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Synthesis and cytotoxic activity of 2-dialkylaminoethylamino substituted xanthenone and thioxanthenone derivatives

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Abstract

The synthesis and biological evaluation of some new pyranoxanthenones and pyranothioxanthenones, substituted with flexible amino side-chains, and their evaluation as potential antitumor agents is described. The cytotoxic activity of the compounds and their eventual selective effect on a phase of the cell cycle were evaluated in vitro, using the murine lymphocytic L1210 leukemia cell line. The new aminoderivatives exhibited highly potent cytotoxicity against the leukemia L1210 cell line when compared to acronycine. All the compounds induced a partial accumulation of cells in the G2 + M phase of the cell cycle. © 2000 Elsevier Science S.A. All rights reserved.

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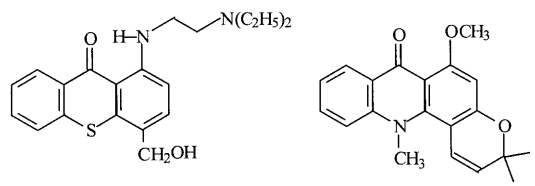
1. Introduction

Xanthones and thioxanthones have been reported to possess interesting cytotoxic activities [1–3]. A well examined example is the thioxanthone derivative hycanthone (Fig. 1), as well as a number of 1-dialkylaminoalkylthioxanthenone derivatives structurally related to it [4]. The basic side chain seems to be an important component for the expression of cytotoxicity, not only for the above mentioned class of compounds, but also for numerous amino derivatives of acridone [5,6] and anthraquinone [7] as well.

In our laboratories we have initiated a research program dealing with the preparation and pharmacological evaluation of some new compounds structurally related to the acridone alkaloid acronycine (Fig. 1) [8], a broad-spectrum antitumor agent, whose clinical development has been hindered, in part, due to its poor water solubility characteristics. Although the mode of action of this alkaloid at both cellular and molecular level has not yet been unambiguously established, it is suggested that interaction with DNA, either by intercalation, or by some other non-covalent processes, is crucial and responsible for its high antitumor potency [9]. We have previously synthesized a series of pyranoxanthenones and thioxanthenones, bearing the isosteric replacement of an oxygen or a sulfur atom, instead of the nitrogen atom of acronycine, as well as various substituents on the pyran ring [10]. A number of these



Hycanthone Acronycine

Fig. 1.

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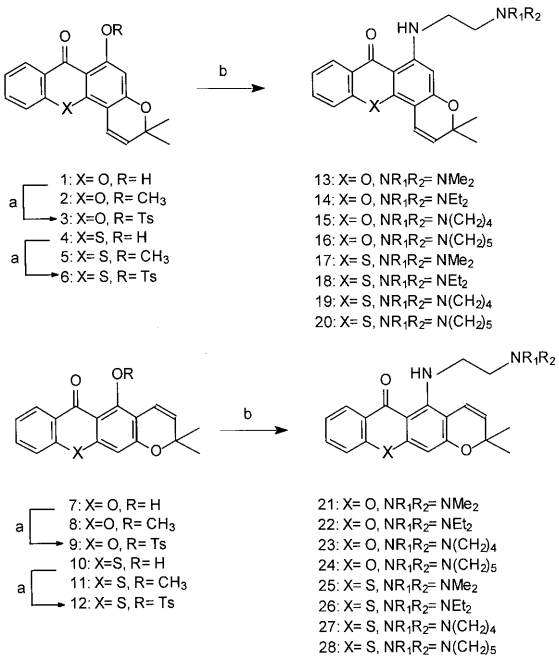

Scheme 1.

compounds possessed interesting activity against the murine L1210 leukemia cell line.

In a search for new xanthenone and thioxanthenone derivatives with improved potency, we turned our attention towards the preparation and investigation of a number of amino substituted analogs, in order to establish if the introduction of a basic side chain in the tetracyclic ring system could effect an increase of cytotoxic activity. We here report the synthesis and the cytotoxicity screening tests of these compounds.

2. Chemistry

The synthetic approach for the preparation of the new derivatives is depicted in Scheme 1. We used 6-hydroxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (**1**), 5-hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**7**) and the corresponding thioxanthenones **4** and **10** as starting materials. These compounds were prepared by condensation of 1,3,5-trihydroxybenzene with salicylic or thiosalicylic acid, etherification of the 3-OH of the resulting 1,3-dihydroxyxanthenones or thioxanthenones with 3-chloro-3-methyl-1-butyne and subsequent thermal cyclization of the ethers in boiling *N*,*N*-diethylaniline [10–12]. Upon treatment with tosyl chloride in acetone, the tosylates **3**, **6**, **9** and **12** were prepared. Displacement of the leaving group with the appropriately substituted amines provided the target compounds **13**–**28**, which were converted into the corresponding fumarates or hydrochlorides.

3. Experimental

3.1. Chemistry

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 400 instrument in deuterated solvents and were referenced to TMS (δ scale). Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. Elemental analyses were within $\pm 0.4\%$ of the theoretical values.

3.2. General procedure for the preparation of tosylates (*3*, *6*, *9*, *12*)

A solution of the appropriate hydroxy derivatives **1**, **4**, **7** or **10** (0.51 mmol) in dry acetone (10 ml) was treated at 0°C with a mixture of *p*-toluenesulfonyl chloride (1.73 mmol) and potassium carbonate (8.89 mmol) and the resulting mixture was heated at reflux under Ar for 3 h. The inorganic precipitate was then filtered off and the filtrate was evaporated to dryness. The residue was purified by column chromatography (dry-pack, silica gel 20×2 cm) using a mixture of cyclohexane:ethyl acetate (7:3) as the eluent, to give the corresponding tosylate.

3.2.1. 3,3-Dimethyl-6-[[(4-methyl)benzenesulfonyl]-oxy]-3H,7H-pyrano[2,3-c]xanthen-7-one (*3*)

Yield: 83%. Mp: 188–190°C (Et_2O–*n*-pentane). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 6H, 2 × gemCH_3), 2.35 (s, 3H, 4′-CH_3), 5.66 (d, 1H, $J = 10.5$ Hz, H-2), 6.55 (s, 1H, H-5), 6.81 (d, 1H, $J = 10.5$ Hz, H-1), 7.30 (d, 2H, $J = 8$ Hz, H-2′, H-6′), 7.46 (m, 3H, H-9, H-10, H-11), 7.91 (d, 2H, $J = 8$ Hz, H-3′, H-5′), 8.16 (dd, 1H, $J = 8$ Hz, 2Hz, H-8).

3.2.2. 3,3-Dimethyl-6-[[(4-methyl)benzenesulfonyl]-oxy]-3H,7H-pyrano[2,3-c]thioxanthen-7-one (*6*)

Yield: 97%. Mp: 187–189°C (Et_2O–*n*-pentane). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 6H, 2 × gemCH_3), 2.30 (s, 3H, 4′-CH_3), 5.78 (d, 1H, $J = 10$ Hz, H-2), 6.74 (s, 1H, H-5), 6.61 (d, 1H, $J = 10$ Hz, H-1), 7.31 (d, 2H, $J = 8$ Hz, H-2′, H-6′), 7.49 (m, 3H, H-9, H-10, H-11), 7.82 (d, 2H, $J = 8$ Hz, H-3′, H-5′), 8.25 (dd, 1H, $J = 8$ Hz, 1Hz, H-8).

3.2.3. 2,2-Dimethyl-5-[[(4-methyl)benzenesulfonyl]oxy]-2H,6H-pyrano[3,2-b]xanthen-6-one (*9*)

Yield: 87%. Mp: 215–217°C (Et_2O–*n*-pentane). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 6H, 2 × gemCH_3), 2.41 (s, 3H, 4′-CH_3), 5.53 (d, 1H, $J = 10$ Hz,

H-3), 6.34 (d, 1H, $J = 10$ Hz, H-4), 6.77 (s, 1H, H-12), 7.30 (d, 2H, $J = 8$ Hz, H-2′, H-6′), 7.46 (m, 3H, H-8, H-9, H-10), 7.90 (d, 2H, $J = 8$ Hz, H-3′, H-5′), 8.17 (dd, 1H, $J = 8$ Hz, 2 Hz, H-7).

3.2.4. 2,2-Dimethyl-5-[[(4-methyl)benzenesulfonyl]-oxy]-2H,6H-pyrano[3,2-b]thioxanthen-6-one ***(12)***

Yield: 92%. Mp: 218–220°C (Et_2O–*n*-pentane). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 6H, 2 × gemCH_3), 2.39 (s, 3H, 4′-CH_3), 5.64 (d, 1H, $J = 10$ Hz, H-3), 6.52 (d, 1H, $J = 10$ Hz, H-4), 6.86 (s, 1H, H-12), 7.25 (d, 2H, $J = 9$ Hz, H-2′, H-6′), 7.47 (m, 3H, H-8, H-9, H-10), 7.80 (d, 2H, $J = 9$ Hz, H-3′, H-5′), 8.16 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-7).

3.3. General procedure for the synthesis of derivatives ***13–28***

To a solution of the tosylate (0.12 mmol) in dry DMSO (10 ml), was added the appropriate 2-dialkylaminoethyl amine (0.24 mmol) and the reaction was heated at 150°C under Ar for 1 h. The mixture was then poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, 20 × 1.5 cm) using a mixture of dichloromethane : methanol (9:1) as the eluent, to give the corresponding amine as an oil.

3.3.1. 3,3-Dimethyl-6-[[2-(dimethylamino)ethyl]-amino]-3H,7H-pyrano[2,3-c]xanthen-7-one ***(13)***

Yield: 62%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 6H, 2 × gemCH_3), 2.30 (s, 6H, $N(CH_3)_2$), 2.62 (t, 2H, $J = 7$ Hz, $CH_2N(CH_3)_2$), 3.29 (q, 2H, $J = 7$ Hz, 6Hz, $HNCH_2$), 5.47 (d, 1H, $J = 10$ Hz, H-2), 5.86 (s, 1H, H-5), 6.79 (d, 1H, $J = 10$ Hz, H-1), 7.39 (m, 3H, H-9, H-10, H-11), 8.16 (dd, 1H, $J = 8$ Hz, 0.5 Hz, H-8), 9.71 (br.s, 1H, NH). Hydrochloride, Mp: 227–229°C (EtOH–Et_2O). *Anal.* $C_{22}H_{24}N_2O_3$·HCl: (C, H, N).

3.3.2. 3,3-Dimethyl-6-[[2-(diethylamino)ethyl]-amino]-3H,7H-pyrano[2,3-c]xanthen-7-one ***(14)***

Yield: 69%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.08 (t, 6H, $J = 7$ Hz, $N(CH_2CH_3)_2$), 1.44 (s, 6H, 2 × gemCH_3), 2.63 (q, 4H, $J = 7$ Hz, $N(CH_2CH_3)_2$), 2.80 (t, 2H, $J = 7$ Hz, CH_2NEt_2), 3.30 (q, 2H, $J = 7$ Hz, 6Hz, $HNCH_2$), 5.49 (d, 1H, $J = 10$ Hz, H-2), 5.89 (s, 1H, H-5), 6.83 (d, 1H, $J = 10$ Hz, H-1), 7.42 (m, 3H, H-9, H-10, H-11), 8.18 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 9.60 (br.s, 1H, NH). Fumarate, Mp: 240°C (dec) (EtOH–Et_2O). *Anal.* $C_{24}H_{28}N_2O_3$·$C_4H_4O_4$: (C, H, N).

3.3.3. 3,3-Dimethyl-6-[[2-(1-pyrrolidinyl)ethyl]-amino]-3H,7H-pyrano[2,3-c]xanthen-7-one ***(15)***

Yield: 58%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 6H, 2 × gemCH_3), 1.80 (m, 4H, pyrrolidine H), 2.64 (m, 4H, pyrrolidine H), 2.83 (t, 2H, $J = 7$ Hz, $CH_2N(CH_2)_4$), 3.39 (q, 2H, $J = 7$ Hz, 5 Hz, $HNCH_2$), 5.46 (d, 1H, $J = 10$ Hz, H-2), 5.91 (s, 1H, H-5), 6.82 (d, 1H, $J = 10$ Hz, H-1), 7.43 (m, 3H, H-9, H-10, H-11), 8.18 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 9.76 (br.s, 1H, NH). Hydrochloride, Mp: 240–241°C (EtOH–Et_2O). *Anal.* $C_{24}H_{26}N_2O_3$·HCl: (C, H, N).

3.3.4. 3,3-Dimethyl-6-[[2-(1-piperidinyl)ethyl]amino]-3H,7H-pyrano[2,3-c]xanthen-7-one ***(16)***

Yield: 85%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 6H, 2 × gemCH_3), 1.46 (m, 2H, piperidine H), 1.62 (m, 4H, piperidine H), 2.50 (m, 4H, piperidine H), 2.70 (t, 2H, $J = 7$ Hz, $CH_2N(CH_2)_5$), 3.34 (q, 2H, $J = 7$ Hz, 6 Hz, $HNCH_2$), 5.49 (d, 1H, $J = 10$ Hz, H-2), 5.88 (s, 1H, H-5), 6.80 (d, 1H, $J = 10$ Hz, H-1), 7.44 (m, 3H, H-9, H-10, H-11), 8.20 (dd, 1H, $J = 8$ Hz, 1 Hz, H-8), 9.72 (br.s, 1H, NH). Hydrochloride, Mp: 248°C (dec) (EtOH–Et_2O). *Anal.* $C_{25}H_{28}N_2O_3$·HCl: (C, H, N).

3.3.5. 3,3-Dimethyl-6-[[2-(dimethylamino)ethyl]amino]-3H,7H-pyrano[2,3-c]thioxanthen-7-one ***(17)***

Yield: 71%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 6H, 2 × gemCH_3), 2.30 (s, 6H, $N(CH_3)_2$), 2.62 (t, 2H, $J = 7$ Hz, $CH_2N(CH_3)_2$), 3.33 (q, 2H, $J = 7$ Hz, 5Hz, $HNCH_2$), 5.32 (d, 1H, $J = 10$ Hz, H-2), 6.05 (s, 1H, H-5), 6.62 (d, 1H, $J = 10$ Hz, H-1), 7.38 (m, 3H, H-9, H-10, H-11), 8.41 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 10.50 (br.s, 1H, NH). Fumarate, Mp: 272°C (dec) (EtOH–Et_2O). *Anal.* $C_{22}H_{24}N_2O_2S$·$C_4H_4O_4$·H_2O: (C, H, N).

3.3.6. 3,3-Dimethyl-6-[[2-(diethylamino)ethyl]amino]-3H,7H-pyrano[2,3-c]thioxanthen-7-one ***(18)***

Yield: 68%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.11 (t, 6H, $J = 7$ Hz, $N(CH_2CH_3)_2$), 1.47 (s, 6H, 2 × gemCH_3), 2.65 (q, 4H, $J = 7$ Hz, $N(CH_2CH_3)_2$), 2.87 (t, 2H, $J = 7$ Hz, CH_2NEt_2), 3.32 (q, 2H, $J = 7$ Hz, 5 Hz, $HNCH_2$), 5.59 (d, 1H, $J = 10$ Hz, H-2), 6.05 (s, 1H, H-5), 6.72 (d, 1H, $J = 10$ Hz, H-1), 7.41 (m, 3H, H-9, H-10, H-11), 8.50 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 8.07 (br.s, 1H, NH). Fumarate, Mp: 290°C (dec) (EtOH–Et_2O). *Anal.* $C_{24}H_{28}N_2O_2S$·$C_4H_4O_4$·$2H_2O$: (C, H, N).

3.3.7. 3,3-Dimethyl-6-[[2-(1-pyrrolidinyl)ethyl]amino]-3H,7H-pyrano[2,3-c]thioxanthen-7-one ***(19)***

Yield: 70%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 6H, 2 × gemCH_3), 1.87 (m, 4H, pyrrolidine H), 2.79 (m, 4H, pyrrolidine H), 2.97 (t, 2H, $J = 7$ Hz, $CH_2N(CH_2)_4$), 3.44 (q, 2H, $J = 7$ Hz, 4 Hz, $HNCH_2$), 5.61 (d, 1H, $J = 10$ Hz, H-2), 6.06 (s, 1H, H-5), 6.66 (d, 1H, $J = 10$ Hz, H-1), 7.45 (m, 3H, H-9, H-10, H-11), 8.46 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 10.61 (br.s, 1H, NH). Fumarate, Mp: 276°C (dec) (EtOH–Et_2O). *Anal.* $C_{24}H_{26}N_2O_2S$·$C_4H_4O_4$: (C, H, N).

*3.3.8. 3,3-Dimethyl-6-[[2-(1-piperidinyl)ethyl]amino]-3H,7H-pyrano[2,3-c]thioxanthen-7-one (**20**)*

Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 6H, 2 × gemCH_3), 1.44 (m, 2H, piperidine H), 1.68 (m, 4H, piperidine H), 2.54 (m, 4H, piperidine H), 2.74 (t, 2H, $J = 7$ Hz, CH_2N(CH_2)$_5$), 3.39 (q, 2H, $J = 7$ Hz, 4 Hz, HNCH_2), 5.58 (d, 1H, $J = 10$ Hz, H-2), 6.07 (s, 1H, H-5), 6.67 (d, 1H, $J = 10$ Hz, H-1), 7.46 (m, 3H, H-9, H-10, H-11), 8.47 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 8.00 (br.s, 1H, NH). Fumarate, Mp: 292°C (dec) (EtOH–Et_2O). *Anal.* $C_{25}H_{28}N_2O_2S{\cdot}C_4H_4O_4$: (C, H, N).

*3.3.9. 2,2-Dimethyl-5-[[2-(dimethylamino)ethyl]amino]-2H,6H-pyrano[3,2-b]xanthen-6-one (**21**)*

Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46 (s, 6H, 2 × gemCH_3), 2.29 (s, 6H, N(CH_3)$_2$), 2.55 (t, 2H, $J = 7$ Hz, CH_2N(CH_3)$_2$), 3.53 (q, 2H, $J = 7$ Hz, 6 Hz, HNCH_2), 5.46 (d, 1H, $J = 10$ Hz, H-3), 6.18 (s, 1H, H-12), 6.58 (d, 1H, $J = 10$ Hz, H-4), 7.41 (m, 3H, H-8, H-9, H-10), 8.19 (dd, 1H, $J = 8$ Hz, 0.5 Hz, H-7), 9.67 (br.s, 1H, NH). Hydrochloride, Mp: 198–200°C (EtOH–Et_2O). *Anal.* $C_{22}H_{24}N_2O_3{\cdot}$HCl: (C, H, N).

*3.3.10. 2,2-Dimethyl-5-[[2-(diethylamino)ethyl]amino]-2H,6H-pyrano[3,2-b]xanthen-6-one (**22**)*

Yield: 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.04 (t, 6H, $J = 7$ Hz, N(CH_2CH_3)$_2$, 1.47 (s, 6H, 2 × gemCH_3), 2.60 (q, 4H, $J = 7$ Hz, N(CH_2CH_3)$_2$), 2.70 (t, 2H, $J = 7$ Hz, CH_2NEt_2), 3.53 (q, 2H, $J = 7$ Hz, 5 Hz, HNCH_2), 5.47 (d, 1H, $J = 10$ Hz, H-3), 6.19 (s, 1H, H-12), 6.51 (d, 1H, $J = 10$ Hz, H-4), 7.39 (m, 3H, H-8, H-9, H-10), 8.18 (dd, 1H, $J = 8$ Hz, 0.5 Hz, H-7), 9.60 (br.s, 1H, NH). Hydrochloride, Mp: 195–197°C (EtOH–Et_2O). *Anal.* $C_{24}H_{28}N_2O_3{\cdot}$HCl: (C, H, N).

*3.3.11. 2,2-Dimethyl-5-[[2-(1-pyrrolidinyl)ethyl]amino]-2H,6H-pyrano[3,2-b]xanthen-6-one (**23**)*

Yield: 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 6H, 2 × gemCH_3), 1.71 (m, 4H, pyrrolidine H), 2.52 (m, 4H, pyrrolidine H), 2.70 (t, 2H, $J = 7$ Hz, CH_2N(CH_2)$_4$), 3.52 (q, 2H, $J = 7$ Hz, 5 Hz, HNCH_2), 5.49 (d, 1H, $J = 10$ Hz, H-3), 6.12 (s, 1H, H-12), 6.51 (d, 1H, $J = 10$ Hz, H-4), 7.36 (m, 3H, H-8, H-9, H-10), 8.11 (dd, 1H, $J = 8$ Hz, 0.5 Hz, H-7), 9.53 (br.s, 1H, NH). Hydrochloride, Mp: 248–250°C (EtOH–Et_2O). *Anal.* $C_{24}H_{26}N_2O_3{\cdot}$HCl: (C, H, N).

*3.3.12. 2,2-Dimethyl-5-[[2-(1-piperidinyl)ethyl]amino]-2H,6H-pyrano[3,2-b]xanthen-6-one (**24**)*

Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46 (s, 6H, 2 × gemCH_3), 1.47 (m, 2H, piperidine H), 1.58 (m, 4H, piperidine H), 2.44 (m, 4H, piperidine H), 2.61 (t, 2H, $J = 7$ Hz, CH_2N(CH_2)$_5$), 3.57 (q, 2H, $J = 7$ Hz, 5 Hz, HNCH_2), 5.48 (d, 1H, $J = 10$ Hz, H-3), 6.20 (s, 1H, H-12), 6.61 (d, 1H, $J = 10$ Hz, H-4), 7.44 (m, 3H, H-8, H-9, H-10), 8.19 (dd, 1H, $J = 8$ Hz, 1 Hz, H-7), 9.62 (br.s, 1H, NH). Hydrochloride, Mp: 298°C (dec) (EtOH–Et_2O). *Anal.* $C_{25}H_{28}N_2O_3{\cdot}$HCl: (C, H, N).

*3.3.13. 2,2-Dimethyl-5-[[2-(dimethylamino)ethyl]amino]-2H,6H-pyrano[3,2-b]thioxanthen-6-one (**25**)*

Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 6H, 2 × gemCH_3), 2.77 (s, 6H, N(CH_3)$_2$), 3.18 (t, 2H, $J = 7$ Hz, CH_2N(CH_3)$_2$), 3.93 (q, 2H, $J = 7$ Hz, 4 Hz, HNCH_2), 5.62 (d, 1H, $J = 10$ Hz, H-3), 6.48 (d, 1H, $J = 10$ Hz, H-4), 6.49 (s, 1H, H-12), 7.42 (m, 3H, H-8, H-9, H-10), 8.41 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-7), 9.91 (br.s, 1H, NH). Fumarate, Mp: 254°C (dec) (EtOH–Et_2O). *Anal.* $C_{22}H_{24}N_2O_2S{\cdot}C_4H_4O_4$: (C, H, N).

*3.3.14. 2,2-Dimethyl-5-[[2-(diethylamino)ethyl]amino]-2H,6H-pyrano[3,2-b]thioxanthen-6-one (**26**)*

Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.01 (t, 6H, $J = 7$ Hz, N(CH_2CH_3)$_2$, 1.47 (s, 6H, 2 × gemCH_3), 2.60 (q, 4H, $J = 7$ Hz, N(CH_2CH_3)$_2$), 2.65 (t, 2H, $J = 7$ Hz, CH_2NEt_2), 3.48 (q, 2H, $J = 7$ Hz, 4 Hz, HNCH_2), 5.48 (d, 1H, $J = 10$ Hz, H-3), 6.38 (s, 1H, H-12), 6.54 (d, 1H, $J = 10$ Hz, H-4), 7.41 (m, 3H, H-8, H-9, H-10), 8.47 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-7), 9.98 (br.s, 1H, NH). Fumarate, Mp: 235–237°C (EtOH–Et_2O). *Anal.* $C_{24}H_{28}N_2O_2S{\cdot}C_4H_4O_4{\cdot}H_2O$: (C, H, N).

*3.3.15. 2,2-Dimethyl-5-[[2-(1-pyrrolidinyl)ethyl]amino]-2H,6H-pyrano[3,2-b]thioxanthen-6-one (**27**)*

Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 6H, 2 × gemCH_3), 1.97 (m, 4H, pyrrolidine H), 2.94 (m, 4H, pyrrolidine H), 3.19 (t, 2H, $J = 7$ Hz, CH_2N(CH_2)$_4$), 3.81 (q, 2H, $J = 7$ Hz, 4 Hz, HNCH_2), 5.61 (d, 1H, $J = 10$ Hz, H-3), 6.42 (s, 1H, H-12), 6.47 (d, 1H, $J = 10$ Hz, H-4), 7.42 (m, 3H, H-8, H-9, H-10), 8.37 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-7), 10.85 (br.s, 1H, NH). Fumarate, Mp: 280°C (dec) (EtOH–Et_2O). *Anal.* $C_{24}H_{26}N_2O_2S{\cdot}C_4H_4O_4$: (C, H, N).

*3.3.16. 2,2-Dimethyl-5-[[2-(1-piperidinyl)ethyl]amino]-2H,6H-pyrano[3,2-b]thioxanthen-6-one (**28**)*

Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 6H, 2 × gemCH_3), 1.52 (m, 6H, piperidine H), 2.47 (m, 4H, piperidine H), 2.54 (t, 2H, $J = 7$ Hz, CH_2N(CH_2)$_5$), 3.51 (q, 2H, $J = 7$ Hz, 4 Hz, HNCH_2), 5.49 (d, 1H, $J = 10$ Hz, H-3), 6.38 (s, 1H, H-12), 6.61 (d, 1H, $J = 10$ Hz, H-4), 7.44 (m, 3H, H-8, H-9, H-10), 8.44 (dd, 1H, $J = 8$ Hz, 1.5 Hz, H-8), 10.05 (br.s, 1H, NH). Fumarate, Mp: 231–233°C (EtOH–Et_2O). *Anal.* $C_{25}H_{28}N_2O_2S{\cdot}C_4H_4O_4$: (C, H, N).

3.4. Typical procedure for the preparation of the hydrochloride and fumarate salts

3.4.1. Preparation of the hydrochloride salts

A saturated solution of HCl in dry diethyl ether was added dropwise to a solution of the amine in dry diethyl ether, until no precipitation occurred. The pre-

cipitate was filtered and recrystallized (EtOH–Et_2O), to yield the hydrochloride in approximately 90% yield.

3.4.2. Preparation of the fumarate salts

A solution of fumaric acid (0.21 mmol) in absolute ethanol was added to a stirred solution of the amine (0.2 mmol) in absolute ethanol and the reaction mixture was refluxed for 12 h under Ar. After cooling, the precipitate was filtered, washed with dry diethyl ether and recrystallized (EtOH–Et_2O), to yield the fumarate in 82–87% yield.

3.5. Cytotoxicity

The potential cytotoxic activity of all the synthesized compounds and their eventual selective effect on a phase of the cell cycle were evaluated in vitro using the microculture tetrazolium assay essentially as described [13]. Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). Cells were exposed for 48 h to nine graded concentrations of the test compound. Results are expressed as IC_{50} (mean, $n = 3$), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

Table 1
Cytotoxicity and cell cycle selectivity

Comp.	IC_{50} (μM)	Cell cycle effect (% of cells in the indicated phase) [a]
13	7.1	G2+M (43% at 10 μM)
14	8.4	G2+M (39% at 10 μM)
15	2.1	G2+M (46% at 10 μM)
16	9.3	G2+M (47% at 25 μM)
17	5.3	G2+M (39% at 10 μM)
18	9.9	G2+M (55% at 25 μM)
19	9.5	G2+M (46% at 25 μM)
20	10.2	G2+M (49% at 25 μM)
21	5.8	G2+M (40% at 10 μM)
22	5.1	G2+M (42% at 10 μM)
23	3.5	G2+M (49% at 10 μM)
24	8.2	G2+M (47% at 25 μM)
25	9.6	G2+M (45% at 25 μM)
26	9.6	G2+M (51% at 25 μM)
27	5.1	G2+M (50% at 10 μM)
28	12.4	G2+M (46% at 25 μM)
2	17.2	not specific
5	36.9	not specific
8	14	not specific
11	48.3	not specific
Acronycine	21.0	G2+M (46% at 50 μM)

[a] 25% of untreated cells were in the G2+M phase of the cell cycle.

3.6. Cell cycle analysis

L1210 cells (2.5×10^5/ml) were incubated for 21 h (approximately two doubling times) with various concentrations of cytotoxic drugs. Cells were then fixed by 70% v/v ethanol, washed twice with phosphate-buffered saline (PBS) and incubated in PBS containing 100 μg/ml RNase and 25 μg/ml propidium iodide (PI) for 30 min at 20°C. For each sample, 10 000 cells were analyzed on an Epics XL Coulter flow cytometer. Results are expressed as the percentage of cells accumulated in the G2 + M phase of the cell cycle.

4. Results and discussion

The IC_{50} and the cell cycle effect of the new compounds are presented in Table 1. We used acronycine as the reference compound and the cytotoxic activity was also compared with that reported for the xanthenone and thioxanthenone derivatives **2**, **5**, **8** and **11** [10].

On the basis of the first results, it is obvious that the introduction of a basic flexible side chain at position 6 of the angular or at position 5 of the linear tetracyclic ring system exerts a profound effect on the cytotoxic activity. In fact, all the derivatives tested have shown significant activity, being 2 to 10 times more potent than acronycine in inhibiting L1210 cell proliferation. They have also expressed markedly improved cytotoxicity towards the methoxy xanthenone and thioxanthenone analogs **2**, **5**, **8** and **11**.

The most cytotoxic compound, among all compounds synthesized, is the angular 2-(1-pyrrolidinyl) derivative **15**, with an $IC_{50} = 2.1$ μM. It is also noticeable that, among the linear derivatives **21**–**28**, the 5-[2-(1-pyrrolidinyl)ethyl]amino substituted compounds **23** and **27** exert comparatively higher antitumor potency. In the linear series, for identical (*N*,*N*-dialkylamino)ethylamino substitution, changing from the xanthenone to the thioxanthenone ring system results in a reduced cytotoxicity, while in the angular series it appears to be a slight and variable effect, following the oxygen for sulfur replacement.

The perturbation of the cell cycle induced by these compounds was studied on the same cell line. In contrast with the methoxy xanthenone and thioxanthenone analogs **2**, **5**, **8** and **11**, all the new compounds induced a partial accumulation of cells in the G2 + M phase of the cell cycle. Since it has been reported that the same type of perturbation was observed with acronycine [14], this result could suggest a similar mechanism of action at the molecular level.

Further biological evaluation of the new compounds is currently in progress.

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