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Structural characteristics of novel symmetrical diaryl derivatives with nitrogenated functions. Requirements for cytotoxic activity

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Abstract—In an attempt to discover the essential features that would allow us to explain the differences in cytotoxic activity shown by a series of symmetrical diaryl derivatives with nitrogenated functions, we have studied by molecular modelling techniques the variation in Log *P* and conformational behaviour, in terms of structural modifications. The Log *P* data—although they provide few clues concerning the observed variability in activity—suggest that an initial separation of active and inactive compounds is possible based on this parameter. The subsequent study of the conformational behaviour of the compounds, selected according to their Log *P* values, showed that the active compounds preferentially display an extended conformation and inactive ones are associated with a certain type of folding, with a triangular-type conformation adopted in these cases.

1. Introduction

Our research team is currently[1,2] focusing on finding novel anti-tumoral compounds whose cytotoxic activity is related to a mechanism of action based on the induction of apoptosis. Apoptosis is a unique type of cell death characterised by cytoskeletal disruption, cellular shrinkage, membrane blebbing, nuclear condensation and internucleosomal DNA fragmentation.[3] This is a genetically controlled process and, as such, dysregulation of the apoptotic machinery is frequently observed in numerous types of cancers. Because of the close correlation between tumorigenesis and dysregulation of apoptosis, any therapeutic strategy aimed at specifically triggering apoptosis—the principal mechanism employed by the immune system and chemotherapeutic drugs in eradicating tumour cells—have potential therapeutic applications.[4–6] In addition, it has been observed that resistant tumour cells evade the action of anticancer agents by increasing their apoptotic threshold.[7–10] However, details of the biology underlying the action of some drugs and the reasons why some cancers are drug-resistant are not well understood.[11] This increasing prevalence of multi-drug-resistant cancers continues to provide impetus for the discovery of novel anti-tumoral drugs.

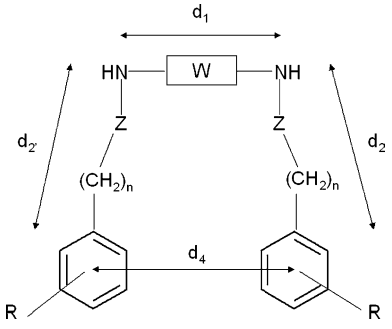

Figure 1. Preliminary model used for the design of compounds, according to data collected from the bibliography.

The objective of the present study was to obtain structure–activity relationships (SAR) using an array of new compounds [symmetrical diaryl derivatives with nitrogenated functions (Figs. 1 and 2)] in order to

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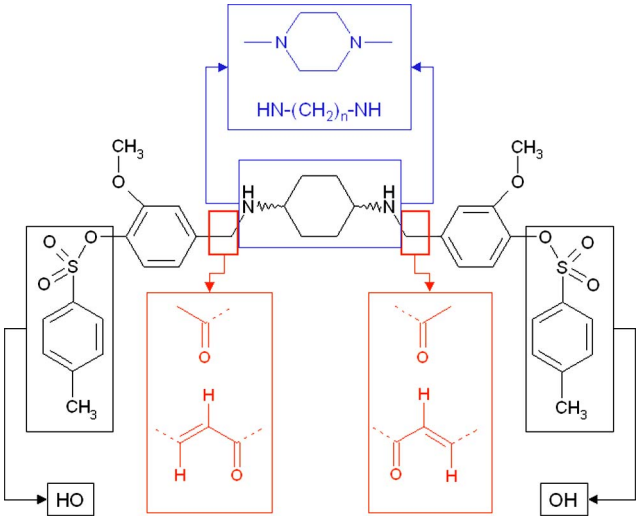


Figure 2. Overview of structural variations carried out for the studied nitrogenated symmetrical diaryl derivatives (with compound **9** as a template).

analyse their ability to induce cytotoxicity in different cell lines (Table 1). Our interest in this subject stems from our desire to understand and to rationalise the biological activity results obtained and, subsequently, to use these data in the design of new compounds that would allow us to optimise the target biological activity.

In earlier studies, we focused on the ability of compounds to induce apoptosis and considered structures based on a model system consisting of three entities whose chemical nature could be varied. These units were linked together in such a way that the resulting model had a central nucleus connected to two equivalent arms that formed a variable angle (Fig. 1).

The chemical structures of the entities were selected according to the literature (see references cited in Ref. 2), in which they appear as fragments in molecules whose biological activity is consistent with our overall objective. Our compounds have been described previously[2] and showed both cytotoxic and apoptotic activities. However, the intimate mechanism of such biological activity remains unknown in these systems, although the data obtained to date suggest the involvement of the caspase cascade. The structural variations carried out for this series are outlined in Figure 2, with *N*,*N*′-di[3-methoxy-4-(4-methylphenylsulfonyloxy) benzyl]cyclohexane-1,4-diamine (compound **9**, Table 1), the most active compound, taken as a template.

Initial results did not allow us to establish the structure–activity relationships for these compounds in a satisfactory way. Indeed, it is remarkable how some structurally very similar molecules, which fulfil the original design parameters, are inactive, while others are active (cf. compound **5b** with compounds **5a** or **5c**). Another remarkable result is the increase in target activity that is observed on reducing the carbonyl groups in **3c** to give compound **9**, which is the most active compound in the series (Table 1).

In the present work, two approaches were applied in an effort to gain an insight into the structural requirements for activity in these compounds. First, it was planned to obtain some descriptive parameter at a molecular level, such as the log of the partition coefficient (Log *P*), surface area or molecular volume, that would allow discrimination between active and inactive compounds. Second, an approach was desired that would allow an active compound to be characterised by a set of chemical functions with a certain spatial arrangement and molecular shape that could be related to the observed biological activity but would not be associated with the inactive compounds.[12,13] Thus, the presence of certain chemical functions is not only necessary, in agreement with the pharmacophoric approach, but these functions must be exposed in a manner that can be recognised by the associated binding site—a property that requires specific conformational behaviour. In this framework, a compound can be considered as a collection of multiple instances, one for each conformation. An active compound contains at least one active instance, while an inactive compound does not contain any active instance. On the assumption that all the active compounds bind to the same site, we would expect their binding conformations to have similar shapes. For this second approach it is necessary to carry out a study of the conformational behaviour of the compounds and subsequently select and analyse the supposed bioactive conformations of the compounds.

2. Results and discussion

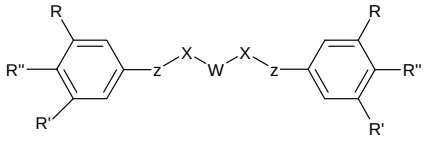
The high conformational freedom of the target compounds encouraged us to use the geometric data obtained for some structurally analogous molecules as a reference; these data were obtained from The Cambridge Structural Database[14] (Table 2).

Compounds CADPON, MEBZAM and SAVZUK were taken as templates for building the 1,3-diaminopropane and 1,4-diaminobutane derivatives, with the central chains maintained in an extended conformation and a *trans* configuration for the amide groups. For the 1,4-diaminocyclohexane derivatives, the configuration is assumed to be *cis* for the central amino groups and *trans* for the amide groups, in agreement with the data collected for GICXAS and its analogues. For the piperazine derivatives the relative configuration for the carbonyl groups was taken as *trans*, as in BrPRBz and SETFOM, but in this study subsequent free rotation of the C–N bonds was allowed. In all cases, the *trans* conformation was assumed for the vinylic fragments, as found in SETFOM and its analogues. The starting configurations are given in Figure 3.

Once the models for all compounds had been constructed, the initial geometries were fully minimised to an energy gradient below 10^{-3} kcal mol^{-1} Å^{-1}.

Attempts were made to correlate the cytotoxic activity of these compounds to the calculated log of the partition coefficient (Log *P*). The value of Log *P* was deter-

Table 1. Cytotoxic screening results of the tested compounds and their Log *P*, surface area and volume



Compound	R	R′	R″	X	Z	W	Log *P*	SA[a]	Vol[b]	Cytotoxicity (IC_{50}, μM)		
										A[c]	B[d]	C[e]
1a	H	H	–OMe	CO	—	1,4-Diaminobutane	2.07	669.02	1107.04	n.a[f]	n.a.	n.a.
1b	H	H	–OMe	CO	—	Piperazine	1.89	612.73	1038.71	n.a.	n.a.	n.a.
1c	H	H	–OMe	CO	—	1,4-Diaminocyclohexane	2.54	663.91	1134.04	n.a.	n.a.	n.a.
1d	H	H	–OMe	CO	—	1,3-Diaminopropane	1.62	641.07	1051.85	n.a.	n.a.	n.a.
2	H	H	–OMe	CH_2	—	1,4-Diaminobutane	2.99	677.87	1106.65	n.a.	53.5	42.3
3a	H	–OMe	–OTs	CO	—	1,4-Diaminobutane	5.20	1067.35	1858.04	n.a.	n.a.	70.1
3b	H	–OMe	–OTs	CO	—	Piperazine	5.02	1010.18	1781.38	47.1	10.4	70.2
3c	H	–OMe	–OTs	CO	—	1,4-Diaminocyclohexane	5.67	1063.16	1883.85	38.1	39.4	74.0
3d	H	–OMe	–OTs	CO	—	1,3-Diaminopropane	4.75	1017.29	1788.86	31.8	n.a.	n.a.
4a	H	–OMe	–OH	CO	—	1,4-Diaminobutane	1.50	683.84	1152.08	n.a.	n.a.	n.a.
4b	H	–OMe	–OH	CO	—	Piperazine	1.32	638.97	1077.73	n.a.	n.a.	n.a.
4c	H	–OMe	–OH	CO	—	1,4-Diaminocyclohexane	1.97	684.87	1172.38	n.a.	n.a.	n.a.
4d	H	–OMe	–OH	CO	—	1,3-Diaminopropane	1.05	653.60	1084.14	n.a.	n.a.	n.a.
5a	H	–OMe	–OTs	CO	–CH=CH–	1,4-Diaminobutane	6.02	1169.78	2037.53	57.3	n.a.	78.7
5b	H	–OMe	–OTs	CO	–CH=CH–	Piperazine	5.83	1115.93	1973.24	n.a.	n.a.	n.a.
5c	H	–OMe	–OTs	CO	–CH=CH–	1,4-Diaminocyclohexane	6.49	1167.83	2064.02	35.2	46.5	n.a.
5d	H	–OMe	–OTs	CO	–CH=CH–	1,3-Diaminopropane	5.57	1121.76	1967.96	n.a.	n.a.	n.a.
6a	H	–OMe	–OH	CO	–CH=CH–	1,4-Diaminobutane	2.32	786.79	1320.32	n.a.	n.a.	n.a.
6b	H	–OMe	–OH	CO	–CH=CH–	Piperazine	2.17	742.82	1264.11	n.a.	n.a.	n.a.
6c	H	–OMe	–OH	CO	–CH=CH–	1,4-Diaminocyclohexane	2.79	789.73	1357.98	n.a.	n.a.	n.a.
6d	H	–OMe	–OH	CO	–CH=CH–	1,3-Diaminopropane	1.87	770.78	1273.17	n.a.	n.a.	n.a.
7a	H	–O-CH_2-O–		CO	—	1,4- Diaminobutane	1.95	662.27	1088.84	n.a.	n.a.	n.a.
7b	H	–O-CH_2-O–		CO	—	Piperazine	1.76	605.37	1020.63	n.a.	n.a.	n.a.
7c	H	–O-CH_2-O–		CO	–CH_2–	1,4-Diaminobutane	1.81	709.34	1190.94	n.a.	n.a.	n.a.
7d	H	–O-CH_2-O–		CO	–CH_2–	Piperazine	1.62	639.24	1099.98	n.a.	n.a.	n.a.
7e	H	–O-CH_2-O–		CO	–CH=CH–	1,4-Diaminobutane	2.76	766.67	1248.67	n.a.	n.a.	n.a.
7f	H	–O-CH_2-O–		CO	–CH=CH–	Piperazine	2.58	717.31	1204.45	n.a.	n.a.	n.a.
8	–OMe	–OMe	–OMe	CO	—	1,4-Diaminobutane	1.06	819.41	1386.07	n.a.	n.a.	n.a.
9	H	–OMe	–OTs	CH_2	—	1,4-Diaminocyclohexane	6.56	1009.00	1844.04	5.7	2.6	1.5
Camptothecin										0.291	0.014	0.009

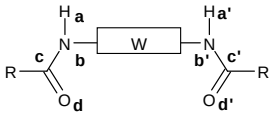
[a] SA, surface area in $Å^2$.
[b] Vol, volume in $Å^3$.
[c] Cell line: MD-MBA-231.
[d] Cell line: HT-29.
[e] Cell line: T-24.
[f] n.a., $IC_{50} > 100$ μM.

mined on preliminary geometries obtained after the first minimisation (Table 1). Although a direct correlation could not be established, the resulting data did shed some light on the biological data. For example, in the case of the arylsulfonyl group (Tosyl derivatives), a structural modification introduced initially as a hydroxyl-protecting group caused an increase in the value of Log *P* (compound **3** versus **4**; compound **5** versus **6**) along with an increase in cytotoxic activity. Reduction of the carbonyl groups also produced a noticeable increase in biological activity (compound **2** versus **1a**) and this could, in principle, be related to the increase in Log *P*. Similarly, the activity of compound **3c** (Log *P* 5.67) increased markedly when the carbonyl group was replaced by a methylene group (compound **9**, Log *P* 6.56) to give the most active compound in the series.

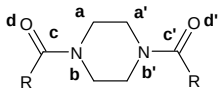
A Log *P* value of 2.99 seems to mark the border between active and inactive compounds.

The Log *P* values were used as selection criteria and the initial geometries of the selected compounds (Log *P* > 2.99) were subjected to a systematic conformational exploration. This study aimed to highlight conformational changes that would allow us to explain certain observations in terms of our hypothesis, which attempts to relate the biological profiles of the compounds with the occurrence of certain types of conformational behaviour in the bioactive conformation of the compounds. For instance, it was hoped that we could explain the lack of activity of compounds **5b** and **5d**, which apparently cannot be related to the structural variability of the compounds, as well as the possibility of explaining other observa-

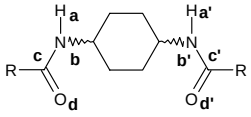
Table 2. Geometrical data taken from reference crystallographic CSD structures



CSD			Dihedral (in °)			Distance (in Å)	
Refcode.	W	R	abcd	a′b′c′d′	dbb′d′	bb′	cc′
CADPON	$(CH_2)_3$	$(CH_2)_{10}CH_3$	−174.75	176.40	−59.62	4.90	7.31
MEBZAM	$(CH_2)_4$	C_6H_5	−173.96	173.96	−174.75	6.22	8.59
SAZVUK	$(CH_2)_4$	$pOCH_3C_6H_4$	−172.32	172.32	−180.00	6.19	8.54



CSD		Dihedral (in °)			Distance (in Å)	
Refcode.	R	abcd	a′b′c′d′	dbb′d′	bb′	cc′
BrPRBz	$(CH_2)_2Br$	168.75	−3.86	180.00	2.84	5.25
SETFOM	–CH=CH–R	162.86	−3.09	180.00	2.82	5.49



CSD		Dihedral (in °)			Distance (in Å)	
Refcode.	R	abcd	a′b′c′d′	dbb′d′	bb′	cc′
GICYAS	$–COOCH_3$	174.24	−174.24	−180.00	5.69	7.86

tions including the increase in activity for compounds **2** and **9**. The dihedral angles and bonds selected for rotation are shown in Figure 4.

The data obtained in the conformational analysis of the selected compounds (**2**, **3**, **5** and **9**) are shown in Table 3. The minimum energy conformers were superimposed, with the nitrogens from the central structure taken as adjusting atoms, along with the oxygens of the methoxy groups and the sulfur atoms of the tosyl groups where applicable. The effectiveness of the superimposed models was evaluated in terms of the root mean square (rms) values obtained. The energy differences between the different conformations analysed for each trajectory were in the range 2–5 kcal.

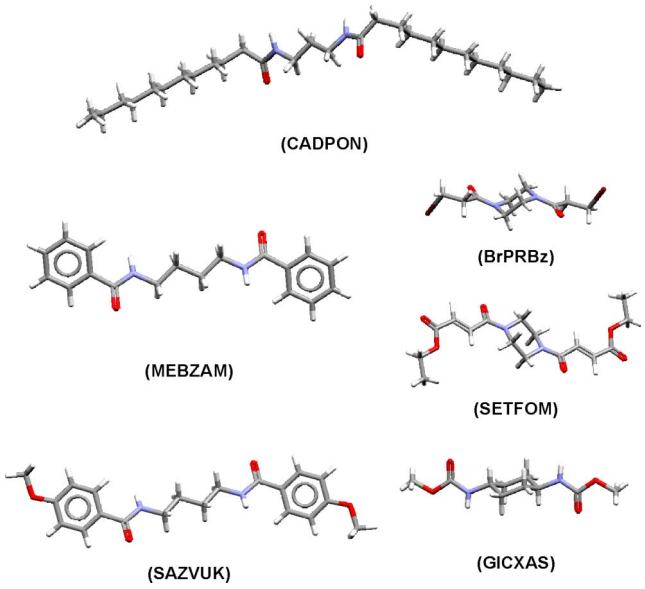


Figure 3. Crystallographic structures taken as geometric references in conformational analysis (CSD Refcode).

First, it can be observed that both compounds **2** and **9** (Fig. 5) preferentially show extended conformations in which the central amino groups (potential hydrogen bond donors) form a dihedral angle that varies between 70° and 120°. The lateral aromatic ring is practically in the same plane and the two methoxy groups are orientated so that one points upwards and the other one downwards below this plane, forming a dihedral of approximately −170°. In the case of compound **9**, the oxygens of the SO_2 groups (potential hydrogen bond acceptors) are orientated towards the same face of the aromatic ring plane. The distances between these structural elements, which are considered important for the target biological activity according to our own experimental results, are given in Table 3.

Taking these observations as a reference point, it can be observed that active compounds **3** and **5** behave in a similar way, although in compound **5c** (Fig. 6) one of the lateral rings is in a plane that is practically perpendicular to that containing the other aromatic ring. On the other hand, compounds **5b** and **5d** are inactive as cytotoxic agents and they show different conformational

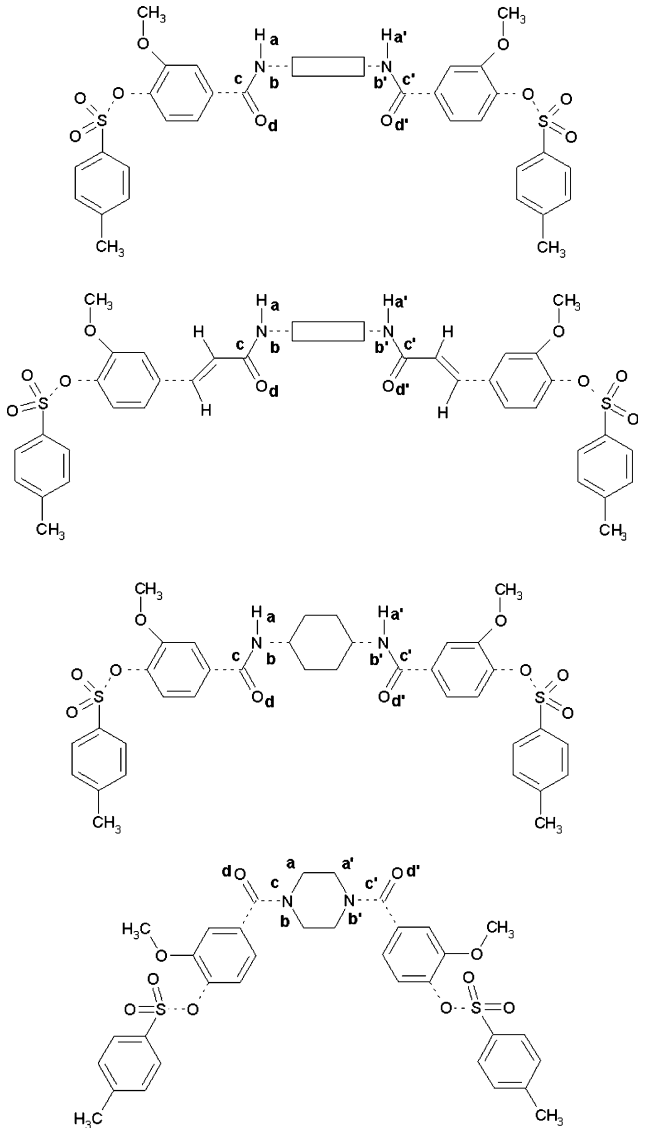


Figure 4. Dihedral angles and bonds (in doted line) selected for conformational analysis (see text for details).

behaviour in that they preferentially adopt more folded conformations (particularly **5d**, Fig. 6), as deduced from the distances given in Table 3.

The differences observed in the biological activities can be explained on the assumption that the inactive compounds, despite containing the structural elements required by the original design (which are also present in the active compounds), have different conformational behaviour. This is manifested in a remarkable preference for less extended conformations, a situation that causes these elements to be in dispositions that are less accessible to their biological target. The result is that these compounds do not show the desired cytotoxic activity.

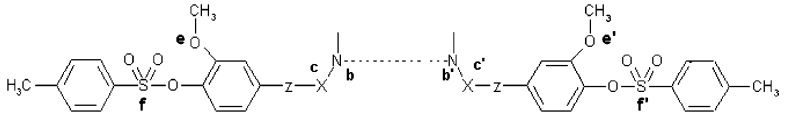
In similar way, the higher activity of **9** in comparison to that of **3c** could also be related to the fact that the replacement of the carbonyl group in **3c** by a methylene group in **9** promotes the preference for extended conformations. The observations discussed above allow us to propose that the bioactive conformation for these compounds is that in which the groups are in extended conformations.

With regard to the data corresponding to surface area and volume (Table 1), it can be observed that the active compounds (with the exception of compound **2**) which have Log $P \geqslant 2.99$, and present adequate conformational behaviour also have surface area values in a range of 1009 and 1170 Å^2, and their volume could be included in a range of 1780 and 2064 Å^3.

3. Conclusions

Two approaches were applied to gain an insight into the structural requirements for biological activity in a series of symmetrical diaryl derivatives with nitrogenated functions. First, a descriptive parameter [log of partition coefficient (Log P)] at a molecular level was obtained and, second, analysis of the conformational behaviour of the active and inactive compounds was carried out.

Table 3. Conformational analysis data



Compound	X	Z	W	Conf[a]	RMS[b]	bb′[c]	ee′[c]	ff′[c]
2	CH_2	—	1,4-Diaminobutane	10	2.98	6.28	15.57	—
3a	CO	—	1,4-Diaminobutane	16	2.02	6.28	16.44	20.30
3b	CO	—	Piperazine	9	4.36	2.95	13.00	16.30
3c	CO	—	1,4-Diaminocyclohexane	12	2.62	5.82	16.31	20.26
3d	CO	—	1,3-Diaminopropane	16	2.37	4.94	15.28	16.58
5a	CO	–CH=CH–	1,4-Diaminobutane	13	1.57	6.28	21.20	24.90
5b	CO	–CH=CH–	Piperazine	8	2.57	3.00	14.87	15.50
5c	CO	–CH=CH–	1,4-Diaminocyclohexane	14	1.71	5.76	20.93	23.56
5d	CO	–CH=CH–	1,3-Diaminopropane	13	3.83	4.94	8.97	6.49
9	CH_2	—	1,4-Diaminocyclohexane	10	1.06	1.06	16.51	20.21

[a] Number of lowest energy conformations in trajectory.
[b] Root mean square for trajectory.
[c] Distances in Å measured on the representative lowest energy conformation.

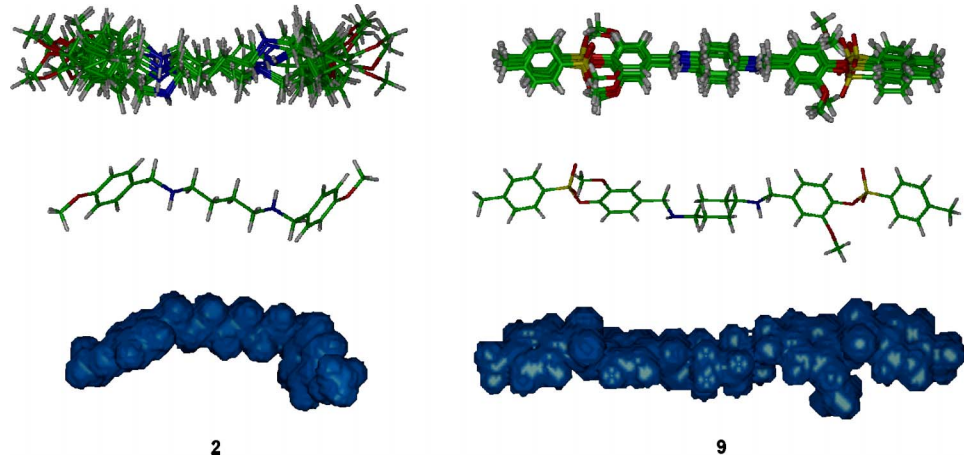


Figure 5. Conformational models, representative lowest energy conformation, and molecular VDW surface (atom radius scale: 1.00) for the most active compounds **2** and **9**.

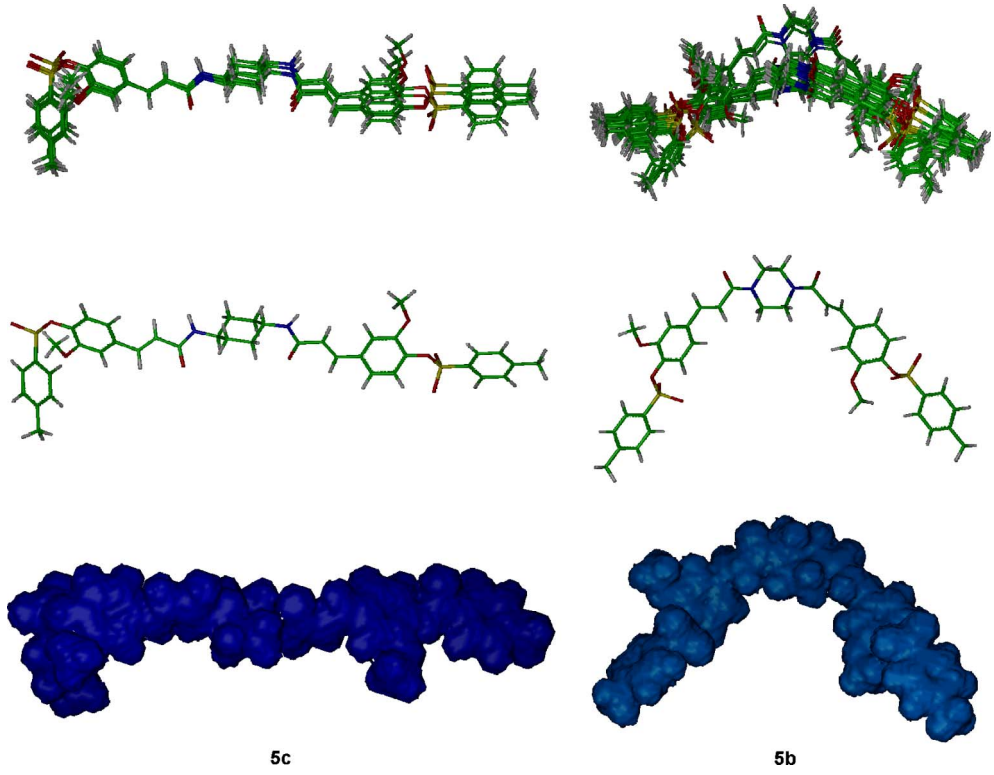


Figure 6. Conformational models, representative lowest energy conformation, and molecular surface (atom radius scale: 1.00) for compounds **5c** (as representative active compound) and **5b** (as representative inactive compound).

The Log *P* data, although not providing many clues to the observed variability in the cytotoxic activity, suggested that the separation of active (Log *P* > 2.99) and inactive (Log *P* < 2.99) compounds is possible. The introduction of tosyl and vinyl groups, as well as the replacement of the carbonyl group with a methylene, causes an increase in Log *P* and improves the cytotoxic activity.

With respect to the study of the conformational behaviour, which was carried out on compounds selected according to the Log *P* values, the lack of activity of compounds such as **5b** and **5d** can be related to the remarkably high preference for less extended conformations. The active compounds, on the other hand, preferentially adopt extended conformations.

4. Experimental

All the computational work was performed on *Silicon-Graphics* Octane2 workstations by applying the software package InsightII[15] or on a PC by applying the software package Hyperchem version 5.1 Pro.[16]

The starting atomic coordinates were obtained from the structurally related CSD structures reported in Table 2 (CSD System[14] version 5.26: search and information retrieval with ConQuest,[17] version 1.7; structure visualization with Mercury,[17] version 1.3). The three-dimensional models of the reported compounds (Table 1) were constructed using as starting fragments the CSD related structures cited above, as well as atoms and structural fragments from the *Builder* module. The protocol can be summed up as follows: (a) initial construction of the model. (b) Hierarchized systematic conformational analysis: determination of the rotation-sensitive bonds; election of a 30° window to check each selected dihedron. First filtration: elimination of the conformations that are non-distinguishable by symmetry. Second filtration: elimination of conformations that present steric impediments. Third filtration: calculation of the energy of conformations and elimination of those conformations whose relative energy is greater than 10 kcal/mol at a global minimum. Fourth filtration: optimisation of the geometry of the conformations and elimination of those whose relative energy is greater than 10 kcal/mol at a global minimum. All of the molecular mechanics calculations were carried out using the consistent valence force field CVFF[18] (*Search and Compare* module, InsightII). (c) Analysis of conformational trajectory (*Analysis* module InsightII) and selection of representative lowest energy conformation. Root mean square (rms) deviations of the structures were monitored. (d) Mechano-quantics optimisation of the conformations obtained in the previous step, with the molecular orbital calculations package *Mopac*, (AM1[19] semi-empirical approach, *AMPAC/MOPAC* InsightII module). (e) Descriptors were obtained.

The lipophilicity was determined through the calculation of Log*P* (the log of its octanol–water partition coefficient) by application of the method implemented in the corresponding QSAR module of Hyperchem version 5.1Pro, using the approach of Ghose and Crippen.[20–22] The determinations were carried out on the initial geometry after the first geometry minimisation.

Computation of solvent-accessible surface area and volume was carried out by the grid method described by Bodor et al.[23] using the atomic radii of Gavezotti,[24] by application of the method implemented in the corresponding QSAR module of Hyperchem version 5.1Pro. The determinations were carried out on the lowest energy representative conformation obtained after conformational analysis.

References and notes

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