Journal of Enzyme Inhibition and Medicinal Chemistry, April 2009; 24(2): 537–544

1-Arylmethyl-2,3-dioxo-2,3-dihydroindole thiosemicarbazones as leads for developing cytotoxins and anticonvulsants

SUBHAS S. KARKI[1], VIVEK SINGH BAHADURIA[1], VIVEK RANA[1], SUJEET KUMAR[1], PRASANNA G. SUBBARO[2], UMASHANKAR DAS[3], JAN BALZARINI[4], ERIK DE CLERCQ[4], & JONATHAN R. DIMMOCK[3,†]

[1]*Department of Pharmaceutical Chemistry India,* [2]*Department of Pharmacology, KLES College of Pharmacy, 2nd Block, Rajajinagar, Bangalore, 560 010, India,* [3]*College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C9, Canada, and* [4]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

(*Received 27 February 2008; accepted 30 April 2008*)

Abstract

Various substituted 1-arylmethyl-2,3-dioxo-2,3-dihydroindole thiosemicarbazones **3a-h**, 1-benzyl-2,3-dioxo-2,3-dihydroindole N^4-aryl thiosemicarbazones **4a-i** and 1-benzyl-2,3-dioxy-2,3-dihydroindole N^4-cyclohexylthiocarbazone **5** were synthesized. All of these compounds were evaluated against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemia cells. Nearly 40% of these compounds possess low micromolar IC_{50} values and some are either more potent than, or equipotent with, melphalan. Various correlations between the structures of these compounds and cytotoxic potencies were obtained which included the use of QSAR and molecular modeling techniques. Representative compounds displayed anticonvulsant properties in rats and were well tolerated by these animals. The encouraging biodata noted affords adequate rationale for outlining guidelines for further development of these molecular scaffolds.

Keywords: *Indoles, thiosemicarbazones, cytotoxicity, molecular modeling, SAR, anticonvulsants, bioassays*

Introduction

An ongoing interest in these laboratories are thiosemicarbazones which display antineoplastic properties [1,2] and also have the capacity to prevent convulsions [1–4]. In particular, various compounds having the general formula **1** (Figure 1) display antineoplastic [5] and anticonvulsant [6] properties; both of these activities are retained in the cyclic analog **2** [2]. A further reason for using **2** as the lead molecule is that while various acyclic thiosemicarbazones are lethal to mice at doses of 25-100 mg/kg [1], administration of 300 mg/kg of **2** was tolerated by these animals [2].

The objectives of the present investigation were to develop analogs of **2** principally as candidate cytotoxins and also for an assessment of their anticonvulsant properties. The thiosemicarbazone **2** displays cytotoxicity to human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells but the IC_{50} values are low, *viz* 205, 83 and 76 μM, respectively [2]. These potencies may reflect the formation of only weak interactions between the ligand and a binding site. Hence the systematic enlargement of the structure of **2** may allow the additional groups to bind to a receptor thereby reinforcing the interactions of the scaffold **2** with biomacromolecules.

A review of the literature revealed that the insertion of a nitrogen atom into the acyclic ring of **2** and conversion of one of the methylene group into a carbonyl function

Correspondence: S. S. Karki, Department of Pharmaceutical Chemistry, KLES College of Pharmacy, 2nd Block, Rajajinagar, Bangalore, 560 010, India. Tel: 91 80 23325611. Fax: 91 80 23425373. E-mail: subhasskarki@hotmail.com

†J. R. Dimmock, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5C9 Canada. Tel: 306-966-6331. Fax: 306-966-6377. E-mail: jr.dimmock@usask.ca

ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802234885

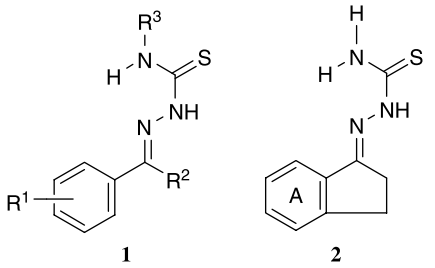


Figure 1. Structures of the thiosemicarbazones **1** and **2**.

gave rise to a series of 2,3-dioxo-2,3-dihydroindoles which display cytotoxic properties [7]. These additional polar atoms may enhance hydrogen bonding at a receptor. In addition, bearing in mind the importance of hydrophobic forces in eliciting a biological response, an arylalkyl group was attached to the heterocyclic nitrogen atom. From this basic design, various substituents were mounted on this scaffold leading to series **3-5**. The decision to prepare this cluster of compounds was reinforced by the report that the related semicarbazones of 2,3-dioxy-2,3-dihydroindoles demonstrate anticonvulsant properties [8].

In summary, the decision was made to prepare series **3-5** principally for cytotoxic evaluation with the aim of discovering compounds with significantly increased potencies compared to **2**. If this objective was achieved, efforts would be made to provide guidelines for subsequent amplification of the project. In addition, the assessment of whether representative compounds have anticonvulsant properties was planned.

Materials and methods

Melting points are uncorrected and recorded in Celsius degrees. R_f values were obtained using silica gel thin layer chromatography plates and a solvent system of chloroform:methanol (1:9). Ultraviolet spectra of **3a**, **4a**,**b**,**e**,**g-i** and **5** in methanol were obtained on a Jasco V-630 instrument. The infrared spectra of all compounds were determined by a diffuse reflectance technique using potassium bromide powder on a Jasco 460 + FTIR machine. ^{1}H NMR spectra (300, 400 or 500 MHz) of all compounds except for **4e** were generated in dimethylsulfoxide $-d_6$ on Bruker Ultraspec spectrophotometers. Electron impact mass spectra were obtained on **3a**, **4a**,**b**,**e**,**g-i** and **5** using a Perkin Elmer instrument.

*Syntheses of **3a-h***

The synthesis of the intermediate 2,3-dioxy-2,3-dihydroindoles was accomplished using a literature methodology [9] and a previously reported procedure was used to convert these compounds to the corresponding 1-arylmethyl-2,3-dioxy-2,3-dihydroindoles [10].

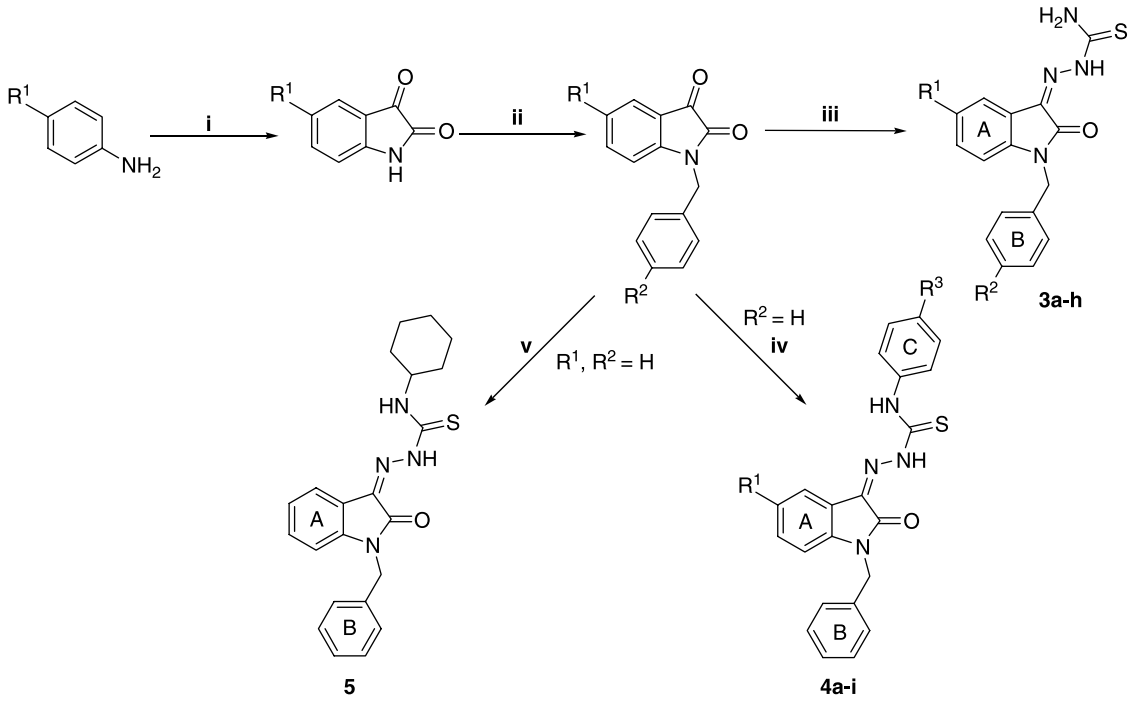
A mixture of the 1-arylmethyl-2,3-dioxy-2,3-dihydroindole (0.005 mol), thiosemicarbazide (0.005 mol), acetic acid (0.5-1.0 mL) and ethanol (100 mL) was heated under reflux until the reaction was completed (~ 4 h). Approximately half of the ethanol was removed *in vacuo* and the solution was left overnight at room temperature. The solid which precipitated was collected, washed with cold ethanol and recrystallized from ethanol:chloroform (9:1) to give the following compounds (melting points and percentage yields in parentheses), namely **3a** (260-263, 85), **3b** (233-238, 69), **3c** (256-260, 74), **3d** (238-239, 72), **3e** (251-253, 70), **3f** (240-242, 75), **3g** (241-243, 68), and **3h** (258-260, 65). The physicochemical data generated for a representative compound **3e** are as follows: R_f: 0.61, FTIR (KBr): 3411 (NH_2), 3237 (NH), 3146, 3045-3015 (CH), 2984-2929 (CH), 1698 (CO), 1596 (CN) cm^{-1}. ^{1}H NMR: δ: 12.21 (1H, s, NH), 9.12 (s, 1H, NH), 8.82 (s, 1H, Ar), 7.90 (m, 1H, Ar), 7.51-7.48 (m, 1H, Ar), 7.22 (2H, d, Ar, J = 8.0 Hz), 7.10 (d, 2H, Ar, J = 8.0 Hz), 6.90 (d, 2H, Ar, J = 8.0 Hz), 4.87 (s, 2H, CH_2), 2.21 (s, 3H, CH_3); EIMS: 403.55 (100).

*Synthesis of **4a-c** and **5***

The N^4-arylthiosemicarbazides and N-cyclohexylthiosemicarbazide required in the preparation of **4a-i** and **5**, respectively, were prepared by a literature methodology [11,12]. The appropriate 1-benzyl-2,3-dioxy-2,3-dihydroindole reacted with a thiosemicarbazide using the methodology for preparing **3a-h**. The melting points and percentage yields of the compounds are as follows; **4a** (160-162, 83), **4b** (208-211, 80), **4c** (215-219, 70), **4d** (144-148, 73), **4e** (204-207, 72), **4f** (145-147, 68), **4g** (185-188, 65), **4h** (200-202, 74), **4i** (228-230, 70) and **5** (184-195, 90). Some physical characteristics of two representative compounds are as follows; **4a**: R_f 0.69; UV (λ max) 254, 368 nm; FTIR (KBr): 3321, 3225 (NH), 2915 (CH), 1686 (CO), 1541 (CN) cm^{-1}; ^{1}H NMR: δ: 13.01 (s, 1H, NH), 9.52 (s, 1H, NH), 7.75 (d, 2H, Ar, J = 8.0 Hz), 7.65 (d, 1H, Ar, J = 7.0 Hz), 7.43-7.26 (t, m, 10H, Ar, J = 15.6 Hz), 7.13-7.09 (m, 1H, Ar), 6.83 (d, 1H, Ar, J = 8.0 Hz), 4.95 (s, 2H, N-CH_2); EIMS: 387.52 (100). **5**: R_f: 0.53; UV (λ max): 253, 257, 371 nm; FTIR (KBr): 3352 (NH), 3030 (CH), 2926-2848 (CH), 1691 (CO), 1571 (CN) cm^{-1}; ^{1}H NMR: δ: 12.36 (s, 1H, NH), 8.83 (d, 1H, NH, J = 8.2 Hz), 7.72 (d, 1H, Ar, J = 7.3 Hz), 7.31-7.20 (m, 6H, Ar), 7.07 (t, 1H, Ar, J = 14.7 Hz), 6.97 (d, 1H, Ar, J = 7.8 Hz), 4.94 (s, 2H, N-CH_2), 1.93-1.11 (m, 11H, cyclohexyl H); EIMS: 393.85 (100).

Molecular modeling

Models of **3a**,**4a**,**b**,**e**,**g-i** and **5** were built using the Spartan programme [13]. The conformers with the



Scheme 1. The synthetic chemical route employed in obtaining the compounds in series **3-5**. The reagents used are as follows: i: $CCl_3CH(OH)_2$ / H_2SO_4 / Na_2SO_4; ii: R^2-$C_6H_4CH_2Cl$ / K_2CO_3 /DMF; iii: $H_2NNHCSNH_2$; iv: $R^3C_6H_4NHCSNHNH_2$; v.: $C_6H_{11}NHCSNHNH_2$. The nature of the R^1, R^2 and R^3 substituents are presented in Table I.

lowest averages were obtained with the CONFLEX programme and optimization took place by mechanics using augmented MM2 parameters.

Determination of log P values

The log P values of **3a-h**, **4a-i**, **5** and melphalan were generated using the JME molecular editor [14].

Statistical analyses

The statistical evaluations were made using a commercial software package [15].

Cytotoxicity assays

The methodology for undertaking the Molt 4/C8, CEM and L1210 assays has been published previously [16]. In brief, varying concentrations of the compounds were incubated at 37°C for 72 h (Molt 4/C8 and CEM T-lymphocytes) or 48 h (L1210 cells).

*Evaluation of **3a, 4a,b,e,g-i** and **5** for anticonvulsant properties*

Adult female Wistar rats (200-250 mg) were acclimatized to laboratory conditions for two or three days prior to experimentation and then fasted overnight. Each of the 2,3-dioxo-2,3-dihydroindole thiosemicarbazones and phenobarbitone were dissolved in saline or suspended in gum acacia solution (2% w/v) and administered orally to six animals. Maximal electroshock seizures were given to the rats by the application of a stimulus of 130 mA for 0.2 s from an electroconvulsometer (INCO, India). The time which elapsed between the administration of the electric current and the abolition of tonic seizures and clonic convulsions as well as recovery from stupor were recorded. The Protocol used for Animal Experimentation followed that of the Institutional Animal Ethics Committee of the KLES College of Pharmacy, Bangalore, Karnataka, India.

Results

The compounds in series **3-5** were prepared by the methodologies outlined in Scheme 1. ^{1}H NMR spectroscopy indicated that the compounds exist as single isomers in solution. Molecular models of **3a** and **4a** revealed that the energies of the *Z* isomers are 394.2 and 556.0 KJ/mol, respectively, while the figures for the *E* isomers are 406.6 and 568.2 KJ/mol, respectively. Hence the assumption was made that the compounds adopted the *Z* conformation. Molecular models of **3a**,**4a**,**b**,**e**,**g-i** and **5** were built and various structural parameters are presented in Table II. The log P values of all of the compounds were computed and are portrayed in Table I.

All of the compounds were assayed against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells. These data are summarized in Table I.

The anticonvulsant properties of **3a**,**4a**,**b**,**e**,**g-i** and **5** were evaluated and the data are portrayed in Table III.

Discussion

Comments will be made initially regarding the antineoplastic properties of **3a-h**, **4a-i** and **5** followed by some considerations of the potential of selected compounds as candidate anticonvulsants.

The choices of the R^1 and R^2 substituents in series **3** and **4** were made on the basis of attempting to discern the contributions of the various physicochemical properties of these groups to cytotoxic potencies. The substituents were principally chloro, bromo, methyl, fluoro and methoxy groups which are found in three of the four quadrants of a Craig plot [17] thereby indicating a range of electronic and hydrophobic properties. In terms of the sizes of these groups, the molecular refractivity (MR) values vary from 0.92 (fluoro) to 8.88 (bromo) [18].

The cytotoxic potential of the compounds in series **3-5** was evaluated against human Molt 4/C8 and CEM T-lymphocytes in order to ascertain their potencies towards human transformed cells. In addition, a number of clinically useful anticancer drugs display significant cytotoxic effects towards murine L1210 cells [19]; this cell line therefore may detect promising lead compounds and was also employed in the bioassays. The results of these evaluations are presented in Table I.

A review of the biodata in Table I reveals that 37% of the compounds have IC_{50} values in the low micromolar range (1-10 μM). Hence various members of series **3** and **4** serve as lead molecules for further structural modifications. In particular the results in the Molt 4/C8 bioassay indicate that **3f** and **4a**,**b**,**h** have statistically significantly greater potencies than melphalan being 1.6, 1.9, 1.4 and 2.3 times, respectively, more potent than this established anticancer drug. Furthermore the following compounds are equipotent with melphalan, namely **4e**,**f** (Molt 4/C8 screen), **3f** and **4a**,**b**,**f**,**h** (CEM test) and **3d**,**f**,**g** (L1210 assay).

The biodata in Table I reveal that in general Molt 4/C8 cells are the most sensitive to the compounds in series **3** and **4**. The following observations were made pertaining to the influence of molecular modifications on cytotoxic potencies in this screen. The unsubstituted compound **3a** has very low potency. However placement of halogens on ring A in series **3** led to **3b** and **3c** which caused a dramatic lowering of the IC_{50} values of 17-fold on average. No alterations in potencies were noted when a methyl group was introduced into ring B of **3b** yielding **3d** nor when either a methyl or fluoro substituent was placed in ring B of **3c** giving rise to **3e** and **3g**, respectively. However retention of the methyl group in ring B and introducing a fluoro substituent in ring A led to the potent cytotoxin **3f**. An interesting observation arose insofar as the structural isomer **3h** is more than 250 times less potent than **3f** which illustrates the remarkable influence of the aryl substituents on cytotoxic properties in series **3**. Thus, in series **3** significant cytotoxic potencies are observed when R^1 is a bromo, chloro or fluoro substituent.

A review of the IC_{50} values of series **4** in the Molt 4/C8 screen revealed the following correlations. First, the

Table I. Evaluation of **3a-h, 4a-i** and **5** towards human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemic cells and clogP values of these compounds.

	Substituents			IC_{50}(μM)*			
Compound	R^1	R^2	R^3	Molt 4/C8	CEM	L1210	clogP
3a	H	H	–	168 ± 66.0	206 ± 44.0	305 ± 7.0	2.78
3b	Cl	H	–	7.4 ± 1.6	12 ± 3	9.0 ± 0.4	3.43
3c	Br	H	–	12 ± 3	29 ± 14	12 ± 5	3.56
3d	Cl	CH_3	–	5.7 ± 1.5	15 ± 9	2.4 ± 1.8	3.88
3e	Br	CH_3	–	11 ± 6	5.6 ± 0.8	2.6 ± 0.4	4.01
3f	F	CH_3	–	2.0 ± 0.3	2.3 ± 1.0	2.2 ± 0.1	2.39
3g	Br	F	–	12.0 ± 0	24 ± 8	3.2 ± 2.4	3.73
3h	CH_3	F	–	>500	>500	164 ± 57	3.37
4a	H	–	H	1.7 ± 0.4	2.5 ± 0.1	32 ± 11	4.21
4b	H	–	CH_3	2.3 ± 0.1	3.2 ± 0.5	118 ± 23	4.66
4c	F	–	CH_3	10 ± 1	12 ± 2	15 ± 1	3.83
4d	CH_3	–	CH_3	14 ± 6	17 ± 6	57 ± 6	5.09
4e	H	–	OCH_3	3.1 ± 0.4	12 ± 6	326 ± 102	4.27
4f	Cl	–	OCH_3	2.9 ± 0.5	2.9 ± 0.5	35 ± 6	4.92
4g	H	–	OC_2H_5	155 ± 33	133 ± 8	60 ± 17	4.65
4h	H	–	Cl	1.4 ± 0.2	2.1 ± 0.2	23 ± 1	4.89
4i	H	–	F	14 ± 1.0	34 ± 3.0	14 ± 2.0	4.38
5	–	–	–	>500	>500	>500	5.06
Melphalan	–	–	–	3.2 ± 0.6	2.5 ± 0.2	2.1 ± 0.02	0.08

* The IC_{50} figures are the concentrations of compounds required to inhibit the growth of the cells by 50%.

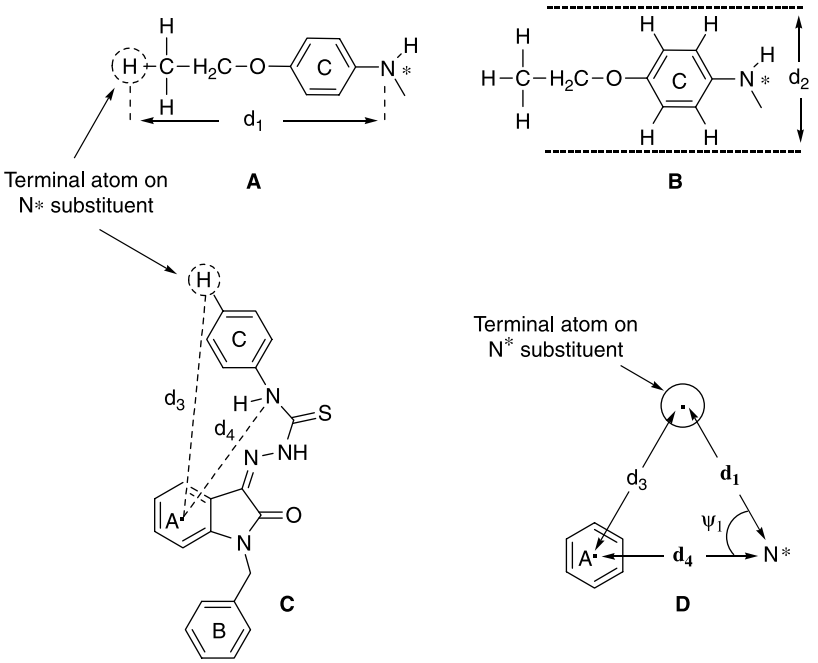


Figure 2. Determination of the spatial requirements of the atom or group attached to the terminal atom N* of the thiosemicarbazono moiety. The measurements are illustrated for **4g**. A. The distance d_1. B. The distance d_2. C. The spans d_3 and d_4. D. The angle ψ_1.

introduction of a phenyl ring onto **3a** led to **4a** with a 99-fold increase in potency. Various groups were placed onto ring C of **4a**. While the 4-chloro analog **4h** was equipotent with **4a**, the introduction of methyl (**4b**), methoxy (**4e**), ethoxy (**4g**) and fluoro (**4i**) substituents led to compounds having 74%, 55%, 1% and 12% of the potency of **4a**, respectively. In order to discern whether the relative biodata for **4a**,**b**, **e**, **g-i** was controlled by the electronic, hydrophobic or steric properties of the atom or group in ring C, linear and semilogarithmic plots were constructed between the IC_{50} values and the Hammett sigma constants, Hansch pi figures and the molar refractivity (MR) values of the R^3 group. A negative correlation was noted between the IC_{50} values and the σ constants ($p = 0.046$) revealing that potency increased as the electron-attracting properties of the aryl substituents rose. In addition, a trend towards a positive correlation with the MR constants was observed in the linear plot ($p = 0.095$) which suggests that potency increased as the size of the R^3 substituent diminished. No other correlations were noted ($p > 0.1$).

Second, replacement of the R^1 proton in ring A of **4b** by fluorine or methyl gave rise to **4c** and **4d**, respectively, which have lower potencies than **4b** while chloro substitution of **4e** yielding **4f** did not increase potency. Thus in contrast to series **3**, halogenation at the R^1 location did not yield compounds with lower IC_{50} figures than the unsubstituted analog ($R^1 = H$). In summary, the location of a phenyl ring C on the terminal nitrogen of the thiosemicarbazono group gave a lead compound **4a** with a low IC_{50} figure. With the exception of the 4-chloro analog **4h**, substitution in ring C is deleterious in terms of cytotoxic potencies.

The dramatic increase in the potency of **4a** compared to **3a** is intriguing. Replacement of aryl ring C in **4a** by a saturated six-membered alicyclic ring gave rise to **5**. This compound is in excess of 294 times less potent than **4a**. In addition, **5** is less potent than **3a**. Hence the aryl ring C in series **4** may well form π-π stacking with a complementary aromatic structure at a binding site.

The general conclusions regarding the effect of molecular modifications on potencies in the Molt 4/C8 bioassay were similar for the CEM and L1210 screens. Thus in series **3**, halogenation of ring A increased potencies compared to the unsubstituted analog **3a** while the R^2 substituents had marginal effects on the IC_{50} values. In the case of series **4**, the conversion of **3a** and **4a** confirmed the importance of the aryl ring C with regard to increased potency while substitution in this ring generally did not lead to lower IC_{50} figures. However halogenation in ring A did increase potencies in general. Compound **5** has IC_{50} values in excess of 500 μM in all three screens. Linear and semilogarithmic plots were made between the IC_{50} values of **4a**,**b**,**e**,**g-i** in the CEM assay and the σ, π and MR constants of the substituents R^3 in ring C. Neither correlations nor trends were noted ($p > 0.1$). The procedure was repeated using the cytotoxicity data derived from the L1210 test. A semilogarithmic plot between the IC_{50} figures and the σ constants revealed a negative correlation ($p = 0.046$), i.e., increasing the electron-attracting properties of the R^3 group led to increases in potencies. No other trends or correlations were found ($p > 0.1$).

The log P values of the compounds in series **3-5** were determined and are presented in Table I. Linear,

semilogarithmic and logarithmic plots were constructed between the IC_{50} Figures (when they were less than 500 μM) and the log P values in each of the three screens. Neither correlations nor trends were found ($p > 0.1$).

On the basis of the biodata generated, consideration was given pertaining to the expansion of this programme. While different avenues could be followed, the huge differential in the IC_{50} values of **3a**, **4a** and **5** in all three screens suggested that an estimate of the spatial requirements of the substituents attached to the terminal nitrogen of the thiosemicarbazono group (subsequently referred to as the N2⋆ atom) would be judicious. In this way compounds could be designed in the future to explore any correlation between the size of the group on the N2⋆ atom and cytotoxic potencies. In other words, a binding area close to the N⋆ atom may well have strict constraints as to the size of the group on the N2⋆ atom which could be accommodated. Thus models of **3a**,**4a**,**b**,**e**,**g–i** and **5** were built and the dimensions of these groups were obtained by measuring the length and breadth of the N2⋆ substituents as illustrated in Figure 2A,B. In addition, the varying sizes of the N⋆ groups may have caused differences in the relative locations of the N2⋆ substituents and the aryl ring A. Hence the spans d_3 and d_4 as illustrated in Figures 2C were determined as well as the angle ψ_1 between the axes represented by d_1 and d_4 (Figure 2D). These data are presented in Table II. From the point of view of clarity, the R^3 substituents in series **4** are tabulated and, in addition, since the trends in cytotoxic potencies are similar in the three bioassays, the IC_{50} figures for only the Molt 4/C8 screen are presented in Table II.

The spatial requirement on the N⋆ atom for each compound was estimated by multiplying d_1 by d_2 revealing an area A which is given in Table II. The spans d_4 show that the size of the N⋆ group does not alter the relative location of the N⋆ atom with respect to the aryl ring A. However the nature of the N⋆ substituent causes changes in the angle ψ_1. The compounds listed in Table II may be classified into three groups G1, G2 and G3 which are potent cytotoxins G1 (IC_{50} values < 15 μM, **4a**,**b**,**e**,**h**,**i**), weakly active compounds G2 (**3a**, **4g**) and G3 namely the inactive thiosemicarbazone **5**. In the case of G1, the figures for A, d_3 and ψ_1 are 27-37 sq Å, 11.4-13.6 Å and 152-160°, respectively. For group G2, the values fall outside of these ranges. Thus for **3a**, the figures are 1.7 sq Å, 7.4 Å and 166°, respectively, while for **4 g** the values are 43 sq Å, 14.8 Å and 163°, respectively. These differences in topological features may well influence cytotoxic potencies. The fact that the inactive cyclohexyl analog **5** has an A coordinate within the same ambit as G1 suggests that it is the aromaticity of ring C in series **4** which is responsible for the marked potency of **4a**,**b**,**e**,**h**,**i** and not the size of the group on the N⋆ atom. Thus in the future, further analogs in series **4** should be prepared for cytotoxic evaluation whose N⋆ substituent should have varying d_1 and d_2 distances but retain A values in the 27-37 sq Å range. A null hypothesis could involve the placement of aromatic groups on the N⋆ atom but whose A figures exceed 37 sq Å, e.g., by forming the corresponding naphthyl and anthryl analogs.

Finally an evaluation of the anticonvulsant potential of representative compounds was undertaken. The compounds chosen have no substituents in ring A (R^1 = H in series **3** and **4**) *viz* **3a**, **4a**, **b**,**e**,**g–i** and **5** which were examined in the maximal electroshock screen. This assay was selected since it has been claimed to be a predictor of compounds which control generalized tonic-clonic and partial seizures [20]. Relatively high doses of 200 and 300 mg/kg were employed in order to observe whether any toxic symptoms (as well as anticonvulsant properties) would be observed. A reduction in the time of tonic convulsions was noted by the following compounds (the percentage decreases in the time of seizures is indicated in parentheses), namely **3a** (37), **4a** (26), **4b** (31), **4e** (34), **4g** (32), **4i** (27), **5** (23) and phenobarbitone (51). Thus protection in this screen was observed for all of the compounds except for **4h** and in the case of **3a** and **4b**,**e**,**g** the reduction in the times of the seizures was the same as phenobarbitone. In the second anticonvulsant screen, the compounds were assessed for reducing the time

Table II. Some data for **3a**, **4a**,**b**,**e**,**g–i** and **5** obtained by molecular modeling.

Compound	R^3 Substituent in Series **4**	Interatomic distances (Å)				A	Angle ψ_1(°)	IC_{50} value (μM) in Molt 4/C8 assay
		d_1	d_2	d_3	d_4	($d_1 \times d_2$: sq Å)		
3a	–	0.99	1.17	7.39	6.42	1.69	165.9	168
4a	H	5.32	5.00	11.39	6.41	26.6	152.1	1.7
4b	CH_3	6.22	5.00	12.43	6.41	31.1	159.6	2.3
4e	OCH_3	7.44	5.01	13.59	6.41	37.3	157.7	3.1
4g	OC_2H_5	8.59	5.01	14.83	6.41	43.0	162.7	155
4h	Cl	5.92	5.01	11.95	6.41	29.7	151.8	1.4
4i	F	5.58	5.01	11.63	6.41	28.0	152.1	14
5	–	5.35	5.00	11.32	6.42	26.8	148.3	>500

Table III. Evaluation of **3a**, **4a**, **b**, **e**, **g–i** and **5** for anticonvulsant properties.

Compound*	Time (mean ± SEM) of		
	Tonic convulsions (s)	Clonic Seizures (s)	Recovery from Stupor (min)
3a	21.5 ± 0.50†	13.8 ± 3.55†,‡	5.83 ± 1.08
4a	25.3 ± 2.16‡,¶	17.2 ± 3.47†	4.33 ± 0.42
4b	23.5 ± 0.67†	15.2 ± 3.15†,‡	3.66 ± 0.80
4e	22.7 ± 1.71†	3.66 ± 1.98†	8.00 ± 1.48¶
4g	23.2 ± 1.80†	9.33 ± 0.30†	14.5 ± 0.85†,§
4h	27.7 ± 1.50§	31.5 ± 4.19	6.16 ± 1.83
4i	25.0 ± 1.32‡,¶	29.0 ± 1.93	2.50 ± 0.34
5	26.3 ± 2.34‡,¶	20.3 ± 1.34§	3.43 ± 1.56
Phenobarbitone	16.7 ± 3.52	1.50 ± 0.56	4.16 ± 0.48
Vehicle‖	34.2 ± 2.77	33.8 ± 4.70	3.83 ± 1.35

⋆ The doses administered to six rats were either 30 mg/kg (phenobarbitone), 200 mg/kg (**3a**,**4a**,**e**,**g**,**h**) or 300 mg/kg (**4b**,**i**); † Result is different from vehicle ($p < 0.01$); ‡ Result is different from phenobarbitone ($p < 0.05$); ¶ Result is different from vehicle ($p < 0.05$); § Result is different from phenobarbitone ($p < 0.01$); ‖ The vehicle (gum acacia solution, 2% w/v, 0.2 mL) was administered to six rats.

of clonic seizures. The data in Table III reveal that the following thiosemicarbazones reduce the time of convulsions (the percentage reductions are given in parentheses), namely **3a** (59), **4a** (49), **4b** (55), **4e** (89), **4g** (72), **5** (40) and phenobarbitone (96).

The conclusion to be drawn from this survey of representative thiosemicarbazones is that, in general, these molecules have anticonvulsant properties. Lead compounds that were identified are **3a** and **4a**,**b**,**e**,**g** which controlled both tonic and clonic seizures. Furthermore the protection afforded by two of these compounds, namely **4e** and **4g**, towards clonic convulsions is noteworthy.

The rats became quiescent after administration of the compounds and the times taken for the animals to recover from this stupor are presented in Table III. Only **4e** and **4g** prolonged the inertia of the rats compared to administration of the vehicle. In all cases, recovery was noted which is a favourable property of these compounds when considering their development as candidate antiepileptic drugs. In fact, the animals were observed for 72 hours after administration of the compounds and appear to have recovered normal functionality. In addition, this tolerance in rats is important in future molecular modifications of these series of compounds with a view to finding promising, novel anticancer agents. In other words, many of the compounds in series **3** and **4** have cytotoxic properties and coupled to low rodent toxicity, afford ample evidence for expansion of this cluster of compounds.

Conclusions

The structure of the prototype molecule **2** was modified leading to series **3** and **4** which, in general, were substantially more cytotoxic to Molt 4/C8, CEM and L1210 cells than **2**. In fact, the IC_{50} values of many of these compounds are in the low micromolar range and in several cases exceed the potency of melphalan or were equipotent with this reference drug. Various correlations between the structures and cytotoxic potencies emerged thereby affording guidelines for expanding the project. The *in vivo* evaluation of representative compounds in rats revealed that protection against seizures was demonstrated and the animals tolerated doses of 200 or 300 mg/kg. A number of new lead compounds have been identified for development which may result in novel antineoplastic and/or anticonvulsant molecules with good tolerability in mammals.

Acknowledgements

The authors thank Professor B. G. Desai of the KLES College of Pharmacy, Bangalore, India for providing the necessary facilities. The Government of India, Ministry of Science and Technology, awarded a BOYSCAST Fellowship to Dr. S. S. Karki which enabled him to work at the University of Saskatchewan as a Visiting Scholar which is gratefully acknowledged. Appreciation is extended to the Belgian Fonds voor Wetenschappelijk Onderzoek (Vlaanderen) which funded the cytotoxicity assays and to Mrs. L. van Berckelaer for excellent technical assistance. Ms. B. McCullough is thanked for typing the manuscript.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Dimmock JR, Smith DC, Brenner JM, Jonnalagadda SS, Sardessai MS, Wood JD, Bigam GE. Antiepileptic and antileukemic thiosemicarbazones and semicarbazones of 4-aryl-3-buten-2-ones. Eur J Med Chem 1986;21:187–192.

[2] Dimmock JR, Pandeya SN, Quail JW, Pugazhenthi U, Allen TM, Kao GY, Balzarini J, De Clercq E. Evaluation of the semicarbazones, thiosemicarbazones and bis-carbohydrazones

of some aryl alicyclic ketones for anticonvulsant and other biological activities. Eur J Med Chem 1995;30:303–314.

[3] Dimmock JR, Jonnalagadda SS, Hussein S, Tewari S, Quail JW, Reid RS, Delbaere LTJ, Prasad L. Evaluation of some thiosemicarbazones of arylidene ketones and analogues for anticonvulsant activities. Eur J Med Chem 1990;25:581–588.

[4] Dimmock JR, Puthucode RN, Smith JM, Hetherington M, Quail JW, Pugazhenthi U, Lechler T, Stables JP. (Aryloxy)aryl semicarbazones and related compounds: A novel class of anticonvulsant agents possessing high activity in the maximal electroshock screen. J Med Chem 1996;39:3984–3997.

[5] Anatoly VE, Rashel SE, Margeris JL, Aina AZ, Anda ZD, Iya MK. Condensation products of a-aminoisopropyl phenyl ketone, via the C=O carbonyl bond, with substituted amines, functionally substituted hydrazines or derivatives of natural amino acids, having anticancer activity. FR 2540868, August 17, 1984 Chem Abstr 1985;102:78567j.

[6] Dimmock JR, Sidhu KK, Thayer RS, Mack P, Duffy MJ, Reid RS, Quail JW, Pugazhenthi U, Ong A, Bikker JA, Weaver DF. Anticonvulsant activities of some arylsemicarbazones displaying potent oral activity in the maximal electroshock screen in rats accompanied by high protection indices. J Med Chem 1993;36:2243–2252.

[7] Karali N, Terzioglu N, Gürsoy A. Syntheses and primary cytotoxicity evaluation of new 5-bromo-3-substituted-hydrazono-1*H*-2-indolinones. Arch Pharm Pharm Med Chem 2002; 8:374–380.

[8] Pandeya SN, Raja AS, Stables JP. Synthesis of isatin semicarbazones as novel anticonvulsants – role of hydrogen bonding. J Pharm Pharmaceut Sci 2002;5:266–271.

[9] Marvel CS, Heirs GS In: Blatt AH, editor. Isatin. Organic syntheses collection. 2nd ed., Vol 1, New York: John Wiley and Sons, Inc; 1941. p 327–330.

[10] Azizian J, Fallah-Bagher-Shaidaei, Kefayati H. A facile one-pot method for the preparation of N-alkyl isatins under microwave irradiation. Synth Commun 2003;33:789–793.

[11] Lieber E, Pillai CN, Hites RD. Reactions of nitrous acid with 4-substituted-thiosemicarbazides. Can J. Chem 1957;35:832–842.

[12] Sen AB, Gupta SK. Possible antiamoebic agents. Part XIX. Synthesis of 1,3,4-thiadiazolines and di-1,3,4-thiadiazolines. J Ind Chem Soc 1962;39:628–634.

[13] Spartan '04 for Windows, Wavefunction, Inc., Irvine, CA 92612, USA.

[14] JME Molecular Editor, April 2006 version; http://www.molinspiration.com

[15] Statistical Package for Social Sciences. 2005. SPSS for Windows, Release 14.0.0, SPSS Inc., Chicago.

[16] Baraldi PB, Nunez MDelC, Tabrizo MA, De Clercq E, Balzarini J, Bermejo J, Estévez F, Romagnoli R. Design, syntheses and biological evaluation of hybrid molecules containing α-methylene-γ-butyrolactones and polypyrrole minor groove binders. J Med Chem 2004;47:2877–2886.

[17] Craig PN. Interdependence between physical parameters and selection of substituent groups for correlation studies. J Med Chem 1971;14:680–684.

[18] Hansch C, Leo AJ. Substituent Constants for Correlation Analysis in Chemistry and Biology. New York: John Wiley and Sons; 1979. p 49.

[19] Suffness M, Douros J. In: De Vita VT, Busch H, editors. Methods in Cancer Research., Vol. XVI, Part A, New York: Academic Press; 1979. p 84.

[20] Isaacson EI. In Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. 10th ed. Philadelphia: Lippincott-Raven Publishers; 1998. p 456.