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# NOVEL IMIDAZOTHIOXANTHONES: SYNTHESIS, DNA BINDING AND CYTOTOXICITY.

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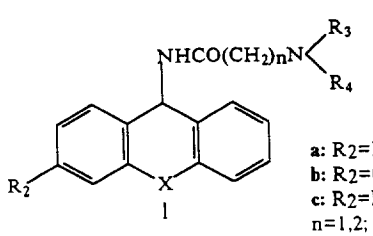
**Abstract:** A new class of DNA-binding ligands (**10a-f**) has been synthesized and examined for DNA-binding affinity using thermal denaturation and for *in vitro* cytotoxicity in a number of cell lines. All the compounds were found to possess significant DNA-binding affinity which correlates with *in vitro* cytotoxicity across eight cell lines. 

In the ongoing search for new effective chemotherapeutic agents, a wide variety of drugs has emerged. These drugs belong to different chemical classes, have different modes of action and are classified into several categories.[1] One of the most important classes of anticancer drugs is that of the DNA-binding agents, [2-5] which can bind either in the major or minor grooves, or can intercalate in between base pairs of double-stranded DNA.[4] Furthermore, these molecules can bind either covalently or non-covalently to DNA.[2]

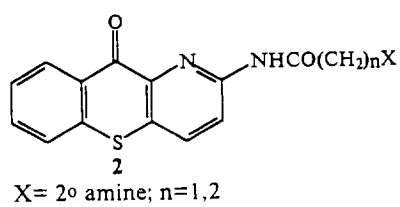
We have previously reported the synthesis and cytotoxicity of a variety of potential intercalators, the *N*-(9*H*-xanthen-9-yl)aminoalkanamides **1a,b** and the *N*-(9*H*-thioxanthen-9-yl)amino-alkanamides **1c** (Figure 1) and demonstrated that the thioxanthene derivatives **1c** are more potent than their oxo-analogs **1a,b**.[6]

**Figure 1**




1

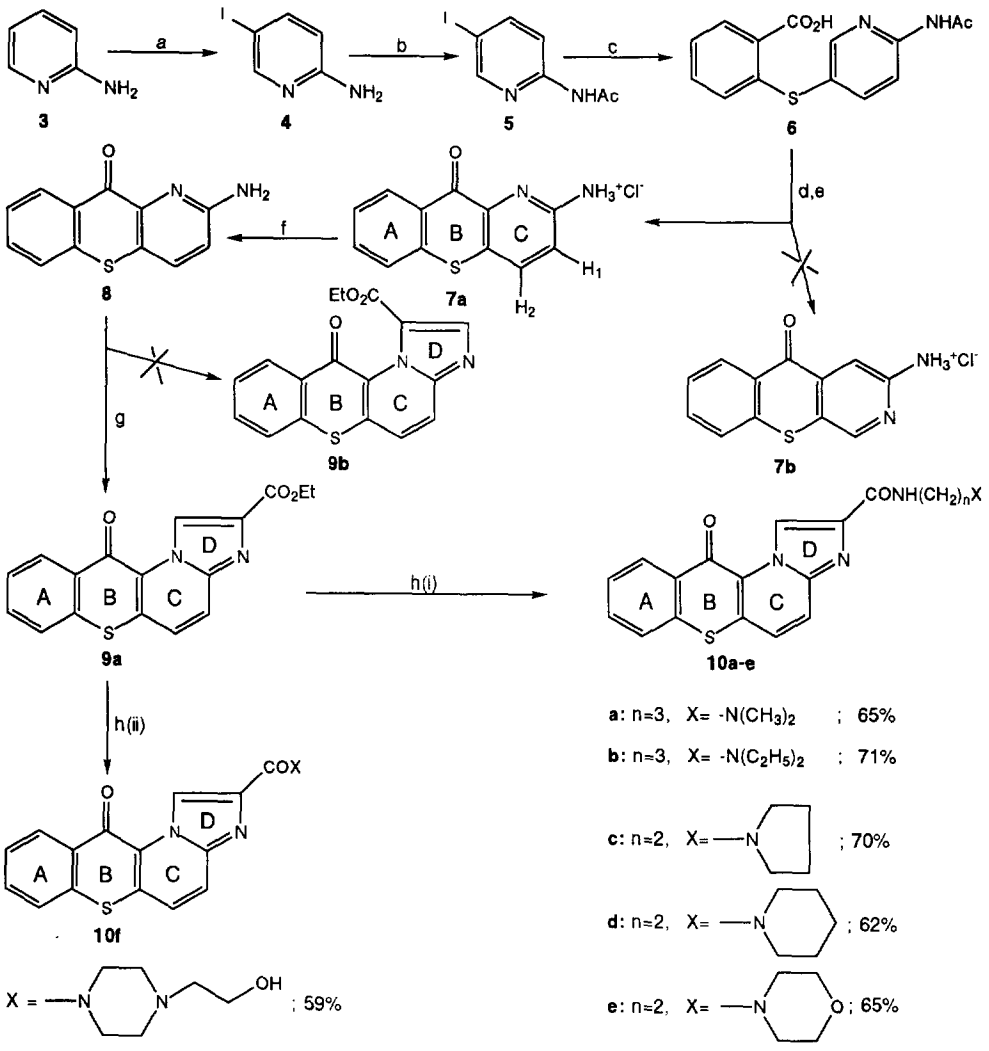
**a:** $R_2$=H, X=O
**b:** $R_2$=Cl, X=O
**c:** $R_2$=H, X=S
n=1,2; $R_3$=$R_4$=alkyl




2

X= 2° amine; n=1,2

**Scheme 1**




**Reagents:** (a) $I_2$/KI, $H_2O$, 60%; (b) $Ac_2O$, AcOH, 82%; (c) *o*-thiosalicylic acid, $K_2CO_3$, Cu/DMF reflux, 16h, 91%; (d) PPA, 120 °C, 16h; (e) HCl (33%), reflux, 10h, 74%; (f) $K_2CO_3$/$CHCl_3$, reflux, 10h, 91%; (g) $BrCH_2C(O)CO_2Et$/THF, RT, 8h and then EtOH, reflux, 8h, 56%; (h) i) $H_2N(CH_2)_nX$, **10a-e** and ii) 1-(2-hydroxyethyl)piperazine, **10f**.

As part of our program of structure-activity relationship (SAR) studies on thioxanthones, we have also communicated our findings regarding the cytotoxicity of a number of substituted aza-thioxanthones **2** (Figure 1). Compounds of type **2** were found to be more cytotoxic than their non-azacounterparts **1a-c**.[7] Encouraged by these results we made further structural modifications by incorporating a fourth ring (D) into the basic A-B-C three-nucleus skeleton, bearing side chains consisting of a variety of *N*-(ω-aminoalkyl) carboxamides. The rationale for the introduction of a fourth ring into the classical pharmacophore was based on the observation that a number of tetracyclic compounds show improved cytotoxicity with respect to their tricyclic counterparts.[8-10] For this reason, tetracyclic ring systems are currently the subject of extensive QSAR studies.[11] We report here the synthesis of the novel imidazothioxanthone series **10a-f** (Scheme 1), and their evaluation using DNA-binding and *in vitro* cytotoxicity techniques.

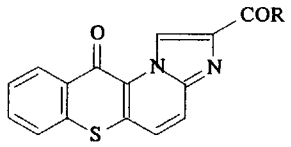
The synthetic strategy followed for the preparation of **10a-f** is shown in Scheme 1. Thus, treatment of the hydrochloride salt of the amino aza-thioxanthone **7a**, obtained by the route we have previously described,[7] with potassium carbonate in chloroform gave the free base **8**. Reaction of **8** with β-bromopyruvic acid ethyl ester[12,13] afforded the hitherto unknown tetracyclic ester **9a**.[14] Although two different isomers, **9a** and **9b**, could be expected from this reaction,[13] only **9a** was obtained, the structure of which was elucidated from NMR spectral data. The desired final products **10a-f** were obtained from the reaction of **9a** with the appropriate *N*,*N*-disubstituted diaminoalkanes.[14,15]

The new imidazothioxanthones **9a** and **10a-f** were examined for DNA-binding affinity using thermal denaturation[16-18] and for *in vitro* cytotoxicity in the following cell lines: MOLT-4, WEHI, HOP-92, SKOV-3, MCF-7, SW-620, MAC-15A, A2780, $A2780_{cis}^{R}$ (Table 1).

In general, all the new imidazothioxanthones, with the exception of the ester **9a**, are more potent than their tricyclic aza counterparts **2**,[7] which may be attributed to the incorporation of the fourth ring. In addition, all new compounds appear to have significant DNA-binding affinity at both of the [drug]:[DNA] ratios examined (Table 1). Furthermore, there appears to be a correlation between DNA-binding affinity and cytotoxicity in all cell lines except the ovarian carcinoma SKOV-3.

The presence of the alkanamido side chains in **10a-f** is possibly responsible for the great enhancement observed in their overall DNA-binding affinity and cytotoxocity compared to the ester analog **9a**. This could be due to electrostatic interactions of the flexible protonated side chains with the deoxyribophosphate backbone of DNA.[19] In addition, all compounds have low relative resistance factor (RF) values in the A2780 cisplatin-resistant line suggesting little or no selectivity toward these cells.

An approximately two-fold increase in the DNA binding affinity of all molecules is noticed when examined in the 1:5 compared to the 1:10 [drug]:[DNA] ratio, as judged from the induced ΔTm values.




| No | Structure | [a]ΔTm / °C for [drug] : [DNA] ratios | | [b]$IC_{50}$ ($\mu$M) cytotoxicities | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | 1 : 10 | 1 : 5 | MOLT-4 [c] | WEHI [d] | HOP-92 [c] | SCOV-3 [c] | MCF-7 [b] | SW-620 [c] | Mac 15A [d] | A2780 [e] | A2780 [e] $cis^R$ | RF [f] |
| 9 | $OC_2H_5$ | 0.5 | 1.2 | >100 | >100 | 87.5 | >100 | 84.4 | >100 | NT | >100 | >100 | - |
| 10e | $NH(CH_2)_2N$⟨O⟩ | 1.6 | 2.9 | 3.30 | NT[g] | 12.8 | 23 | 7.08 | 4.85 | NT | 5.8 | 7.6 | 1.3 |
| 10f | N⟨N⟩$N(CH_2)_2OH$ | 1.9 | 3.5 | NT | NT | NT | 13.5 | NT | NT | 0.42 | 0.74 | 1.8 | 1.8 |
| 10c | $NH(CH_2)_2N$⟨ | 3.4 | 6.5 | 0.47 | NT | 15.9 | 29 | 0.61 | 1.52 | NT | 4.5 | 6.0 | 1.3 |
| 10a | $NH(CH_2)_3N(CH_3)_2$ | 5.7 | 10.4 | 0.42 | 0.30 | 0.36 | 2.15 | 11.2 | 1.44 | 0.50 | 0.54 | 0.76 | 1.4 |
| 10d | $NH(CH_2)_2N$⟨⟩ | 6.0 | 10.5 | NT | 0.004 | NT | 7.7 | NT | NT | 0.18 | 0.56 | 0.65 | 1.2 |
| 10b | $NH(CH_2)_3N(C_2H_5)_2$ | 7.0 | 12.4 | NT | NT | NT | 1.65 | NT | NT | NT | 0.43 | 0.48 | 1 |
| | Proflavine | 6.3 | 11.5 | | | | | | | | 0.47 | | |
| | Mitoxantrone | 15.0 | ≥25 | | | | 0.0053 | | | | 5.5x10-4 | | |

[a]Induced thermal stabilization for CT-DNA: values of 0.1-0.2 °C; [b]Is the concentration of drug needed to inhibit growth of cancer cells in culture to 50% of control values; [c]After 48h exposure; [d]In an MTT assay system; [e]After 96h exposure; [f]Resistance Factor; [g]NT= not tested.

Analogs **10a** and **10d** show binding affinity at both [drug]:[DNA] ratios comparable to that of proflavine, an established DNA intercalant, while **10b** appears to bind slightly more strongly. This behavior may reflect the structural features (e.g. length, basicity) of the side chains present in these analogs, suggesting differences in electrostatic and/or hydrogen bonding and, hence, overall interaction with DNA. The poor binding of **10f** must be due to the different arrangement of the spacer unit $(CH_2)_2$ in the side chain compared to the other analogs. Within the *N*-(3-dialkylaminopropyl) series, the *N,N*-diethyl substituent in the side chain of **10b** leads to generally enhanced DNA binding and improved cytotoxicity compared to the *N,N*-dimethyl group of its counterpart, **10a**, across the cell lines SKOV-3 and A2780/A2780 $_{cis}{}^{R}$. Replacement of the morpholino moiety in **10e** with a piperidino, **10d**, leads to much stronger binding at both 1:10 and 1:5 [drug]:[DNA] ratios and to significantly higher cytotoxicity in the cell lines used. In the mouse leukemia WEHI, **10d** was particularly active, having a very low $IC_{50}$ value (0.004 μM).

In conclusion, the new imidazothioxanthones **10a-f** exhibit cytotoxicity in a broad spectrum of tumor cell lines. The most potent compounds **10a**, **10b**, **10c** and **10d** are currently being examined for *in vivo* antitumor activity. Studies are also underway to try to elucidate the preferred DNA binding mode (s) of these molecules.

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(16) The compounds were studied in DNA thermal denaturation experiments using calf thymus (CT) DNA (type-I, highly polymerized). The CT-DNA had $A_{260}/A_{280}$=1.9 and was satisfactorily free from protein; a molar extinction value at 260 nm of $\varepsilon$=6600 $M^{-1}$ $cm^{-1}$ was used. Aqueous solutions of DNA were prepared in Millipore-purified water buffered at pH 7.00±0.01 using 10 mM sodium phosphate and 1 mM EDTA; no added salt or support electrolyte was used. Working solutions containing 100 μM of DNA alone and in the presence of either 10 or 20 μM of added compound were monitored at 260 nm using a modified Shimadzu UV-2101 PC spectrophotometer fitted with a Shimadzu SPR-8 Peltier heating/cooling accessory. Heating was applied at 1 °C $min^{-1}$ from 40 °C until thermal denaturation of the DNA was complete, as judged from the increase in absorption. The optical absorbance versus temperature curves were sampled, normalized and analyzed. Thermal denaturation temperatures ($T_m$) were determined at a relative absorbance value of 0.50 and are reported as the mean ± s.e.m of three determinations. The change in $T_m$ ($\Delta T_m$) following interaction of CT-DNA with an added compound was evaluated from:
$\Delta T_m = T_{m\,(DNA\text{-}compound)} - T_{m\,(DNA)}$ and is reported (Table 1) for two [DNAp]:[compound] molar ratios, 1:5 and 1:10. These values were selected following trials to establish the saturation concentration of each compound, and from experience with other agents which bind either by intercalation or via the minor groove of duplex DNA. The ratios selected did not lead to saturation of DNA binding.
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