval, 1/2 and 2/2 rats were protected in the scPTZ test using doses of 112.5 and 225 mg/kg. No neurotoxicity was detected after 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h using doses up to and including 450 mg/kg. The ED_{50} figures for mephenytoin in the MES and scPTZ tests were 18.1 (14.0–24.9) and 21.7 (18.0–25.8) mg/kg, respectively, while the TD_{50} value was 85.7 (69.9–93.8) mg/kg.

Compound **1a** was administered intraperitoneally to rats and examined in the MES and neurotoxicity screens using doses of 3 and 50 mg/kg, respectively. The number of animals (out of 4) which were protected in the MES test after 0.25, 0.5, 1, 2 and 4 h were 1, 0, 0, 1 and 1, respectively, while no toxicity was observed in 8 animals after 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h.

Compounds **1a** and **8** were examined in the hippocampal kindled rat model using a literature procedure.[17] The severity of the seizure was estimated using Racine's motor seizure score.[18] In brief, bipolar electrodes were inserted into the ventral hippocampus and after one week, the animals were kindled to stage 5. After 7 days, varying doses of compounds were administered intraperitoneally into rats and the seizure scores and afterdischarge duration times were recorded after 15, 45, 75, 105 and 135 min. The ED_{50} values, i.e., the doses required to protect half of the treated animals against seizures, were determined after 15 min and found to be 40.8 (36.5–44.3) and 43.1 (33.9–55.9) mg/kg for **1a** and **8**, respectively. Statistically significant reductions ($p < 0.05$ determined by the Student's t-test) in afterdischarge durations (in seconds ± SEM) for **1a** were as follows (dose in mg/kg, time in min in parentheses): 98.2 ± 7.64 (25, 105), 96.3 ± 8.42 (37.5, 105), 87.5 ± 4.31 (43, 75), 84.4 ± 2.43 (43, 105) and 91.3 ± 5.92 (43, 135). The comparable data for **8** were 49.9 ± 13.4 (37.5, 15) and 44.3 ± 13.6 (50, 15).

Cytotoxic Evaluation using Murine P388 Cells

Compounds **1a–f**, **2a–f**, **3a–d**, **7** and **8** were evaluated against murine P388D1 cells using a reported procedure.[19] In brief, solutions of different concentrations of the compounds were added to P388 cells and incubated at 37°C for 48 h. The percentage survival of the cells was then noted. All tests and controls were carried out in triplicate at each concentration of the compounds.

Antitubercular Assay

A concentration of 12.5 μg/ml of **1a–f**, **2a–f**, **3a–d**, **7** and **8** was assessed against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue assay.[20] Antitubercular properties were displayed by the following amides (% inhibition in parentheses), namely **1b** (21), **1c** (15), **2b** (6), **3a** (61), **3b** (77), **3c** (48), **3d** (40) and **7** (58). Under the conditions employed, the remaining compounds did not inhibit the growth of this microorganism.

RESULTS

Acylation of 4-piperidone with various aroyl chlorides led to the formation of **1a–f**. Reaction of **1a–f** with semicarbazide gave rise to the corresponding semicarbazones while the synthesis of **3a–d** was achieved by condensing **1a–d** with thiosemicarbazide. ^{1}H NMR spectroscopy revealed that the olefinic double bond of **1–3** adopted the *E*-configuration. The amides **7** and **8** (*vide infra*) were prepared by

TABLE II Interatomic distances (d_1–d_6), bond (ψ_1) and torsion (θ_1) angles in **1a** and **6**[a]

Compound	d_1	d_2	d_3	d_4	d_5	d_6	ψ_1	θ_1
1a	6.20	5.66	2.30	0.77	0.70	0.63	21.7	−36.5
6	4.68	5.66	2.31	−3.57	−2.42	−1.25	23.5	6.00

[a] Positive and negative d_4–d_6 values refer to whether the aryl ring is above or below the plane of the N–O axis, respectively. The positive and negative θ_1 figures refer to torsion angles being clockwise and anticlockwise, respectively.

acylation of piperidine and diethylamine, respectively, with cinnamoyl chloride. Certain aspects of the topography of representative molecules in series **1–3** were compared with those of the established anticonvulsants **4–6** using molecular modeling. These results are summarized in Tables II and III.

Each of the compounds **1a–f**, **2a–f**, **3a–d**, **7** and **8** were evaluated in the MES, scPTZ and NT screens. The results are presented in Table I. Selected compounds were examined in additional tests in order to explore further their potential as anticonvulsant agents. All of the amides **1a–f**, **2a–f**, **3a–d**, **7** and **8** were evaluated in vitro against murine P388 lymphocytic leukemic cells and *Mycobacterium tuberculosis* $H_{37}Rv$.

DISCUSSION

All of the compounds in series **1–3** were evaluated in the MES, scPTZ and NT screens in mice. The MES test was utilized as a predictor of compounds which prevent the spread of seizures while the scPTZ screen was employed to detect those molecules which increase minimal seizure threshold.[21] Neurotoxicity was determined by the rotorod method.[22] These results are presented in Table I. In addition, visual observations of the animals were made for adverse effects in all three screens such as the loss of righting reflex, anesthesia and death.

The data in Table I revealed that protection in the MES screen was demonstrated by **1a,b,d,f** while **1c** was inactive and **1e** was lethal when a dose of 100 mg/kg was administered to mice. In series **2** and **3**, both **2a,c** afforded protection. Evaluation of **1a–f**, **2a–f** and **3a–d** in the scPTZ test indicated that **1a–d**, **f** demonstrated anticonvulsant activity while of the ten compounds in series **2** and **3**, only **2a,c,d** gave protection against seizures. Neurotoxicity was noted in all of the compounds in series **1–3** except **2e**, **3b** and **3d**. The conclusion drawn from this initial screen was that the hypothesis that anticonvulsant activity would be displayed by the compounds in series **1–3** was validated in series **1**, partially verified in series **2** while the thiosemicarbazones **3** were devoid of anticonvulsant properties.

The promising activity of the novel series of anticonvulsants **1** may have been due to the presence of the proposed pharmacophore *vide supra*, namely an aryl ring and an amidic group in a certain spatial arrangement. Consequently the topography of the proposed pharmacophore was determined for the following reasons. First, new series of compounds could be designed having similar and also divergent vectors which would enable further evaluations of the hypothesis. Second, the shape of the pharmacophore may be similar to those found in established antiepileptic drugs. Thus the interatomic distances between the centre of the aryl ring of **1a** and the nitrogen and oxygen atoms (d_1, d_2) as well as the nitrogen-oxygen span (d_3) were determined in addition to the bond angle ψ_1 as illustrated in Figure 3A. Similar measurements were undertaken with mephenytoin **6** (Figure 3B) which, like **1a**, affords protection in both the MES and scPTZ screens.[16] Both **1a** and **6** have an aryl ring separated from an amidic oxygen atom by a three atom spacer. Another consideration to be made pertains to the orientation of the aryl ring in relation to the plane of the amidic bond in **1a** and **6**. In order to obtain this information, the distances between carbon atoms 1 and 4 and the centre of the aryl ring from the nitrogen–oxygen plane were obtained and designated d_4–d_6 as indicated in Figure 3C. Furthermore, the torsion angle θ_1 between the aryl ring and the nitrogen–oxygen plane in **1a** and **6** was obtained (Figure 3D). These data are presented in Table II.

The data for **1a** revealed that the centre of the aryl ring was closer to the oxygen rather than the nitrogen atom of the amidic group, i.e., $d_2 < d_1$. The aryl

TABLE III Interatomic distances (d_7–d_{12}), bond (ψ_2) and torsion (θ_2) angles in **2a**, **3a**, **4** (R^1=R^2=H) and **5** (R^1=R^2=H)[a]

Compound	d_7	d_8	d_9	d_{10}	d_{11}	d_{12}	ψ_2	θ_2
2a	12.11	12.81	2.26	−5.39	−4.42	−3.50	9.90	−37.5
3a	12.12	13.16	2.64	−5.31	−4.35	−3.57	11.00	−37.0
4 (R^1=R^2=H)	9.76	11.41	2.26	1.01	−0.07	−1.14	8.50	50.5
5 (R^1=R^2=H)	10.71	12.25	2.64	−2.49	−1.67	−0.84	10.8	50.5

[a] Positive and negative d_{10}–d_{12} values refer to whether the aryl ring is above or below the plane of the N–O axis, respectively. The positive and negative θ_2 figures refer to torsion angles being clockwise and anticlockwise, respectively.

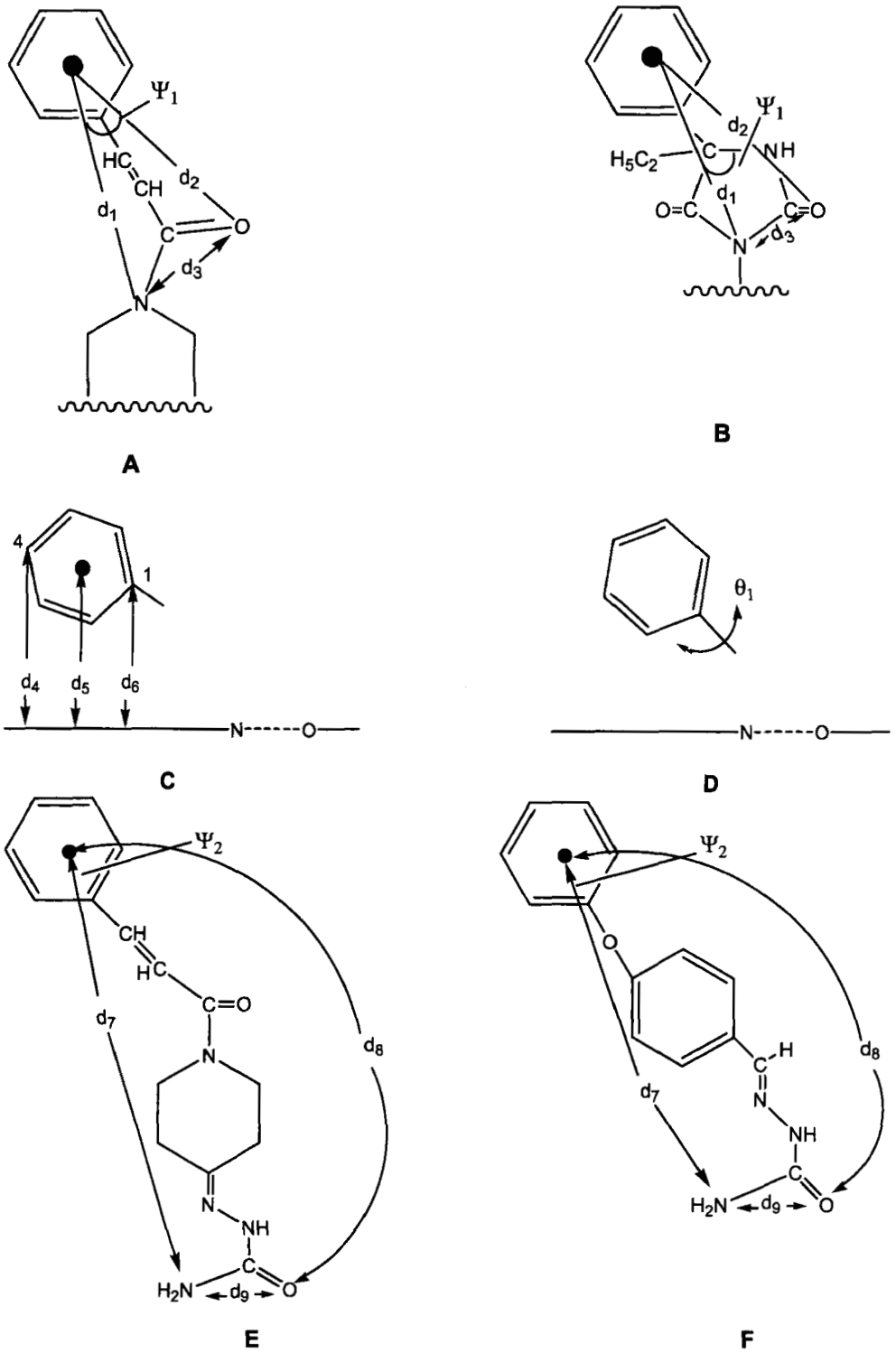


FIGURE 3 **A**. Interatomic distances d_1–d_3 and bond angle ψ_1 in **1a**. **B**. Interatomic distances d_1–d_3 and bond angle ψ_1 in **6**. **C**. Interatomic distances d_4–d_6 in **1a** and **6**. **D**. Torsion angle θ_1 in **1a** and **6**. **E**. Interatomic distances d_7–d_9 and bond angle ψ_2 in **2a**. **F**. Interatomic distances d_7–d_9 and bond angle ψ_2 in **4** (R^1=R^2 = H).

ring of **1a** was titled upwards from the N–O plane ($d_4 > d_5 > d_6$) and created a torsion angle of 36.5° with the adjacent olefinic linkage. In order to consider the effect of altering these figures in regard to eliciting anticonvulsant activity, two series of compounds should be prepared in the future. First, the placement of alkyl groups of varying sizes onto the olefinic linkage may alter the position of the aryl ring in relation to the amidic group. Second, the insertion of ortho substituents into the aryl ring of **1a** having different Taft E_s values should lead to increases in the θ_1 figures which may lead to changes in anticonvulsant profiles. While there were little or no differences between the d_2 and d_3 figures for **1a**

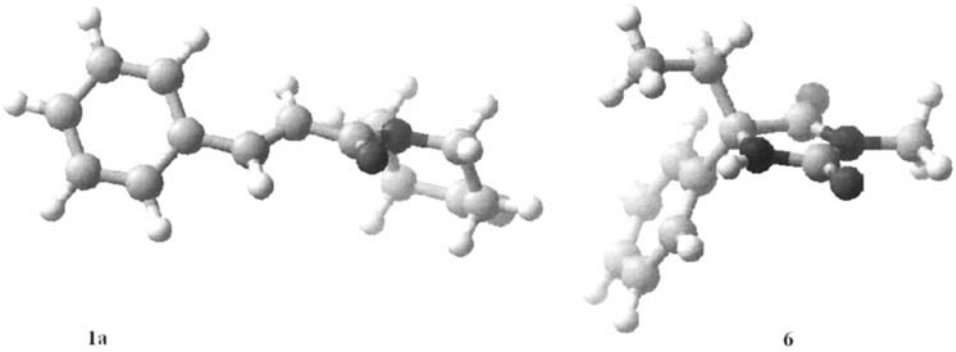
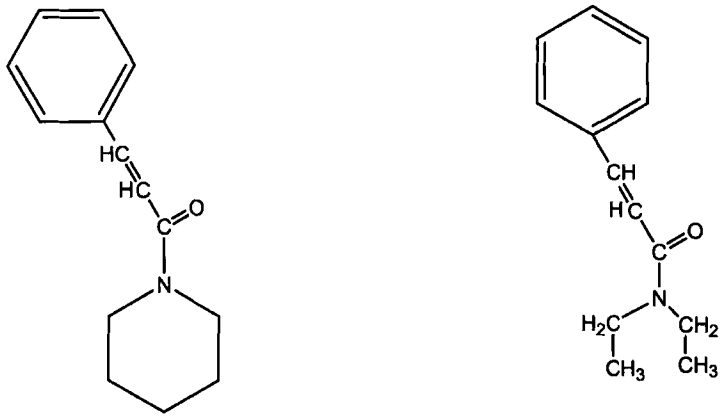


FIGURE 4 Models of **1a** and **6** showing the variations between the distances d_4–d_6 and the torsion angle θ_1 in these two compounds.

and **6**, the d_1 span in mephenytoin was 25% shorter than the datum obtained for **1a**. This result was attributed to the conformational restraints imposed by the heterocyclic ring in **6**. The aryl ring in **6** adopted a markedly different topography in relation to the N–O plane than in **1a** as revealed by the d_4–d_6 distances and the torsion angle θ_1 as illustrated in Figure 4. A conclusion to be drawn from a comparison between the data obtained for **1a** and **6** is that while the d_2, d_3, and ψ_1 figures are similar, there is considerable tolerance in the orientation of the aryl ring in relation to the amidic bonds which permit retention of anticonvulsant properties.

Molecular modeling was utilized in regard to representative compounds in series **2** and **3**, namely **2a** and **3a**. First, the d_1–d_3 and ψ_1 data for **2a** and **3a** were determined and found to be 6.23, 5.66, 2.30 Å, 21.6° and 6.24, 5.66, 2.30 Å, 21.6°, respectively, which are the same or very similar values as the figures generated for **1a** (Table II). In the MES and scPTZ screens, anticonvulsant activity was displayed by the compounds in series **1**, **2** and **3** in 9/12, 5/12 and 0/8 of the bioevaluations, respectively. Clearly the replacement of the 4-oxo group by semicarbazono and thiosemicarbazono substituents was disadvantageous but these results are unlikely to be due to changes in the conformation of the putative pharmacophore. It is conceivable that repulsion between the semicarbazono and thiosemicarbazone substituents and one or more bulky groups adjacent to the binding site accounts for the greater anticonvulsant activity of the compounds in series **1** than **2** or **3**. Second, comparisons of the shapes of **2a** and **3a** with the analogues **4a** and **5a** were made. The interatomic distances d_7–d_9 and bond angle ψ_2 are portrayed in Figures 3E and 3F, respectively, while the spans d_{10}–d_{12} and θ_2 angle (not shown) were determined in an analogous manner as d_4–d_6 and θ_1, respectively. The data are presented in Table III. The results indicated substantial differences in the relative positions of the aryl rings and terminal amidic functions (d_7, d_8 and ψ_2). Furthermore, the orientations of the aryl rings in **2a** and **3a** in relation to the N–O plane differ considerably from the topography of the terminal aryl ring in **4a** and **5a** (d_{10}–d_{12}, θ_2). These differences may explain



7 **8**

FIGURE 5 The structures of the amides **7** and **8**.

the greater anticonvulsant properties of the compounds in series **4** and **5** than were displayed in series **2** and **3**.

A further investigation was undertaken in order to determine whether simplification of the structure of a representative compound in series **1**, namely **1a**, was accompanied by retention of anticonvulsant properties. Thus the 4-oxo substituent attached to the piperidine ring of **1a** was excised leading to **7** while removal of the 4-methylene group of **7** produced the acyclic analogue **8**. The structures of **7** and **8** are portrayed in Figure 5. This decision was encouraged by virtue of the fact that anticonvulsant properties of **7** had been described.[23–26] As revealed in Table I, both compounds possessed anticonvulsant properties. Furthermore, quantification of the anticonvulsant effect of **8** when administered intraperitoneally to mice was undertaken. The ED_{50} figures in the MES and scPTZ screens revealed **8** to have equipotency with mephenytoin in the MES screen and 44% of the potency of this established drug in the scPTZ test. These results demonstrated that the *N,N*-diethylcinnamoylamido group is a pharmacophore. Thus in the future, analogues of this structural unit should be developed inter alia with a view to exaggerating the differential between potency and toxicity.

A valuable feature of candidate antiepileptic drugs is an ability to be active when administered orally, especially since epileptics often use medication for protracted periods of time. Compounds **1a,b,f,2c** and **8** were given to rats *per os* using doses of 30 or 50 mg/kg and examined over the 0.25–4 hour time span in the MES and toxicity screens while **1b**, **2c** and **8** were included in the scPTZ test. Protection in the MES screen was demonstrated in all (**1a** and **8**), some (**1b**, **2c**) or none (**1f**) of the animals while **1b**, **2c** and **8** were inactive in the scPTZ test. None of the compounds displayed overt signs of toxicity.

From the results generated, both **1a** and **8** were considered as lead molecules. Consequently additional bioevaluations were undertaken on these two compounds in order to examine further their potential as candidate anticonvulsants. In the MES screen, the ED_{50} figures of **1a** and **8** when administered orally to rats were 20.7 and 25.3 mg/kg, respectively. In the scPTZ screen, administration of 250 mg/kg of **1a** did not lead to any display of anticonvulsant activity whereas utilization of a dose of 225 mg/kg of **8** afforded complete protection in this screen. In the case of **1a**, while toxicity was absent at 125 mg/kg, neurological deficit was noted in all of the animals when 250 mg/kg was given to rats. On the other hand, doses up to and including 450 mg/kg of **8** were administered to rats without the appearance of toxic symptoms. When given orally to rats, the comparable figures for mephenytoin in the MES, scPTZ and NT screens were 18.1, 21.7 and 85.7 mg/kg, respectively.[21] In regard to the MES test, **1a** and **8** possess 87% and 72% of the potency of mephenytoin. The protection index (P.I.) values in the MES screen are the ratios of the TD_{50} and ED_{50} figures. Thus for **1a**, **8** and mephenytoin, the PI values are > 6.04, > 17.8 and 4.74, respectively, indicating a marked advantage of **1a** and especially **8** over mephenytoin.

Finally, a hippocampal kindling screen in rats was carried out which is a good model for identifying compounds which prevent focal seizures and the spread of seizures.[27] In this test, the ED_{50} values of **1a** and **8** were 40.8 and 43.1 mg/kg, respectively, giving rise to P.I. values of > 3.06 and > 10.4, respectively.

It may be concluded from these bioevaluations that the hypothesis of an aryl ring fixed in close proximity to an amido group confers anticonvulsant properties is worthy of further investigation. Comparisons between the shapes of **1a** and mephenytoin indicated that variation in the topography was tolerated. The relative positions of the aryl ring and the terminal amido or thioamido groups of **2a** and **3a** with **4** and **5**, respectively, were markedly different which may have contributed to the absence of anticonvulsant properties of most of the compounds in series **2** and **3**. Molecular simplification led to the potent anticonvulsant **8** which was well tolerated when given orally to rats. The data generated revealed that further development of series **1** and **8** is clearly warranted.

The final aspect of the study was to ascertain whether the compounds in series **1–3**, **7** and **8** were cytotoxic towards P388 lymphocytic leukemia and *Mycobacterium tuberculosis*. None of the compounds inhibited the growth of the neoplastic cells at 50 μM. This result was somewhat surprising since various substituted 4-piperidones bearing an α,β-unsaturated keto group attached to the piperidyl nitrogen atom displayed potent cytotoxic properties.[28]

Under the conditions employed, antitubercular properties were displayed in series **1** and **2** only by **1b,c** and **2b**; in these cases the potencies were weak (6–21% inhibition). On the other hand, all of the compounds in series **3** inhibited the growth of the microorganism in the 40–77% range which is in accord with the observation noted earlier of the antimycobacterial properties of various thiosemicarbazones.[6] The potency ratios of these four compounds were determined, i.e., percentage inhibitions/concentrations in μM which inhibited microbial growth. Linear and semilogarithmic plots were constructed between the sigma, pi and molar refractivity values of the aryl substituents in **3a–d** and the potency ratios. A trend towards a positive correlation was noted between the sigma values and potency ratios ($p < 0.15$) in the linear but not semilogarithmic plots. No correlations were observed in the other determinations ($p > 0.15$). While **1a** did

not inhibit the growth of *Mycobacterium tuberculosis*, removal of its keto oxygen atom giving rise to **7** led to a compound which inhibited the growth of the bacterium by 58%. All of the remaining compounds, i.e., **1a,d–f,2a,c–f** and **8** were inactive in this screen.

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