acetonitrile in 0.025 M pH 7.5 sodium phosphate buffer. The appropriate fractions were acidified to pH 2.9 to precipitate product 13, which was filtered, washed with water, and dried under reduced pressure: NMR (Me_2SO-d_6) δ 1.20 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.67 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.58 and 3.70 (2 d, 2 H, J = 18 Hz, CH_2S), 3.77 and 3.83 (2 d, 2 H, J = 20 Hz, CH_2CN), 3.82 (m, 1 H, CH), 4.75 and 5.10 (2 d, 2 H, J = 13 Hz, CH_2O), 5.14 (d, 1 H, J = 5 Hz, CH), 5.72 (dd, 1 H, J = 5 and 8 Hz, CH), 7.60 (d, 1 H, J = 8 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.31 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1782, 1722, 1702, 1665 cm^{-1}; MS m/z 655 $(M + H)^+$.

[6*R*-[6α,7β(*R*)]]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (14). Using procedures similar to those used to prepare **15**, **5** was acylated with (*R*)-α-[[(4-ethyl-2,3-dioxopiperazinyl)carbonyl]amino]benzeneacetic acid by the DCC–NHBT method to obtain **14**: NMR (Me_2SO-d_6) δ 1.07 (t, 3 H, J = 7 Hz, Me), 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.25 and 3.65 (2 m, $N[CH_2CH_2]_2N$, CH_2S, and HOD), 3.82 (m, 1 H, CH), 3.91 (q, 2 H, CH_2, J = 7 Hz), 4.72 and 5.04 (2 d, 2 H, J = 13 Hz, CH_2O), 5.02 (d, 1 H, J = 5 Hz, CH), 5.65 (d, 1 H, J = 7 Hz, CH), 5.76 (dd, 1 H, J = 5 and 8 Hz, CH), 7.30–7.47 (2 m, 5 H, Ph), 7.59 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.47 (d, 1 H, J = 8 Hz, NH) 9.86 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1783, 1712, 1690, 1628 cm^{-1}; MS m/z 889 $(M + H)^+$.

[6*R*-[6α,7β(R)]]-3-[[[4-[(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-[(hydroxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (15). A mixture of 310 mg (2.04 mmol) of (*R*)-(−)-mandelic acid, 20 mL of THF, 276 mg (2.04 mmol) of 1-hydroxybenzotriazole (NHBT), and 462 mg (2.24 mmol) of 1,3-dicyclohexylcarbodiimide (DCC) was stirred for 2 h and filtered. The filtrate was added at 0 °C to a solution prepared from 1.10 g (1.57 mmol) of **5**, 4 mL of THF, 14 mL of water, and 396 mg (4.71 mmol) of sodium bicarbonate. The mixture was stirred cold for 20 min and at room temperature for 4 h. Under reduced pressure, the THF was evaporated. The remaining aqueous mixture was adjusted to pH 7.5 with aqueous sodium bicarbonate and washed with ethyl acetate. The aqueous solution was concentrated slightly under reduced pressure to eliminate traces of organic solvents, treated with activated charcoal, and filtered. On acidification to pH 2.9 with 1 N HCl, a precipitate formed. A portion of the crude product obtained by filtration was redissolved at pH 7.5 and purified by chromatography on C_{18}-silica, eluting with a stepwise gradient of 0–50% acetonitrile in 0.025 M pH 7.5 sodium phosphate buffer. The appropriate fractions were combined and acidified to pH 2.90 to precipitate the product. After filtering, washing with water, and drying under reduced pressure, **15** was obtained in 18% yield: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.60 (2 m, $N[CH_2CH_2]_2N$, half of CH_2S, and HOD), 3.53 (d, 1 H, J = 18 Hz, half of CH_2S), 3.82 (m, 1 H, CH), 4.73 and 5.06 (2 d, 2 H, J = 14 Hz, CH_2O), 5.08 (d, 1 H, J = 5 Hz, CH), 5.12 (d, 1 H, J = 5 Hz, CH), 5.72 (dd, 1 H, J = 5 and 9 Hz, CH), 6.16 (d, 1 H, J = 5 Hz, OH), 7.25–7.48 (2 m, 5 H, Ph), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 8.73 (d, 1 H, J = 9 Hz, NH); IR (KBr) 1782, 1700, 1685, cm^{-1}; MS m/z 722 $(M + H)^+$.

Acknowledgment. We gratefully acknowledge the contributions of Anne Bertasso, E. John Duker, Lucy Foppiani, Eugene LaSala, Ellen R. Lipschitz, David H. McGarry, John Pace, Diane A. Russo, and JoAnn Siebelist for experimental work on biological assays, Gino Sasso for interpretation of NMR spectra, and BettyAnn Hedemus for assistance in preparing the manuscript.

Registry No. 1, 127142-95-4; **2**, 52312-27-3; **4**, 127142-84-1; **5**·CF_3CO_2H, 135327-68-3; **6**, 127142-85-2; **7**, 135312-05-9; **8**, 127142-87-4; **9**·CF_3CO_2H, 135312-06-0; **10**, 127142-89-6; **11**, 135312-07-1; **12**, 135312-08-2; **13**, 135312-09-3; **14**, 135312-10-6; **15**, 135339-50-3; $PhOCH_2COCl$, 701-99-5; $NCCH_2COCl$, 16130-58-8; (*R*)-$PhCH(OH)CO_2H$, 611-71-2; ciprofloxacin, 85721-33-1; (*Z*)-2-amino-α-(methoxyimino)-4-thiazoleethanethioic acid, 80756-85-0; (*Z*)-2-[[[1-(2-amino-4-thiazolyl)-2-[(2-benzothiazolyl)thio]-2-oxoethylidene]amino]oxy]-2-methylpropanoic acid 1,1-dimethylethyl ester, 89604-92-2; (*Z*)-2-[[[1-(2-amino-4-thiazolyl)-2-[(2-benzothiazolyl)thio]-2-oxoethylidene]amino]-oxy]ethanoic acid 1,1-dimethylethyl ester, 89605-09-4; (*Z*)-2-[[[1-(2-amino-4-thiazolyl)-2-[(2-benzothiazolyl)thio]-2-oxoethylidene]amino]oxy]ethanamide, 89876-15-3; (*R*)-α-[[(4-ethyl-2,3-dioxopiperazinyl)carbonyl]amino]benzeneacetic acid, 63422-71-9.

Potential Antitumor Agents. 63. Structure–Activity Relationships for Side-Chain Analogues of the Colon 38 Active Agent 9-Oxo-9*H*-xanthene-4-acetic Acid

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Received December 19, 1990

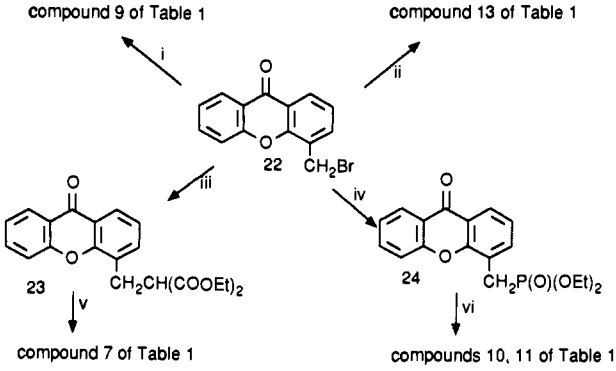
A series of 16 analogues of the solid tumor active compound 9-oxo-9*H*-xanthene-4-acetic acid (XAA), with variations in the acetic acid side chain, have been prepared and evaluated for their ability to cause early haemorrhagic necrosis of colon 38 tumors in mice. The results extend the previous SAR for this class and confirm the necessity for a carboxylic acid group in a fixed disposition with respect to the xanthenone chromophore. None of the compounds showed superior potency to XAA itself, with virtually all alterations in the nature of the anionic center or its geometry with respect to the chromophore greatly reducing or abolishing activity. However, α-methylation of the side chain was permissible, and the two enantiomers of 5-methyl-α-methyl-XAA were separated and tested. Both were active, but the *S*-(+) enantiomer was much more dose-potent than the *R*-(−) enantiomer, in both the in vivo tumor necrosis assay and an in vitro assay measuring the stimulation of nitric oxide production by macrophages. This suggests that the enantiomers have different intrinsic activities, rather than differing in their vivo metabolism.

The drug flavone-8-acetic acid (1) (FAA, NSC 347512) has been reported to have solid-tumor selective activity in experimental animal models,[1,2] involving the induction of selective tumor necrosis,[3] induction of cytokines,[4] vascular collapse leading to blood flow shut-down,[5,6] and

(1) Atassi, G.; Briet, P.; Berthelon, J.-J.; Collonges, F. *Eur. J. Med. Chem.* **1985**, *20*, 393.
(2) Plowman, J.; Narayanan, V. L.; Dykes, D.; Szarvasi, E.; Briet, P.; Yoder, O. C.; Paull, K. D. *Cancer Treat. Rep.* **1986**, *70*, 631.
(3) Smith, G. P.; Calveley, S. B.; Smith, M. J.; Baguley, B. C. *Eur. J. Cancer. Clin. Oncol.* **1987**, *23*, 1209.

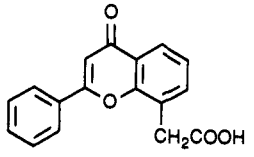
0022-2623/91/1834-2864$02.50/0

Scheme I[a]

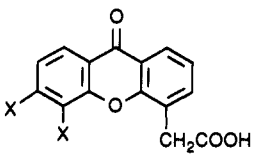


[a] (i) Na_2SO_3; (ii) thiourea; (iii) $CH_2(COOEt)_2/NaH$; (iv) $(EtO)_3P$; (v) AcOH/HCl/Δ; (vi) 2 N NaOH (10):48% HBr/Δ (11).

stimulation of macrophages.[7,8] However, FAA has not shown clinical activity despite extensive trials.[9] Reasons suggested for this include low potency,[10] nonlinear pharmacokinetics leading to a steep dose–response profile,[11] and a mechanism of action against implanted solid tumors in mice that is not relevant for activity against spontaneous tumors in humans.[12]



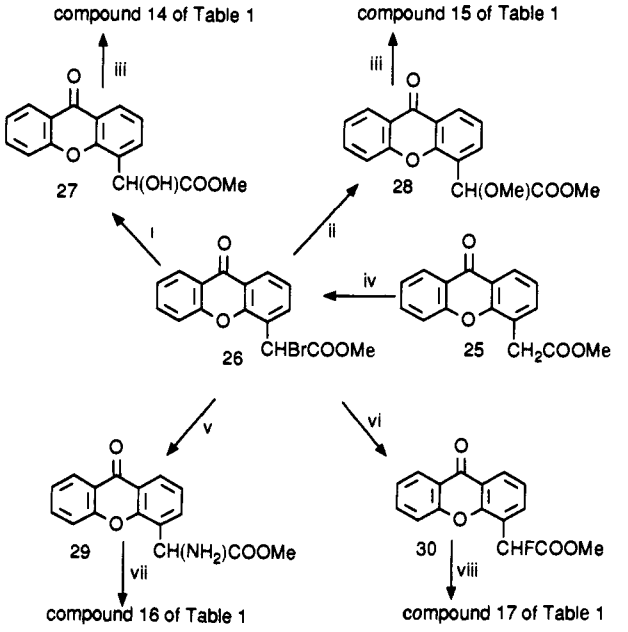
1 : FAA



2 : X = H : XAA
3 : X = Me : DXAA

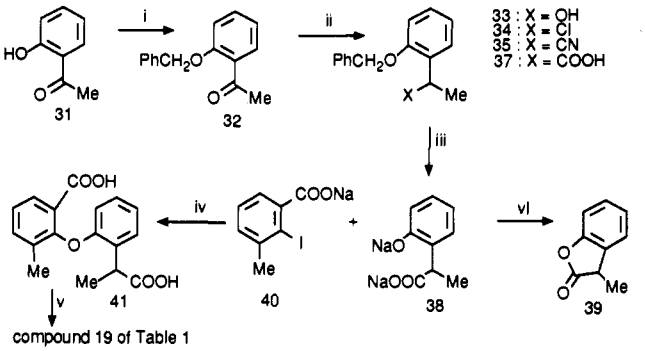
In a search for compounds with higher potency, we have investigated[13–15] a related series with a similar profile of activity, the 9-oxo-9*H*-xanthene-4-acetic acids (XAA compounds, e.g. the parent compound 2). Structure–activity relationships[13,14] for in vivo colon 38 activity among ring-substituted analogues of XAA showed that small lipophilic substituents in the 5- and 6-positions enhance dose potency, with the 5,6-dimethyl derivative (3) being about 11-fold more dose-potent than FAA[15] and also a much more effective inducer of host responses than is FAA.[8,16,17]

Scheme II[a]



[a] (i) KOAc/MeOH/H_2O; (ii) MeONa/MeOH; (iii) NaOH/MeOH/Δ; (iv) NBS; (v) NH_4OH/dioxane; (vi) KF/DMF/Δ; (vii) concentrated HCl/Δ/12 h; (viii) 2 N HCl/dioxane.

Scheme III[a]



[a] (i) $PhCH_2Cl/Bu_4N^+Br^-/CH_2Cl_2$/aqueous NaOH; (ii) $NaBH_4$; $CaCl_2$/concentrated HCl; NaCN/DMSO/Δ; KOH/EtOH/Δ/12 h; (iii) Pd/C/H_2; (iv) Cu^+/TDA-1/dioxane/Δ; (v) 90% H_2SO_4/100 °C/10 min; (vi) *p*-TsOH/toluene.

Recent evidence from this laboratory[18] shows that this compound also has less dose-dependent pharmacokinetics and a longer plasma half-life than FAA.

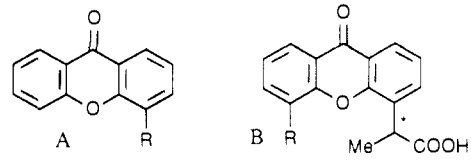
Since other 5,6-disubstituted compounds also show high dose potencies,[15] as well as being highly active (>90% tumor necrosis in the short-term histology assay[19]), it is likely this is the optimal configuration among substituted 9-oxo-9*H*-xanthene-4-acetic acids. Therefore, in further attempts to develop this series (seeking in particular compounds with shallower dose–response curves) we have explored variations in the side chain of the 9-oxo-9*H*-xanthene-4-acetic acids and report this work here.

Chemistry

Most of the side-chain variants (4–13) of Table I were synthesized from either the parent compound itself (2) or

(4) Mace, K. F.; Hornung, R. W.; Wiltrout, R. H.; Young, H. A. *Cancer Res.* **1990**, *50*, 1742.
(5) Zwi, L. J.; Baguley, B. C.; Gavin, J. B.; Wilson, W. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1005.
(6) Hill, S.; Williams, K. B.; Denekamp, J. *Eur. J. Cancer* **1990**, *25*, 1419.
(7) Ching, L.-M.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1512.
(8) Thomsen, L. L.; Ching, L.-M.; Baguley, B. C. *Cancer Res.* **1990**, *50*, 6966.
(9) Kerr, D. J.; Kaye, S. B. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1271.
(10) Kerr, D. J.; Kaye, S. B.; Cassidy, J.; Bradley, C.; Rankin, E. M.; Adams, L.; Setanoians, A.; Young, T.; Forrest, G.; Soukop, M.; Clavel, M. *Cancer Res.* **1987**, *47*, 6776.
(11) Chabot, G. G.; Bissery, M.-C.; Corbett, T. H.; Rutkowski, K. *Cancer Chem. Pharm.* **1989**, *24*, 15.
(12) Cummings, J.; Smyth, J. F. *Cancer Chem. Pharm.* **1989**, *24*, 269.
(13) Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Calveley, S. B.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 793.
(14) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1375.
(15) Rewcastle, G. W.; Atwell, G. J.; Zhuang, L.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1991**, *34*, 217.
(16) Ching, L.-M.; Joseph, W. R.; Zhuang, L.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. *Eur. J. Cancer* **1991**, *27*, 79.
(17) Thomsen, L. L.; Ching, L.-M.; Zhuang, L.; Gavin, J. B.; Baguley, B. C. *Cancer Res.* **1991**, *51*, 77.
(18) McKeage, M.; Kestell, P.; Denny, W. A.; Baguley, B. C. *Cancer Chemother. Pharmacol.*, in press.
(19) Baguley, B. C.; Calveley, S. B.; Crowe, K. K.; Fray, L. M.; O'Rourke, S. M.; Smith, G. P. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 263.

Table I. Physicochemical Properties and Biological Activities of Side-Chain Analogues of 9-Oxo-9*H*-xanthene-4-acetic Acid



no.	formula	R	mp (°C)	formula	analyses	R_m[a]	colon 38 OD[b]	colon 38 activity[c]	colon 38 range[d]
2	A	CH_2COOH (XAA)				0.64	220	++	
3	5,6-dimethyl-XAA					0.49	30	++	30–20
4	A	$CH_2COO(CH_2)_2NMe_2$	221–222	$C_{19}H_{19}NO_4$·HCl	C, H, N, Cl	−0.24	330	+	
5	A	$CH_2CONH(CH_2)_2NMe_2$	217.5–218.5	$C_{19}H_{20}N_2O_3$·HCl	C, H, N, Cl	−0.27	750	+	
6	A	COOH	289–290		289[e]	0.49	220	−	
7	A	CH_2CH_2COOH	190–191	$C_{16}H_{12}O_4$	C, H	0.70	500	+	
8	A	OCH_2COOH	240–240.5	$C_{15}H_{10}O_5$	C, H	0.21	750	−	
9	A	CH_2SO_3H	>360	$C_{14}H_9O_5S$·Na	C, H, S	−0.59	330	−	
10	A	$CH_2P(O)(OEt)OH$	212–214	$C_{16}H_{15}O_5P$	C, H, P	−0.04	750	−	
11	A	$CH_2P(O)(OH)_2$	272–275	$C_{14}H_{11}O_5P$	C, H, P	−0.48	750	−	
12	A	$CH_2CONHSO_2Ph$	236–238	$C_{21}H_{15}NO_5S$	C, H, N, S	0.51	750	−	
13	A	$CH_2SC(NH)NH_2$	261–262	$C_{15}H_{12}N_2O_2S$·HBr	C, H, N, S	−0.20	220	−	
14	A	CH(OH)COOH[e]	223–225	$C_{18}H_{14}O_6$	C, H	0.04	750	+	
15	A	CH(OMe)COOH[e]	162–164	$C_{16}H_{12}O_5$	C, H	0.49	750	−	
16	A	$CH(NH_2)COOH$[e]	250 (dec)	$C_{15}H_{11}NO_4$·HCl·H_2O	C, H, N	−0.53	750	+	
17	A	CH(F)COOH	191–193	$C_{15}H_9FO_4$	C, H, F	0.75	750	−	
18	B	H (racemate)	205–206	$C_{16}H_{12}O_4$	C, H	0.85	500	++	500–330
19	B	Me (racemate)	225–226[f]	$C_{17}H_{14}O_4$	C, H	0.88	150	++	(150–100)
20	B	Me (*S* enantiomer)	213–214				65	++	
21	B	Me (*R* enantiomer)	213–214				220	++	(220–150)

[a] R_m = relative measure of drug lipophilicity, determined by liquid–liquid chromatography as detailed in ref 35, 4′-(9-acridinylamino)-methanesulfonanilide (AMSA) as internal standard. [b] OD = optimal dose: the minimum dose of drug in milligrams/kilogram, administered intraperitoneally as the sodium salt in water in a single dose, to achieve the level of activity cited. [c] Animals bearing subcutaneous colon 38 tumors (4–8 mm in diameter) were treated with drug at the optimal dose (determined by carrying out a full dose profile at 1.5-fold dose intervals). Tumors were removed after 24 h, fixed in formalin, stained in hematoxylin/eosin, and examined histopathologically as detailed in the text. ++ = >90% necrosis across examined sections, + = 50–90% necrosis, − = <50% necrosis. [d] Range of doses (highest nontoxic to minimum effective) over which 50–90% necrosis was achieved (if no range is given, effects were seen only at a single dose. [e] Reference 36. [f] Reference 20.

from 4-(bromomethyl)xanthenone[13] (**22**). Simple esterification or amidation of (**2**) gave compounds **4**, **5**, and **12**, respectively. The oxyacetic acid (**8**) was prepared from 4-hydroxyxanthenone and ethyl bromoacetate, and the (phenylsulfonyl)acetamide (**12**) was prepared from the acid chloride of **2** by direct treatment with phenylsulfonamide sodium salt. Condensation of the bromomethyl compound **22** with diethyl malonate followed by acid-catalyzed hydrolysis of the diester **23** and decarboxylation gave the propanoic acid **7**, while treatment with triethyl phosphite and controlled base- or acid-catalyzed hydrolysis of the resulting diester **24** gave **10** and **11**, respectively (Scheme I). The sulfonic acid **9** and the isothiouronium compound **13** were made by treatment of **22** with Na_2SO_4 and thiourea respectively (Scheme I).

The α-substituted compounds **14–17** were prepared (as racemic mixtures) from the α-bromo ester **26** (Scheme II), which was prepared from methyl 9-oxo-9*H*-xanthene-4-acetate (**25**) by treatment with NBS. The α-methyl compound (**18**) was prepared (as a racemate) by a published method[20] via bromination of 4-ethylxanthenone and conversion of the resulting 4-(1-bromoethyl)xanthenone to acid **18** via the nitrile. It has been tested previously as an antiinflammatory agent.[20]

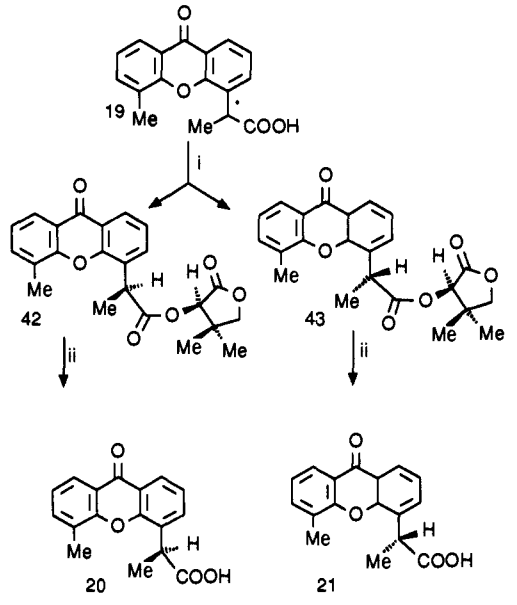
However, this method could not be used for the corresponding α-methyl derivative of the 5-methylxanthenone, due to competing bromination of the nuclear methyl. This compound (**19**) (as a racemic mixture) was instead prepared by the method outlined in Scheme III. Reaction of 2′-hydroxyacetophenone (**31**) with benzyl chloride under phase-transfer conditions gave 2′-benzyloxyacetophenone (**32**), which was converted successively to the alcohol (**33**) with $NaBH_4$ and the chloride (**34**) with anhydrous $CaCl_2$ in concentrated HCl at room temperature. Reaction of this chloride with NaCN in DMSO gave a 3:2 mixture of 2-(2-(benzyloxy)phenyl)propanenitrile (**35**) and the isomeric isocyanide (**36**). This mixture was selectively hydrolyzed in ethanolic KOH to give 2-(2-(benzyloxy)phenyl)propanoic acid (**37**), which was demasked to give the desired 2-(2-hydroxyphenyl)propanoic acid (**38**). This was used in the usual copper/TDA-catalyzed condensation with 2-iodo-3-methylbenzoic acid (**40**), and the resulting diacid **41** was ring-closed in 90% H_2SO_4 to give racemic 2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid (**19**).

Resolution of racemic acid **19** was achieved by a modification of the published procedure,[21] which employs chiral α-hydroxy esters as the resolving agent. Thus the acid **19** was converted via its acid chloride to the ketene, which was then reacted with a slight excess of (*R*)-(−)-pantolactone, in the expectation that the *R,R* diastereoisomeric ester would be preferentially formed (Scheme IV).[21] In the event, a mixture of diastereomers **42** and **43** was formed and was separated by chromatography on silica gel. The faster eluting minor isomer (mp 155.5–156 °C) was shown by X-ray crystallography to be the *R,S* diastereomer (**42**), proving the major compound (mp 150–151 °C) to be the expected *R,R* diastereomer (**43**). Hydrolysis of the esters under non-enolizing (acid) conditions gave the respective chiral (*S*)-(+) and (*R*)-(−) acids **20** and **21**.

(20) Nakanishi, M.; Oe, T.; Tsuruda, M. Matsuo, H.; Sakuragi, S.; Maruyama, Y. *Yakugaku Zasshi* **1976**, *96*, 99; *Chem. Abstr.* **1976**, *84*, 135515b.

(21) Larsen, R. D.; Corley, E. G.; Davis, P.; Reider, P. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1989**, *111*, 7650.

Scheme IV[a]



[a] (i) $(COCl_2)$/*N*-methylpyrrolidine/(*R*)-(−)-pantolactone; (ii) 2 N HCl/AcOH/85 °C.

Results and Discussion

Table I gives results for the in vivo evaluation of the compounds against subcutaneously implanted C38 colon tumor in mice, using the short-term histology assay[19] to measure antitumor effect. Within the XAA series, this assay is predictive for tumor growth delay effects (determined independently in longer term assays).[8] Compounds were given as a single intraperitoneal injection of the sodium salt in water (the hydrochloride salts of **4**, **5**, and **16**, and the bromide salt of **13**), over a range of doses increasing at 1.5-fold intervals up to the maximum tolerated dose, with at least two (and usually three) independent determinations at each dose level, using groups of five mice per experiment. For most of the compounds here, activity was seen only at one dose level: for those where activity was seen at more than one dose level, the range of doses (highest nontoxic to minimum effective) over which 50–90% necrosis was achieved is recorded in Table I. The XAA analogues of Table I can be divided into four groups.

(1) Derivatives of the Acetic Acid Itself. Experimental studies with isomers of both FAA[1] and XAA[13] showed there was little positional tolerance, with only the (topologically equivalent) 8-acetic acid (**1**) and 4-acetic acid (**2**) showing significant activity. Early studies[1] with derivatives of FAA showed that the esters of the acid were equally active against colon 38, and in fact the (*N*,*N*-dimethylamino)ethyl ester was also evaluated clinically along with FAA.[22] However, the corresponding amide was inactive in the C38 growth inhibition assay,[1] and it was soon decided that the ester was only a prodrug form, being rapidly converted to the acetic acid.[9,22] In the present study, the ester **4** did show activity but had lower potency than XAA itself. The amide **5** had a low level of activity in the short-term histology assay (Table I), but is was not possible to decide if this was intrinsic or due to release of the free acid.

(2) Carboxylic Acid Analogues of Varying Chain Length. Compounds **6**–**8** explored variation in the position of the acid functionality by varying the chain length. Studies with FAA analogues[1] showed that compounds where the chain was extended by one atom (specifically the OCH_2COOH and CH_2CH_2COOH compounds) lost virtually all activity in the C38 growth inhibition assay. Similar results were seen in the present study, where all the chain length homologues **6**–**8** were essentially inactive, although the propionic acid **7** did show low activity at 500 mg/kg (Table I).

(3) Compounds with Acetic Acid Isosteres. It is difficult to measure the aqueous pK_a values of these compounds because of their insolubility, and accurate estimation from analogous compounds is also difficult due to the possible H-bonding interactions which can occur with the xanthenone ether oxygen. However, the similar pK_a values of phenylacetic acid and 2-methoxyphenylacetic acid (ca. 4.30)[23] suggest that the parent 4-acetic acid (**2**) has a pK_a of ca. 4–5, which means it will be essentially completely ionized at physiological pH. Compounds **9**–**12** bear various groups designed to place an anionic charge in approximately the same spatial relationship with the xanthenone chromophore as does the 4-acetic acid. The sulfonic and phosphonic acid compounds **9**–**11** are all very strong acids (pK_a values ca. 1–3, 100% ionized at physiological pH)[24] whereas the sulfonylacetamide **12** is a much weaker acid (pK_a ca. 6–7).[25] However, despite covering the appropriate range of pK_a values, none of these acetic acid isosteres showed any activity. The isothiouronium compound **13** does not really belong in this group, but was made because the analogous FAA compound was the only nonacid analogue reported[1] to show colon 38 activity in the growth inhibition assay. However **13** showed no activity in the short-term histology assay for tumor necrosis.

(4) α-Substituted Acetic Acids. Our interest in these compounds stemmed from the fact that the rapid clearance of FAA in humans takes place primarily by metabolism, with all the metabolites so far detected being glucuronidates of the acetic acid side chain.[26] Tolerated modifications to the side chain might therefore be beneficial if they served to slow down or otherwise alter this metabolism. Much work on the metabolism of α-methylarylacetic acid nonsteroidal antiinflammatory drugs has shown this to be enantioselective,[27] with preferential glucuronidation of *S* enantiomers observed,[28] as well as metabolic isomerization of *R* into *S* enantiomers.[29]

While most modifications to the acetic acid chain abolish antitumor activity (see above), the α-methyl derivative of FAA has been reported to retain activity, albeit with reduced potency.[1] This was confirmed in the present study, where the α-methyl-XAA derivative **18** showed good activity but reduced potency. However, all the other α-substituted XAA analogues **14**–**17** proved inactive. The reasons for this are unclear: it seems unlikely to be a steric effect, given the activity of **18**. Compounds **14** and **16** are

(22) Dodion, P. F.; Abrams, J.; Gerard, B.; Crespeigne, N.; Peters, B.; Von Berchum, C.; Kenis, Y. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 837.

(23) Kortum, G.; Vogel, W.; Andrussov, K.; *Dissociation constants of organic acids in aqueous solution*; Butterworths: London, 1961.

(24) Albert, A.; Serjeant, E. P. *The determination of ionization constants* 3rd ed.; Chapman & Hall: New York, 1984.

(25) Albright, J. D.; De Vries, V. G.; Du, M. T.; Largis, E. E.; Miner, T. G.; Reich, M. F.; Shepherd, R. G. *J. Med. Chem.* **1983**, *26*, 1393.

(26) Cummings, J.; Double, J. A.; Bibby, M. C.; Farmer, P.; Evans, S.; Kerr, D. J.; Kaye, S. B.; Smyth, J. F. *Cancer Res.* **1989**, *49*, 3587.

(27) El Mouelhi, M.; Ruelius, H. W.; Fenselau, C.; Dulik, D. M. *Drug Met. Disp.* **1987**, *15*, 767.

(28) Lee, E. J. D.; Williams, K.; Day, R.; Graham, G.; Champion, D. *Br. J. Clin. Pharm.* **1985**, *19*, 669.

(29) Yamaguchi, T.; Nakamura, Y. *Drug Met. Disp.* **1985**, *13*, 614.

considerably more hydrophilic than XAA, a property known[14] not to favour activity in the XAA series.

The 5-methyl derivative **19** also showed high activity but considerably reduced potency compared with the parent 5-methyl-XAA itself.[14] Thus the effect of an α-methyl side chain in both the FAA and XAA series is to reduce potency (compared with that of the unsubstituted parent) by about 2–3-fold. Since these compounds have a chiral center in the side chain, it was possible that only one of the two enantiomers of **19** was active, with the other serving merely to reduce the potency of the racemate. It is known, for example, that only the *S*-(+) enantiomers of most aryl-α-methylacetic acids show antiinflammatory activity.[28] The enantiomers of **19** were therefore separated and tested separately. Both proved equally active in the short-term histology assay, but with greatly varying potency. The *S*-(+) enantiomer **20** had an optimal (minimum effective) dose of 65 mg/kg and the *R*-(−) enantiomer one of 220 mg/kg. Thus the *S*-(+) enantiomer is approximately as potent as the parent 5-methyl-XAA (optimal dose 45 mg/kg),[14] with the *R*-(−) enantiomer much less effective. The optimal dose for the enantiomeric mixture **19** lies, within error, between those of the two enantiomers (**20** and **21**), suggesting that the enantiomers have no antagonistic effects.

In order to determine whether the difference in potency of the two enantiomers was a result of differences in intrinsic activity or due to changes in vivo metabolism, their ability to stimulate activated murine macrophages in vitro to form nitrite/nitrate was determined. This is a measure of the stimulation of oxidation of L-arginine to nitric oxide.[30] The optimal dose for the *S*-enantiomer **20** was 160 μM with a maximum rate of nitrate production of 10.8 ± 1.0 mmol/10^6 cells. In contrast, the maximal rate of nitrite/nitrate production by the *R* enantiomer **21**, achieved at a concentration of 1300 nM, was significantly lower, being only marginally above the background level at 2.7 ± 0.5 nmol/10^6 cells). The corresponding values for 5-methyl-XAA are 9.3 nmol/10^6 cells at 170 μM.[8]

Conclusions

This work further defines the SAR for the solid tumor activity (induction of haemorrhagic necrosis) of the XAA class of compounds[13-16] and confirms the necessity for a carboxylic acid group in a fixed disposition with respect to the xanthenone chromophore. While altering the nature of the anionic center (compounds **9–12**) or its gross geometry with respect to the chromophore (compounds **6–8**) is not permissible, limited modification of the side chain is possible (compounds **18**, **19**), although none of the side-chain variations provided compounds more dose-potent than XAA itself. The differences in the activities of enantiomers **20** and **21** appear to be due to different intrinsic activities of the compounds rather than to differing metabolism, since similar differences are seen in both the in vivo histology assays for haemorrhagic necrosis and the in vitro assays for macrophage stimulation.

Experimental Section

Where analyses are indicated by the symbols of the elements, results were within ±0.4% of the theoretical and were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal IA 9200 with digital thermometer. All compounds had NMR spectra (measured on a Bruker WP-60, Me_4Si) consistent with their assigned structure. Quoted NMR data was acquired on a Bruker AM-400 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

9-Oxo-9*H*-xanthene-4-propanoic Acid (7). Diethyl malonate (1.92 g, 12 mmol) was added at 20 °C to a stirred suspension of NaH (0.57 g of 50% dispersion, 12 mmol) in benzene (12 mL). After being stirred for a further 30 min the homogeneous mixture was cooled to 0 °C and 4-(bromomethyl)xanthenone[13] (**22**) (3.18 g, 11 mmol) was added portionwise. After 1 h at 20 °C and a further 1 h at 50 °C, excess solvent was removed under reduced pressure, and the residue was shaken with 0.01 N AcOH and extracted with CH_2Cl_2. Workup of the organic phase and crystallization of the residue twice from petroleum ether gave diethyl 2-[(9-oxo-9*H*-xanthen-4-yl)methyl]propanedioate (**23**) (2.28 g, 54% yield), mp 73–74 °C. Anal. ($C_{21}H_{20}O_6$) C, H. The diester **23** (1.5 g) was heated under reflux for 6 h in a mixture of AcOH (24 mL) and concentrated HCl (12 mL). Solvents were removed under reduced pressure, and the residue was shaken with water and extracted with warm aqueous $KHCO_3$. The filtrate was carefully neutralized with dilute AcOH and filtered again, and the filtrate was acidified with HCl to give the desired propanoic acid **7** (0.9 g, 72% yield). Crystallization from MeOH gave prisms, mp 190–191 °C. Anal. Table I.

((9-Oxo-9*H*-xanthen-4-yl)oxy)acetic Acid (8). A mixture of 4-hydroxyxanthenone[31] (2.54 g, 12 mmol), powdered K_2CO_3 (1.8 g, 13 mmol), and KI (0.1 g) in methyl isobutyl ketone (60 mL) was stirred at 75 °C for 1 h. Ethyl bromoacetate (2.67 g, 16 mmol) was added, and the mixture was stirred under reflux for 2 h and filtered hot, with the insolubles being washed with additional hot methyl isobutyl ketone. The combined filtrates were evaporated, and the residue was triturated with water and crystallized from petroleum ether to give crude ethyl (9-oxo-9*H*-xanthen-4-yl)-oxy)acetate (2.2 g, 61% yield), mp 78–79 °C. Hydrolysis of this with 1 N KOH gave the desired acid **8**, which crystallized from EtOH as colorless needles, mp 240–240.5 6C. Anal. Table I.

9-Oxo-9*H*-xanthene-4-methanesulfonic Acid (9). Powdered 4-(bromomethyl)xanthenone (1.45 g, 5 mmol) was added to a hot solution of Na_2SO_3 (1.26 g, 10 mmol) in water (25 mL) and DMSO (7 mL), and the mixture was stirred under reflux for 1 h. The residue remaining after evaporation of the solvents was extracted with MeOH, and the filtrate was concentrated and crystallized from MeOH/EtOAc. Further crystallization of this product from 6 N aqueous HCl and then MeOH/EtOAc gave the pure acid **9** as colorless prisms (1.0 g, 71% yield). The sodium salt crystallized from MeOH/EtOAc, mp >360 °C. Anal. Table I.

Ethyl Hydrogen 9-Oxo-9*H*-xanthene-4-methanephosphonate (10) and 9-Oxo-9*H*-xanthene-4-methanephosphonic Acid (11). A mixture of 4-(bromomethyl)xanthenone (2.02 g, 7 mmol) and triethyl phosphite (1.17 g, 7 mmol) was heated at 135–140 °C for 1 h, cooled, and triturated with petroleum ether. The resulting solid was collected and recrystallized twice from petroleum ether to give diethyl 9-oxo-9*H*-xanthene-4-methanephosphonate (**24**) as colorless needles (1.81 g, 75% yield), mp 94–95 °C. Anal. ($C_{18}H_{19}O_5P$) C, H, P. This diester (**24**) (1.5 g) was heated under reflux in excess 2 N NaOH in EtOH/water (7:3) (80 mL) until reaction was complete (as monitored by TLC). The mixture was then diluted with water, concentrated under reduced pressure to remove the EtOH, filtered, and acidified with HCl. The solid that separated on prolonged cooling at 0 °C was collected and crystallized from EtOH to give ethyl hydrogen 9-oxo-9*H*-xanthene-4-methanephosphonate (**10**) (0.95 g, 69% yield), mp 212–214 °C. Anal. Table I.

The diester **24** (2.0 g) was heated under reflux in 48% HBr (15 mL) for 3 h, and the mixture was then cooled and diluted with water. The solid that separated was washed well with cold water, dried, and crystallized from MeOH/EtOAc to give 9-oxo-9*H*-xanthene-4-methanephosphonic acid (**11**) (1.44 g, 86% yield) as white prisms, mp 272–275 °C. Anal. Table I.

***N*-(Phenylsulfonyl)-9-oxo-9*H*-xanthene-4-acetamide (12).** A suspension of 9-oxo-9*H*-xanthene-4-acetic acid (**2**) (1.27 g, 5 mmol) and PCl_5 (1.25 g, 6 mmol) in benzene (30 mL) was heated under reflux with stirring until homogeneous, and then exhaustively evaporated under reduced pressure on the water bath. The residue was kept under vacuum over solid KOH for 12 h and then treated in one portion with an ice-cold solution of phenyl-

(30) Marletta, M. A. *Trends Biochem. Sci.* **1989**, *14*, 488.

(31) Kostanecki, W.; Rutishausen, R. *Ber. Dtsch. Chem. Ges.* **1892**, *25*, 1648.

sulfonamide sodium salt (1.07 g, 6 mmol) in dry dioxan (30 mL). The mixture was stirred at 20 °C for 4 h, and the solvent was then removed under reduced pressure. The residue was dissolved in warm 2 N aqueous Na_2CO_3 and clarified by filtration. Careful neutralization with dilute AcOH precipitated the crude product, which was collected and crystallized twice from MeOH to give the desired acetamide **12** (0.81 g, 41% yield), mp 236–238 °C. Anal. Table I.

2-[(9-Oxo-9*H*-xanthen-4-yl)methyl]isothiouronium Bromide (13). A mixture of 4-(bromomethyl)xanthenone (**22**) (2.89 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in dry DMF (20 mL) was stirred at 95 °C for 30 min and then diluted with benzene. The resulting precipitate was collected, dissolved in hot water, and filtered. The filtrate was evaporated to dryness and the residue was crystallized from MeOH to give the desired salt **13** as white prisms (2.6 g, 71% yield), mp 261–262 °C. Anal. Table I.

2-Hydroxy-2-(9-oxo-9*H*-xanthen-4-yl)acetic Acid (14). A mixture of methyl 9-oxo-9*H*-xanthene-4-acetate (**25**) (3.8 g, 14 mmol) and *N*-bromosuccinimide (3.0 g, 17 mmol) in 200 mL of CCl_4 was heated under reflux for 4 h with a catalytic amount of benzoyl peroxide. After cooling, the mixture was diluted with CH_2Cl_2 and washed with water. After drying ($CaCl_2$), the solvent was removed, and the solid residue was recrystallized from EtOAc to give fine white prisms of methyl 2-bromo-2-(9-oxo-9*H*-xanthen-4-yl)acetate (**26**) (4.19 g, 85%), mp 140–141 °C. Anal. ($C_{16}H_{11}BrO_4$) C, H, Br. ^{1}H NMR ($CDCl_3$); δ 8.36 (d, 1 H, *J* = 8.0 Hz, H-1′), 8.33 (d, 1 H, *J* = 7.9 Hz, H-8′), 8.10 (d, 1 H, *J* = 7.6 Hz, H-3′), 7.76 (t, 1 H, *J* = 7.8 Hz, H-6′), 7.55 (d, 1 H, *J* = 7.89 Hz, H-5′), 7.43 (t, 1 H, *J* = 7.8 Hz, H-2′), 7.42 (t, 1 H, *J* = 7.6 Hz, H-7′), 6.16 (s, 1 H, CHBr), and 3.84 (s, 3 H, CH_3O). Reaction of the above bromo ester (**26**) with excess potassium acetate in MeOH containing a trace of water under reflux gave crude methyl 2-hydroxy-2-(9-oxo-9*H*-xanthen-4-yl)acetate (**27**). ^{1}H NMR ($CDCl_3$); δ 8.32 (m, 2 H, H-1′,8′), 7.78 (d, 1 H, *J* = 7.4 Hz, H-5′), 7.72 (t, 1 H, *J* = 7.8 Hz, H-6′), 7.48 (d, 1 H, *J* = 8.5 Hz, H-5′), 7.38 (m, 2 H, H-2′,7′), 5.74 (s, 1 H, H-2), 3.95 (m, 1 H, OH), and 3.76 (s, 3 H, CH_3O). Hydrolysis of this with NaOH in refluxing aqueous methanol gave 2-hydroxy-2-(9-oxo-9*H*-xanthen-4-yl)acetic acid (**14**), mp (MeOH) 223–225 °C. Anal. Table I.

2-Methoxy-2-(9-oxo-9*H*-xanthen-4-yl)acetic Acid (15). Reaction of the above bromo ester **26** with sodium methoxide in MeOH under reflux gave crude methyl 2-methoxy-2-(9-oxo-9*H*-xanthen-4-yl)acetate (**28**). ^{1}H NMR ($CDCl_3$): δ 8.35 (m, 2 H, H-1′,8′), 7.88 (d, 1 H, *J* = 7.4 Hz, H-3′), 7.75 (t, 1 H, *J* = 7.8 Hz, H-6′), 7.55 (d, 1 H, *J* = 8.4 Hz, H-5′), 7.41 (m, 2 H, H-2′,7′), 5.53 (s, 1 H, H-2), 3.75 (s, 3 H, CH_3O), and 3.53 (s, 3 H, CH_3O). Hydrolysis of this ester with NaOH in aqueous MeOH gave 2-methoxy-2-(9-oxo-9*H*-xanthen-4-yl)acetic acid (**15**), mp (MeOH) 162–164 °C, in 75% overall yield. Anal. Table I.

2-Amino-2-(9-oxo-9*H*-xanthen-4-yl)acetic Acid (16). A solution of the above bromo ester **26** (2.5 g, 7.2 mmol) in dioxane (200 mL) was treated with concentrated NH_4OH (30 mL), and the mixture was stirred for 24 h at room temperature. The volatiles were removed under reduced pressure, and the residue was extracted into EtOAc and washed with water. The organic layer was extracted twice with 1 M HCl and discarded. The aqueous layer was treated with concentrated NH_4OH solution until basic and extracted with EtOAc. After drying (Na_2SO_4), the solvent was removed to give crude methyl 2-amino-2-(9-oxo-9*H*-xanthen-4-yl)acetate (**29**) as an oil (1.71 g, 84%). ^{1}H NMR ($CDCl_3$): δ 8.27–8.33 (m, 2 H, H-1′,8′), 7.71–7.77 (m, 2 H, H-3′6′), 7.50 (d, 1 H, *J* = 8.4 Hz, H-5′), 7.35–7.41 (m, 2 H, H-2′,7′), 5.18 (s, 1 H, H-2), 3.72 (s, 3 H, CH_3), and 2.35 (br s, 2 H, NH_2). The above ester was dissolved in concentrated HCl and heated under reflux for 12 h. The mixture was cooled and the product was collected by filtration and washed with concentrated HCl. Recrystallization from MeOH gave the hydrochloride salt of 2-amino-2-(9-oxo-9*H*-xanthen-4-yl)acetic acid (**16**), mp 250 °C dec. Anal. Table I.

2-Fluoro-2-(9-oxo-9*H*-xanthen-4-yl)acetic Acid (17). A mixture of the above bromo ester (**26**) (4 g, 11.5 mmol) and anhydrous KF (16 g) in DMF (100 mL) was heated under reflux for 24 h, and the solvent was removed under vacuum. The residue was extracted with EtOAc, washed with water, and dried (Na_2SO_4). Chromatography on SiO_2 (CH_2Cl_2–hexanes, 1:1) gave crude methyl 2-fluoro-2-(9-oxo-9*H*-xanthen-4-yl)acetate (**30**) (1.1 g, 33%). ^{1}H NMR ($CDCl_3$): δ 8.43 (d, 1 H, *J* = 8.0 Hz, H-1′), 8.35 (d, 1 H, *J* = 7.9 Hz, H-8′), 7.87 (d, 1 H, *J* = 7.4 Hz, H-3′), 7.77 (t, 1 H, *J* = 7.6 Hz, H-6′), 7.54 (d, 1 H, *J* = 8.1 Hz, H-5′), 7.44 (t, 1 H, *J* = 8.4 Hz, H-2′), 7.43 (t, 1 H, *J* = 8.4 Hz, H-7′), 6.44 (d, 1 H, *J* = 46.7 Hz, CHF), and 3.83 (s, 1 H, CH_3O). This ester was hydrolyzed with 2 N HCl in refluxing dioxane to give a white solid, which was recrystallized from MeOH to give 2-fluoro-2-(9-oxo-9*H*-xanthen-4-yl)acetic acid (**17**), mp 191–193 °C. Anal. Table I.

Racemic 2-(5-Methyl-9-oxo-9*H*-xanthen-4-yl)propanoic Acid (19). Reaction of 2′-hydroxyacetophenone (**31**) (68 g, 0.5 mol) with benzyl chloride (68 mL, 0.60 mol) was performed under phase-transfer conditions, using tetrabutylammonium bromide (16 g, 0.05 mol) in CH_2Cl_2 and aqueous NaOH for 24 h at room temperature. The organic layer was separated, washed three times with water, and dried ($CaCl_2$). The solution was distilled to remove solvent and excess benzyl chloride, and the residual oil was allowed to cool to give 2′-(benzyloxy)acetophenone (**32**) (107 g, 95%), mp (hexane) 41–41.5 °C (lit.[32] mp 40 °C). ^{1}H NMR ($CDCl_3$): δ 7.75 (d, 1 H, *J* = 7.6 Hz, H-6′) , 7.32–7.46 (m, 6 H), 6.98–7.03 (m, 2 H, H-3′,5′), 5.16 (s, 2 H, CH_2), and 2.60 (s, 3 H, CH_3).

A solution of the above ketone (**32**) (50 g, 0.22 mol) in EtOH (200 mL) containing $NaBH_4$ (4.2 g, 11 mmol) was heated under reflux for 1 h. Excess $NaBH_4$ was neutralized by the addition of acetone, the solvent was concentrated, and after dilution with water the residue was extracted into EtOAc. Drying (Na_2SO_4) and removal of solvent gave crude 1-(2-(benzyloxy)phenyl)ethanol (**33**) as an oil (49.3 g, 98%). ^{1}H NMR ($CDCl_3$): δ 7.28–7.41 (m, 5 H), 7.20 (dd, 1 H, *J* = 7.5, 8.0 Hz, H-4′), 6.96 (t, 1 H, *J* = 7.5 Hz, H-5′), 6.91 (d, 1 H, *J* = 8.2 Hz, H-3′), 5.15 (q, 1 H, *J* = 6.5 Hz, H-1), 5.06 (s, 2 H, CH_2), 2.73 (br s, 1 H, OH), and 1.49 (d, 3 H, *J* = 6.5 Hz, CH_3).

A solution of the above alcohol (**33**) (25.3 g, 0.11 mol) in dioxane (75 mL) was added to a mixture consisting of 25 g of finely powdered anhydrous $CaCl_2$ in 50 mL of concentrated HCl at room temperature. The viscous mixture was stirred for 1 h and diluted with a mixture of EtOAc and ice. The organic layer was washed with water and 2 N NaOH and dried (Na_2SO_4) to give crude 1-(benzyloxy)-2-(1-chloroethyl)benzene (**34**) as an oil (25.8 , 95%). ^{1}H NMR ($CDCl_3$): δ 7.68 (d, 1 H, *J* = 7.7 Hz, H-3), 7.45 (d, 2 H, *J* = 7.4 Hz, H-2′,6′), 7.39 (t, 2 H, *J* = 7.3 Hz, H-3′,5′), 7.32 (t, 1 H, *J* = 7.2 Hz, H-4′), 7.24 (t, 1 H, *J* = 7.8 Hz, H-5), 6.99 (t, 1 H, *J* = 7.6 Hz, H-4), 6.92 (d, 1 H, *J* = 8.2 Hz, H-6), 5.66 (q, 1 H, *J* = 6.9 Hz, HCCl), 5.10 (s, 2 H, CH_2), and 1.81 (d, 3 H, *J* = 6.9 Hz, CH_3).

The above chloride (**34**) was dissolved in DMSO (100 mL) containing NaCN (10 g, 2 equiv), and the mixture was heated at reflux for 1 h. After cooling and diluting with water, the product was extracted into EtOAc. Drying (Na_2SO_4) and removal of solvent gave 20.6 g of a crude oil, which was shown by ^{1}H NMR and TLC to consist of two main products, identified as the desired 2-(2-(benzyloxy)phenyl)propanenitrile (**35**) (ca. 60%) and the isomeric isocyanide (**36**) (ca. 40%). ^{1}H NMR ($CDCl_3$): δ 7.18–7.44 (m, 7 H), 6.91–7.01 (m, 2 H, H-3′,5′), 5.17 (q, 0.4 H, *J* = 6.5 Hz, HCNC), 4.09 (s, 2 H, CH_2), 4.28 (q, 0.6 H, *J* = 7.2 Hz, HCCN), 1.57 (d, 1.8 H, *J* = 7.2 Hz, CH_3CCN), and 1.51 (d, 1.2 H, *J* = 6.5 Hz, CH_3CNC).

The above crude nitrile mixture was dissolved in a mixture of EtOH and 2 N aqueous KOH and heated under reflux overnight. The EtOH was boiled off, and, after cooling, the pH was adjusted to 8–9 by the addition of dilute HCl. The mixture was extracted with EtOAc, and the organic layer was discarded. The aqueous layer was acidified with 2 N HCl and extracted again with EtOAc. Drying (Na_2SO_4) and removal of solvent gave 2-(2-(benzyloxy)-phenyl)propanoic acid (**37**), mp (aqueous EtOH) 66–67.5 °C (11.2 g, 40% yield based on starting ketone). ^{1}H NMR ($CDCl_3$): δ 9.0–10.5 (br s, 1 H, OH), 7.19–7.42 (m, 7 H), 6.95 (t, 1 H, *J* = 7.5 Hz, H-5′), 6.92 (d, 1 H, *J* = 8.2 Hz, H-3′), 5.05 and 5.06 (2 s, 2 H, diastereotopic CH_2), 4.12 (q, 1 H, *J* = 7.2 Hz, H-2), and 1.49 (d, 3 H, *J* = 7.2 Hz, CH_3). Anal. ($C_{16}H_{16}O_3$) C, H.

Hydrogenation of the above acid with Pd/C in EtOH gave a quantitative yield of 2-(2-hydroxyphenyl)propanoic acid (**38**) as

(32) Pandit, U. K.; Bruice, T. C. *J. Am. Chem. Soc.* **1960**, *82*, 3386.

an oil. ^{1}H NMR ($CDCl_3$): δ 7.70 (br s, 2 H, OH), 7.15 (d, 1 H, J = 7.6 Hz, H-6′), 7.11 (dd, 1 H, J = 6.8, 8.0 Hz, H-6′), 6.88 (t, 1 H, J = 7.5 Hz, H-5′), 6.81 (d, 1 H, J = 8.0 Hz, H-3′), 3.95 (q, 1 H, J = 7.2 Hz, H-2), 1.51 (d, 3 H, J = 7.2 Hz, CH_3). On treatment with catalytic p-toluenesulfonic acid in toluene, the above acid readily underwent ring closure to give the known[33] noncrystalline lactone, 3-methyl-2(3*H*)-benzofuran-2-one (**39**). ^{1}H NMR ($CDCl_3$): δ 7.24–7.30 (m, 2 H, H-4,6), 7.15 (t, 1 H, J = 7.5 Hz, H-5), 7.09 (d, 1 H, J = 8 Hz, H-7), 3.72 (q, 1 H, J = 7.6 Hz, H-3), and 1.56 (d, 3 H, J = 7.6 Hz, CH_3).

A stirred mixture of the finely ground disodium salt of the (hydroxyphenyl)propanoic acid (**38**) (3.89 g, 18.5 mmol) and the sodium salt of 2-iodo-3-methylbenzoic acid (**40**) (4.80 g, 16.9 mmol) in dry dioxane (200 mL) containing tris[2-(2-methoxyethoxy)-ethyl]amine (TDA-1; 1.1 g, 3.4 mmol) and CuCl (0.2 g) was heated under reflux for 12 h. The solvent was removed under reduced pressure, and the residue was dissolved in 2 N NaOH and filtered through Celite. The solution was then added to a 2 N HCl solution to give a precipitate, which was collected and washed well with hot water. Recrystallization from EtOAc gave 2-[2-(methylcarboxymethyl)phenoxy]-3-methylbenzoic acid (**41**) (2.16 g, 53%), mp 210–212 °C. Anal. ($C_{17}H_{16}O_5$) C, H. Ring closure of the above diacid in 90% H_2SO_4 at 100 °C for 10 min gave an 80% yield of racemic 2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid (**19**), mp (EtOH) 225–226 °C. Anal. Table I.

(*R*)- and (*S*)-2-(5-Methyl-9-oxo-9*H*-xanthen-4-yl)-propanoic Acid (20 and 21). To a suspension of racemic 2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid (**19**) (2.67 g, 9.46 mmol) in toluene (100 mL) was added oxalyl chloride (5 mL), and the mixture was heated under reflux for 1 h. The volatiles were removed under reduced pressure, and the residue was dissolved in toluene (50 mL). The solution was cooled to −78 °C, and *N*-methylpyrrolidine (0.44 mL, 3 equiv) was added with stirring. After 30 min a solution of (*R*)-(−)-pantolactone (3.7 g, 28 mmol) in toluene was added, and the mixture was allowed to warm slowly to room temperature overnight. The mixture was diluted with EtOAc and washed with water, dilute HCl, water, and $NaHCO_3$ solution. Removal of the solvent gave an oil, which was chromatographed on SiO_2. Elution with CH_2Cl_2–hexane (4:1) gave first (*R*)-tetrahydro-4,4-dimethyl-2-oxo-3-furanyl (*S*)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoate (**42**), mp (MeOH) 155.5–156.0 °C, $[\alpha]_D^{24}$ +58.7° (*c* 9.16 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.29 (dd, 1 H, J = 1.6, 7.9 Hz, H-1′), 8.17 (dd, 1 H, J = 1.6, 8.0 Hz, H-8′), 7.74 (dd, 1 H, J = 1.6, 7.4 Hz, H-3′), 7.59 (br d, 1 H, J = 7.2 Hz, H-6′), 7.39 (t, 1 H, J = 7.7 Hz, H-2′), 7.30 (t, 1 H, J = 7.6 Hz, H-7′), 5.39 (s, 1 H, H-3″), 4.58 (q, 1 H, J = 7.2 Hz, H-2), 3.91 (dd, 2 H, J = 9.0, 19.5 Hz, H-5″), 2.58 (s, 3 H, 5′-CH_3), 1.75 (d, 1 H, J = 7.2 Hz, CH_3), 0.89 (s, 3 H, 4″-CH_3), and 0.59 (s, 3 H, 4″-CH_3). Anal. ($C_{23}H_{22}O_6$) C, H.

Later eluates gave (*R*)-tetrahydro-4,4-dimethyl-2-oxo-3-furanyl (*R*)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoate (**43**), mp (MeOH) 150–151 °C, $[\alpha]_D^{24}$ −53.7° (*c* 10.4 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.29 (dd, 1 H, J = 1.6, 8.0 Hz, H-1′), 8.17 (dd, 1 H, J = 1.1, 7.9 Hz, H-8′), 7.76 (dd, 1 H, J = 1.4, 7.4 Hz, H-3′), 7.57 (br d, 1 H, J = 6.9 Hz, H-6′), 7.40 (t, 1 H, J = 7.7 Hz, H-2′), 7.28 (dd, 1 H, J = 7.4, 7.7 Hz, H-7′), 5.36 (s, 1 H, H-3″), 4.56 (q, 1 H, J = 7.3 Hz, H-2), 3.95 (ABq, 2 H, J = 2.9 Hz, 5″-H_2), 2.57 (s, 3 H, 5-CH_3), 1.80 (d, 3 H, J = 7.3 Hz, CH_3), 1.06 (s, 3 H, 4″-CH_3), and 0.85 (s, 3 H, 4″-CH_3). Anal. ($C_{23}H_{22}O_6$) C, H.

Hydrolysis of the above esters with 2 N HCl in acetic acid at 85 °C gave the analogous acids (*S*)-(+)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propionic acid (**20**), mp (EtOH) 213–214 °C, $[\alpha]_D^{24}$ +46.6° (*c* 2.73, Na salt in water), and (*R*)-(−)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propionic acid (**21**), mp (EtOH) 213–214 °C, $[\alpha]_D^{24}$ −47.1° (*c* 4.16, Na salt in water).

Measurement of Nitrite/Nitrate Production by Macrophages. The method used has been published previously.[8] Briefly, C3H/HeN mice were injected intraperitoneally with 10^8 BCG organisms per mouse and pertioneal exudate cells were collected 14–15 days later. Adherent macrophages were prepared by plating these cells in culture medium (α-MEM) in 96-well plates and incubating for 2 h at 37 °C in 95% air/5% CO_2. The nonadherent cells and supernatant were then removed, and the adherent cells (macrophages) were washed twice with phosphate-buffered saline. The macrophages were then covered with 200 μL of culture medium containing the antitumor agents at concentrations between 17 and 1300 μM and incubated for a further 20 h. Nitrite concentrations in the medium were measured by the Griess reaction.[34]

Equal volumes of culture medium supernatant and Griess reagent (0.5% sulfanilamide, 0.05% naphthylenediamine dihydrochloride in 2.5% H_3PO_4) were added to Eppendorf tubes and incubated at 20 °C for 10 min. The tubes were centrifuged for 5 min at 8000g, the supernatants transferred to 96-well microplates, and absorbances at 550 nm determined on a plate reader. The nitrite concentration in medium alone (0.3–0.4 nmol/well) was subtracted and the drug concentrations (tested in triplicate) were expressed as the mean ± SEM.

In Vivo Colon 38 Testing. With the exception of **4**, **5**, **13**, and **16**, drugs were converted to their sodium salts. Colon 38 fragments were implanted subcutaneously in BDF_1 mice and allowed to grow to a diameter of 4–8 mm, when drug was given as a single intraperitoneal dose of the sodium salt in water. Each compound was tested (in triplicate) over a range of doses escalating by 1.5-fold up to a maximum of 750 mg/kg (or to the maximum nonlethal dose if this was lower). After 24 h the tumor was surgically removed and fixed in formalin. Sections were stained and examined histologically for evidence of necrosis. Flavoneacetic acid (**1**) was used as a standard and given at a dose of 330 mg/kg. Compounds causing necrosis across 90–100% of the tumor section were scored as (++), compounds showing lesser but still extensive necrosis (50–90%) were scored as (+), and compounds consistently showing less than 50% necrosis at all dose levels were scored as negative (−).

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Registry No. **2**, 35614-21-2; **3**, 117570-53-3; **4**, 134940-06-0; **4**·HCl, 134940-33-3; **5**, 134940-07-1; **5**·HCl, 134940-34-4; **6**, 42073-77-8; **7**, 129095-12-1; **8**, 93866-57-0; **9**, 134940-08-2; **9**·Na, 134940-38-8; **10**, 134940-09-3; **11**, 134940-10-6; **12**, 134940-11-7; **13**, 134940-12-8; **13**·HBr, 134940-35-5; **14**, 134940-13-9; **15**, 134940-14-0; **16**, 134940-15-1; **16**·HCl, 134940-36-6; **17**, 134940-16-2; **18**, 134940-17-3; **19**, 134940-18-4; **20**, 135029-18-4; **20**·Na, 135029-20-8; **21**, 135029-19-5; **21**·Na, 135029-21-9; **22**, 26539-21-9; **23**, 134967-24-1; **24**, 114089-59-7; **25**, 88521-87-3; **26**, 134940-19-5; **27**, 134940-20-8; **28**, 134940-21-9; **29**, 134940-22-0; **30**, 134940-23-1; **31**, 118-93-4; **32**, 31165-67-0; **33**, 134940-24-2; **34**, 134940-25-3; **35**, 134940-26-4; **36**, 134940-27-5; **37**, 134940-28-6; **38**, 134940-37-7; **39**, 134940-29-7; **40**, 126158-00-7; **41**, 134940-30-0; **42**, 134940-31-1; **43**, 134940-32-2; **52**, 135029-20-8; **53**, 135029-21-9; diethyl malonate, 105-53-3; 4-hydroxyxanthenone, 14686-63-6; ethyl ((9-oxo-9*H*-xanthen-4-yl)oxy)acetate, 93322-29-3.

Supplementary Material Available: X-ray crystallographic data for compound **42** (perspective diagram, tables of coordinates, etc.) (10 pages.) Ordering information is given on any current masthead page.

(33) Shibata, S.; Shoji, J.; Tokutake, N.; Kaneko, Y.; Shimizu, H.; Chiang, H. C. *Chem. Pharm. Bull.* **1962**, *10*, 477.

(34) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131.

(35) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1976**, *19*, 722.

(36) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 707.