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Synthesis, biological evaluation, and molecular modeling of novel thioacridone derivatives related to the anticancer alkaloid acronycine

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Abstract—The well-reported, but moderate antitumor activity of the acronycine alkaloid led us to synthesize a novel series of thioacridone compounds related to acronycine, as potential anticancer agents. Compounds were designed either as DNA intercalating agents, or as DNA intercalating agents with covalent bond forming potential. Bathochromic shifts of the compounds upon complexation with salmon testis DNA suggested intercalation as the mode of DNA binding. The binding interaction of the compounds was found to be approximately $10^2\,M^{-1}$, with that of the most potent compound 1-(2-dimethylaminoethylamino)-9(10H)-thioacridone, $10^4\,M^{-1}$. In vitro cytotoxic activity (IC_{50}) against HL-60 cells was found to range between 3.5 and 22 μg/mL. QSAR analyses yielded a multiple linear regression equation with an r^2 of 0.847 for DNA binding and an r^2 of 0.575 for cytotoxicity. The physicochemical parameters used in the QSAR analyses were log *P*, polar surface area, and calculated molar refractivity. Docking studies were also performed to compare the binding of the most potent and least potent compounds in the study in order to predict desirable chemical characteristics for further exploitation in drug design efforts. The thioacridone compounds in this series demonstrate cytotoxic activity in vitro that merit future in vivo evaluation.

1. Introduction

Acronycine (Fig. 1), also known as acronine, is an acridone alkaloid which was first isolated in 1948 from the bark of the Australian tree *Acronychia baueri*.[1] In subsequent investigations it was found that acronycine possess anticancer activity against a wide spectrum of experimental neoplasms in laboratory (murine) animals.[2] Dorr et al.[3] studied the in vitro cytotoxicity of acronycine in a variety of solid malignancies against human tumor cells isolated from patients. The results of early studies showed that acronycine possesses activity against a variety of solid human tumors, including ovarian cancer and metastatic tumors of unknown origin. These findings were further developed and reviewed in elegant studies by Tillequin and his group.[4]

The structure–activity relationship (SAR) in the acronycine group of compounds appears to be very inflexible considering that most of the acronycine derivatives tested to date against cancer, showed much lower activity than acronycine itself. Schneider and co-workers[5] attempted to synthesize structural analogs by modifying the side chain in the 6-position on acronycine. However, compound **1** (Fig. 1), containing an oxy-dimethylaminoethyl side chain in lieu of the methoxy group in the 6-position of acronycine, exhibited significant antitumor activity. This compound was, therefore, a strong motivating factor in the design of the compounds reported in the current study. Furthermore, in addition to compound **1**, several acronycine analogs, in which the methoxy group at C-6 has been replaced by dialkylaminoalkylamino side chains (including a dimethylaminoethyl analog) have recently been tested in a

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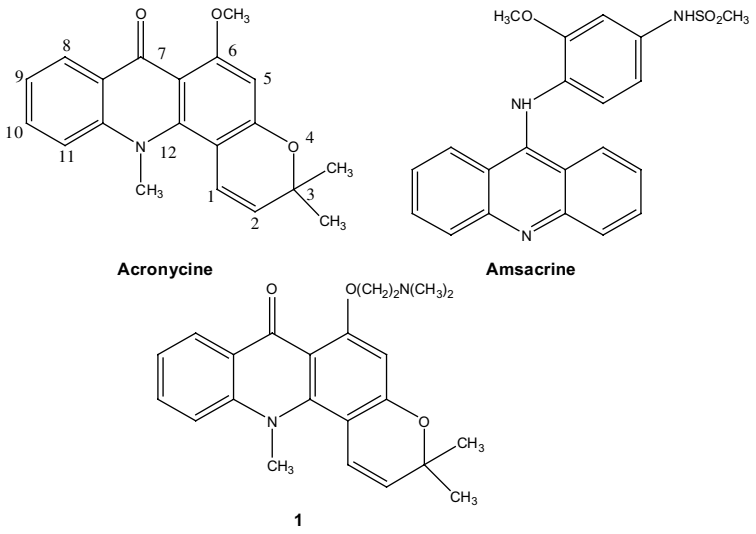
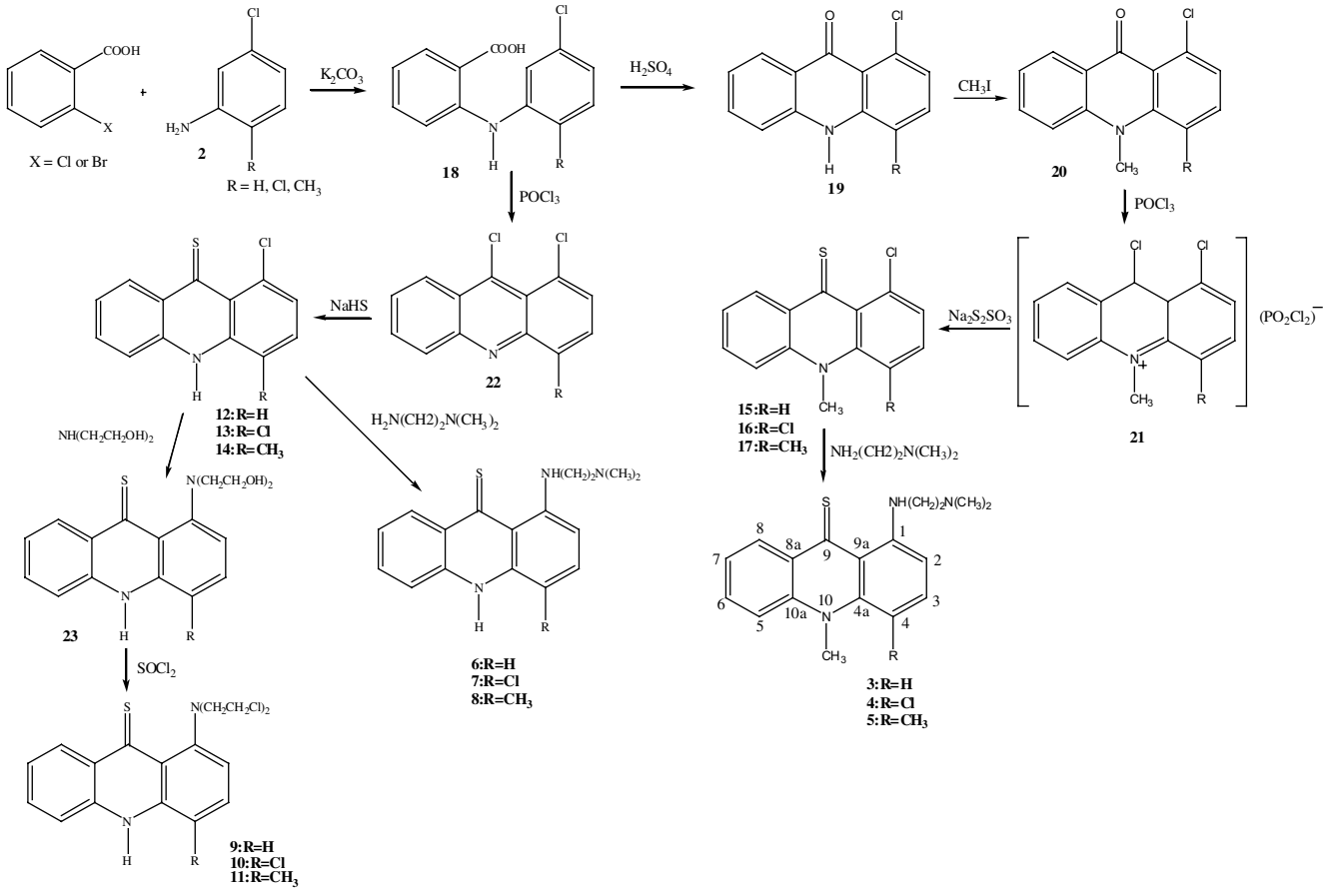


Figure 1. Structure of compounds discussed in the text, including the lead compound, **1**.[5]



Scheme 1. Synthesis of compounds used in this study.

cytotoxic assay.[6] This modification resulted in a significant increase of the cytotoxic activity toward L-1210 cells compared with acronycine.[6]

We were interested to introduce structural modifications into the acronycine molecule with two modes of action in mind: DNA intercalation (compounds **3**–**8** in Scheme 1), as well as alkylation (compounds **9**–**11** in Scheme 1). There are two characteristics common to many DNA intercalators, a planar polycyclic chromophore able to intercalate into DNA, and the presence of a basic side chain.[7] This side chain can both increase DNA binding affinity and, depending on its hydrophilicity, may increase the solubility of a compound. The fused planar

tricyclic moiety contained in acronycine that is necessary for DNA binding by intercalation was, therefore, maintained and a basic side chain was introduced in the 6-position by replacing the methoxy group with a 2-(dimethylamino)ethylamino chain. The nonplanar portion of the polycyclic ring structure (the pyran ring) was eliminated since it was reported not to be essential for the intercalation process,[8] although Elomri et al.[9] did suggest that the 1,2-double bond on the pyran ring may be an essential structural requirement for cytotoxic activity.

Furthermore, the carbonyl oxygen in the 7-position was replaced with sulfur. The replacement of oxygen (as found in the acronycine-derived series reported by other investigators, including Smolders et al.[10]) with sulfur, introduces a new element in the structure–activity equation. Sulfur is an atom with a larger atomic radius than that of oxygen. Consequently, the presence of sulfur imparts lower electronegativity and lower ionization energy to the molecule, and such molecules have a lowered tendency to form ionic bonds. Therefore, the C=S group is less polar than the C=O group. Thus, thioketone compounds are expected to be less water soluble than their corresponding carbonyl congeners. However, replacement of oxygen by sulfur as proposed above, results in compounds that are not merely weakly basic acridines, but such modification also drastically alters many electronic properties including the molecular dipole moment and electronic charge distribution. These changes might have a considerable influence on the biological activity of the resulting compounds. Steric and electronic influences were also investigated using chloro and methyl substituent groups at the 4-position. Compounds with the general structure of **9**–**11**, were synthesized with a side chain that likely will display DNA cross-linking characteristics (Scheme 1).

2. Chemistry

The substrate *N*-phenylanthranilic acids **18**, used as starting compounds (Scheme 1) were prepared through condensation of an appropriate 2-halobenzoic acid with an excess amount of the appropriate aromatic amine. Cyclization of compounds **18** with phosphoryl chloride yielded the dichloroacridines **22**, which upon reaction with sodium hydrogen sulfide afforded the 1-chlorothioacridones **12**–**14**. Treatment of the latter compounds with an excess amount of dimethylaminoethylamine then afforded the target compounds **6**–**8**. The preparation of compounds **3**–**5** involved the reaction of the appropriate 1-chloro-*N*-methylthioacridones (**15**–**17**) with 2-dimethylaminoethylamine. The necessary starting thioacridone compounds **15**–**17** were obtained via the reaction of sodium thiosulfate with 9-chloroacridium salts **21**, which were produced from 1-chloro-*N*-methylacridones (**20**) and phosphoryl chloride. The required *N*-methylacridones **20** in turn were obtained by *N*-methylation of the corresponding 1-chloroacridones **19** using methyl iodide. The 1-chloroacridones required for this study were prepared via cyclization of the appropriate *N*-phenylanthranilic acids **18** with sulfuric acid following previously described methods.[11,12]

3. Results and discussion

3.1. Spectral shift determination

UV–vis absorption spectroscopy represents one of the most convenient and facile ways to identify compounds that intercalate with DNA. The technique involves measuring the bathochromic shift (wavelength absorption to longer wavelengths) that is encountered as a compound intercalates with DNA. The values of the bathochromic shifts measured, are listed in Table 1 and an example shown in Figure 2.

Compounds **3**–**11** underwent bathochromic shifts similar to that of the reference compound acriflavine. The spectra in the visible range of the new compounds showed bathochromic shifts in the order of 4–22 nm when the compounds bound to DNA. These results suggest that these compounds interact with DNA through intercalation. The 1-chlorothioacridone intermediates **12**–**14** and **15**–**17** showed virtually no bathochromic shift after binding with DNA, suggesting that these compounds do not intercalate with DNA.

3.2. DNA binding and intercalation studies

The results of ethidium binding are given in Table 2 as a C_{50} value of the compounds. The C_{50} value of a drug is

Table 1. Bathochromic shifts ($\Delta\lambda$) of 1-aminothioacridones upon addition of DNA

Compound	λ_{max} (nm)	$\Delta\lambda$ (nm)
3	418	+4
4	410	+5
5	431	+8
6	403	+12
7	385	+10
8	418	+13
9	454	+4
10	463	+7
11	479	+22
Acriflavine	445	+19

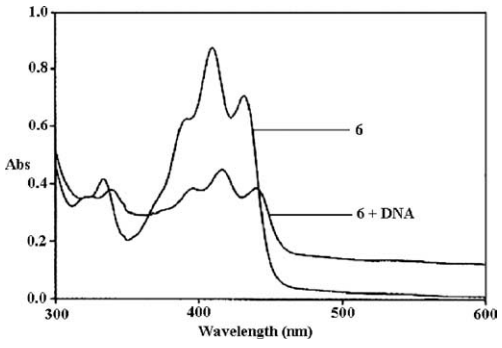


Figure 2. UV absorption spectrum showing the bathochromic effect of DNA addition on the spectrum of **6**.

Table 2. C_{50} values of 1-aminothioacridones

Compound	C_{50} (μM)
3	23.2
4	42.6
5	38.2
6	8.7
7	35.3
8	26.8
9	43.1
10	46.4
11	50.2

the concentration necessary to reduce the fluorescence of initially DNA-bound ethidium by 50% under standard assay conditions.[13] A reduction in the fluorescence of DNA–ethidium complexes was found upon addition of the 1-aminothioacridone compounds **3**–**11**. Such a reduction in fluorescence is indicative of intercalative binding into DNA. Only marginal displacement of ethidium was observed with the 1-chlorothioacridone intermediates **12**–**17**. As a result, the C_{50} values of these compounds could not be determined. The results in Table 1 show that compound **6** binds to DNA with the highest affinity. The results also show that compounds **3**–**8**—carrying an $NH(CH_2)_2N(CH_3)$ side chain—bind to DNA with higher affinities than compounds **9**–**11**, which all contain a nitrogen mustard moiety. This is in qualitative agreement with the binding constant values obtained for these compounds, as shown in Table 3 below.

The binding constant of the thioacridone compounds with DNA was measured to determine the degree of intercalation of the compounds with DNA in vitro. Scatchard plots were used to determine the DNA binding constants (*K*, Table 3). The results show that all target compounds examined (**3**–**11**), interact with DNA, albeit with different affinities. The results further indicate that 1-chlorothioacridone intermediates **12**–**17** are also capable of forming complexes with DNA. Their binding affinities, however, were much lower than those measured for the compounds carrying a side chain (compare **12**–**14** with **6**–**8**, or **15**–**17** with **3**–**5**). The addition of a basic chain to the thioacridone chromophore improved the DNA binding properties of the system, likely by stabilization of the intercalation through ionic interaction between the protonated side chain nitrogen and the ionic phosphate groups of DNA. On the other hand, the ring nitrogen of the 1-chlorothioacridone precursors **12**–**17** is not basic and, therefore, not protonated at pH 7.0, and is also not available to stabilize the intercalation of the tricyclic ring.

Among compounds with a side chain, those carrying a substituent at the 4-position of the thioacridone structure bind to DNA less strongly than those in which the 4-position is unsubstituted (compare **7** and **8** with **6**). This observation may be the result of a possible impeding effect experienced by rings substituted with either a chloro or methyl group at the 4-position upon intercalative entry of the thioacridone nucleus into the DNA molecule. The N-methylated (**3**–**5** and **15**–**17**) compounds seem to have lower binding affinities to DNA than their corresponding desmethyl (**6**–**8** and **12**–**15**) congeners.

With the exception of compound **6**, the interaction of the target compounds with DNA was found to be relatively weak, with binding affinity constants in the region of $10^2\,M^{-1}$ compared with most other DNA intercalating drugs which show binding affinities in the range of 10^5–$10^{10}\,M^{-1}$.[8] This attenuated DNA binding characteristic could impart a broad-spectrum anticancer activity to these compounds. Anticancer drugs require optimization of the distributive properties of the drug in order to penetrate to remote tumor sites in sufficient concentrations to be effective. For DNA intercalation agents, compounds that will distribute most efficiently are likely to be those with the lowest DNA binding constants, since a larger proportion of unbound drug will be available at equilibrium. Consequently, the new 1-aminothioacridone compounds are expected to have a broad scope of anticancer activity including solid tumors, as well as leukemia.

3.3. Cytotoxicity evaluation

The results of the cytotoxic evaluation are given in Table 3, expressed as the concentration of an individual compound that is required to kill 50% of the cells (IC_{50}) after 4 days of drug exposure. The cytotoxicity of the new thioacridone compounds was compared with that of amsacrine (see Fig. 1), a potent cytotoxic agent and also structurally related to the target compounds **3**–**11** (Scheme 1, Fig. 1). Amsacrine has been shown to bind to DNA both by intercalation and by external binding.[15]

Our results show that compounds **3**–**11** exhibit significant in vitro cytotoxic activity with IC_{50} values ranging from ~2 to 15 μg/mL. Compound **6** was the most active with an IC_{50} value of 2.3 μg/mL. The activity of this compound, however, was still lower than that of amsa-

Table 3. Binding constants (*K*) for 1-aminothioacridones **3**–**11** and their 1-chlorothioacridone precursors **12**–**17**, and in vitro cytotoxicity against HL-60 human promyelocytic leukemia cells

Compound	*K* (M^{-1})	IC_{50} (μg/mL)
3	8.6×10^2	3.5
4	1.1×10^2	5.6
5	2.3×10^2	4
6	2.6×10^4	2.3
7	1.2×10^2	8.5
8	2.7×10^2	15
9	1.1×10^2	8.7
10	0.9×10^2	10.2
11	0.6×10^2	17
12	0.008×10^2	22.6
13	0.006×10^2	>26
14	0.007×10^2	>26
15	0.003×10^2	8.9
16	0.004×10^2	6.7
17	0.005×10^2	6
Amsacrine	—	0.01[a]

[a] Taken from Su et al.[14]

crine, which has a reported IC_{50} value of 0.01 μg/mL against human leukemia HL-60 cells.[14] It must be noted, though, that the IC_{50} value for amsacrine was obtained using a different technique and under different conditions[13] than those utilized in the current study.

Compared with the target compounds **3**–**8**, the corresponding 1-chlorothioacridone precursors **12**–**17** exhibited decreased cytotoxic activity (Table 3). These results suggest that a 2-(dimethylamino)ethylamino side chain at the 1-position of the thioacridone structure is essential for high selectivity in this series of compounds. The $NH(CH)_2N(CH_3)_2$ side chain seems to confer higher activity than does the nitrogen mustard moiety (**9**–**11**). This finding suggests that there may be differences in the mechanism of interaction of these compounds with DNA or that these compounds may interact with additional, or alternative, biochemical targets.

No direct correlation could be found between DNA binding and cytotoxicity. For example, based on IC_{50} values, compound **6** (IC_{50} = 2.3 μg/mL) displayed the highest toxicity of all compounds tested, and also displayed the highest binding affinity ($K = 2.6 \times 10^4$). This might suggest that DNA binding is a factor in the cytotoxic activity of compound **6**. In contrast, compound **8**, which binds more strongly to DNA than compounds **4**, **5**, and **7**, has much less cytotoxic potency than the latter compounds. It would appear that affinity for DNA is not the sole determinant of cytotoxic potency in this series of compounds. It is possible that there exists a dual mechanism whereby DNA binding contributes as one mechanism only. Further study is needed to determine more precisely the exact mechanism of action of these compounds. SAR for cytotoxicity observed indicated that the presence of a 2-(dimethylamino)ethylamino side chain at the 1-position of the thioacridone structure significantly increases the cytotoxic activity of the basic intercalation system (compounds **3**–**8**). The nitrogen mustard moiety is less effective in this respect (compounds **12**–**14**). Substitution at the 4-position with either chloro or methyl decreases cytotoxicity of the 1-aminothioacridone compounds **3**–**11**.

3.4. QSAR analysis

$$\log(1/K) = 0.0331\ \mathrm{PSA} - 0.331\ \log P + 1.051\ \mathrm{CMR} - 8.89$$
$$R^2 = 0.847 \quad \mathrm{SE} = 0.665 \qquad (1)$$

$$IC_{50} = 0.454\ \log P + 8.943\ \mathrm{PSA} - 2.741\ \mathrm{CMR} - 23.692$$
$$R^2 = 0.575 \quad \mathrm{SE} = 5.9106 \qquad (2)$$

A classical QSAR study was performed using our data. Both the DNA binding and cytotoxic activities were correlated with molecular descriptors (Table 4), including polar surface area (PSA), log *P*, and the calculated molar refractivity (CMR). The results are given as Eq. 1 and 2 (above) as well as in the graphs represented in Figures 3 and 4. Multiple linear regression analysis was needed, since no linear correlation could be found for one single descriptor, and biological activity. In the case of DNA binding, Eq. 1 suggests that logP, PSA, and CMR affect the antitumor activity. A resultant r^2 of

Table 4. QSAR descriptors of the compounds studied

Compound	PSA	logP	CMR
3	56.865	2.95	9.979
4	55.242	3.51	10.470
5	55.028	3.44	10.443
6	79.739	2.71	9.5154
7	75.659	3.27	10.006
8	64.261	3.20	9.979
9	39.316	4.92	10.129
10	40.590	5.48	10.620
11	32.989	5.41	10.593
12	66.547	3.78	7.414
13	65.349	4.34	7.905
14	55.924	4.26	7.878
15	43.371	4.01	7.878
16	41.347	4.57	8.369
17	41.347	4.50	8.341

Abbreviations are: polar surface area (PSA) and calculated molar refractivity (CMR).

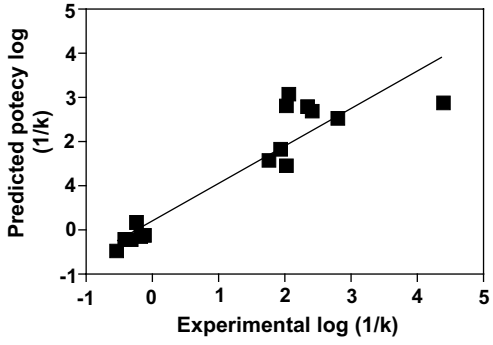


Figure 3. Correlation between experimental and predicted potency.

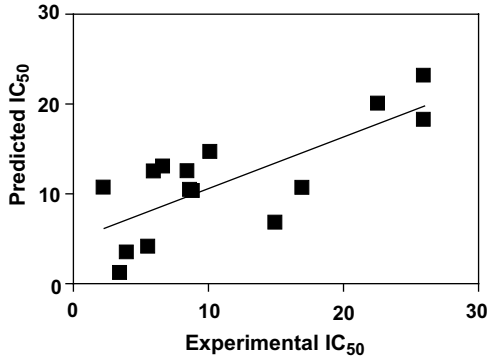


Figure 4. Correlation between experimental and predicted cytotoxicity.

0.847 was found for Eq. 1. The negative sign of log *P* suggests that the more hydrophilic compounds bind better to DNA. This finding correlates with the notion that a common characteristic of intercalative drugs is that they need at least one basic side chain to increase DNA binding affinity.[7] Eq. 2 suggests that log *P*, PSA, and CMR affect the cytotoxicity of these compounds. The resultant r^2 was found to be 0.575. This equation predicts that a more negative CMR will influence cytotoxic activity in this group of compounds.

3.5. Docking studies

Docking studies were also performed to investigate the DNA binding differences of the most potent (**6**), and the least potent (**15**) compounds. The results are shown in Figure 5. The planar ring system of compound **6** fits comfortably between the DNA base pairs (Fig. 5A,B), with the basic side chain oriented in a favorable position to undergo hydrogen bonding. In contrast, compound **15** can still undergo intercalation, but the methyl group may result in steric hindering (Fig. 5C,D) and consequently a decreased binding potential. In addition, the absence of the basic side chain in compound **15**, when compared with **6**, may also explain the lower potency of this compound. The relative binding energies calculated in FLEXIDOCK® for the two compounds were found to be −375 for compound **6** and −75 for **15**, correlating well with the experimental DNA binding values reported in Table 3. The more negative the relative binding energy, the more potent the binding is between ligand and target.

4. Conclusions

The current study was performed to evaluate a new series of 1-aminothioacridone compounds for cytotoxic activity. Our results demonstrate that these compounds possess cytotoxic activity in vitro. However, no clear relationship between cytotoxicity and DNA binding could be established, suggesting that DNA might not be the sole molecular target for the compounds in this series. Of all the compounds tested, the dimethylaminoethylamino-thioacridone compound **6** exhibited the most promising biological activity and may be useful as a lead compound in the search for more potent anticancer agents.

5. Experimental

5.1. Spectral shift determinations

Solutions of the test compounds at specified concentrations were prepared in a 7.5 mM phosphate buffer, pH 7.0, containing 1 mM ethylenediaminetetraacetic acid (EDTA). For compounds **3**–**11**, compounds were first dissolved in the least amount of ethanol, and then 2–5 drops of 1 M HCl were added followed by buffer. In the case of **12**–**17**, 2–5 drops of 1 M NaOH were added to the ethanolic solution before the addition of buffer. The DNA solution was prepared by dissolving salmon testis DNA in the buffer to give a solution of 1 mg/mL. Absorption spectrum of the test solution in the absence of DNA was recorded over a wavelength

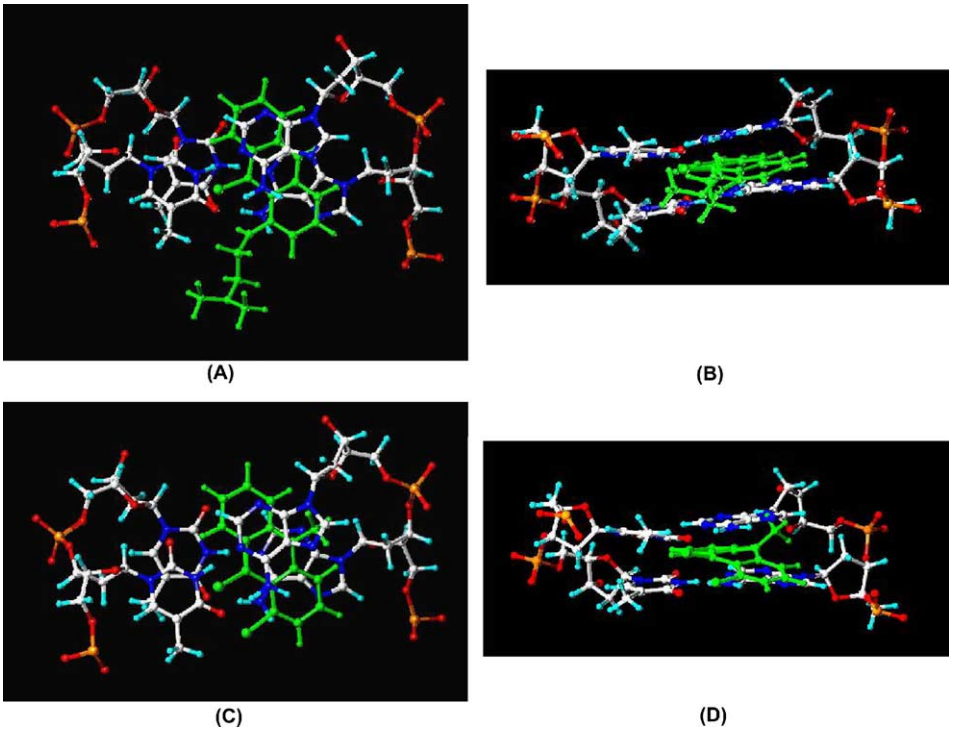


Figure 5. Docking studies of the most potent DNA binding compound, **6** (A,B), and least potent, **15** (C,D), into DNA base pairs (AT). The thioacridone compounds are shown in green, to distinguish them from the DNA base pairs.

range of 600–300 nm. This gave the absorbance of totally free ligand A1. Spectrophotometric titration was then carried out by sequential addition of aliquots of the DNA solution to 3 mL of the test compound in buffer at room temperature. The absorbance A of the mixture was recorded after each addition. The titration was continued until no further decrease in absorption could be observed. This value was taken as the absorbance of totally bound ligand A2.

5.2. DNA binding constant determination

The fraction of ligand bound to DNA (α) after each addition of DNA during the titration was calculated from the expression $\alpha = A1-A/A1-A2$.[16] If α is known, the values c (molar concentration of free ligand) and r (molar concentration of bound ligand/DNAP) can then be calculated. DNAP refers to the molar concentration of DNA expressed in terms of phosphate. A Scatchard plot ($r/c = K_n - K_r$) was then constructed for each compound, where K is the binding constant for formation of the bound ligand/DNA complex, and n is the number of binding sites/DNAP. The slope of the line on the Scatchard plot is $-K$. Acriflavine, a known DNA intercalator, was used as a standard under identical experimental conditions.

5.3. Ethidium displacement determination

Ethidium displacement determination was carried out according to Cain et al.[13] Briefly, ethidium bromide was added to 3 mL buffer solution (2 mM HEPES, 8 mM NaCl, 0.05 mM EDTA, pH 7.0) to obtain a final concentration of 2 μM. The optimal amount of DNA was determined by slowly adding DNA (calf thymus) to the solution to obtain near saturation of ethidium bromide with DNA as determined by the enhancement of fluorescence. The test compound was then added in 1 μL portions and the fluorescence recorded after each addition. The temperature was maintained at 25 °C during the assays. Titrations were performed in duplicate and the C_{50} values (representing the concentration of compound needed to decrease the DNA-bound ethidium fluorescence by 50%) were determined from a plot of fluorescence (% max) against added test compound concentration (μM).

5.4. Cell culture

HL-60 cells were routinely maintained as exponentially proliferating suspension cultures in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) containing penicillin (100 IU/mL) and streptomycin (100 μg/mL). Incubations were carried out at 37 °C in a humidified 5% CO_2 incubator.

5.5. Cytotoxicity

Cytotoxicity studies were carried out in 5 mL liquid cultures established at 2×10^5 cells/mL. The compounds tested were dissolved in DMSO and the same volume of DMSO was used as control, not exceeding 0.2% for all the treatments. For compounds **3**–**11**, compounds were first dissolved in the least amount of DMSO, and then 2–5 drops of 1 M HCl were added followed by the buffer. In the case of **12**–**17** 2–5 drops of 1 M NaOH were added to the DMSO solution before the addition of the buffer. To determine their cytotoxic activity, compounds were added to the cells in microplates and incubated for 4 days in the humidified incubator. Cytotoxicity was assessed by determining the cell numbers with a Coulter® counter, and their viability by microscopic examination for exclusion of 0.1% trypan blue. The IC_{50} for each compound was determined to be the drug concentration that produced 50% growth inhibition. Each experiment was done in duplicate.

5.6. Chemistry

All melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. The ^1H and ^{13}C spectra were recorded on a Varian VXR 300 spectrometer. Samples were dissolved in a deuterated solvent (either DMSO-d_6 or $CDCl_3$, as indicated) with tetramethylsilane (Me_4Si) as an internal standard. Infrared spectra were recorded as a neat film on KBr plates with a Shimadzu FT IR 4200 spectrophotometer. Mass spectra and HR-MS were recorded at 70 eV (EI), or in FAB mode, on a VG 7070E spectrometer. Elemental analyses were performed on a Perkin–Elmer model 240 instrument at the Department of Chemistry, Cape Town University and the Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa, and data were all within acceptable limits (±0.04%). Reactions were monitored by thin layer chromatography on Merck Silica gel 60 F_{254} precoated aluminum plates, using a $CHCl_3/CH_3OH$ (4:1) mobile phase system. Spots were visualized under UV or by iodine vapor.

5.6.1. 1-(2-Dimethylaminoethylamino)-10-methyl-9(10*H*)-thioacridone (3). A mixture of 1-chloro-10-methylthioacridone (**15**) (500 mg, 1.9 mmol), anhydrous potassium carbonate (150 g, 1.1 mmol), and 2-dimethylaminoethylamine (2 mL, 18 mmol) was stirred for 30 min and then left at room temperature for 24 h. Diethyl ether (50 mL) was added and the product extracted with 1 M HCl (3×100 mL). The combined aqueous layer was filtered and the solution made basic with 1 M NaOH. The product was then extracted with diethyl ether (3×50 mL). The combined organic layer was dried over anhydrous calcium chloride, and filtered. Removal of the solvent by evaporation gave 220 mg (36% yield) of **3** as a viscous yellow liquid with darkens on exposure to light. IR: $\nu_{max} = 1569$, 1219, 1069, 752, 720 cm^{-1}; FAB-MS: $m/z = 314$ $(M+3H)^+$, 276, 269, 255, 241, 233, 228, 219; ^1H NMR ($CDCl_3$): $\delta = 10.46$, 8.47, 7.67–7.18, 6.48, 6.33, 3.38, 2.71, 2.36; ^{13}C NMR ($CDCl_3$): $\delta = 152.62$, 145.19, 141.87, 134.98, 132.98, 127.09, 123.01, 120.73, 114.10, 108.19, 100.75, 99.17, 58.10, 45.65, 41.15, 34.30. Elemental analysis, calcd for $C_{18}H_{21}N_3S$: C, 69.42; H, 6.80; N, 13.49. Found: C, 69.47; H, 6.92; N, 13.52.

Compounds **4** and **5** were obtained in an analogous manner from **16** (500 mg, 1.7 mmol) and **17** (500 mg, 8 mmol), respectively.

5.6.2. 1-(2-Dimethylaminoethylamino)-4-chloro-10-methyl-9(10*H*)-thioacridone (4). Compound **4** was isolated as a yellow viscous liquid, which darkens on exposure to air; yield 32%; IR: ν_{max} = 1565, 1240, 1195, 1066, 750, 723 cm^{-1}; MS: m/z = 348 $(M+3H)^+$, 265, 172, 159; ^1H NMR ($CDCl_3$): δ = 10.35, 8.30, 7.61, 7.45–7.19, 6.33, 3.90, 3.35, 2.68, 2.35; ^{13}C NMR ($CDCl_3$): δ = 150.96, 145.32, 144.85, 137.76, 133.13, 126.38, 123.82, 121.55, 116.12, 111.31, 105.55, 103.14, 57.97, 45.41, 43.97, 41.15. Elemental analysis, calcd for $C_{18}H_{20}ClN_3S$: C, 62.61; H, 5.80; N, 12.17. Found: C, 62.52; H, 5.97; N, 12.23.

5.6.3. 1-(2-Dimethylaminoethylamino)-4,10-dimethyl-9(10*H*)-thioacridone (5). Compound **5** was isolated as a pale yellow viscous liquid, which darkens on exposure to air; yield 30%; IR: ν_{max} = 1597, 1306, 1250, 1167, 1075, 753, 723 cm^{-1}; MS: m/z = 328 $(M+3H)^+$, 311, 282, 268, 236, 222; ^1H NMR ($CDCl_3$): δ = 10.15, 8.34, 7.62, 7.37–7.17, 6.35, 3.77, 3.37, 2.70, 2.46, 2.36; ^{13}C NMR ($CDCl_3$): δ = 150.38, 147.80, 145.60, 139.29, 126.46, 123.81, 120.94, 115.81, 110.71, 109.84, 102.19, 58.15, 45.64, 43.48, 41.15, 22.04. Elemental analysis, calcd for $C_{19}H_{23}N_2S$: C, 70.15; H, 7.08; N, 12.92. Found: C, 70.16; H, 7.12; N, 12.87.

5.6.4. 1-(2-Dimethylaminoethylamino)-9(10*H*)-thioacridone (6). A mixture of 1-chloro-9(10*H*)-thioacridone (**12**) (100 mg, 0.41 mmol), anhydrous potassium carbonate (30 mg, 0.21 mmol), and 2-dimethylaminoethylamine (2 mL, 18 mmol) was magnetically stirred for 15 min and then left at room temperature for 10 h (6 h in the case of **7**). Diethyl ether (20 mL) was added and the product extracted with 1 M HCl (3 × 50 mL). The combined extracts were filtered and excess ice cold 1 M NaOH (200 mL) was added to the filtrate with shaking. The precipitate was collected by filtration, washed with water, and air dried to give **6** as a yellow fine powder in a yield of 50 mg (41%); mp >360 °C; IR: ν_{max} = 3409, 1607, 1559, 1516, 1456, 1262 cm^{-1}; FAB-MS: m/z = 300 $(M+3H)^+$, 255, 241, 229, 214; ^1H NMR ($CDCl_3$): δ = 8.11–7.99, 7.65, 7.34, 7.22, 3.83, 2.59; ^{13}C NMR ($CDCl_3$): δ = 152.01, 149.96, 135.96, 130.47, 129.50, 127.94, 124.93, 123.61, 123.20, 122.80, 116.54, 114.62, 58.49, 46.64, 44.84. Elemental analysis, calcd for $C_{17}H_{19}N_3S$: C, 68.65; H, 6.44; N, 14.13. Found: C, 68.43; H, 6.59; N, 14.18.

5.6.5. 1-(2-Dimethylaminoethylamino)-4-chloro-9(10*H*)-thioacridone (7). Compound **7** was isolated as a fine yellow powder; yield 52 mg (44%); mp 80–82 °C; IR: ν_{max} = 3422, 1547, 1507, 1408, 1329 cm^{-1}; FAB-MS: m/z = 334 $(M+3H)^+$, 298, 289, 275, 263, 253, 248; ^1H NMR ($CDCl_3$): δ = 8.05–7.92, 7.64–7.54, 7.28–7.12, 3.82, 2.56, 2.35; ^{13}C NMR ($CDCl_3$): δ = 154.55, 132.51, 130.80, 129.58, 128.25, 127.11, 125.31, 125.24, 125.17, 123.98, 122.02, 116.14, 58.71, 48.62, 44.84. Elemental analysis, calcd for $C_{17}H_{18}ClN_3S$: C, 61.53; H, 5.47; N, 12.66. Found: C, 61.68; H, 5.32; N, 12.62.

5.6.6. 1-(2-Dimethylaminoethylamino)-4-methyl-9(10*H*)-thioacridone (8). Compound **8** was isolated as a yellow powder; yield 48 mg (40%); mp 312–314 °C; IR: ν_{max} = 3308, 1600, 1551, 1505, 1460, 1325 cm^{-1}; FAB-MS: m/z = 314 $(M+3H)^+$, 276, 269, 255, 241, 233, 228, 219, 205; ^1H NMR ($CDCl_3$): δ = 8.13, 8.04, 7.79, 7.64, 7.36–7.22, 3.75, 2.80, 2.50, 2.32; ^{13}C NMR ($CDCl_3$): δ = 14.09, 149.48, 136.69, 130.04, 129.48, 128.07, 125.60, 125.27, 125.06, 121.78, 116.62, 113.74, 59.05, 49.05, 44.97, 19.26. Elemental analysis, calcd for $C_{18}H_{21}N_3S$: C, 69.42; H, 6.80; N, 13.49. Found: C, 69.57; H, 6.82; N, 13.52.

5.6.7. 1-Bis(2-chloroethyl)-amino-9(10*H*)-thioacridone (9). Thionyl chloride (5 mL, 68.5 mmol) was carefully added to 1-bis(2-hydroxyethyl)-amino-9(10*H*)-thioacridone (**23**, R=H, 200 mg, 0.637 mmol) in an ice-cooled flask. The mixture was allowed to stir overnight at room temperature and then poured into anhydrous diethyl ether (25 mL). An orange precipitate formed. The solvent was distilled off, more ether was added, and the product filtered. The product was washed several times with ether to remove any residual thionyl chloride. The product was then dissolved in hot absolute ethanol and precipitated with ether. Filtration and vacuum desiccation afforded 170 mg (60%) of the product as a yellow orange powder; mp 237 °C; IR: ν_{max} = 3409, 1607, 1559, 1516, 1456, 1262 cm^{-1}; EI-MS: m/z = 350 (M^+), 265, 172, 159, 119, 105, 91; ^1H NMR ($CDCl_3$): δ = 12.22, 8.05–7.92, 7.64–7.54, 7.28–7.12, 3.78, 3.62; ^{13}C NMR ($CDCl_3$): δ = 150.96, 145.32, 144.85, 137.76, 133.19, 126.38, 123.82, 121.55, 116.12, 111.31, 105.55, 103.14, 55.61, 41.15. Elemental analysis, calcd for $C_{17}H_{16}Cl_2N_2S$: C, 58.29; H, 4.57; N, 8.00. Found: C, 57.96; H, 4.92; N, 8.32.

Compounds**10** and **11** were obtained in an analogous manner from their corresponding precursors **23**.

5.6.8. 1-Bis(2-chloroethyl)-amino-4-chloro-9(10*H*)-thioacridone (10). Compound **10** was isolated as an orange powder; yield 85%; mp 222 °C; IR: ν_{max} = 3422, 1547, 1507, 1408, 1329 cm^{-1}; MS: m/z = 384 (M^+), 357, 292, 264, 188, 159, 131; ^1H NMR ($CDCl_3$): δ = 12.20, 8.53, 8.32, 7.73, 7.47–7.34, 3.91, 3.76; ^{13}C NMR ($CDCl_3$): δ = 152.62, 145.19, 141.87, 134.98, 134.98, 132.98, 127.09, 123.01, 114.10, 108.19, 100.75, 99.17, 55.56, 41.15. Elemental analysis, calcd for $C_{17}H_{15}Cl_3N_2S$: C, 53.13; H, 3.91; N, 7.29. Found: C, 53.22, H, 4.03; N, 7.30.

5.6.9. 1-Bis(2-chloroethyl)-amino-4-methyl-9(10*H*)-thioacridone (11). Compound **11** was isolated as an orange powder; yield 85%; mp 254 °C; IR: ν_{max} = 3308, 1600, 1551, 1505, 1460, 1325 cm^{-1}; MS: m/z = 364 (M^+), 292, 264, 188, 159; ^1H NMR ($CDCl_3$): 12.28, 8.54, 8.0, 7.82, 7.57–7.37, 6.50, 3.88, 3.68; ^{13}C NMR ($CDCl_3$): δ = 150.38, 147.80, 145.60, 139.29, 132.85, 126.46, 123.81, 120.94, 115.81, 110.71, 109.84, 102.19, 55.60, 41.15, 18.80. Elemental analysis, calcd for $C_{18}H_{18}Cl_2N_2S$: C, 59.34; H, 4.95; N, 7.69. Found: C, 59.34; H, 5.03; N, 7.73.

5.6.10. 1-Chloro-9(10*H*)-thioacridone (12). NaHS (24 g, 0.43 mol) and 96% ethanol (500 mL) was added to 1,9-dichloroacridine (**22**, R=H, 10 g, 0.04 mol). The mixture

was heated under reflux for 30 min, filtered whilst warm, and acidified with dilute HCl until complete precipitation of the product was effected. The crude product (8 g, 81%) was recrystallized from pyridine/water to give light brown crystals (mp 210–212 °C); IR: ν_{max} = 3438, 3260, 1617, 1578, 1466, 1210 cm^{-1}; MS: *m/z* = 245 (M^+), 210, 201, 177, 166, 140, 105; ^1H NMR (DMSO-d_6): δ = 13.62, 9.38–9.28, 7.64–7.48, 7.37–7.26; ^{13}C NMR (DMSO-d_6): δ = 199.21, 139.42, 137.18, 136.31, 135.51, 133.61, 132.60, 130.26, 128.80, 118.02, 116.50. Elemental analysis, calcd for $C_{13}H_8ClNS$: C, 63.54; H, 3.28; N, 5.70. Found: C, 63.75; H, 3.19; N, 5.72.

Compounds **13** and **14** were obtained according to the same procedure from their corresponding 1,9-dichloroacridines **22**.

5.6.11. 1,4-Dichloro-9(10*H*)-thioacridone (13). Compound **13** was isolated as light brown crystals: yield 86%: mp 198–200 °C (from xylene); IR: ν_{max} = 3439, 3296, 2924, 2359, 2359, 1607, 1474, 1192 cm^{-1}; MS: *m/z* = 279 (M^+), 263, 244, 209, 200, 164; ^1H NMR (DMSO-d_6): δ = 11.30, 8.44, 4.40, 8.17, 7.74, 7.31; ^{13}C NMR (DMSO-d_6): δ = 202.11, 135.40, 134.18, 133.97, 133.57, 132.73, 131.90, 128.75, 127.27, 126.06, 124.11, 120.20, 118.50. Elemental analysis, calcd for $C_{13}H_7Cl_2NS$: C, 55.73; H, 2.52; N, 5.00. Found: C, 55.77; H, 2.51; N, 5.01.

5.6.12. 1-Chloro-4-methyl-9(10*H*)-thioacridone (14). Compound **14** was isolated as dark brown crystals; yield 91%; mp 228–230 °C (from xylene); IR: ν_{max} = 3316, 2922, 1611, 1514, 1226, 754 cm^{-1}; MS: *m/z* = 259 (M+), 243, 225, 180; ^1H NMR: δ = 10.87, 8.51, 7.92, 7.69, 7.43, 7.26, 3.32; ^{13}C NMR: δ = 201.65, 137.32, 134.35, 133.35, 132.94, 132.55, 131.21, 128.77, 126.46, 125.87, 123.34, 118.17, 17.92. Elemental analysis, calcd for $C_{14}H_{10}ClNS$: C, 64.74; H, 3.88; N, 5.39. Found: C, 65.04; H, 3.91; N, 5.35.

5.6.13. 1-Chloro-10-methylthioacridone (15). A mixture of 1-chloro-10-methylacridone (**20**, R=H, 2.5 g, 0.01 mol) and phosphoryl chloride (10 mL, 0.11 mol) was heated under reflux for 1 h. The solution that formed was then cooled with ice after which 150 mL of a 1 M sodium thiosulfate solution was added. A brownish sticky material formed almost immediately. The material was stirred vigorously for about 5 min. Dilute ammonia solution (50 mL) was added and stirring continued for another 5 min. The granular precipitate that formed was then filtered off, dried, and recrystallized twice from ethanol to give a light brown powder, mp 114–116 °C; IR: ν_{max} = 1570, 1480, 1215, 755 cm^{-1}. MS: *m/z* = 259 (M^+), 244, 215, 201, 168; ^1H NMR ($CDCl_3$): δ = 8.60, 7.71–7.22, 3.65; ^{13}C NMR ($CDCl_3$): δ = 204.99, 140.05, 136.83, 134.58, 132.87, 131.22, 129.39, 126.30, 122.84, 114.10, 113.58, 36.03. Elemental analysis, calculated for $C_{14}H_{10}ClNS$: C, 64.74; H, 3.88; N, 5.39. Found: C, 64.71; H, 3.92; N, 5.40.

Compounds **16** and **17** were obtained in an analogous manner from their corresponding 1-chloro-methylacridones **20**.

5.6.14. 1,4-Dichloro-10-methylthioacridone (16). Compound **16** was synthesized by mixing, at room temperature, 1,4-dichloro-10-methylacridone (**20**, R=Cl, 2.5 g, 0.009 mol), phosphoryl chloride (15 mL, 0.175), and 150 mL of a 1 M sodium thiosulfate solution. Yield 70%; mp 125–126 °C; IR: ν_{max} = 1570, 1483, 1218, 758 cm^{-1}; MS: *m/z* = 277, 258, 242, 164; ^1H NMR ($CDCl_3$): δ = 8.60, 7.71–7.23, 3.87; ^{13}C NMR ($CDCl_3$): δ = 205.26, 140.17, 137.00, 136.18, 134.71, 132.90, 131.26, 129.48, 126.48, 126.37, 122.90, 114.08, 113.55, 96.98, 36.08. Elemental analysis, calcd for $C_{14}H_9C_{12}NS$: C, 57.16; H, 3.08; N, 4.76. Found: C, 57.17; H, 3.12; N, 4.62.

5.6.15. 1-Chloro-4,10-dimethylthioacridone (17). Compound **17** was synthesized by mixing, at room temperature, 1-chloro-4,10-dimethylacridone (**20**, R=Me, 25 g, 0.01 mol), phosphoryl chloride (10 mL, 0.11 mol), and 150 mL of 1 M sodium thiosulfate solution. Yield 75%; mp 120–122 °C; IR: ν_{max} = 1566, 1469, 1240, 751 cm^{-1}; MS: *m/z* = 274 (M+H), 238, 224, 154, 136; ^1H NMR (DMSO-d_6): δ = 8.33, 7.61; 7.37–7.17, 3.79, 2.65; ^{13}C NMR (DMSO-d_6): δ = 208.17, 142.59, 139.51, 139.38, 135.02, 133.41, 132.46, 130.77, 128.26, 127.16, 126.02, 122.87, 115.50, 43.73, 22.68. Elemental analysis, calcd for $C_{15}H_{12}ClNS$: C, 65.81; H, 4.42; N, 5.12. Found: C, 65.84; H, 4.31; N, 5.16.

5.7. Molecular modeling

Molecular modeling studies were either performed on a Silicon Graphics Octane® computer using SYBYL® 6.9.1[17] software, or on a Dell Dimension® P4 desktop running ChemDraw® 8.[18] With SYBYL®, the compounds were drawn using SKETCH® and energy minimized (100 iterations) using the Tripos® force field with atomic charges added using the Gasteiger–Hückel method. SYBYL® was used to calculate polar surface area (PSA) and perform the docking studies with FLEXIDOCK®. The DNA molecule was built using the BIOPOLYMER/BUILD® module and energy minimized using the AMBER7 force field. ChemDraw® 8 was used to calculate log*P* as well as the molecular refractivity.

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