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Original article

Cytotoxic 3,5-bis(benzylidene)piperidin-4-ones and *N*-acyl analogs displaying selective toxicity for malignant cells

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Abstract

A series of 3,5-bis(benzylidene)piperidin-4-ones **1**, 1-acryloyl-3,5-bis(benzylidene)piperidin-4-ones **2** and adducts of **2** with sodium 2-mercaptoethanesulfonate (mesna), namely series **3**, were prepared as candidate cytotoxic agents. These compounds were examined against neoplastic HSC-2, HSC-4 and HL-60 cells as well as HGF, HPC and HPLF normal cell lines and many of the compounds displayed selective toxicity for malignant cells. The CC_{50} values of the analogs in series **2** towards the cancer cell lines were mainly submicromolar. The relative potencies, selectivity and log *P* values were in the order of **2** > **1** > **3**. The sulfonic acid group of a representative compound in series **3** was replaced by a thiol function to produce **4** leading to substantial increases in cytotoxic potencies and hydrophobicity indicating that the presence of a hydrophilic sulfonic acid group was disadvantageous in terms of potency. Molecular modeling suggested that the superior cytotoxicity of various members of series **1**–**3** over an acyclic analog **5** may have been due to the greater torsion angles θ_1 and θ_2 created between the arylidene aryl rings and the adjacent olefinic groups in series **1**–**3**.

Keywords: Piperidin-4-ones; Enones; Sodium 2-mercaptoethanesulfonate; Mesna; Cytotoxicity; Molecular modeling; X-ray crystallography; Selective cytotoxicity

1. Introduction

One of the major interests in this laboratory is the study of the antineoplastic properties of conjugated arylidene ketones. This decision is based on their preferential affinity towards thiols rather than hydroxy or amino groups [1–3]. Hence such molecules may not interact with nucleic acids and thus be bereft of the genotoxic effects of many anticancer drugs used today [4]. Originally only one conjugated arylidene keto group was present in the molecules. However, recently the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore has been incorporated into various cyclic systems thereby permitting two successive alkylations of thiols to occur [5–7]. This sequential attack with cellular thiols is illustrated in Fig. 1. The rationale behind this latter molecular design is as follows. A number of studies have demonstrated that depletion of thiol concentrations prior to treatment with various anticancer drugs has increased cell killing compared to the use of the drug alone [8,9]. In addition, various conjugated enones inhibit the π isozyme of glutathione-*S*-transferase [10] and such inhibition has been shown to enhance the cytotoxicity of the cancer chemotherapeutic alkylating agent thiotepa [11]. Hence the initial alkylation may create a selective chemosensitivity in the malignant cells to a further interaction with cellular thiols.

However, while these previous studies [5–7] demonstrated the cytotoxic properties of such molecules, the bioevaluations

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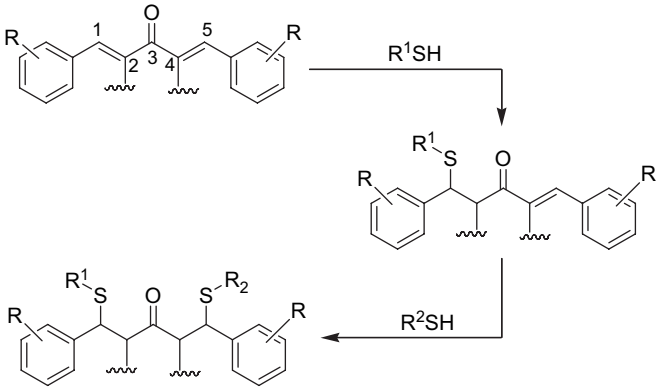


Fig. 1. Sequential interaction of the cellular thiols HSR^1 and HSR^2 with the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore.

used neoplastic and transformed cells. Hence little or no indication was gleaned as to whether these molecules possessed selective toxicity to malignant cells. In order to address this issue, the decision was reached to examine various cyclic compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore against both malignant and normal cells. An initial study using alicyclic scaffolds demonstrated clearly that selectivity and potency were both dependent on the nature of the scaffold [12].

The objectives of the present investigation were as follows. First, an evaluation of whether the incorporation of the 1,5-diaryl-3-oxo-1,4-pentadienyl group into a heterocyclic rather than alicyclic ring would lead to compounds possessing selective toxicity to malignant cells was planned. Previous studies from our laboratories demonstrated that a series of 3,5-bis (benzylidene)piperidin-4-ones **1** possessed excellent cytotoxicity towards human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 lymphoid leukemia cells [6]. In order to increase the extent of interactions with cellular thiols, the *N*-acryloyl analogs **2** were prepared and in general they possessed greater potencies than **1** [6]. Second, selective toxicity may be achieved by the use of chemoprotectants of normal cells. In particular, the administration of sodium 2-mercaptoethanesulfonate (mesna) with various anticancer drugs led to retention of the drug's neoplastic properties but amelioration of its side effects by the thiol [13–15]. The potential exists for the compounds in series **3**, to undergo a retro-Michael reaction liberating the analogs **2** plus a cytoprotective thiol.

In summary, the initial experimentation, aimed at the discovery of candidate cytotoxics with selective toxicity for neoplastic cells, involved the synthesis and bioevaluation of the compounds in series **1**–**3** towards both malignant and normal cell lines. If this quest was successful, attempts to ascertain why this phenomenon occurred were planned.

2. Chemistry

The compounds in series **1**–**3** were prepared by the synthetic chemical routes presented in Scheme 1. Acid catalyzed reaction of various aryl aldehydes with 4-piperidone led to the preparation of series **1** which on treatment with acryloyl chloride produced the corresponding amides **2a**–**e**. Acylation of 4-piperidone with acryloyl chloride produced 1-acryloylpiperidin-4-one which condensed with mesna to provide the sodium salt of the thiol adduct which, in the presence of acid, was condensed with various aryl aldehydes to give the desired products **3a**–**e**. As a result of the marked variation in the cytotoxic properties of the compounds in series **1**–**3** vide infra, two analogs **4** and **5** were synthesized in order to assist in the interpretation of the biodata. The preparation of **4** is indicated in Scheme 1 whereby 1-acryloylpiperidin-4-one was condensed with 1,2-ethanedithiol which gave rise to 1-[3-(2-mercaptoethylsulfanyl)-propionyl]piperidin-4-one which reacted with 4-methoxybenzaldehyde to form the desired product. The reaction of 4-nitrobenzaldehyde with acetone leading to the isolation of **5** is portrayed in Scheme 2.

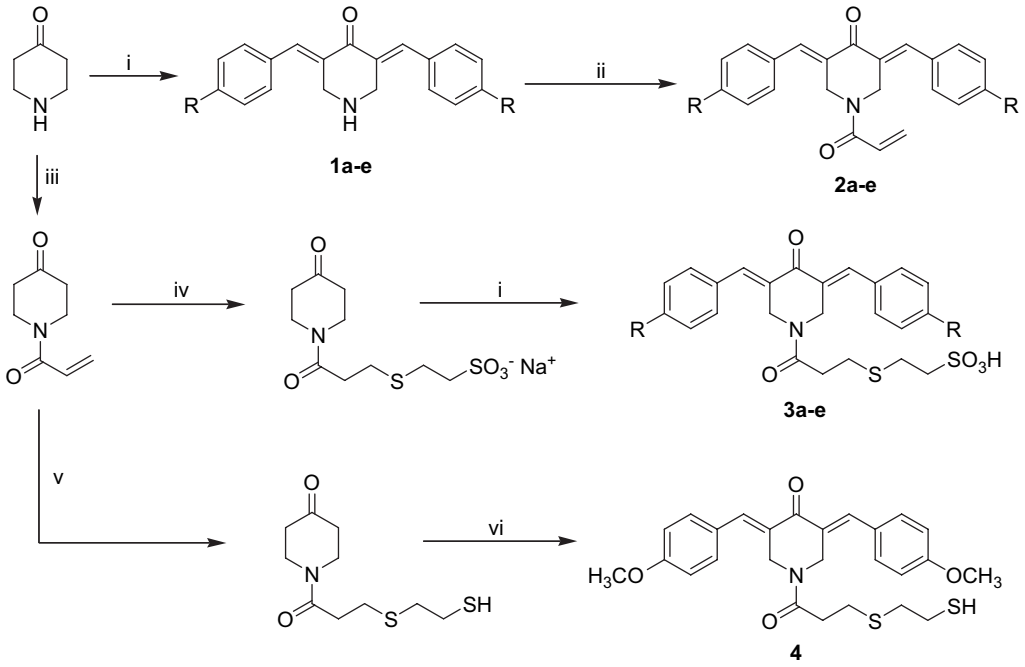
^{1}H NMR spectroscopy indicated that the compounds in series **1**–**5** were stereoisomerically pure. Previous X-ray crystallographic studies revealed the *E*,*E* configuration of the 3,5-arylidene groups in representative compounds in series **1** [16] and **2** [6]. Additionally, in this study the X-ray crystallographic structure of **4** showed that the compounds adopted the *E*,*E* configuration. In the case of **5**, the *J* values of the olefinic protons confirmed the *E* stereochemistry of the olefinic groups.

3. Bioevaluations

All of the compounds **1a**–**e**, **2a**–**e**, **3a**–**e**, **4** and **5** as well as mesna, 1,2-ethanedithiol and the anticancer alkylating agent melphalan were evaluated for cytotoxic properties. The assays used the following human malignant cell lines, namely two squamous cell carcinomas (HSC-2, HSC-4) and a promyelocytic leukemia neoplasm (HL-60). The three normal human cells used were a gingival fibroblast (HGF), pulp cells (HPC) and a peridontal ligament fibroblast (HPLF). These data are presented in Table 1.

4. Results and discussion

The biodata in Table 1 reveal that most of the compounds in series **2** are potent inhibitors of the growth of various malignant cells. The *N*-acyl analogs **2** have submicromolar IC_{50} values in most cases and, on average, are 23 times more potent to HSC-2, HSC-4 and HL-60 cells than **1a**–**e**. On the other hand, the mesna adducts **3a**–**e** have approximately one-third of the potencies of the compounds in series **1**. In order to identify novel compounds possessing a preferential toxicity for neoplasms, selectivity index (SI) figures were calculated for the compounds in series **1**–**3** and are presented in Table 1. A SI value of 10 was arbitrarily chosen as an indication of noteworthy selectivity and from the results obtained, the percentage of compounds meeting this criterion in series **1**, **2** and **3** were 60, 100 and 0, respectively. The conclusions to be drawn from these observations are that the *N*-acryloylpiperidines **2** are potent cytotoxins which exert a selective toxicity for malignant cells. In general, these properties are lower in



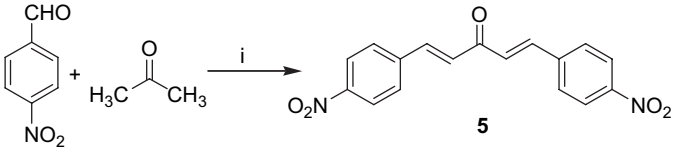
Scheme 1. Synthetic chemical pathway to the compounds in series **1**–**4**. The reagents used in the syntheses were as follows: (i) $2RC_6H_4CHO/CH_3COOH/HCl$; (ii) $CH_2{=}COCl/K_2CO_3$; (iii) $CH_2{=}CHCOCl/K_2CO_3$/tetrabutyl ammonium bromide; (iv) $HSCH_2CH_2SO_3Na/N(C_2H_5)_3$; (v) $HSCH_2CH_2SH/N(C_2H_5)_3$; (vi) $2C_6H_4(4\text{-}OCH_3)CHO/CH_3COOH/HCl$. The aryl substituents in series **1**–**3** were as follows: **a**: R = H; **b**: R = Cl; **c**: R = NO_2; **d**: R = CH_3; **e**: R = OCH_3.

series **1** while the mesna adducts are substantially less promising than series **1** and **2** in terms of both potency and selectivity.

Further experimentation was undertaken with a view to determine some of the factors which contributed to the variation in potencies and especially to the differing SI values. In terms of potencies, the greater toxicity of series **2** than **1** was attributed to the additional electrophilic group in **2a**–**e** enabling these compounds to act as trifunctional alkylating agents. The lower potencies of **3a**–**e** may have been due to a number of factors including the presence of the highly polar sulfonic acid group which could impede transport via cell membranes. In order to examine this possibility, compound **4** was prepared which is identical to **3e** except that the sulfonic acid group was replaced by a mercapto function. The data in Table 1 strengthen this hypothesis insofar as the substantially greater potency of **4** compared to **3e** is revealed. Second, the 4-nitro analogs **1c**, **2c** and **3c** possess the highest potencies in each of the series **1**–**3**. In order to examine whether the compression of part of the 1,5-bis(4-nitrophenyl)-3-oxo-1,4-pentadienyl group into a piperidine ring influenced cytotoxicity, the open chain analog **5** was assayed towards the same cell lines. The data in Table 1 reveal that the average potencies of **1c**, **2c** and **3c** range from 20 to 362 times that of **5**, confirming the important contribution that the rigidity of the 4-piperidones makes to potent cytotoxic properties.

In order to discover some of the factors that influence SI values, the following determinations were undertaken. First, the physicochemical properties of the aryl substituents in series **1**–**3** were considered. These groups were chosen with different Hammett sigma (σ), Hansch pi (π) and molar refractivity (MR) values in order to provide variation in the electronic, hydrophobic and steric properties, respectively, of these groups. Thus in addition to the unsubstituted compounds **1a**, **2a** and **3a**, substituents with both positive (+) and negative (−) σ and π figures were utilized, namely the 4-chloro (+, +), 4-nitro (+, −), 4-methyl (−, +) and 4-methoxy (−, −) groups. Furthermore, the MR values ranged from 1.03 for hydrogen to 7.87 for the 4-methoxy group. In order to ascertain whether one or more of these physicochemical constants correlated with the SI values, linear and semilogarithmic plots were made between the SI values and the σ, π and MR constants of the aryl substituents in **1a**–**d**, **2a**–**e** and **3a**–**e**. However, no correlations were noted ($p > 0.05$). In addition, the calculated log P values of the compounds in series **1**–**5** are presented in Table 1. The average figures for **1a**–**e**, **2a**–**e** and **3a**–**e** are 3.81, 4.37 and 1.61, respectively, indicating that the relative hydrophobicities are **2** > **1** > **3**. In general, this sequence is the same as noted for cytotoxic potencies (the average CC_{50} figures against human tumors for **1**, **2** and **3** are 29.4, 1.27 and 81.3 μM, respectively), and SI values (the average SI values are >11.8, 17.6 and 3.88 for **1**, **2** and **3**, respectively). The substantially lower log P values of series **3** compared to **1a**–**e** and **2a**–**e** indicates clearly that the polar sulfonic acid group in **3** diminishes the hydrophobic properties of the molecules (e.g., substitution of the sulfonic acid group by a mercapto function increased the log P value by 3.23) which likely has an adverse effect on membrane permeability and cytotoxic potencies.

Second, the SI values of the compounds in series **1** and **2** are impressive in general but they are substantially lower in **3a**–**e**. In order to investigate whether this observation



Scheme 2. Synthesis of compound **5**; (i) NaOH.

pertaining to series **3** was associated with the stabilities of the compounds, a solution of a representative compound **3c** was incubated at 37 °C for 24 h which were the temperature and time of the cytotoxicity assays. The compound was stable under these conditions. Thus the probability exists that **3c** does not release **2c** and 2-mercaptoethanesulfonic acid, which may account for the lower cytotoxic potency and selective toxicity of **3c** compared to **2c**. Presumably other members of series **3** were also stable which accounts for their generally lower cytotoxicity than is found in series **1** and **2**.

Third, the lower potency of **5** compared to **1c**, **2c** and **3c** may have been due to differences in the relative positions of important atoms and groups of the 1,5-bis(4-nitrophenyl)-3-oxo-1,4-pentadienyl toxophore. In particular, possible differences between the orientation and relative positions of the aryl rings were considered and in order to address this issue, molecular models of these four compounds were built. The aryl rings were designated A and B. Ring B and the substituent R in **2c** and **3c** are located on the same side of axis 1 as indicated in Fig. 2A. The torsion angles θ_1 and θ_2 reflect the lack of coplanarity of rings A and B, respectively, with the adjacent olefinic linkages (Fig. 2A). A comparison of the locations of rings A and B was made by measuring the distances d_1–d_3 which are the spans between the centres of the aryl rings and the keto oxygen atoms (Fig. 2B). In addition, the bond angles ψ_1 and ψ_2 were obtained. These data for θ_1, θ_2, d_1–d_3, ψ_1 and ψ_2 are presented in Table 2.

The data in Table 2 indicate that in the case of the 4-piperidones **1c**, **2c** and **3c**, the torsion angles θ_1 and θ_2 vary between 42° and 61°. The lack of coplanarity between aryl rings and the adjacent olefinic linkages in 4-piperidones in which two arylidene rings are conjugated with a keto function has been attributed to nonbonded interactions between one of the *ortho* hydrogen atoms of the aryl rings with the equatorial protons at positions 3 and 5 of the piperidine ring [6,16]. In contrast, the aryl rings in **5** are virtually coplanar with the adjacent unsaturated groups. The other parameters of **1c**, **2c** and **3c**, namely the interatomic distances d_1–d_3 as well as the bond angles ψ_1 and ψ_2, are similar to the figures obtained for **5**. Thus one reason for the marked disparity in cytotoxic potencies between the piperidones in series **1**–**3** and the acyclic analog **5** may be that the marked noncoplanarity of rings A and B in series **1**–**3** which permits a favorable topography for alignment at a binding site.

The X-ray crystallographic structure of **4** revealed that this molecule crystallized with two nonequivalent conformations in the asymmetric unit designated **4A** and **4B**. The ORTEP-3 diagram [17] of **4A** is presented in Fig. 3. The principal difference between the two conformers **4A** and **4B** is the relative locations of the terminal 2-mercaptoethyl group. As indicated in

Table 1
The cytotoxicity and calculated log *P* values of the compounds in series **1**–**5**

Compound	CC_{50}[a] (μM)								SI[b]	log *P*
	Human tumor cells				Human normal cells					
	HSC-2	HSC-4	HL-60	Average	HGF	HPC	HPLF	Average		
1a	2.2	4.4	1.5	2.7	20	38	24	27	10	3.36
1b	18	20	22	20	69	130	56	85	4.3	4.71
1c	0.90	6.1	3.6	3.5	13	88	39	47	13	3.27
1d	3.9	9.3	4.5	5.9	150	200	170	173	29	4.25
1e	100	75	170	115	>400	>400	160	>320	>2.8	3.47
2a	0.63	1.2	0.74	0.86	6.0	15	8.2	9.7	11	3.92
2b	0.63	0.99	0.55	0.72	13	6.0	6.3	8.4	12	5.27
2c	0.094	0.56	0.13	0.26	4.6	3.9	3.6	4.0	15	3.84
2d	0.57	0.75	0.81	0.71	5.6	18	11	11.5	16	4.81
2e	1.5	8.1	1.9	3.8	100	190	97	129	34	4.03
3a	200	71	190	154	330	290	250	290	1.9	1.15
3b	32	24	33	30	200	140	150	163	5.4	2.51
3c	5.0	5.2	4.0	4.7	31	48	30	36	7.7	1.07
3d	150	89	140	126	300	210	220	243	1.9	2.05
3e	88	57	130	92	250	270	180	233	2.5	1.27
4	4.0	3.5	2.6	3.4	8.1	9.4	17	11.5	3.4	4.50
5	41	83	158	94	>400	>400	>400	>400	>4.3	4.10
Mesna	>400	>400	>400	>400	>400	>400	>400	>400	~1.0	–
1,2-Ethanediol	>400	385	221	>335	>400	>400	>400	>400	~1.2	–
Melphalan	35	81	6.0	40.7	>200	>200	>200	>200	>4.9	–

[a] The CC_{50} figure indicates the concentration of compound required to reduce the number of viable cells by 50%. Data were obtained from dose response curves while the CC_{50} values are the mean figures from duplicate determinations. The differences between two determinations were within 5%. The maximum concentration of the compounds was 400 μM except in the case of melphalan in which solubility problems necessitated a higher concentration of 200 μM.

[b] SI indicate the selectivity index which is the ratio of the average CC_{50} figures for the normal cells and tumor cells.

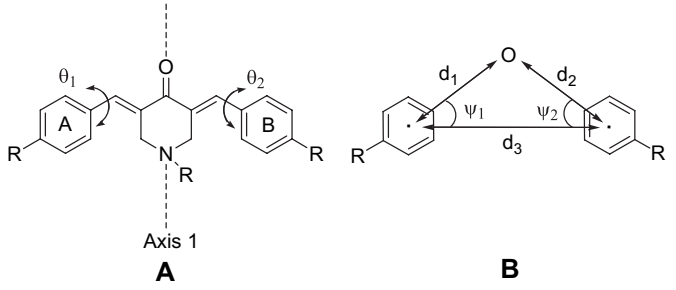


Fig. 2. (A) Designation of the torsion angles θ_1 and θ_2 in representative compounds. (B) The positions of rings A and B in relation to the keto oxygen atom determined by measurements of the interatomic distances d_1–d_3 and bond angles ψ_1 and ψ_2. The distance d_4 is the span between the centre of ring B and axis 2 which is the plane of the centre of ring A and the keto oxygen atom.

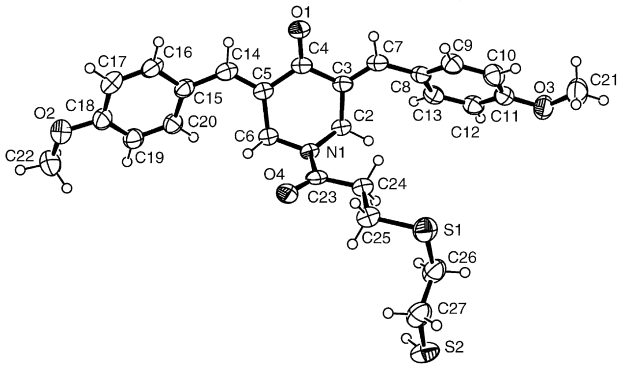


Fig. 3. ORTEP-3 diagram of **4A**.

Fig. 3, the C26–C27–S2 substituent is approximately perpendicular to ring B. On the other hand, this group in **4B** is twisted towards ring B. The shape of **4** determined by molecular modeling was similar to **4A** and not **4B**. The X-ray determination was undertaken for two reasons. In the first place, the data confirm the proposed structure for **4** in terms of the integrity of the olefinic double bonds, i.e., while a free mercapto group is present in **4**, no intramolecular thiolation at the olefinic carbon atoms occurred. In addition, as noted with analogs of **4**, the olefinic double bonds in this compound adopted the *E* configuration. Furthermore, X-ray crystallography confirmed the marked lack of coplanarity between rings A and B and the adjacent olefinic groups. The C5–C14–C15–C20 (θ_1) and C3–C7–C8–C13 (θ_2) torsion angles are −24.7° and 38.0°, respectively. Interatomic distances of less than 2.5 Å are indicative of nonbonded interactions. In the case of **4A**, the C6He–C20H and C2He–C13H spans are 2.271 Å and 2.353 Å, respectively, which are believed to account for the θ_1 and θ_2 torsion angles being greater than 0°.

5. Conclusions

This study revealed that a number of 3,5-bis(benzylidene)-piperidin-4-ones and *N*-acyl analogs display selective toxicity for malignant cells. In particular, series **2** are potent cytotoxins with noteworthy SI values. The additional site for thiolation in series **2**, compared to series **1** and **3**, for example, permits a greater number of sequential interactions with cellular nucleophiles which may contribute significantly to these favorable properties. The data generated in this project enable suggestions to be made for further avenues to pursue with a view of increasing potency and selectivity. For example, the design of further analogs of series **1**–**3** should be undertaken in order to determine the importance of hydrophobicity. In addition, substituents of varying sizes should be placed in the *ortho* positions of the arylidene rings to find whether the magnitude of the θ_1 and θ_2 values correlate with potency and selectivity. Furthermore, the sulfonic acid group in series **3** should be esterified which may facilitate penetration of the cell wall which, on subsequent hydrolysis and dethiolation, could lead to mesna and 1-acryloyl-3,5-bis(benzylidene)piperidin-4-one. Future studies will also be directed to design compounds which will liberate the cytoprotective thiol and antineoplastic agent rapidly and completely. Since high levels of thiol are required to protect normal cells [15], the liberated α,β-unsaturated ketones must be well tolerated in animals and hence in vivo evaluations will need to be undertaken.

Table 2
Some torsion angles (θ_1, θ_2), interatomic distances (d_1–d_3) and bond angles (ψ_1, ψ_2) present in **1c**, **2c**, **3c**, **4** and **5** determined by molecular modeling

Compound	θ_1	θ_2	d_1	d_2	d_3	ψ_1	ψ_2
1c	60.1	−61.1	7.12	7.12	12.16	31.4	31.4
2c	−43.8	41.8	7.02	7.04	12.90	23.4	23.3
3c	−41.5	43.6	7.10	7.08	12.44	28.7	28.7
4	−39.2	61.5	7.05	7.07	12.59	26.9	26.9
5	−0.1	−0.3	7.13	7.13	12.12	31.7	31.7

6. Experimental protocols

6.1. Chemistry

Melting points were determined using a Gallenkamp instrument and are uncorrected. Elemental analyses (C, H, N) were undertaken using an Elementer analyzer and were within 0.4% of the calculated values. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 FT machine while the mass spectrum was obtained with a VG 7OSEa instrument. The X-ray crystallographic diffractions were determined using a Nonius machine.

6.1.1. Synthesis of 3,5-bis(benzylidene)piperidin-4-ones (**1a**–**e**) and 1-acryloyl-3,5-bis(benzylidene)piperidin-4-ones (**2a**–**e**)

The synthesis of the 4-piperidones **1a**–**e** and **2a**–**c**, **e** has been described previously [6]. Compound **2d** was prepared by a reported method [6] and recrystallized from 95% ethanol. Yield: 88%; m.p. 147 °C; ^{1}H NMR ($CDCl_3$) δ: 2.42 (s, 6H), 4.68 (s, 2H), 4.82 (s, 2H), 5.57 (d, 1H, $J = 10.30$ Hz), 6.19 (d, 1H, $J = 16.75$ Hz), 6.28 (m, 1H), 7.28 (m, 8H), 7.84 (s, 2H). Anal. calcd. for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92%. Found: C, 80.79; H, 6.30; N, 4.06%.

*6.1.2. General method for the synthesis of 2-{3-[3,5-bis(benzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acids (**3a–e**)*

A mixture of 4-piperidone (0.01 mol), acryloyl chloride (0.012 mol), potassium carbonate (0.02 mol) and tetrabutyl ammonium bromide (0.001 mol) in acetone (75 ml) was stirred at room temperature overnight. The reaction mixture was filtered and after evaporation of the solvent, the residue was dissolved in chloroform and washed with water (100 ml). The organic layer was dried over sodium sulfate, filtered and removal of the solvent in vacuo gave 1-acryloylpiperidin-4-one as viscous oil which was used without any purification.

^{1}H NMR ($CDCl_3$) δ: 2.49 (br s, 4H), 3.75 (br s, 2H), 3.91 (br s, 2H), 5.75 (dd, 1H), 6.31 (dd, 1H), 6.66 (dd, 1H).

A mixture of 1-acryloylpiperidin-4-one vide supra, sodium mercaptoethanesulfonate (0.005 mol), triethylamine (0.001 mol), chloroform (25 ml) and methanol (25 ml) was stirred at room temperature overnight. The precipitate was collected, washed with a mixture of chloroform and methanol (1:1, 10 ml, previously cooled to 5 °C) and dried to produce sodium 2-[3-(4-oxopiperidin-1-yl)-3-oxo- propylsulfanyl]ethanesulfonate. The crude product, which was prepared in 55% yield with respect to 4-piperidone, was used without purification in the synthesis of series **3**. ^{1}H NMR (D_2O) δ: 2.78 (m, 8H), 3.06 (t, 2H), 3.52 (t, 2H), 3.77 (t, 2H), 3.81 (t, 2H).

A mixture of sodium 2-[3-(4-oxopiperidin-1-yl)-3-oxopropylsulfanyl]ethanesulfonate (0.0032 mol) and aryl aldehyde (0.0066 mol) in glacial acetic acid (15 ml) was acidified with dry hydrogen chloride and stirred at room temperature for 8 h. The precipitate was collected, washed with glacial acetic acid (5 ml) and dried under vacuum overnight at 45 °C. The product obtained was crystallized from ethanol.

*6.1.2.1. 2-{3-[3,5-Bis(benzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (**3a**).* Yield: 73%; m.p. 125 °C; ^{1}H NMR ($CDCl_3$) δ: 2.27 (br s, 2H), 2.58 (br s, 2H), 2.68 (br s, 2H), 3.12 (br s, 2H), 4.51 (s, 2H), 4.81 (s, 2H), 7.11 (m, 10H), 7.75 (s, 1H), 7.89 (s, 1H). Anal. calcd. for $C_{24}H_{25}NO_5S_2\cdot 2H_2O$: C, 56.75; H, 4.92; N, 2.96%. Found: C, 56.75; H, 5.00; N, 2.50%.

*6.1.2.2. 2-{3-[3,5-Bis(4-chlorobenzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (**3b**).* Yield: 85%; m.p. 142 °C; ^{1}H NMR ($CDCl_3$) δ: 2.59 (t, 2H), 2.68 (t, 2H), 2.78 (t, 2H), 3.15 (t, 2H), 4.79 (s, 2H), 4.91 (s, 2H), 7.29 (m, 8H), 7.87 (s, 1H), 7.94 (s, 1H). Anal. calcd. for $C_{24}H_{23}Cl_2NO_5S_2\cdot 1.5\ H_2O$: C, 50.75; H, 4.05; N, 2.46%. Found: C, 50.76; H, 4.23; N, 2.32%.

*6.1.2.3. 2-{3-[3,5-Bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (**3c**).* Yield: 75%; m.p.135 °C; ^{1}H NMR ($CDCl_3$) δ: 2.65 (br s, 2H), 2.76 (m, 4H), 3.23 (br s, 2H), 4.76 (s, 2H), 4.89 (s, 2H), 7.57 (m, 4H), 8.00 (s, 1H), 8.06 (s, 1H), 8.34 (m, 4H). Anal. calcd. for $C_{24}\ H_{23}N_3O_9S_2\cdot 2H_2O$: C, 48.19; H, 3.84; N, 7.02%. Found: C, 48.29; H, 3.81; N, 7.00%.

*6.1.2.4. 2-{3-[3,5-Bis(4-methylbenzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (**3d**).* Yield: 80%; m.p. 143 °C; ^{1}H NMR ($CDCl_3$) δ: 2.42 (d, 6H, $J = 12.20$ Hz), 2.67 (t, 4H), 2.82 (t, 2H), 3.22 (t, 2H), 4.87 (s, 2H), 4.98 (s, 2H), 7.29 (m, 8H), 7.95 (s, 1H), 8.01 (s, 1H). Anal. calcd. for $C_{26}H_{29}NO_5S_2\cdot 1.5H_2O$: C, 59.24; H, 5.52; N, 2.85%. Found C, 59.36; H, 5.52; N, 2.85%.

*6.1.2.5. 2-{3-[3,5-Bis(4-methoxybenzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (**3e**).* Yield: 82%; m.p. 157 °C; ^{1}H NMR (D_2O) δ: 2.17 (br s, 2H), 2.31 (br s, 2H), 2.43 (t, 2H), 2.74 (t, 2H), 3.49 (s, 3H), 3.57 (s, 3H), 4.29 (s, 2H), 4.36 (s, 2H), 6.58 (d, 2H, $J = 7.71$ Hz), 6.70 (d, 2H, $J = 7.79$ Hz), 7.01 (m, 4H), 7.32 (s, 1H), 7.41 (s, 1H). ^{13}C NMR (D_2O): δ 26.46, 27.22, 33.27, 43.80, 46.58, 51.38, 55.59, 55.74, 114.67, 114.85, 126.91, 127.21, 129.03, 129.36, 133.18, 133.55, 137.65, 138.34, 160.72, 160.88, 171.79, 186.35. Anal. calcd. for $C_{26}H_{29}NO_7S_2\cdot 2H_2O$: C, 54.96; H, 5.10; N, 2.46%. Found C, 55.22; H, 4.63; N, 2.41%.

*6.1.3. Synthesis of 1-[3-(2-mercaptoethylsulfanyl) propionyl]-3,5-bis(4-methoxybenzylidene)piperidin-4-one (**4**)*

This piperidone was prepared from 1-acryloylpiperidin-4-one by the same methodology employed for the synthesis of the compounds in series **3** except that 1,2-ethanedithiol was used in place of sodium 2-mercaptoethanesulfonate. The crude product was crystallized from ethanol. Yield: 82%; m.p. 128 °C; ^{1}H NMR ($CDCl_3$) δ: 2.47 (t, 2H), 2.73 (m, 4H), 3.52 (m, 2H), 3.85 (d, 6H, $J = 12.89$ Hz), 4.72 (s, 2H), 4.94 (s, 2H), 6.90 (m, 4H), 7.36 (d, 2H, $J = 8.41$ Hz), 7.46 (d, 2H, $J = 8.45$ Hz), 7.80 (s, 1H), 7.85 (s, 1H). Mass (LCMS): (M + 2+ 1): 486. Anal. calcd. for $C_{26}H_{29}NO_4$: C, 64.57; H, 6.04; N, 2.90%. Found: C, 64.31; H, 5.92; N, 3.09%.

*6.1.4. Synthesis of 1,5-bis-(4-nitrophenyl)-1,4-pentadien-3-one (**5**)*

A solution of sodium hydroxide (10% w/v, 1 ml) was added to a solution of acetone (0.01 mol) and 4-nitrobenzaldehyde (0.02 mol) in ethanol (20 ml). The mixture was stirred at room temperature for 10 min. The precipitate was collected and crystallized from acetonitrile to give **5**. Yield: 62%; m.p. 145–146 °C; ^{1}H NMR (DMSO-d_6) δ: 7.56 (d, 1H, $J = 16.10$ Hz), 7.93 (d, 1H, $J = 16.10$ Hz), 8.07 (d, 2H, $J = 8.55$ Hz), 8.31 (d, 2H, $J = 8.55$ Hz). Anal. calcd. for $C_{17}H_{12}N_2O_5$: C, 62.96; H, 3.73; N, 8.64%. Found: C, 62.84; H, 3.71; N, 8.72%.

*6.1.5. X-ray crystallographic determination of 1-[3-(2-mercaptoethylsulfanyl)propionyl]-3,5-bis (4-methoxybenzylidene)piperidin-4-one (**4**)*

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge

Crystallographic Data Centre as supplementary publication no. CCDC 630874. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or email deposit@ccdc.cam.ac.uk).

6.2. Cytotoxicity evaluations

The methodology of the assays using HSC-2, HSC-4, HL-60, HGF, HPC and HPLF cells has been described previously[18]. In brief, the cells were incubated at 37 °C for 24 h in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum except HL-60 cells which were cultured in RPMI1640 containing 10% fetal bovine serum. Cell viability was assessed using the MTT method except for the HL-60 cells in which case the trypan blue exclusion procedure was used.

6.3. Stability study of *3c*

A solution of **3c** in deuterium oxide (10 mM) was incubated at 37 °C for 24 h. The ^{1}H NMR spectra on dissolution and after incubation were identical.

6.4. Determination of calculated log *P* values of *1–5*

The log *P* figures were obtained from Online Cheminformatics Services provided by Molinspiration Cheminformatics [19].

6.5. Statistical evaluations

The Hammett sigma, Hansch pi and MR values were obtained from the literature [20].

6.6. Molecular modeling

Molecular modeling was undertaken using BioMedCache software [21].

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