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NEW AZA-THIOXANTHONES: SYNTHESIS AND CYTOTOXICITY

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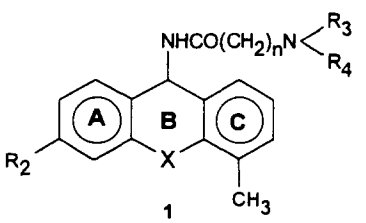
Abstract: A new series of aza-thioxanthones has been synthesized and tested for *in vitro* cytotoxicity in a number of cell lines. Almost all the compounds were found to exhibit significant cytotoxicity in the leukemic MOLT-4 line, whereas some of them showed pronounced activity in the non-small lung cancer cell line HOP-92.

DNA-binding agents constitute one of the most important classes of anticancer drugs in clinical use today whose antineoplastic effectiveness depends upon the mode and intensity of binding. Three types of binding to DNA have been described: a) covalent binding,[1,2] b) nonintercalative groove binding,[3,4] and c) intercalation.[5-7]

We have recently reported on the synthesis and biological evaluation of a variety of potential intercalators, the *N*-(9*H*-xanthen-9-yl)aminoalkanamides **1a, b** and the *N*-(9*H*-thio-xanthen-9-yl)amino-alkanamides **1c** (Figure 1).[8] In this series the thioxanthene derivatives were found to be more potent than their oxo-analogs. In addition, analogs bearing dialkylaminopropanamido side chains were more active than their corresponding dialkylaminoethanamides.

Figure 1



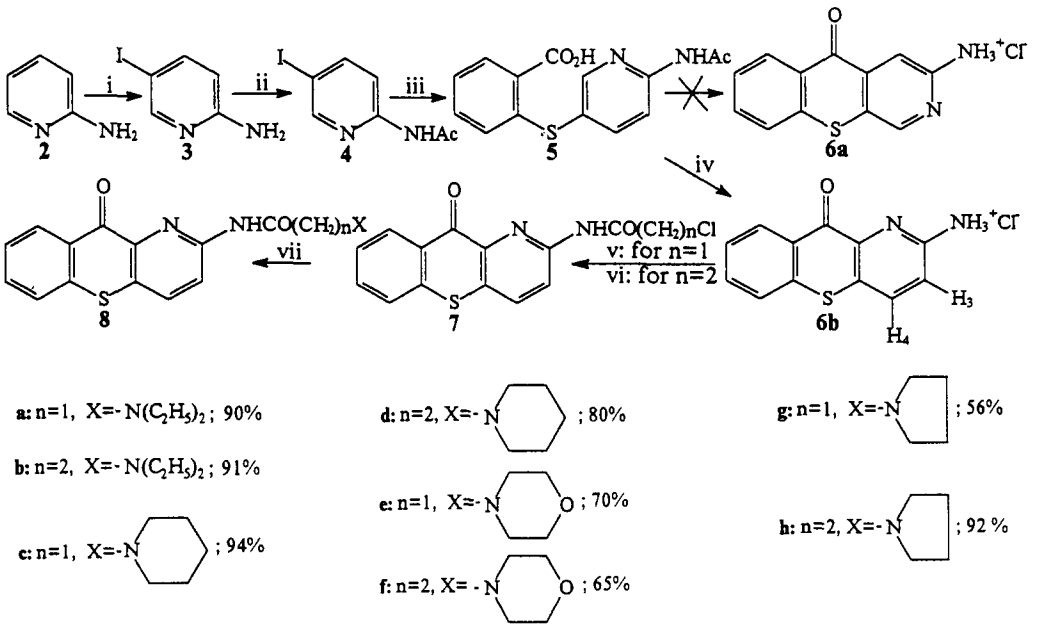
1

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

Continuing these investigations, we have synthesized several new aza-thioxanthones **8a-h** (Scheme 1). In this series, we have: i) relocated the alkylaminoalkanamido side chain from ring B to C and ii) changed ring C from benzene to pyridine. The latter change was made because of the improved

cytotoxicity and lower toxicity when pyridine replaces benzene in anthraquinone and ellipticine derivatives.[9a-c] To the best of our knowledge, there are only two reports in the literature where the pyridine ring has been incorporated into the thioxanthene chromopohore.[10,11]

Scheme 1



Reagents: (i) I_2/KI, H_2O, 60%; (ii) Ac_2O, AcOH, 82%; (iii) *o*-thiosalicylic acid, K_2CO_3, Cu / DMF reflux, 16h, 91%; (iv) PPA, 120 °C, 16h and then reflux with HCl (33%); (v) **8a**, **8c**, **8e** and **8g**: $K_2CO_3/CHCl_3$, 10h reflux and then $K_2CO_3/ClCH_2COCl$, RT, 15h; (vi) **8b**, **8d**, **8f** and **8h**: K_2CO_3/toluene, 10h reflux and then $K_2CO_3/ClCOCH_2CH_2Cl$, RT for 15h and then 3h reflux; (vii) XH, benzene, 40-80 °C, 9-16h.

The synthetic pathway followed for the preparation of the target molecules **8a-h** is depicted in Scheme 1. Commercially available 2-aminopyridine **2** was iodinated in the presence of potassium iodide to give 2-amino-5-iodopyridine **3**.[12] This was then acylated to **4** followed by Ullmann condensation[13] with *o*-thiosalicylic acid to give the thioether **5**. This in turn, was converted to **6b** by Friedel-Crafts intramolecular ring closure.[14] Although two different types of isomer, **6a** and **6b**, could be expected from this reaction[15,16] only the isomer **6b** was obtained. The structure of **6b** was confirmed by the ^1H NMR spectrum which showed an *ortho* coupling (J=8.96 Hz) between the aromatic H_3 and H_4 protons of ring C.

Treatment of the salt **6b** with the appropriate α,ω-chloroacylchlorides in the presence of potassium carbonate gave the corresponding chloroalkanamides **7**,[17,18] which upon reaction with the suitable secondary amines afforded the final products **8a-h**.[18,19]

The new aza-thioxanthones **8b, d, f, g** and **h** were examined for their *in vitro* cytotoxicity by the National Cancer Institute, USA using standard NCI protocols. The cell lines used cover a broad spectrum of commonly occcuring tumors (Table 1).

In general, all of the tested new aza-thioxanthones appear to be more cytotoxic than both the non aza derivatives **1a-c** (Figure 1)[8] and some of their aza counterparts of lucanthone.[11] In the breast cancer MCF-7 cell line none of the compounds showed any appreciable activity. Conversely, the new compounds exhibited cytotoxicity in the leukemic cell line MOLT-4. The *N,N*-diethylamino derivative **8b**, showed a remarkable activity in the non-small lung cancer cell line HOP-92 (IC_{50}=0.002 μM). The potency of the piperidino analog **8d** in the same cell line is also noteworthy (IC_{50}=0.41 μM). A substantial decrease in the activity of **8d** in the HOP-92 was, however, observed upon removal of one methylene unit from its side chain. Thus, compound **8c** with an IC_{50} value of 15.1 μM appears to be 37 times less cytotoxic than its homolog **8d**. In the SW-620 colon cell line, compounds **8d, 8c** and **8g** showed a promising cytotoxicity, the latter compound being, as in the case of the MOLT-4 cell line, the most potent of all (IC_{50}=4.96 μM). Finally, in the ovarian carcinoma SKOV-3 cell line, all compounds exhibited little activity except the pyrrolidino analog **8h**, which is almost 9 fold more cytotoxic than its morpholino counterpart **8f**.

TABLE 1 : Cytotoxicities (IC^a_{50}, μM) of the new aza-thioxanthones

No	X	n	MOLT-4	HOP-92	SW-620	SCOV-3	MCF-7
8g		1	1.91	27.3	4.96	20.6	27.2
8h		2	3.42	18.1	15.5	5.23	17.0
8f		2	NT[b]	45.3	27.3	47.6	49.8
8c		1	2.69	15.1	5.42	21.4	19.4
8d		2	2.56	0.41	8.31	23.2	15.9
8b	$N(C_2H_5)_2$	2	7.77	0.002	12.0	20.5	13.3

[a]IC_{50} is the concentration of drug needed to inhibit growth of cancer cells in culture to 50% of control values, after 48h exposure; [b]NT=not tested.

In conclusion, the presence of morpholine in the side chain of the aza-thioxanthones examined, leads to decreased cytotoxicity. Substitution of the pyrrolidino group in **8h** by its piperidino counterpart, **8d**, leads to improved potency across the MOLT-4, HOP-92, SW-620 and MCF-7 cell lines. This improvement was not observed with respect to **8g** and **8c**. This can probably be attributed to the absence of the second (CH_2) spacer unit in the side chains of the latter. Finally, the presence of the *N,N*-diethylamino substituent in the side chain of **8b** leads to substantially enhanced potency in the case of the non-small cell lung cancer HOP-92.

These findings suggest that more analogs of aza-thioxanthones need to become available and tested as potential antitumor agents. We are now exploring the incorporation of a suitably substituted fourth ring into the three (A-B-C) aza-thioxanthone skeleton.[20]

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