Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2793–2802

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis, Cytotoxic Activity, NMR Study and Stereochemical Effects of Some New Pyrano[3,2-*b*]thioxanthen-6-ones and Pyrano[2,3-*c*]thioxanthen-7-ones

Ioannis K. Kostakis,[a] Nicole Pouli,[a] Panagiotis Marakos,[a] Emmanuel Mikros,[a] Alexios-Leandros Skaltsounis,[b,*] Stephane Leonce,[c] Ghanem Atassi[c] and Pierre Renard[c]

[a]*Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis-Zografou, Athens 17345, Greece*

[b]*Division of Pharmacognosy, University of Athens, Panepistimiopolis-Zografou, Athens 17345, Greece*

[c]*Institut de Recherches SERVIER, 11 Rue des Moulineaux, 92150 Suresnes, France*

Received 31 July 2000; accepted 18 April 2001

Abstract—Some new substituted pyrano[3,2-*b*]thioxanthen-6-ones and pyrano[2,3-*c*]thioxanthen-7-ones were prepared and their cytotoxic activity was evaluated using acronycine as the reference compound. The conformation of the molecules was also investigated in an effort to correlate this parameter with the biological activity. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

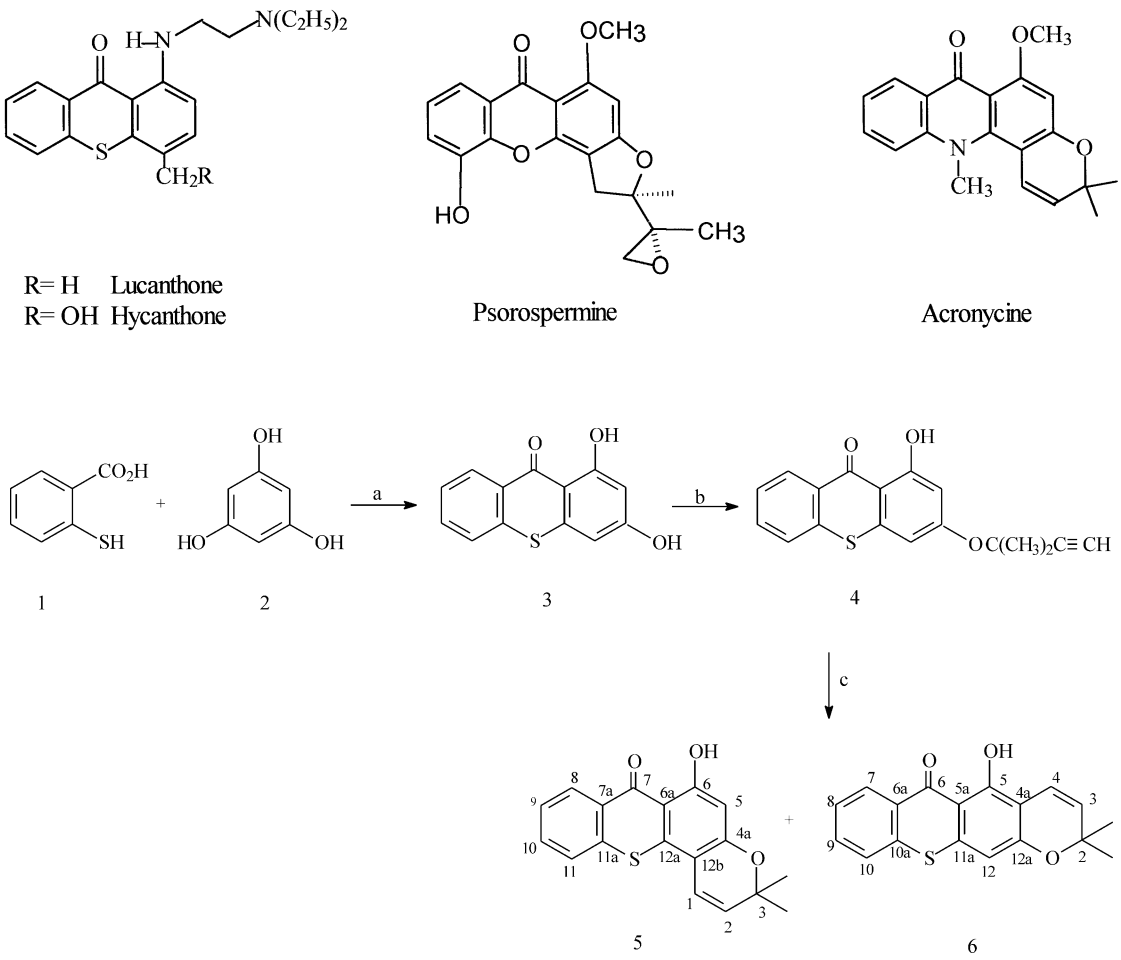
Thioxanthones, as well as xanthones, possess a number of interesting pharmacological activities.[1–3] Certain members of these classes of compounds exhibit significant antitumor and cytotoxic effects, well examined examples being lucanthone (Fig. 1), a thioxanthone analogue and its active metabolite hycanthone.[4,5] A series of hycanthone derivatives have been recently reported to display high levels of in vivo activity versus murine pancreatic adenocarcinoma (Panc 03).[6] Concerning the xanthone analogues, some derivatives of 9-oxo-9*H*-xanthene-4-acetic acid are among the most potent compounds yet reported against colon 38 tumors in mice.[7] The dihydrofuranoxanthone psorospermin (Fig. 1), was found to possess significant cytotoxicity against a variety of human tumor cell lines and excellent in vivo activity against P-388 mouse lymphocytic leukemia, mammary (CD) and colon (CG) experimental animal models.[8]

On the other hand, the acridone alkaloid acronycine (Fig. 1) shows cytotoxity against numerous solid tumors, including sarcoma, myeloma, carcinoma, and melanoma.[9,10] Despite the interest in psorospermin and acronycine, which share structural similarity, since both contain a linear tricyclic ring system and a fourth, oxygen containing ring, fused to it, little chemical and, consequently biological investigation, has been conducted concerning the development of structure–activity relationships for related compounds. Recent examples of new synthetic agents include the diacetate of *cis*-1,2-dihydroxy-1,2-dihydroacronycine, which exhibits promising antitumor properties, when compared to the parent compound (Scheme 1).[11]

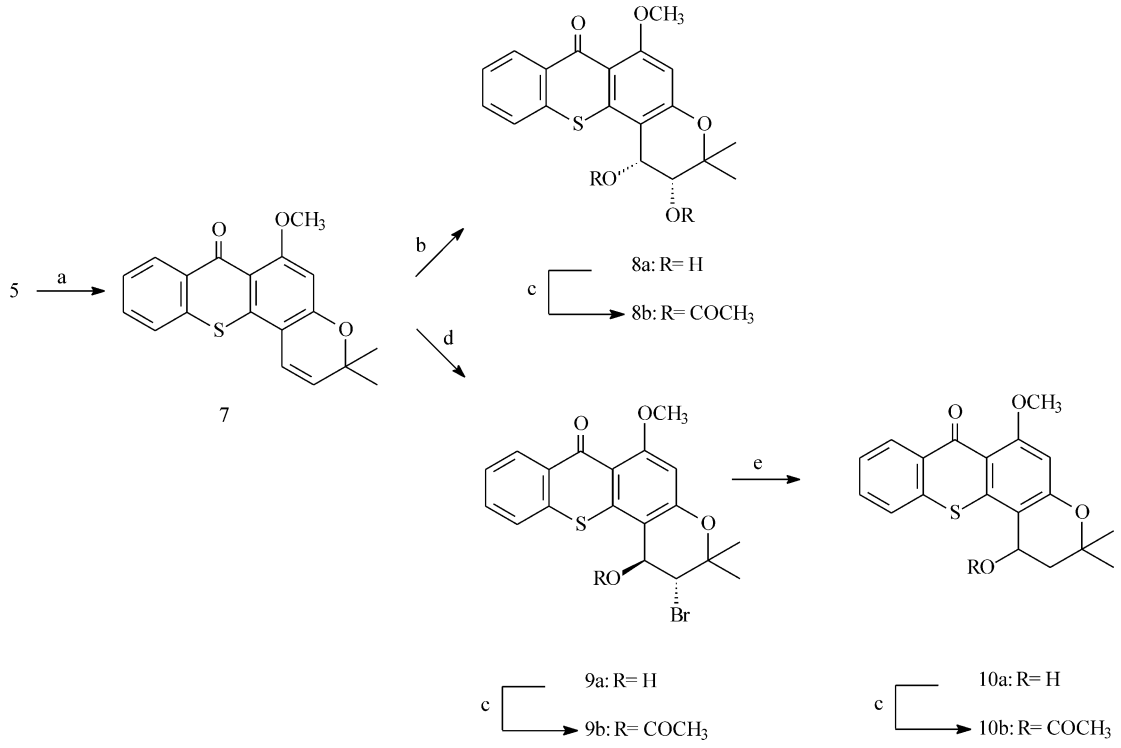
On the basis of these considerations and in connection with our research on acronycine structural manipulation, we synthesized the pyrano[2,3-*c*]thioxanthen-7-one **7** (Scheme 2), bearing the isosteric replacement of a sulfur atom instead of the nitrogen atom of acronycine. In further attempts to develop this series, we have also prepared the isomeric pyrano[3,2-*b*]thioxanthen-6-one **11** (Scheme 3), and adducts in the double bond of the new compounds, in order to investigate the potential cytotoxic activity of these derivatives. The conformation of the pyran ring of the most active compounds is analyzed on the basis of data provided from $^{3}J_{H1-H2}$ coupling constants as well as of NOE's observed between H-1, H-2 and the methyl groups at C-3. A conformational analysis using molecular mechanics calculations in comparison with experimental data gives evidence for

*Corresponding author. Tel.: +30-1-727-4598; fax: +30-1-727-4594; e-mail: skaltsounis@pharm.uoa.gs

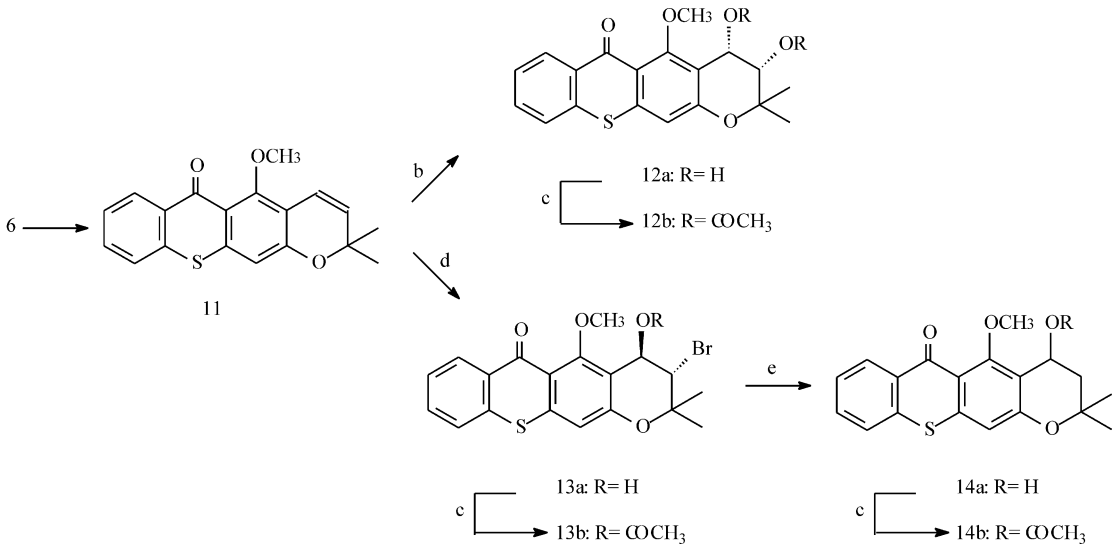
0968-0896/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(01)00130-4



Scheme 1. (a) Methanesulfonic acid, P_2O_5, Δ; (b) 3-chloro-3-methyl-1-butyne, CuI, K_2CO_3, NaI, DMF; (c) *N*,*N*-DEA, Δ.



Scheme 2. (a) (1) NaH, THF; (2) $(CH_3)_2SO_4$, Δ; (b) (1) OsO_4, *N*-methylmorpholine *N*-oxide; (2) $NaHSO_3$; (c) Ac_2O, Py; (d) NBS, H_2O–THF; (e) AIBN, Bu_3SnH, toluene, Δ.



Scheme 3. (a) (1) NaH, THF; (2) $(CH_3)_2SO_4$, Δ; (b) (1) OsO_4, *N*-methylmorpholine *N*-oxide; (2) $NaHSO_3$; (c) Ac_2O, Py; (d) NBS, H_2O–THF; (e) AIBN, Bu_3SnH, toluene, Δ.

the predominant conformation adopted by the D ring. A correlation between the conformation and the biological activity of these compounds is discussed.

Results

Chemistry

The reaction of thiosalicylic acid (**1**) with 1,3,5-trihydroxybenzene (**2**) in methanesulfonic acid in the presence of phosphorus pentoxide, provided 1,3-dihydroxy-9*H*-thioxanthen-9-one (**3**) (Scheme 1). The deactivation of the 1-hydroxyl group of **3**, due to its low acidity, because of hydrogen bonding to the 9-carbonyl, made feasible the selective etherification of the 3-hydroxyl group with 3-chloro-3-methyl-1-butyne to give the corresponding ether **4**. Subsequent thermal cyclization of this ether in boiling *N*,*N*-diethylaniline, afforded a mixture of two four-ring isomers, namely 6-hydroxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]thioxanthen-7-one (angular isomer **5**) and 5-hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]thioxanthen-6-one (linear isomer **6**). The isomers were separated from the mixture by column chromatography. Methylation of the free hydroxyl group of the two isomers was then carried out, with dimethyl sulphate in the presence of sodium hydride in THF, to afford the corresponding methylethers **7** (Scheme 2) and **11** (Scheme 3).

The structures of compounds **7** and **11** were unambiguously established by ^1H and ^{13}C NMR spectroscopy, using both direct and long-range heteronuclear correlation experiments (HMBC and HMQC sequences). Structural discrimination between the two isomers resulted from the observation that while both the 5-carbon atom and the 6-carbon atom of the linear and angular isomers respectively exhibit ^3J coupling with the methoxyl hydrogen atoms, it is only in the case of the linear isomer, that a ^3J coupling exists between the carbon that bears the methoxyl group and the 4-hydrogen atom. On the other hand, the 6-carbon atom of the angular isomer possesses a ^2J coupling with the aromatic 5-hydrogen atom.

Catalytic syn-hydroxylation of the unsaturated pyranothioxanthenone **7**, with osmium tetroxide and *N*-methylmorpholine-*N*-oxide as oxidizing reagent, yielded the corresponding *cis*-diol **8a** (Scheme 2). On the other hand, reaction of the derivative **7** with *N*-bromosuccinimide (NBS) in water resulted to the *trans*-bromohydrin **9a**. Reductive debromination of the above mentioned bromohydrin, via free radical reaction with tri-*n*-butyltinhydride in the presence of 2,2′-diazodiisobutylnitrile (AIBN), furnished the 1-hydroxy analogue **10a**. Furthermore, the acetates of compounds **8a**, **9a** and **10a** were prepared by reaction with acetic anhydride, in the presence of pyridine.

The corresponding linear derivatives **12a**–**14b** were prepared by analogous procedures, as shown in Scheme 3, starting from the isomer **11**.

Biological evaluation

The cytotoxic activity of the new compounds, was carried out in vitro on the L1210 leukemic cell line, with acronycine as the reference compound. The data, presented in Table 1, imply that compounds **8a**, **9b**, **10b**, **11**, **12a** were 2–3 times less potent than acronycine and compounds **13a**,**b** and **14a**,**b**, were devoid of antiproliferative activity. On the other hand, compound **9a** exhibited interesting cytotoxicity, being twice more potent than acronycine, while compounds **7**, **8b**, **10a**, and **12b**, were about as potent as acronycine. In general, we observe that the angular isomers, that possess a structural similarity to acronycine, appear to be more potent than the linear analogues, suggesting that the angular orientation of the D ring is important for the biological activity. The cytotoxicity of the angular derivatives **8a**,**b**, **9a**,**b** and **10a**,**b** differs according to their stereochemistry and the substitution on the D ring.

These factors seem to play an important role and worth to be investigated.

NMR study and molecular calculations

The pyrane-D ring of the abovementioned compounds can adopt two distinct half chair conformations, which can be identified from the interproton ^3J coupling constants and NOE's in conjunction with molecular models. The calculated relative energies of conformer I and II as resulted by MM + calculations along with the calculated dihedral angles Θ≡H1–C1–C2–H1 for angular compounds **8a**,**b**, **9a**,**b** and **10a**,**b**, are summarized in Table 2. The calculated $^3J_{H1-H2}$ coupling constants derived from Θ and the appropriate Karplus equation proposed by Altona et al.,[12] compared to the experimental values are presented in the same table.

The two conformers I and II of the most active compound **9a** are depicted in Figure 2. In the case of conformer I, the OH-1 group and Br-2 atom are in a pseudoequatorial position, while, concerning conformer II, both substituents adopt a pseudoaxial orientation. Conformer I appears to be more stable, by 0.7 kcal/mol and the theoretical coupling constant $^3J_{H1-H2}$ is 8.3 Hz in I and 1.9 Hz in II (Table 2). The experimental coupling of 6.6 Hz suggests that conformer I is predominant, but the comparison with the theoretical value implies that a percentage of conformer II should be also present in solution. The NOESY spectrum shows that the CH_3-3β group has connectivities with H-1 and H-2, whereas the CH_3-3α group has connectivities only with H-2, which confirms the J couplings data. When the OH-1 group is acetylated, as in **9b,** the $^3J_{H1-H2}$ coupling constant is 2.6 Hz suggesting that conformer II is predominant in solution. This is also confirmed by NOESY data and calculations, as no NOE connectivities were observed between H-1 and methyl groups in 3 and conformer II seems to be theoretically more stable by 0.5 kcal/mol.

Table 1. Inhibition of L1210 cell proliferation

Compound	IC_{50} (μM)
7	36.9±3.6
8a	65.5±5.7
8b	36.6±2.9
9a	12.1±1.3
9b	47.2±4.2
10a	27.7±2.8
10b	46.6±3.3
11	48.3±3.7
12a	64.1±4.5
12b	33.0±3.2
13a	>80
13b	>80
14a	>80
14b	>80
Acronycine	25.0±4.1

Table 2. Calculated properties for conformer I and II for compounds **8a**,**b**, **9a**,**b**, **10a**,**b** in comparison with experimental J_{exp} coupling constant between H-1 and H-2

Compd	Conformer I				Conformer II				J_{exp}		ΔE
	Θ		J_{calc}		Θ		J_{calc}				
8a	44		4.3		−45		4.2		4.9		−0.6
8b	47		4		−451		4.3		5		−0.6
9a	158		8.3		74		1.9		6.6		−0.7
9b	158		8.4		76		1.6		2.6		0.5
10a	155[a]	37[b]	9.0[a]	7.5[b]	77[a]	−45[b]	1.9[a]	4.1[b]	4.1[a]	5.4[b]	0.7
10b	160[a]	42[b]	9.8[a]	6.8[b]	77[a]	−43[b]	1.9[a]	4.5[b]	2.7[a]	4.6[b]	0.1

ΔE (kcal/mol) represents the energy difference $\Delta E = E_I - E_{II}$, between energy of conformer I E_I, and energy of conformer II E_{II}, calculated by MM + ; Θ is the resulted from MM + models H_1–C–C–H_2 dihedral angle, J_{calc} is the H_1–H_2 coupling constant calculated from Θ using the appropriate Karplus equation.
[a]These values correspond to the Θ, J_{calc} and J_{exp} for α-H2.
[b]These values correspond the Θ, J_{calc} and J_{exp} for β-H2.

The NMR spectral data, concerning the mono substituted derivative **10a**, indicate that this compound should adopt conformation II. The experimental coupling constants were found to be $^3J_{H2a-H1} = 4.1$ Hz and $^3J_{H2b-H1} = 5.4$ Hz and are relatively close to those calculated for conformer II that is 2.3 and 4.1 Hz, while the calculated couplings for conformer I were 9.5 and 7.1 Hz, respectively. A simple calculation of the populations revealed that conformer II should predominate (by more than 70%). The existing NOE between the CH_3-3β with both H-2 and between the CH_3-3α and the H-2α as well, consent with the predominance of conformer II and this was confirmed by molecular calculations, which presume that the later is more stable by 0.7 kcal/mol. However, the weak intensity cross-peak correlating CH_3-3α with H-1 argue in favor of the co-existence of conformer I in solution. On the other hand, the acetylated compound **10b** seems to adopt only conformation II, since the experimental ^3J values are in perfect agreement with the theoretical ones.

In the case of the *cis*-dihydroxy compound **8a**, the coupling constant $^3J_{H1-H2}$ was found to be 4.9 Hz and the NOESY spectrum shows connectivities between the CH_3-3β group with both H-1 and H-2 and between CH_3-3α group and H-2 only. The relative intensities of the cross-peaks suggest that an equilibrium should exist between the two conformers, although conformer I seems to be more stable theoretically by 0.5 kcal/mol. Similar spectral data were observed concerning the diacetate **8b**. Unfortunately, the theoretical coupling constants $^3J_{H1-H2}$ are similar for both conformers I and II, concerning compounds **8a** and **8b**, and a more detailed analysis, to elucidate the equilibrium between them, would require a quantitative analysis of NOE, which is out of the scope of this article.

Discussion

From the abovementioned stereochemical remarks, we assume that among the angular compounds we observe differences concerning the conformational behavior of the D pyran ring. In correlation with the biological activity, the presence of an unhindered OH-1 group favors the cytotoxic activity. Compound **9a**, that exhibits the highest cytotoxicity, adopts conformation I,

whereas for all the other compounds conformer II is more stable, or an equilibrium exist between the two conformers. In conjunction with the *trans* configuration of C-1 and C-2 substituents, OH-1 group is in pseudo-equatorial orientation and consequently it is easily accessible. Compound **10a**, exhibiting the second higher cytotoxicity, has no substituent in C-2, but, as conformer II is the more stable and it is predominant in solution, the OH-1 has a pseudoaxial orientation and is sterically hindered by the homoaxial CH_3-3α group.

The accessibility of OH-1 on compound **9a**, compared to the others, is demonstrated by the possibility of the formation of an intramolecular hydrogen bond, according to the following observations. The NMR spectra of compound **9a** in various temperatures reveal that the $^3J_{H1-H2}$ coupling constant increases, lowering the temperature from 320 to 230 K. In these spectra it appears that the chemical shifts of protons H-5 and OCH_3 were progressively shifted up by 0.6 and 0.1 ppm respectively, by lowering the temperature, while the signals of H-2, OH-1 and CH_3-α were deshielded by 0.2–0.5 ppm. Similar shifts of the protons resonances were observed increasing the concentration or changing the solvent from CD_3OD to $CDCl_3$. Moreover, an NOE connectivity between H-8 and H-2 was observed in the NOESY spectrum. These phenomena, which have already been reported for acronycine analogues,[13] can be interpreted with the formation of a dimer through a hydrogen bond between OH-1 of a molecule and the carbonyl group of a second one, as depicted in Figure 3. This dimer is probably formed by two enantiomeric structures, which are present in solution. According to the calculations, it can be formed only if the D ring adopts conformation I. In the case that conformation II is adopted, the axial orientation of OH-1 and the presence of the bulky homoaxial CH_3-3ß in the same direction, hinders the formation of the hydrogen bond. This can explain the fact that lowering the temperature, where the hydrogen bonds are stabilized, the equilibrium between the two conformers is shifted towards I and the observed coupling constant increases. We did not observe any changes when we recorded the spectra of **9b** in various temperatures, suggesting that in contrast to **9a**, no aggregation takes place.

As already mentioned, only a small percentage of the monohydroxy derivative **10a** seems to adopt conformation I in solution and thus, it is likely to consider the formation of dimers, which could be in equilibrium with the monomers I and II. This is not consistent with the very small differences, which were observed varying the temperature, the solvent and the concentration and therefore it is difficult to demonstrate the existence of dimeric species, via the formation of a hydrogen bond, where the OH-1 is involved. On the contrary, our data suggest that these aggregates, if they exist, are not considerably populated in solution.

The alterations observed in the spectra of **8a** varying the temperature or the concentration were less important, for example the shielding of H-5 was 0.2 ppm, suggesting

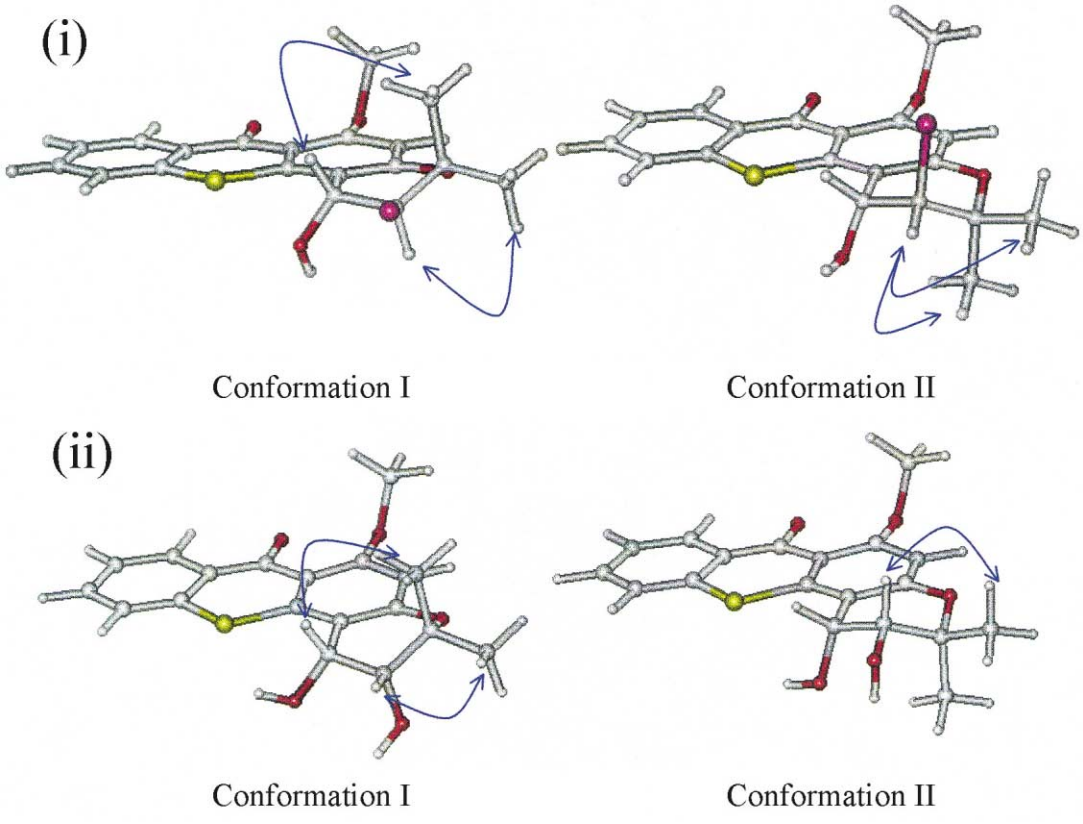


Figure 2. Representation of the low energy conformations for derivative (i) **9a** and (ii) **8a** derived from molecular mechanics calculations. Blue arrows show the expected NOE between D ring protons for each conformation.

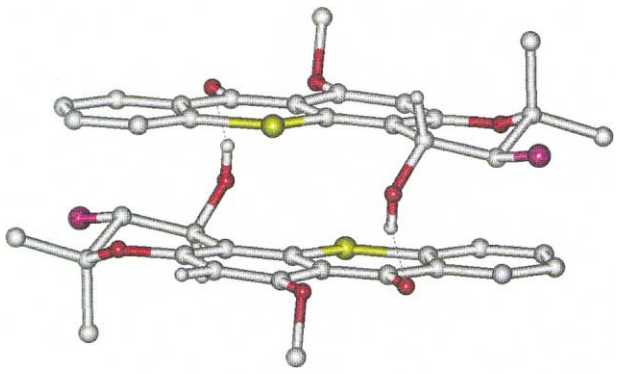


Figure 3. Representation of the dimmer form of derivative **9a** derived from NMR data and molecular mechanics geometry optimisation.

that the formation of the dimer is less extended although two hydroxyl groups in positions 1 and 2 are present. It is probable that the formation of the hydrogen bond is hindered by the presence of the hydroxyl group in position 2, due to an intramolecular hydrogen bond between the two hydroxyl groups. As expected, we did not observe any important changes in the spectra of **8b**, by varying the temperature, since there is no free hydroxyl group present in this molecule.

Conclusion

In this study, we have prepared a series of angular and linear pyranothioxanthenones. The biological evaluation of these compounds revealed that the angular analogues possessed interesting antiproliferative activity and among them **9a** was twice more potent than the reference compound acronycine. The conformation of the D-pyran ring is different among the angular derivatives and this fact could be correlated with the observed biological activity.

The most active compound **9a** possesses a D ring with the OH-1 arranged in a pseudoequatorial orientation. This compound was proven to be the only one that possesses an accessible hydroxyl group at position 1, which could participate in hydrogen bond formation. Therefore, it is reasonable to assume that the existence of an unprotected OH-1 and/or the ability of hydrogen bond formation involving this substituent, seems to be favourable for the biological activity of this class of compounds. This observation could be significant in order to elucidate their mode of action at the molecular level.

Experimental

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra and 2-D spectra were recorded on a Bruker Avanche 400 instrument, whereas ^{13}C NMR specra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2-D NMR techniques: ^{1}H^{1}H COSY, NOESY HMQC and HMBC. 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 ±0.4% of the theoretical values.

1,3-Dihydroxy-9*H*-thioxanthen-9-one (3). A mixture of phosphorous pentoxide (5.49 g, 38.7 mmol) and methanesulfonic acid (40 mL), was heated with vigorous stirring at 90 °C until a clear solution was obtained (approximately 1 h). Thiosalicylic acid (**1**, 1.54 g, 10 mmol) and phloroglycinol (**2**, 1.51 g, 12 mmol) were then added in one portion and the reaction mixture was heated at 90 °C for 15 min. The mixture was poured into ice, stored at 4 °C for 12 h and the precipitate was filtered, washed with water and dried over $CaCl_2$, to give **3** (2.05g, 84%). Mp 228–229 °C (methanol). ^{1}H NMR (DMSO-d_6, 400 MHz), δ (ppm) 6.3 (d, 1H, J = 2.4 Hz, H-2), 6.6 (d, 1H, J = 2.4 Hz, H-4), 7.5-7.8 (m, 3H, H-6, H-7, H-8), 8.4 (dd, 1H, J = 8 Hz, 0.5 Hz, H-9), 11.1 (br.s, 1H, D_2O exch., OH-3), 14.4 (s, 1H, D_2O exch., OH-1). ^{13}C NMR (DMSO-d_6, 50 MHz) δ (ppm) 101.09 (C-2), 103.10 (C-4), 107.53 (C-10a), 125.91 (C-8), 126.74 (C-6), 127.31 (C-9a), 128.49 (C-9), 133.30 (C-7), 136.35 (C-5a), 140.07 (C-4a), 163.94 (C-3), 166.64 (C-1), 183.12 (C-10). Anal. calcd for $C_{13}H_8O_3S$. calcd (%): C: 63.92, H: 3.30. Found (%): C: 63.95, H: 3.28.

1-Hydroxy-3-(1,1-dimethyl-2-propynoxy)-9*H*-thioxanthen-9-one (4). To a solution of **3** (0.3 g, 1.2295 mmol), in dry DMF (10 mL), were added under argon, 3-chloro-3-methyl-1-butyne (0.30 mL, 2.74 mmol), anhydrous potassium carbonate (0.8 g) and anhydrous sodium iodide (44 mg) and the mixture was heated at 60 °C for 10 h. The mixture was then poured into ice water, extracted with CH_2Cl_2 and the combined extracts were washed with a 2 N NaOH solution, water and dried (Na_2SO_4). The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel, 30×1 cm), using a mixture of cyclohexane/EtOAc (15:5) as the eluent, to give **4**. (0.2 g, 53%). Mp 186–188 °C (EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.7 (s, 6H, 2×CH_3), 2.7 (s, 1H, C≡CH), 6.8 (d, 1H, J = 2 Hz, H-4), 6.9 (d, 1H, J = 2 Hz, H-2), 7.4–7.7 (m, 3H, H-6, H-7, H-8), 8.5 (dd, 1H, J = 8 Hz, 0.5 Hz, H-9), 14.4 (s, 1H, D_2O exch., OH). ^{13}C

NMR ($CDCl_3$, 50 MHz) δ (ppm) 29.60 (2×gem CH_3), 74.30 ($\underline{C}(CH_3)_2C{\equiv}CH$), 75.39 ($C{\equiv}\underline{C}H$), 85.60 ($C(CH_3)_2\underline{C}{\equiv}CH$), 104.19 (C-2), 105.96 (C-4), 110.29 (C-10a), 124.90 (C-9a), 125.37 (C-8), 126.19 (C-6), 129.18 (C-9), 132.57 (C-7), 135.55 (C-5a), 139.12 (C-4a), 164.71 (C-3), 166.64 (C-1), 182.74 (C-10). Anal. calcd for $C_{18}H_{14}O_3S$. Calcd (%): C: 69.66, H: 4.55. Found (%): C: 69.45, H: 4.24.

6-Hydroxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-6-one (5) and 5-hydroxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (6). A solution of **4** (0.1 g, 0.3226 mmol) in *N*,*N*-diethylanilline (2 mL) was heated under argon at 200 °C for 90 min. Upon cooling, the reaction mixture was poured into water and extracted with dichloromethane. The combined extracts were washed successively with a 5% HCl solution, a 10% NaOH solution, brine and water. The organic phase was dried (Na_2SO_4) and the solvent was vacuum-evaporated to result to a mixture of the isomers **5** and **6**, which was separated by column chromatography (silica gel, 21×3 cm), using a mixture of cyclohexane/CH_2Cl_2 (10:4) as the eluent, to give pure **5** (34 mg, 34%) and **6** (23 mg, 23%).

6-Hydroxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-6-one (5). Mp 197–199 °C (EtOAc). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.4 (s, 6H, 2×CH_3), 5.7 (d, 1H, $J=10$ Hz, H-2), 6.3 (s, 1H, H-5), 6.5 (d, 1H, $J=10$ Hz, H-1), 7.5 (td, 1H, $J=8$ Hz, 1 Hz, H-10), 7.55 (dd, 1H, $J=8$ Hz, 1 Hz, H-11), 7.6 (td, 1H, $J=8$ Hz, 1 Hz, H-9), 8.5 (dd, 1H, $J=8$ Hz, 1 Hz, H-8), 14.6 (s, 1H, D_2O exch., OH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 28.16 (2×gem CH_3), 77.38 (C-3), 102.40 (C-5), 108.63 (C-12b), 116.69 (C-1), 117.43 (C-6a), 125.64 (C-9), 126.42 (C-11), 128.65 (C-2), 128.99 (C-8), 131.77 (C-7a), 132.51 (C-10), 134.02 (C-11a), 136.37 (C-12a), 157.06 (C-4a), 162.48 (C-6), 178.99 (C-7). Anal. calcd for $C_{18}H_{14}O_3S$. Calcd (%): C: 69.66, H: 4.55. Found (%): C: 69.88, H: 4.34.

5-Hydroxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (6). Mp 163–165 °C (EtOAc), ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.5 (s, 3H, CH_3), 1.6 (s, 3H, CH_3), 5.6 (d, 1H, $J=10$ Hz, H-3), 6.5 (s, 1H, H-12), 6.8 (d, 1H, $J=10$ Hz, H-4), 7.3–7.45 (m, 2H, H-9, H-10), 7.6 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.5 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-7), 14.7 (s, 1H, D_2O exch., OH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 28.46 (2×gem CH_3), 78.19 (C-2), 103.27 (C-12), 107.62 (C-4a), 112.34 (C-5a), 115.79 (C-4), 125.32 (C-8), 126.19 (C-10), 128.06 (C-3), 129.08 (C-7), 131.27 (C-6a), 132.43 (C-9), 134.63 (C-10a), 139.44 (C-11a), 158.60 (C-12a), 161.46 (C-5), 184.07 (C-6). Anal. calcd for $C_{18}H_{14}O_3S$. Calcd (%): C: 69.66, H: 4.55. Found (%): C: 69.39, H: 4.73.

6-Methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-6-one (7). To a solution of **5** (100 mg, 0.3226 mmol) in dry THF (5 mL) was added NaH (80% in hexanes, 19 mg, 0.625 mmol) and the mixture was stirred under argon for 30 min. A solution of dimethyl sulfate (42 mg, 0.33 mmol) in dry THF (3 mL) was then added dropwise and the mixture was refluxed for 3 h. After cooling, the excess of NaH was destroyed with a small amount of EtOH and the precipitate was filtered off. The filtrate was concentrated and purified by column chromatography (silica gel, 10×1 cm), using a mixture of cyclohexane/EtOAc (4:1) as the eluent, to give **7** (99 mg, 95%). Mp 140 °C (Et_2O–*n*-hexane). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.4 (s, 6H, 2×gem CH_3), 3.9 (s, 3H, OCH_3), 5.6 (d, 1H, $J=10$ Hz, H-2), 6.4 (s, 1H, H-5), 6.7 (d, 1H, $J=10$ Hz, H-1), 7.45 (td, 1H, $J=8$ Hz, 0.5 Hz, H-10), 7.50–7.65 (m, 2H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 27.97 (2×gem CH_3), 56.34 (OCH_3), 77.98 (C-3), 98.75 (C-5), 108.57 (C-12b), 113.97 (C-6a), 117.06 (C-1), 125.00 (C-9), 126.30 (C-11), 128.53 (C-2), 129.30 (C-8), 131.32 (C-10), 131.87 (C-7a), 133.84 (C-11a), 136.52 (C-12a), 157.06 (C-4a), 163.81 (C-6), 179.89 (C-7). Anal. calcd for $C_{19}H_{16}O_3S$. Calcd (%): C: 70.35, H: 4.97. Found (%): C: 69.98, H: 4.66.

5-Methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (11). This compound was prepared by an analogous procedure to **7**. Yield: 96%. Mp 158–160 °C (Et_2O–*n*-hexane). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.5 (s, 6H, 2×gem CH_3), 3.9 (s, 3H, OCH_3), 5.7 (d, 1H, $J=10$ Hz, H-3), 6.7 (s, 1H, H-12), 6.75 (d, 1H, $J=10$ Hz, H-4), 7.3–7.5 (m, 2H, H-9, H-10), 7.6 (td, 1H, $J=8$Hz, 0.5 Hz, H-8), 8.45 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 28.33 (2×gem CH_3), 62.66 (OCH_3), 77.78 (C-2), 108.24 (C-12), 114.86 (C-4a), 116.40 (C-4), 117.21 (C-5a), 124.93 (C-8), 126.11 (C-10), 129.49 (C-7), 130.74 (C-3), 131.25 (C-6a), 131.47 (C-9), 135.22 (C-10a), 140.00 (C-11a), 156.98 (C-12a), 158.75 (C-5), 179.19 (C-6). Anal. calcd for $C_{19}H_{16}O_3S$. Calcd (%): C: 70.35, H: 4.97. Found (%): C: 70.23, H: 5.12.

(±)-*cis*-1,2-Dihydro-1,2-dihydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (8a). To a solution of osmium tetroxide (2.5% in isopropanol) (0.71 mL 0.0111 mmol) and *N*-methylmorpholine *N*-oxide (45.63 mg 0.3056 mmol) in a mixture of *t*-BuOH/THF/H_2O (10:3:1.5 mL) was added compound **7** (90 mg, 0.2778 mmol). The reaction mixture was stirred at rt for 2 days. A saturated $NaHSO_3$ solution (0.5 mL) was then added, the mixture was stirred at rt for 90 min and the bulk of THF was vacuum-evaporated. The residue was then extracted with CH_2Cl_2, the organic phase was washed with a saturated $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified by column chromatography (silica gel 25×1 cm), with a mixture of CH_2Cl_2/CH_3OH (98:2) as the eluent to give **8a** (69.5 mg, 70%). Mp 155–157 °C (CH_2Cl_2–*n*-pentane). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.4 (s, 3H, 1×gem CH_3), 1.5 (s, 3H, 1×gem CH_3), 3.1–3.3 (br. s., 2H, D_2O exch., OH-1, OH-2), 3.85 (d, 1H, $J=5$ Hz, H-2), 3.9 (s, 3H, OCH_3), 5.01 (d, 1H, $J=5$ Hz, H-1), 6.25 (s, 1H, H-5), 7.4 (td, 1H, $J=8$Hz, 1.5 Hz, H-10), 7.5-7.6 (m, 2H, H-9, H-11), 8.5 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 25.11 (CH_3), 29.01 (CH_3), 56.25 (OCH_3), 63.58 (C-1), 72.00 (C-2), 78.68 (C-3), 98.92 (C-5), 109.49 (C-12b), 114.77 (C-6a), 124.99 (C-9), 126.53 (C-11), 129.17 (C-8), 131.33 (C-10), 131.82 (C-7a), 133.67 (C-11a), 142.74 (C-12a), 156.48 (C-4a), 163.58

(C-6), 179.58 (C-7). Anal. calcd for $C_{19}H_{18}O_5S$. Calcd (%): C: 63.67, H: 5.06. Found (%): C: 63.81, H: 5.29.

(±)-*cis*-3,4-Dihydro-3,4-dihydroxy-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (12a). This compound was prepared by an analogous procedure to **9a**. Yield: 68%. Mp 150–152 °C (CH_2Cl_2–*n*-pentane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.35 (s, 3H, 1×gem CH_3), 1.5 (s, 3H, 1×gem CH_3), 3.3 (br.s, 2H, D_2O exch., OH-3, OH-4), 3.8 (d, 1H, $J=5$ Hz, H-3), 4.0 (s, 3H, OCH_3), 5.1 (d, 1H, $J=5$ Hz, H-4), 6.8 (s, 1H, H-12), 7.3–7.5 (m, 2H, H-9, H-10), 7.55 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 22.82 (CH_3), 24.37 (CH_3), 62.37 (C-4), 62.56 (OCH_3), 70.57 (C-3), 78.83 (C-2), 109.05 (C-4a), 109.31 (C-12), 116.25 (C-5a), 124.74 (C-8), 125.95 (C-10), 129.52 (C-7), 130.99 (C-6a), 131.50 (C-9), 135.29 (C-10a), 140.62 (C-11a), 156.57 (C-12a), 163.37 (C-5), 179.10 (C-6). Anal. calcd for $C_{19}H_{18}O_5S$. Calcd (%): C: 63.67, H: 5.06. Found (%): C: 63.32, H: 4.88.

(±)-*cis*-1,2-Diacetoxy-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (8b). To a solution of **8a** (30 mg, 0.0838 mmol) in dry pyridine (2 mL), was added acetic anhydride (0.4 mL) and the reaction mixture was stirred at rt for 12 h. Water was then added and the reaction mixture was extracted with CH_2Cl_2, washed with a 5% HCl solution, water and dried (Na_2SO_4). The solvent was vacuum evaporated and the residue was purified by column chromatography (silica gel, 7×1 cm), using a mixture of CH_2Cl_2/CH_3OH (98:2) as the eluent, to give **8b** (34 mg, 90%). Mp 186–188 °C (Et_2O–*n*-pentane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.3 (s, 3H, 1×gem CH_3), 1.4 (s, 3H, 1×gem CH_3), 2.09 (s, 3H, $C\underline{H}_3CO$), 2.15 (s, 3H, $C\underline{H}_3CO$), 3.9 (s, 3H, OCH_3), 5.3 (d, 1H, $J=5$ Hz, H-2), 6.3 (d, 1H, $J=5$ Hz, H-1), 6.45 (s, 1H, H-5), 7.3 (td, 1H, $J=8$ Hz, 1.5 Hz, H-10), 7.4–7.6 (m, 2H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 20.54 ($\underline{C}H_3CO$), 20.63 ($\underline{C}H_3CO$), 21.35 (CH_3), 26.35 (CH_3), 56.32 (OCH_3), 62.95 (C-1), 71.25 (C-2), 76.62 (C-3), 98.95 (C-5), 105.30 (C-12b), 114.89 (C-6a), 125.10 (C-9), 126.61 (C-11), 129.13 (C-8), 131.34 (C-10), 131.82 (C-7a), 133.58 (C-11a), 142.32 (C-12a), 157.13 (C-4a), 163.92 (C-6), 168.74 ($CH_3\underline{C}O$), 170.20 ($CH_3\underline{C}O$), 179.87 (C-7). Anal. calcd for $C_{23}H_{22}O_7S$. Calcd (%): C: 62.43, H: 5.01. Found (%): C: 62.27, H: 5.25.

(±)-*cis*-3,4-Diacetoxy-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (12b). This compound was prepared by an analogous procedure to **8b**. Yield: 91%. Mp 149–151 °C (Et_2O-*n*-pentane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.35 (s, 3H, 1×gem CH_3), 1.4 (s, 3H, 1×gem CH_3), 2.08 (s, 3H, $C\underline{H}_3CO$), 2.12 (s, 3H, $C\underline{H}_3CO$), 3.9 (s, 3H, OCH_3), 5.2 (d, 1H, $J=5$ Hz, H-3), 6.4 (d, 1H, $J=5$ Hz, H-4), 6.86 (s, 1H, H-12), 7.4–7.5 (m, 2H, H-9, H-10), 7.6 (td, 1H, $J=8$ Hz, 1.5 Hz, H-8), 8.4 (dd, 1H, $J=8.5$ Hz, 1 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 20.66 ($\underline{C}H_3CO$), 20.86 ($\underline{C}H_3CO$), 21.73 (CH_3), 26.13 (CH_3), 60.89 (C-4), 62.44 ($\underline{O}CH_3$), 71.25 (C-3), 77.63 (C-2), 108.95 (C-12), 113.00 (C-4a), 117.07 (C-5a), 124.98 (C-8), 126.27 (C-10), 129.57 (C-7), 131.12 (C-6a), 131.80 (C-9), 135.07 (C-10a), 141.58 (C-11a), 156.81 (C-12a), 164.02 (C-5), 169.86 ($CH_3\underline{C}O$), 170.01 ($CH_3\underline{C}O$), 180.06 (C-6). Anal. calcd for $C_{23}H_{22}O_7S$. Calcd (%): C: 62.43, H: 5.01. Found (%): C: 62.12, H: 5.17.

(±)-*trans*-2-Bromo-1,2-dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (9a). *N*-bromosuccinimide (48.27 mg, 0.2712 mmol) was added to a solution of **7** (90 mg, 0.2778 mmol) in a 2:1 mixture of THF/H_2O (9 mL), at −20 °C and the reaction mixture was stirred at this temperature for 10 min. At the end of this time, a saturated NaCl solution was added (2 mL) and stirring was continued for 10 min. The organic solvent was then vacuum-evaporated, the residue was extracted with EtOAc, the organic phase was dried (Na_2SO_4) and the solvent was evaporated to dryness to afford a solid which was purified by column chromatography (silica gel, 30×2 cm), using a mixture of cyclohexane/EtOAc (70:30) as the eluent, to give pure **9a** (87 mg, 75%). Mp 222–224 °C (CH_2Cl_2–Et_2O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.4 (s, 3H, 1×gem CH_3), 1.6 (s, 3H, 1×gem CH_3), 3.8 (s, 3H, OCH_3), 4.05 (d, 1H, $J=7$ Hz, D_2O exch., OH), 4.55 (d, 1H, $J=7$ Hz, H-2), 5.1 (t, 1H, $J=7$ Hz, 7 Hz, H-1), 6.1 (s, 1H, H-5), 7.45 (td, 1H, $J=8$ Hz, 1 Hz, H-10), 7.55–7.62 (m, 2H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 21.57 (CH_3), 27.57 (CH_3), 56.05 (OCH_3), 60.31 (C-2), 69.58 (C-1), 79.54 (C-3), 98.24 (C-5), 110.44 (C-12b), 114.01 (C-6a), 124.85 (C-9), 126.29 (C-10), 128.89 (C-8), 131.47 (C-10), 131.98 (C-7a), 133.44 (C-11a), 135.51 (C-12a), 156.09 (C-4a), 162.56 (C-6), 180.08 (C-7). Anal. calcd for $C_{19}H_{17}O_4SBr$. Calcd (%): C: 54.16, H: 4.07. Found (%): C: 54.29, H: 4.27.

(±)-*trans*-3-Bromo-3,4-dihydro-4-hydroxy-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (13a). This compound was prepared by an analogous procedure to **9a**. Yield: 77%. Mp 150–152 °C (CH_2Cl_2–Et_2O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.5 (s, 3H, 1×gem CH_3), 1.5 (s, 3H, 1×gem CH_3), 3.9 (br.s, 1H, D_2O exch., OH), 4.0 (s, 3H, OCH_3), 4.35 (d, 1H, $J=8$ Hz, H-3), 5.22 (d, 1H, $J=8$ Hz, H-4), 6.8 (s, 1H, H-12), 7.4-7.55 (m, 2H, H-9, H-10), 7.6 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.44 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 23.49 (CH_3), 26.79 (CH_3), 57.30 (C-3), 62.51 (OCH_3), 68.29 (C-4), 79.18 (C-2), 109.38 (C-12), 114.01 (C-4a), 116.83 (C-5a), 125.05 (C-8), 126.31 (C-10), 129.54 (C-7), 131.00 (C-6a), 131.89 (C-9), 135.21 (C-10a), 140.84 (C-11a), 155.74 (C-12a), 162.68 (C-5), 179.19 (C-6). Anal. calcd for $C_{19}H_{17}O_4SBr$. Calcd (%): C: 54.16, H: 4.07. Found (%): C: 53.82, H: 3.81.

(±)-*trans*-1-Acetoxy-2-bromo-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (9b). This compound was prepared by an analogous procedure to **8b**. Yield: 97%. Mp 208–210 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.52 (s, 3H, 1×gem CH_3), 1.55 (s, 3H, 1×gem CH_3), 2.12 (s, 1H, $C\underline{H}_3CO$), 3.8 (s, 3H, OCH_3), 4.4 (d, 1H, $J=2$ Hz, H-2), 6.28 (d, 1H, $J=2$ Hz, H-1), 6.5 (s, 1H, H-5), 7.4 (td, 1H,

$J=8$ Hz, 1.5 Hz, H-10), 7.5–7.6 (m, 2H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 20.75 ($\underline{C}H_3CO$), 24.24 (CH_3), 29.51 (CH_3), 53.37 (C-1), 56.41 ($\overline{O}CH_3$), 69.53 (C-2), 77.65 (C-3), 99.09 (C-5), 102.99 (C-12b), 114.23 (C-6a), 125.13 (C-9), 126.80 (C-11), 129.29 (C-8), 131.43 (C-10), 132.13 (C-7a), 133.57 (C-11a), 142.79 (C-12a), 156.94 (C-4a), 163.92 (C-6), 170.06 ($CH_3\underline{C}O$), 179.87 (C-7). Anal. calcd for $C_{21}H_{19}O_5SBr$. Calcd (%): C: 54.43, H: 4.13. Found (%): C: 54.67, H: 4.39.

(±)-*trans*-4-Acetoxy-3-bromo-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (13b). This compound was prepared by an analogous procedure to **8b**. Yield: 95%. Mp 188–190 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.5 (s, 3H, 1×gem CH_3), 1.6 (s, 3H, 1×gem CH_3), 2.1 (s, 3H, $C\underline{H}_3CO$), 3.9 (s, 3H, OCH_3), 4.3 (d, 1H, $J=2$ Hz, H-3), 6.4 (d, 1H, $J=2$ Hz, H-4), 6.85 (s, 1H, H-12), 7.3–7.6 (m, 2H, H-9, H-10), 7.65 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.5 (dd, 1H, $J=8$ Hz, 1 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 21.04 ($\underline{C}H_3CO$), 25.11 (CH_3), 27.85 (CH_3), 54.12 (C-3), 62.44 ($\overline{O}CH_3$), 67.17 (C-4), 77.19 (C-2), 109.07 (C-12), 111.18 (C-4a), 116.03 (C-5a), 125.00 (C-8), 126.29 (C-10), 129.56 (C-7), 131.44 (C-6a), 131.83 (C-9), 135.17 (C-10a), 140.00 (C-11a), 156.74 (C-12a), 163.32 (C-5), 169.71 ($CH_3\underline{C}O$), 179.17 (C-6). Anal. calcd for $C_{21}H_{19}O_5SBr$. Calcd (%): C: 54.43, H: 4.13. Found (%): C: 54.77, H: 4.08.

(±)-1,2-Dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (10a). A mixture of tributyltin hydride (0.22 mL, 0.83 mmol) and 2,2′-diazodiisobutylnitrile (AIBN, 8.85 mg, 0.054 mmol) in dry toluene (3 mL) was added dropwise under argon, to a solution of **9a** (92.1 mg, 0.2188 mmol) in dry toluene (12 mL), preheated at 40 °C and the reaction mixture was refluxed for 4 h. The solvent was then vacuum-evaporated and the residue was purified by column chromatography (dry pack, silica gel 25×1 cm), using a mixture of cyclohexane/EtOAc (1:1) as the eluent, to give **10a** (57.6 mg, 77%). Mp 178–180 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.4 (s, 3H, 1×gem CH_3), 1.52 (s, 3H, 1×gem CH_3), 2.12 (dd, 1H, $J=14$ Hz, 4 Hz, H-2α), 2.50 (dd, 1H, $J=14$ Hz, 5 Hz, H-2β), 3.1 (d, 1H, $J=6$ Hz, D_2O exch., OH), 3.85 (s, 3H, OCH_3), 5.0 (ddd, 1H, $J=6$ Hz, 5 Hz, 4 Hz, H-1), 6.2 (s, 1H, H-5), 7.3 (td, 1H, $J=8$ Hz, 1.5 Hz, H-10), 7.45–7.54 (m, 3H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 26.11 (CH_3), 29.27 (CH_3), 41.31 (C-2), 56.23 (OCH_3), 62.27 (C-1), 75.55 (C-3), 99.17 (C-5), 110.01 (C-12b), 114.74 (C-6a), 125.04 (C-9), 126.58 (C-11), 129.26 (C-8), 131.40 (C-10), 131.87 (C-7a), 133.05 (C-11a), 142.01 (C-12a), 157.06 (C-4a), 163.99 (C-6), 179.57 (C-7). Anal. calcd for $C_{19}H_{18}O_4S$. Calcd (%): C: 66.65, H: 5.30. Found (%): C: 66.38, H: 5.23.

(±)-3,4-Dihydro-4-hydroxy-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (14a). This compound was prepared by an analogous procedure to **10a**. Yield: 73%. Mp 150–152 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.45 (s, 3H, 1×gem CH_3), 1.55 (s, 3H, 1×gem CH_3), 2.1 (m, 2H, H-3), 3.6 (br.s, 1H, D_2O exch., OH), 4.0 (s, 3H, OCH_3), 5.1 (td, 1H, $J=5$ Hz, 0.5 Hz, H-4), 6.8 (s, 1H, H-12), 7.4–7.5 (m, 2H, H-9, H-10), 7.55 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.5 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 27.77 (CH_3), 28.01 (CH_3), 39.99 (C-3), 60.13 (C-4), 62.07 (OCH_3), 77.62 (C-2), 109.05 (C-12), 115.77 (C-4a), 118.51 (C-5a), 125.04 (C-8), 126.17 (C-10), 129.67 (C-7), 131.13 (C-6a), 131.62 (C-9), 135.00 (C-10a), 138.36 (C-11a), 156.70 (C-12a), 163.00 (C-5), 179.64 (C-6). Anal. calcd for $C_{19}H_{18}O_4S$. Calcd (%): C: 66.65, H: 5.30. Found (%): C: 66.91, H: 5.47.

(±)-1-Acetoxy-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]thioxanthen-7-one (10b). This compound was prepared by an analogous procedure to **8b**. Yield: 94%. Mp 102–104 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.40 (s, 3H, 1×gem CH_3), 1.43 (s, 3H, 1×gem CH_3), 2.1 (s, 3H, $C\underline{H}_3CO$), 2.22 (dd, 1H, $J=15$ Hz, 4.5 Hz, H-2a), 2.28 (dd, 1H, $J=15$ Hz, 3 Hz, H-2b), 3.95 (s, 3H, OCH_3), 6.1 (dd, 1H, $J=4.5$ Hz, 3 Hz, H-1), 6.4 (s, 1H, H-5), 7.4 (td, 1H, $J=8$ Hz, 1.5 Hz, H-10), 7.5–7.65 (m, 3H, H-9, H-11), 8.4 (dd, 1H, $J=8$ Hz, 1 Hz, H-8). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 21.04 ($\underline{C}H_3CO$), 25.36(CH_3), 29.59 (CH_3), 38.67 (C-2), 56.31 ($\overline{O}CH_3$), 64.07 (C-1), 75.12 (C-3), 99.17 (C-5), 105.71 (C-12b), 114.62 (C-6a), 125.07 (C-9), 126.55 (C-11), 129.23 (C-8), 131.27 (C-10), 131.94 (C-7a), 133.89 (C-11a), 142.54 (C-12a), 158.13 (C-4a), 163.87 (C-6), 170.50 ($CH_3\underline{C}O$), 180.24 (C-7). Anal. calcd for $C_{21}H_{20}O_5S$. Calcd (%): C: 65.61, H: 5.24. Found (%): C: 65.44, H: 4.93.

(±)-4-Acetoxy-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]thioxanthen-6-one (14b). This compound was prepared by an analogous procedure to **8b**. Yield: 95%. Mp 138 °C (Et_2O–*n*-hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.40 (s, 3H, 1×gem CH_3), 1.45 (s, 3H, 1×gem CH_3), 2.1 (s, 3H, $C\underline{H}_3CO$), 2.13 (dd, 1H, $J=15$ Hz, 4 Hz, H-2a), 2.15 (dd, 1H, $J=15$ Hz, 3 Hz, H-2b), 3.9 (s, 3H, OCH_3), 6.2 (dd, 1H, $J=4$ Hz, 3 Hz, H-4), 6.8 (s, 1H, H-12), 7.3–7.45 (m, 3H, H-9, H-10), 7.5 (td, 1H, $J=8$ Hz, 0.5 Hz, H-8), 8.45 (dd, 1H, $J=8$ Hz, 0.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 21.88 ($\underline{C}H_3CO$), 25.75 (CH_3), 28.02 (CH_3), 38.67 (C-3), 61.57 (C-4), 62.42 (OCH_3), 78.00 (C-2), 109.09 (C-12), 110.17 (C-4a), 115.64 (C-5a), 124.98 (C-8), 126.17 (C-10), 129.59 (C-7), 131.27 (C-6a), 131.70 (C-9), 134.99 (C-10a), 139.05 (C-11a), 158.09 (C-12a), 162.74 (C-5), 171.20 ($CH_3\underline{C}O$), 179.57 (C-6). Anal. calcd for $C_{21}H_{20}O_5S$. Calcd (%): C: 65.61, H: 5.24. Found (%): C: 65.57, H: 5.25.

Cellular pharmacology. Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD, USA) 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). The cytotoxicity was measured by the microculture tetrazolium assay essentially as described.[14] 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.

NMR spectra

These were performed on a Bruker DRX 400 spectrometer at 400.13 and 100.6 for ^{1}H and ^{13}C experiments, respectively, equipped with an inverse 5 mm broadband probe and B_0 gradients. All spectra were recorded using 5 mg of each product dissolved in 0.6 mL of $CDCl_3$ in a 5 mm tube at ambient temperature (20 °C) unless specially noted. Concentration studies were performed by diluting the initial solution by a factor of 5, three consecutive times.

The 2-D experiments were carried out with the following parameters: (a) COSY spectral width 4000 Hz in both dimensions, 16 transients for each FID; 512 t_2 points, 256 t_1 increments; recycling delay 2 s; (b) The phase-sensitive NOESY was obtained with time-proportional phase incrementation (TPPI), $t_m = 1.5$ s, a recycling delay of 2 s; spectral with of 4000 Hz in both dimensions, 512 t_1 increments, a 512×512 data matrix and 32 transients for each FID; a $\pi/2$ shifted sine-squared weighting function was applied prior to Fourier transformation; (c) C–H correlation spectra (HMQC) was obtained using B_0 gradient pulses for selection of ^{1}H coupled to carbons. A sweep width of 10 ppm for ^{1}H and 200 ppm for ^{13}C was used with 128 FIDs in the t_1 domain and 1K data points in the t_2 domain, 32 transients for each t_1 increment and recycling delay 1.5 s. The HMBC experiment was performed using a low pass *J*-filter (3.4 ms) and a delay in order to observe the long range couplings (60 ms). As in the HMQC experiment, B_0 gradient pulses were applied in order to select ^{1}H coupled to ^{13}C nuclei, 128 transients with 128 increments in the t_1 domain and 1K data points in the t_2 domain.

Molecular calculations

These performed using the MM+ force field of the Hyperchem program.[15] The Polak-Ribiere (conjugate gradient) minimisation method with an energy convergence criterion of 0.01 *K*cal/mol was used for geometry optimisation.

Calculation of the H(1)–H(2) coupling constant has been performed using the equation:

$$J_{cal} = A\cos(2\Theta) + B\cos(\Theta) + C\sin(2\Theta) + D$$

according to Altona et al.[12] Parameters A, B, C, D for the different derivatives are summarized as following:

	8a	8b	9a	9b	10a	10b
A	4.042	4.106	4.373	4.405		4.781
B	−0.91	−0.91	−0.91	−0.91		−0.99
C	−0.031	−0.031	1.265	1.174		1.157
D	4.812	4.902	5.136	5.181		5.912

References and Notes

1. Horwitz, J. P.; Massova, I.; Wiese, T. E.; Besler, B. H.; Corbett, T. H. *J. Med. Chem.* **1994**, *37*, 781.
2. Hostettman, K.; Wagner, H. *Phytochemistry* **1977**, *16*, 821.
3. Banerji, A.; Deshpande, A. D.; Prabhu, B. R.; Pradhan, P. *J. Nat. Prod.* **1994**, *57*, 396.
4. Rosi, D.; Peruzzoti, G.; Dennis, E. W.; Berberian, D. A.; Freele, H.; Tullar, B.; Archer, S. *J. Med. Chem.* **1967**, *10*, 867.
5. Archer, S.; Pica-Mattoccia, L.; Cioli, D.; Seyed-Mozzaffari, A.; Zayed, A. H. *J. Med. Chem.* **1988**, *31*, 254.
6. Perni, R. B.; Wentland, M. P.; Huang, J. I.; Powles, R. G.; Aldous, S.; Klingbeil, K. M.; Peverly, A. D.; Robinson, R. G.; Corbett, T. H.; Jones, J. L.; Mattes, K. C.; Rake, J. B.; Coughlin, S. A. *J. Med. Chem.* **1998**, *41*, 3645.
7. Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Boyd, M.; Thomsen, L. L.; Zhuang, L.; Denny, W. A. *J. Med. Chem.* **1988**, *34*, 2864.
8. Habib, A. M.; Ho, D. K.; Masuda, S.; McCloud, T.; Reddy, K. S.; Abu-Shoer, M.; McKenzie, A.; Byrn, S. R.; Chang, C. J.; Cassady, J. M. *J. Org. Chem.* **1987**, *52*, 412.
9. Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Boder, G. B. *J. Pharm. Sci.* **1966**, *55*, 758.
10. Dorr, R. T.; Liddil, J. D.; Von Hoff, D. D.; Soble, M.; Osborne, C. K. *Cancer Res.* **1989**, *49*, 340.
11. Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pierre, A.; Guilbaud, N.; Leonce, S.; Kraus-Berthier, S.; Rolland, Y.; Atassi, G. *J. Med. Chem.* **1996**, *39*, 4762.
12. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
13. Mikros, E.; Mitaku, S.; Skaltsounis, A. L.; Libot, F.; Tillequin, F.; Koch, M. *Magn. Reson. Chem.* **1999**, *37*, 498.
14. Pierre, A.; Kraus-Berthier, L.; Atassi, G.; Cros, S.; Poupon, M. F.; Lavielle, G.; Berlion, M.; Bizzari, J. P. *Cancer Res.* **1991**, *51*, 2312.
15. HyperChem is developed and licensed from Hypercube Inc. The MM+ force field used in this software for molecular mechanics calculations is an extension of MM2. (Allinger, N.L. *J. Am. Chem Soc.*, **1977**, *99*, 8127, using the MM2(1991) parameters and atom types with the 1977 functional form.