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Xanthone Derivatives as Potential Anti-cancer Drugs

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Abstract

Xanthone derivatives have been shown to be potent inhibitors of tumour growth. Oxygenated xanthones and [3-(dialkylamino)-2-hydroxypropoxy]xanthones have been prepared and tested for in-vitro inhibition of human PLC/PRF/5, KB and 212 cells.

Structure-activity analysis indicated epoxidation of the hydroxyxanthone increased cytotoxicity against tumour cells but ring-opening of the epoxide group with dialkylamine did not enhance the anti-tumour activity. Further evaluation of three of the most active compounds 2, 6-, 3, 6-, and 3, 5-di(2,3-epoxypropoxy)xanthone (compounds **10a**, **11a**, and **12a**, respectively) in DNA, RNA and protein synthesis of tumour cells showed potent inhibitory activity. The 3,5-di(2,3-epoxypropoxy)xanthone also showed potent inhibitory activity against 212 cells, a Ha-*ras* oncogene-transformed NIH 3T3 cell line.

The results indicated that compounds **10a** and **12a** are potent anti-tumour agents which not only suppressed cellular DNA, RNA and protein synthesis but also specifically inhibited the Ha-*ras* oncogene in 212 cells.

Natural and synthetic xanthone derivatives have been shown to be potent inhibitors of the growth of human hepatoma PLC/PRF/5 and epidermoid carcinoma KB cells in-vitro (Liou et al 1993) and a novel series of diaminoanthraquinones showed potent inhibition of the in-vitro growth of tumour cells without cross-resistance to adriamycin (Jiang et al 1992). In a continuing study of the structure-activity relationships of various xanthone derivatives and their design as new chemotherapeutic agents with novel biochemical mechanisms for treating cancer, we have synthesized more oxygenated xanthones, xanthone epoxides and [3-(dialkylamino)-2-hydroxypropoxy]xanthones and studied the cytotoxic effects and structure-activity relationships of various synthetic xanthone derivatives against human PLC/PRF/5 and KB cells. Because 212 cells are designated as a Ha-*ras* oncogene transformed NIH 3T3 cell line and activated Ha-*ras* gene correlated with tumorigenesis (Liu et al 1992), we have also evaluated the anti-tumour activity of various synthetic xanthone derivatives against 212 cells in-vitro.

Experimental

General procedures

Melting points (uncorrected) were determined with a Yanaco Micro-Melting Point apparatus. IR spectra were determined with a Hitachi model 260-30 IR spectrophotometer. ^1H and ^{13}C NMR spectra [δ (ppm), J (Hz)] were determined with a Varian Gemini 200 MHz FT-NMR spectrometer. Mass spectra were determined with a Jeol JMS-D-100 mass spectrometer. Elemental analyses were within $\pm 0.4\%$ of the theoretical values, unless otherwise noted. The structure of 2,3-epoxypropoxyxanthones and 3-(dialkylamino)-2-hydroxypropoxyxanthones were identified by IR, MS, NMR and elemental analysis.

NMR spectra were assigned by comparison with those for parent hydroxyxanthones and data reported in the literature (Chaudhuri et al 1978; Frahm & Chaudhuri 1979; Biemann 1989; Liou et al 1993; Lin et al 1996). Compound **1** (Table 1) was obtained from Tokyo Kasei Kogyo Co. Ltd. Compounds **2**, **3**, **3a**, **4**, **5**, **6**, **7**, **7a**, **8**, **9**, **10**, **10a**, **11**, **11a**, **12**, **13**, **14**, **15** and **16** were synthesized and identified as described elsewhere (Liou et al 1993, 1994; Lin et al 1992, 1993, 1996).

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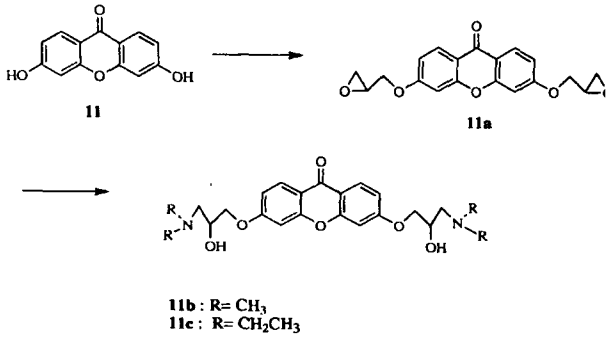
Synthesis

Epoxypropoxyxanthones and xanthonoxypropanolamines were synthesized by a method described elsewhere (Liou et al 1994). Briefly, appropriate hydroxyxanthones were reacted with one equivalent of NaOH in aqueous 2-propanol and an excess of epichlorohydrin to yield the appropriate epoxypropoxyxanthones. Ring-opening of the epoxides with various *N,N*-dialkylamines in THF afforded various xanthonoxypropanolamines. Scheme 1 depicts a typical example of the general synthesis of xanthonoxypropanolamines **11b** and **11c**.

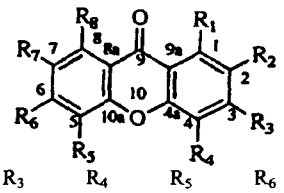


SCHEME 1. Synthesis of xanthonoxypropanolamines **11b** and **11c**.

2-(2,3-Epoxypropoxy)xanthone (2a)

To a solution of 0·13 g (2·32 mmol) of KOH in 0·5 mL water was added 10 mL 2-propanol and then 0·5 g (2·36 mmol) of **2**.

Table 1. Chemical data and cytotoxicity (ED50 values, $\mu g\ mL^{-1}$).



Cmpd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	mp (°C)	Cell line*		
										PLC/PRF/5	KB	212
1[a]	H	H	H	H	H	H	H	H	175	NS	5·85	NS
2	H	OH	H	H	H	H	H	H	189–190	NS	7·90	NS
2a	H	[b]	H	H	H	H	H	H	123–124	1·81	0·22	NS
2b	H	[c]	H	H	H	H	H	H	128–130	NS	7·34	6·90
3	H	H	OH	H	H	H	H	H	241–242[‡]	3·75[†]	0·77[†]	NS
3a	H	H	[b]	H	H	H	H	H	157–158[‡]	1·43[†]	0·85[†]	1·31
3b	H	H	[c]	H	H	H	H	H	95–97	NS	6·87	NS
4	H	OH	OH	H	H	H	H	H	293–295[‡]	NS	NS	6·00
5	H	H	OH	OH	H	H	H	H	238–240[‡]	NS	NS	5·35
6	OH	H	OH	H	H	H	H	H	257–258[‡]	6·95	7·69	6·78
6a	OH	H	[b]	H	H	H	H	H	166–167	1·75	0·42	0·32
6b	OH	H	[c]	H	H	H	H	H	197–198	6·46	1·57	3·72
7	OH	H	H	H	H	OH	H	H	242–243[‡]	NS	9·63	NS
7a	OH	H	H	H	H	[b]	H	H	153–154[†]	3·20	0·81	0·41
7b	OH	H	H	H	H	[c]	H	H	189–190	NS	2·91	3·24
8	OH	H	H	H	H	H	OH	H	194–195	NS	NS	NS
9	H	OH	H	H	OH	H	H	H	224–225	6·60	5·25	6·97
10	H	OH	H	H	H	OH	H	H	> 300[‡]	7·94	7·75	NS
10a	H	[b]	H	H	H	[b]	H	H	170–171[†]	0·23[†]	0·0043[†]	0·031[¶]
11	H	H	OH	H	H	OH	H	H	> 300[‡]	7·04	3·91	NS
11a	H	H	[b]	H	H	[b]	H	H	187–188[†]	0·24[†]	0·11[†]	NS
11b	H	H	[c]	H	H	[c]	H	H	219–220	1·00	0·25	1·49
11c	H	H	[d]	H	H	[d]	H	H	waxy	1·88	0·43	5·59
12	H	H	OH	H	OH	H	H	H	> 300[‡]	3·66	3·54	3·34
12a	H	H	[b]	H	[b]	H	H	H	156–157	0·066	0·0049	0·056
13	OH	H	OH	H	H	H	OH	H	246–247	NS	NS	NS
13a	OH	H	[b]	H	H	H	[b]	H	164–165	0·10	0·35	NS
14	OH	H	OH	H	H	H	H	OH	210–211	NS	NS	NS
14a	OH	H	[b]	H	H	H	H	OH	166–167	1·28	0·69	NS
14b	OH	H	[c]	H	H	H	H	OH	197–198	5·29	0·78	0·11
15	H	H	OH	OH	H	OH	OH	H	> 300[§]	NS[†]	NS[†]	NS
16	OH	H	OH	H	H	OH	OH	H	> 300[§]	NS[†]	NS[†]	6·24
Cisplatin										5·29	0·16	1·27

[a] Obtained from Tokyo Kasei Kogyo Co., Ltd. [b] 2,3-epoxypropoxy-. [c] (3-dimethylamino-2-hydroxy)propoxy-. [d] (3-diethylamino-2-hydroxy)propoxy-. * NS, no significant activity; for significant activity of the pure compound, ED50 $< 4\ \mu g\ mL^{-1}$, n = 8. [†] Data from Liou et al (1993). [‡] Data from Lin et al (1993). [§] Data from Lim et al (1992).
[¶] Our unpublished data.

Epichlorohydrin (1·88 mL, 23·43 mmol) was added to the above mixture, which was then heated at 70°C for 3 h with stirring. The hot reaction mixture was filtered to remove a dimeric by-product (a glycidyl ether). The filtrate was concentrated under reduced pressure at 50–60°C. The semi-solid residue was heated under reflux with 2-propanol (10 mL) and more of the dimer was filtered from the mixture. The clear filtrate, on cooling, yielded a solid. This was collected, washed with 3 mL 2-propanol, and air-dried to yield a tan-coloured product, which was purified by column chromatography (silica gel-$CHCl_3$) and crystallized from methanol to give a white powder **2a** (0·47 g, 1·77 mmol, 75%); MS, m/z (%) 268 (100, M^+); IR (KBr) 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ2·79 (dd, J = 4·8, 2·7 Hz, 1H, CH_2 in the epoxide ring), 2·92 (t, J = 4·8 Hz, 1H, CH_2 in the epoxide ring), 3·40 (m, 1H, CH in the epoxide ring), 3·98 (dd, J = 11, 6 Hz, 1H, OC*H*H), 4·37 (dd, J = 11, 2.7 Hz, 1H, OCH*H*), 7·36 (m, 4H, aromatic proton), 7·68 (m, 2H, aromatic proton), 8·29 (dd, J = 8, 1·8 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ44·5 (CH_2 in the epoxide ring), 49·9 (CH in the epoxide ring), 69·3 (OCH_2), 106·7 (C-1), 115·1 (C-9a), 117·9 (C-5), 119·5 (C-4), 122·0 (C-8a), 123·7 (C-7), 125·2 (C-3), 126·6 (C-8), 134·6 (C-6), 151·1 (C-4a), 154·8 (C-2), 156·1 (C-10a), 176·9 (CO); Anal ($C_{16}H_{12}O_4$) C, H.

2-[(3-dimethylamino-2-hydroxy)propoxy]xanthone hydrochloride salt (2b)

Compound **2a** (0·2 g, 0·75 mmol) in THF (25 mL) was treated with 40% aqueous dimethylamine (4 mL, 35·5 mmol) under an argon atmosphere and thoroughly stirred at room temperature. After 24 h, the solvent and the excess reagent were removed under reduced pressure. The residue was purified by column chromatography ($CHCl_3$-methanol, 4 : 1) to give the free base of **2b** as a yellow oil. This was dissolved in 20% HCl-ethyl acetate solution and yielded a colourless powder **2b** (0·15 g, 0·43 mmol, 57%) on standing; MS, m/z (%) 313 (3, M^+); IR (KBr) 3340, 3270, 1680, 1660 cm^{-1}; ^1H NMR (CD_3OD): δ 3.00 (s, 6H, 2 × CH3), 3·40 (d, J = 6·9 Hz, 2H, $-CH_2-N(CH_3)_2$), 4·14 (d, J = 4·9 Hz, 2H, $-OCH_2-$), 4·43 (m, 1H, $-CHOH-$), 7·40–7·59 (m, 4H, aromatic proton), 7·67 (m, 1H, aromatic proton), 8·24 (dd, J = 8·0, 1·7 Hz, 1H, H-8); ^{13}C NMR (CD_3OD): δ 44·2

(2 × CH_3), 61·1 (−CH_2−$N(CH_3)_2$), 65·6 (−CHOH), 72·0 (OCH_2), 108·3 (C-1), 119·6 (C-5), 121·3 (C-4), 122·3 (C-9a), 123·3 (C-8a), 125·6 (C-7), 126·6 (C-3), 127·5 (C-8), 136·8 (C-6), 153·0 (C-4a), 156·7 (C-2), 157·9 (C-10a), 178·9 (CO); Anal ($C_{18}H_{19}O_4N$.HCl) C, H, N.

3-[(3-Dimethylamino-2-hydroxy)propoxy]xanthone hydrochloride salt (3b)

Compound **3a** (2·0 g, 7·5 mmol) was treated as for **2b** to yield a colourless powder (ethyl acetate) **3b** (2·2 g, 4·9 mmol, 65·3%); MS, m/z (%) 314 (18, M^+); IR (KBr) 3350, 1630, 1610 cm^{-1}; ^1H NMR (CD_3OD): δ 2·99 (s, 6H, 2 × CH_3), 3·40 (d, J = 7·8 Hz, 2H, −CH_2−$N(CH_3)_2$), 4·21 (d, J = 4·8 Hz, 2H, −OCH_2−), 4·45 (m, 1H, −CHOH−), 7·09 (dd, J = 8·7, 2·0 Hz, 1H, H-2), 7·13 (d, J = 2·0 Hz, 1H, H-4), 7·40–7.59 (m, 2H, H-6 and H-7), 7·82 (m, 1H, H-5), 8·18 (d, J = 8·7 Hz, 1H, H-1), 8·24 (dd, J = 8·7, 2·0 Hz, 1H, H-8); ^{13}C NMR (CD_3OD): δ 42·6 (CH_3), 45·6 (CH_3), 60·6 (−CH_2−$N(CH_3)_2$), 65·1 (−CHOH), 71·6 (OCH_2), 102·1 (C-4), 114·7 (C-2), 116·5 (C-9a), 118·9 (C-5), 122·4 (C-8a), 125·2 (C-7), 126·9 (C-8), 128·7 (C-1), 136·0 (C-6), 157·3 (C-10a), 159·0 (C-4a), 165·4 (C-3), 177·6 (CO); Anal ($C_{18}H_{19}O_4N$.HCl) C, H, N.

3-(2,3-Epoxypropoxy)-1-hydroxyxanthone (6a)

Compound **6** (0·5 g, 2·19 mmol) was treated as for **2a** to yield colourless needles ($CHCl_3$) of **6a** (0·51 g, 1·80 mmol, 82%); MS, m/z (%) 284 (100, M^+); IR (KBr) 3400, 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ2·81 (dd, J = 4·8, 2·6 Hz, 1H, CH_2 in the epoxide ring), 2·95 (t, J = 4·8 Hz, 1H, CH_2 in the epoxide ring), 3·40 (m, 1H, CH in the epoxide ring), 4·02 (dd, J = 11, 5·9 Hz, 1H, OC*H*H), 4·38 (dd, J = 11·0, 5·9 Hz, 1H, OCH*H*), 6·37 (d, J = 2·3 Hz, 1H, H-2), 6·48 (d, J = 2·3 Hz, 1H, H-4), 7·34–7·46 (m, 2H, H-6 and H-7), 7·72 (m, 1H, H-5), 8·27 (dd, J = 7·9, 1·8 Hz, 1H, H-8), 12·9 (s, 1H, 1 -OH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$): δ 44·6 (CH_2 in the epoxide ring), 49·7 (CH in the epoxide ring), 69·2 (OCH_2), 93·4 (C-4), 97·5 (C-2), 104·2 (C-9a), 117·6 (C-5), 120·6 (C-8a), 124·1 (C-7), 125·9 (C-8), 135·1 (C-6), 156·0 (C-10a), 157·7 (C-4a), 163·6 (C-1), 165·4 (C-3), 180·9 (CO); Anal ($C_{16}H_{12}O_5$) C, H.

3-[(3-Dimethylamino-2-hydroxy)propoxy]-1-hydroxyxanthone hydrochloride salt (6b)

Compound **6a** (0·2 g, 0·70 mmol) was treated as for **2b** to yield a colourless powder (ethyl acetate) **6b** (0·19 g, 0·53 mmol, 75%); MS, m/z (%) 329 (3) (M^+); IR (KBr) 3340, 3250, 1690, 1640, 1610 cm^{-1}; ^1H NMR (CD_3OD): δ 2·96 (s, 6H, 2 × CH_3), 3·33 (d, J = 6·7 Hz, 2H, −CH_2−$N(CH_3)_2$), 4·12 (d, J = 4·9 Hz, 2H, −OCH_2−), 4·40 (m, 1H, −CHOH−), 6·37 (d, J = 2·3 Hz, 1H, H-2), 6·55 (d, J = 2·3 Hz, 1H, H-4), 7·44 (m, 2H, H-6 and H-7), 7·80 (m, 1H, H-5), 7·88 (dd, J = 7·9, 1·8 Hz, 1H, H-8); ^{13}C NMR (CD_3OD): δ 44·2 (CH_3), 60·9 (−CH_2−$N(CH_3)_2$), 65·4 (−CHOH), 71·9 (OCH_2), 94·6 (C-4), 98·8 (C-2), 105·3 (C-9a), 119·1 (C-5), 121·9 (C-8a), 125·6 (C-7), 126·9 (C-8), 137·0 (C-6), 157·7 (C-10a), 159·5 (C-4a), 164·9 (C-1), 167·4 (C-3), 182·4 (CO); Anal ($C_{18}H_{19}O_5N$.HCl) C, H, N.

6-[(3-Dimethylamino-2-hydroxy)propoxy]-1-hydroxyxanthone hydrochloride salt (7b)

Compound **7a** (0·2 g, 0·70 mmol) was treated as for **2b** to yield a colourless powder (ethyl acetate) **7b** (0·18 g, 0·50 mmol, 72%); MS, m/z (%) 329 (0·4, M^+); IR (KBr) 3340, 3330, 1650, 1620 cm^{-1}; ^1H NMR (CD_3OD): δ 2·98 (s, 6H, 2 × CH_3), 3·37 (d, J = 6·9 Hz, 2H, −CH_2−$N(CH_3)_2$), 4·18 (d, J = 4·9 Hz, 2H, −OCH_2−), 4·43 (m, 1H, −CHOH−), 6·76 (dd, J = 8·8, 1·0 Hz, 1H, H-2), 6·94 (dd, J = 8·8, 1·0 Hz, 1H, H-4), 7·02–7·08 (m, 2H, H-5 and H-7), 7·62 (t, J = 8·4 Hz, 1H, H-3), 8·13 (d, J = 9·5 Hz, 1H, H-8); ^{13}C NMR (CD_3OD): δ 44·3 (CH_3), 60·9 (−CH_2−$N(CH_3)_2$), 65·4 (−CHOH), 72·0 (−OCH_2−), 102·5 (C-5), 108·3 (C-4), 109·9 (C-9a), 111·7 (C-2), 115·2 (C-7), 116·0 (C-8a), 128·7 (C-8), 138·1 (C-3), 158·1 (C-4a), 159·8 (C-10a), 163·3 (C-1), 166·4 (C-6), 182·9 (CO); Anal ($C_{18}H_{19}O_5N.\frac{1}{2}H_2O$.HCl) C, H, N.

3,6-Di[(3-dimethylamino-2-hydroxy)propoxy]xanthone dihydrochloride salt (11b)

Compound **11a** (0·2 g, 0·59 mmol) was treated as for **2b** to yield a colourless powder (ethyl acetate) **11b** (0·12 g, 0·25 mmol, 42%); MS, m/z (%) 429 (0·3, $(M-1)^+$); IR (KBr) 3350, 3320, 1650, 1610 cm^{-1}; ^1H NMR (CD_3OD): δ 2·97 (s, 12H, 4 × CH_3), 3·37 (d, J = 7·0 Hz, 4H, 2 × −CH_2−$N(CH_3)_2$), 4·19 (d, J = 4·8 Hz, 4H, 2 × −OCH_2−), 4·32 (m, 2H, 2 × −CHOH−), 7·06 (d, J = 1·4 Hz, 2H, H-4 and H-5), 7·09 (dd, J = 7·8, 1·4 Hz, 2H, H-2 and H-7), 8·17 (d, J = 7·8 Hz, 2H, H-1 and H-8); ^{13}C NMR (CD_3OD): δ 44·3 (4 × CH_3), 60·9 (2 × −CH_2−$N(CH_3)_2$), 65·5 (2 × −CHOH), 72·0 (2 × −OCH_2−), 102·7 (C-4 and C-5), 115·0 (C-2 and C-7), 117·1 (C-8a and C-9a), 129·2 (C-1 and C-8), 159·8 (C-4a and C-10a), 165·8 (C-3 and C-6), 177·5 (CO); Anal ($C_{23}H_{30}O_6N_2$.2HCl) C, calcd: 54·96, found: 52·81, H, N.

3,6-Di[(3-diethylamino-2-hydroxy)propoxy]xanthone dihydrochloride salt (11c)

Compound **11a** (0·4 g, 1·18 mmol) in THF (50 mL) was reacted with diethylamine (8·34 mL, 80 mmol) and treated as for **2b** to yield a colourless powder (ethyl acetate) **11c** (0·056 g, 0·12 mmol, 10%); MS, m/z (%) 458 (0·4, M^+); IR (KBr) 3350, 3320, 1640, 1610 cm^{-1}; ^1H NMR (CD_3OD): δ 1·39 (t, J = 7·3 Hz, 12H, 4 × CH_3), 3·36 (m, 12H, 6 × −CH_2−N<), 4·21 (d, J = 5·0 Hz, 4H, 2 × −OCH_2−), 4·46 (m, 2H, 2 × −CHOH−), 7·07 (m, 4H, aromatic protons), 8·14 (d, J = 9·5 Hz, 2H, H-1 and H-8). Combustion analysis was not performed because of the extremely hygroscopic nature of this compound.

3,5-Di(2,3-epoxypropoxy)xanthone (12a)

Compound **12** (0·5 g, 2·19 mmol) was treated as for **2a** to yield colourless needles (methanol) **12a** (0·42 g, 1·24 mmol, 57%); MS, m/z (%) 340 (25, M^+); IR (KBr) 1660, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2·81 (dd, J = 4·8, 2·6 Hz, 1H, C*H*H in the epoxide ring), 2·86 (dd, J = 4·8, 2·6 Hz, 1H, CH*H* in the epoxide ring), 2·95 (d, J = 4·2 Hz, 1H, C*H*H in the epoxide ring), 2·98 (d, J = 4·2 Hz, 1H, CH*H* in the epoxide ring), 3·39–3·54 (m, 2H, 2 × CH in the epoxide ring), 4·00–4·15 (m, 2H, OCH_2), 4·39 (dd, J = 11·0, 2·9 Hz, 1H, OC*H*H), 4·48 (dd, J = 11·0, 2·9 Hz, 1H, OCH*H*), 6·98 (dd, J = 9·2, 2·4 Hz, 1H, H-2), 6·99 (d, J = 2·4 Hz, 1H, H-4), 7·25–7·28 (m, 2H, H-6 and H-7), 7·92 (m, 1H, H-8), 8·24 (d, J = 9·2 Hz, 1H, H-1); ^{13}C NMR ($CDCl_3$): δ 44·5, 44·6 (2 × CH_2 in the epoxide ring), 49·7, 50·1 (2 × CH in the epoxide ring), 69·3, 70·5 (2 × OCH_2 in the epoxide ring), 101·1 (C-4), 114·0 (C-2), 116·0 (C-9a), 117·2 (C-8), 118·6 (C-6), 123·0 (C-8a), 128·3 (C-1), 146·8 (C-10a), 147·4 (C-5), 157·7 (C-4a), 163·8 (C-3), 176·1 (CO); Anal ($C_{19}H_{16}O_6.\frac{1}{2}H_2O$) C, H.

3,7-Di(2,3-epoxypropoxy)-1-hydroxyxanthone (13a)
Compound **13** (1·3 g, 5·33 mmol) was treated as for **2a** to yield pale yellow powder (methanol) **13a** (0·80 g, 2·24 mmol, 42%); MS, m/z (%) 356 (100) (M^+); IR (KBr) 3400, 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2·80 (m, 2H, CH_2 in the epoxide ring), 2·95 (t, J = 4·58 Hz, CH_2 in the epoxide ring), 3·40 (m, 2H, 2 × CH in the epoxide ring), 3·97 (dd, J = 6·0, 2·7 Hz, 1H, OC*H*H), 4·02 (dd, J = 6·0, 2·7 Hz, 1H, OCH*H*), 4·37 (dt, J = 11, 2·7, OCH_2), 6·35 (d, J = 2·3 Hz, 1H, H-2), 6·44 (d, J = 2·3 Hz, 1H, H-4), 7·36 (m, 2H, H-5 and H-6), 7·59 (d, J = 2·3, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 44·5, 44·6 (2 × CH_2 in the epoxide ring), 49·7, 49·9 (2 × CH in the epoxide ring), 69·2, 69·4 (2 × OCH_2), 93·1 (C-4), 97·4 (C-2), 103·9 (C-9a), 106·2 (C-8), 119·1 (C-5), 120·8 (C-8a), 125·2 (C-6), 151·0 (C-10a), 154·9 (C-7), 157·6 (C-4a), 163·3 (C-1), 165·3 (C-3), 180·4 (CO); Anal ($C_{19}H_{16}O_7$) C, calcd: 64·03, found: 63·30, H

3-(2,3-Epoxypropoxy)-1,8-dihydroxyxanthone (14a)
Compound **14** (0·5 g, 2·05 mmol) was treated as for **2a** to yield colourless needles (methanol) **14a** (0·36 g, 1·19 mmol, 58%); MS, m/z (%) 300 (100) (M^+); IR (KBr) 3400, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2·78 (dd, J = 4·8, 2·7 Hz, 1H, CH*H* in the epoxide ring), 2·95 (t, J = 4·8 Hz, 1H, C*H*H in the epoxide ring), 3·39 (m, 1H, CH in the epoxide ring), 3·99 (dd, J = 11, 6·0 Hz, 1H, OCH*H*), 4·34 (dd, J = 11, 2·8 Hz, 1H, OC*H*H), 6·33 (d, J = 2·3 Hz, 1H, H-2), 6·41 (d, J = 2·3 Hz, 1H, H-4), 6·76 (dd, J = 8·4, 1·0 Hz, 1H, H-5), 6·85 (dd, J = 8·4, 1·0 Hz, 1H, H-7), 7·55 (t, J = 8·4 Hz, 1H, H-6), 11·86, 11·96 (s, 2 × OH, C_1–OH and C_8–OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 44·5 (CH_2), 49·6 (CH), 69·3 (OCH_2), 93·6 (C-4), 97·8 (C-2), 102·9 (C-9a), 106·9 (C-8a), 107·5 (C-5), 110·9 (C-7), 136·8 (C-6), 156·1 (C-10a), 157·7 (C-4a), 161·3 (C-8), 162·9 (C-1), 165·9 (C-3), 184·6 (CO); Anal ($C_{16}H_{12}O_6$) C, H.

3-[(3-Dimethylamino-2-hydroxy)propoxy]-1,8-dihydroxyxanthone hydrochloride salt (14b)
Compound **14a** (0·2 g, 0·67 mmol) was treated as for **2b** to yield pale yellow powder (ethyl acetate) **14b** (0·19 g, 0·50 mmol, 75%); MS, m/z (%) 345 (0·23, M^+); IR (KBr) 3450, 3330, 1660, 1640 cm^{-1}; ^1H NMR (CD_3OD): δ 2·97, 3·00 (2s, 6H, 2 × CH_3), 3·37 (d, J = 6·4 Hz, 2H, NCH_2), 4·12 (d, J = 4·4 Hz, 2H, OCH_2), 4·40 (m, 1H, CHOH), 6·36 (d, J = 1·8 Hz, 1H, H-2), 6·53 (d, J = 1·8 Hz, 1H, H-4), 6·74 (d, J = 8·4 Hz, 1H, H-5), 6·89 (d, J = 8·4 Hz, 1H, H-7), 7·62 (d, J = 1·8 Hz, 1H, H-6); ^{13}C NMR (CD_3OD): δ 42·6, 45·9 (2 × CH_3), 60·8 (NCH_2), 65·3 (CHOH), 71·9 (OCH_2), 94·8 (C-4), 99·2 (C-2), 104·1 (C-9a), 108·3 (C-8a), 108·7 (C-5), 112·1 (C-7), 138·6 (C-6), 157·8 (C-10a), 159·5 (C-4a), 162·6 (C-8), 164·3 (C-1), 167·9 (C-3), 186·2 (CO); Anal ($C_{18}H_{19}O_5N.3/2H_2O$) C, H, N.

Tumour cell growth inhibition
The microassay for anticellular effect was performed as described previously (Ito 1984; Lin et al 1991). The ED50 values were calculated from a semi-log plot of drug concentration against the percentage of viable cells on day 4.

PLC/PRF/5 cells were established from human hepatoma and are known to produce HBs Ag continuously in culture fluids (Nakajima et al 1982). Human hepatoma PLC/PRF/5 and epidermoid carcinoma KB cells, purchased from American Type Cell Collection, were maintained in Dulbecco's modified Eagles medium (DMEM, Gibco, BRL, Grand Island, NY, USA) containing 10% foetal bovine serum (FBS, Gibco, BRL), 2 mM L-glutamine, 100 units mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin. The 212 cells (a NIH 313 derivative, transformed by Ha-*ras* oncogene) were maintained in minimum essential alpha medium (MEM, Gibco, BRL), containing 10% calf serum (Gibco, BRL) and antibiotics (Liu et al 1992). For microassay, the growth medium was supplemented with 10 mM HEPES buffer pH 7·3 and incubated at 37°C in a CO_2 incubator.

Inhibition of macromolecular synthesis
The synthesis of macromolecules was measured by the incorporation of [^{3}H]thymidine (6·7 Ci $mmol^{-1}$), [^{3}H]uridine (25·9 Ci $mmol^{-1}$) or [^{3}H]methionine (194·9 mCi $mmol^{-1}$, New England Nuclear, Du Pont, Boston, MA, USA) into the tumour cells as described previously (Bhuyan et al 1982). Briefly, the tumour cells (1–1.5 × 10^4/100 μL/well) maintained in growth medium with or without drugs in microplates (Nunc) were incubated at 37°C. After pulsed labelling with [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]methionine for 18 h at day 4 post-treatment, the cells were harvested and loaded onto glass filter paper (Skatron, VA, USA). The incorporation activities of the cells were measured by liquid scintillation counting (LS-5000 TA, Beckman CA, USA). All treatments were conducted in quadruplicate and the mean values were used for analysis. Each experiment was repeated at least three times. Data shown are mean counts $min^{-1} \pm$ s.e.m. Percent inhibition of [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]methionine incorporation was calculated as follows:

$$\% \text{ inhibition} = (1 - \text{test activity/control activity}) \times 100$$

Results and Discussion

Several natural prenylflavonoids and synthetic xanthone derivatives have been shown to be potent inhibitors of human PLC/PRF/5 and KB cells in-vitro (Liou et al 1993) and members of a novel series of diaminoanthraquinones were found to be protein kinase C inhibitors with potent tumour cell growth inhibitory activity (Jiang et al 1992). As a continuation of the evaluation of xanthone derivatives as anti-tumour agents and study of structure–activity relationships, the inhibitory activity of several compounds against human PLC/PRF/5 and KB cells has been studied in-vitro. The structures of the compounds and the results obtained are listed in Table 1. Compounds **2a**, **6a**, **11b**, **11c**, **12a**, **13a** and **14a** showed strong cytotoxic activity against hepatoma PLC/PRF/5 and KB cells in-vitro, and compounds **6b**, **7a** and **14b** showed strong cytotoxic activity against KB cells in-vitro. As shown in Table 1 hydroxylation of **2** at C-3 (**4**), C-5 (**9**) and C-6 (**10**) or **3** at C-1 (**6**), C-4 (**5**), C-5 (**12**), C-6 (**11**), C-1 and C-7 (**13**), and C-1 and C-8 (**14**) did not enhance in-vitro cytotoxic activity against human PLC/PRF/5 and KB cells. Epoxidation of hydroxyxanthones markedly increased the in-vitro cytotoxic activity against human hepatoma PLC/PRF/5 and KB cells; ring-opening of the epoxides with dimethylamine or diethylamine did not. As shown in Table 1 compounds **10a** and **12a** showed the most potent in-vitro cytotoxic activity against human PLC/PRF/5 and KB cells and 3-oxygenated xanthones showed selective inhibitory activity against human PLC/PRF/5 cells.

The anticancer activity of the xanthone derivatives was also

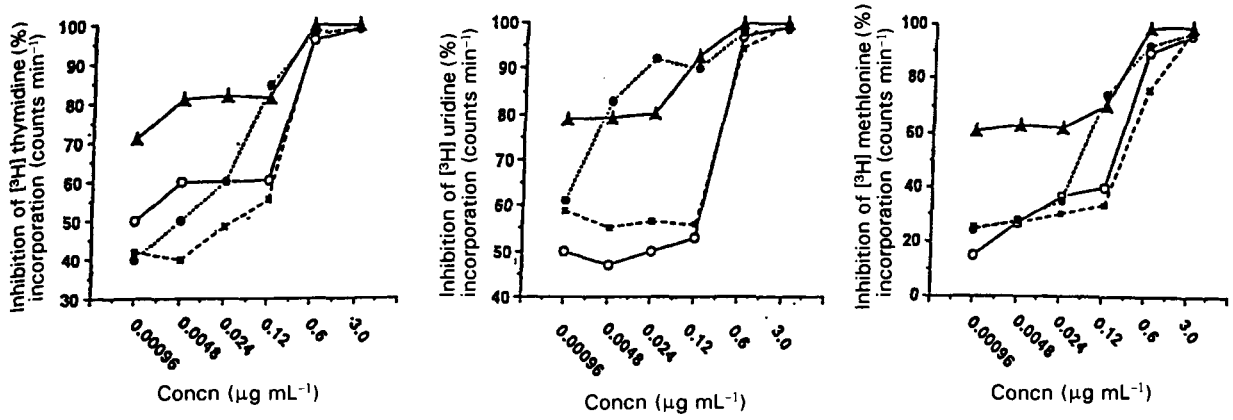


FIG. 1. Inhibitory effect of different concentrations of **12a** on the macromolecular synthesis of human PLC/PRF/5 cells. The cells were grown in the medium with the appropriate concentration of **12a** for 1, 2, 3 or 4 days before addition of A, [^{3}H]thymidine; B, [^{3}H]uridine; or C, [^{3}H]methionine to the medium. The cells were harvested 18 h later and incorporation measured as described in the experimental section. The values are expressed as the mean ± s.e. from three separate experiments. Day 1 (■); day 2 (○); day 3 (●); day 4 (▲).

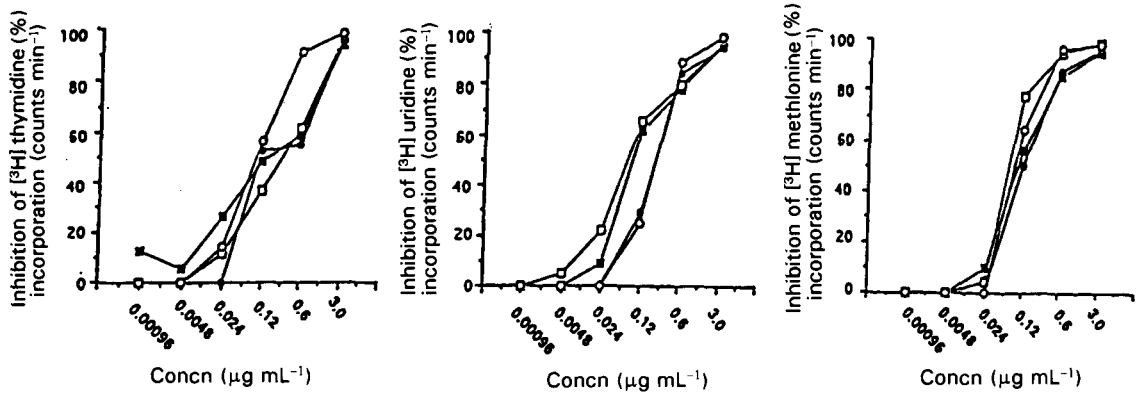


FIG. 2. Inhibitory effect of different concentrations of **12a** on the macromolecular synthesis of KB cells. The cells were grown in the medium with the desired concentrations of **12a** for 1, 2, 3 or 4 days before addition of A, [^{3}H]thymidine; B, [^{3}H]uridine; or C, [^{3}H]methionine to the medium. The cells were harvested 18 h later and incorporation measured as described in the experimental section. The values are expressed as the mean ± s.e. from three separate experiments. Day 1 (■); day 2 (○); day 3 (●); day 4 (▲).

studied by evaluation of their inhibitory activity against 212 cells, a derivative of the mouse fibroblast NIH 3T3 cell containing the oncogenic Ha-*ras* transgene (Liu et al 1992). As shown in Table 1, compounds **3a**, **6a**, **7a**, **10a**, **11b**, **12a** and **14b** showed potent inhibitory activity against 212 cells and the anti-tumour activity of these compounds against proliferation of these cells was comparable with that against proliferation of human PLC/PRF/5 or KB cells.

Cisplatin was used in this study as a positive control. Cisplatin showed potent in-vitro anti-tumour activity against KB and 212 cells but marginal activity against human PLC/PRF/5 cells.

The effects of **10a**, **11a**, and **12a** on macromolecular synthesis were determined by measuring the isotopic incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]methionine in human PLC/PRF/5 and KB cells. Figs 1 and 2 indicate that low concentrations ($9{\cdot}6 \times 10^{-4}$ μg mL^{-1} to 0·12 μg mL^{-1}) of **12a** (data for **10a** and **11a** not shown) have a time-dependent effect on the synthesis of DNA, RNA and protein in human PLC/PRF/5 cells, but not in KB cells. It is also clearly apparent that inhibition of macromolecular biosynthesis was correlated with the concentration and the time of drug treatment in PLC/PRF/5 cells but with concentration only for KB cells.

These results indicated that **10a** and **12a** are potent anti-tumour agents which not only suppressed cellular DNA, RNA and protein synthesis but also specifically inhibited the Ha-*ras* oncogene in 212 cells.

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