(m, 4 H), 3.65 (m, 4 H), 3.20–3.00 (m, 12 H), 2.10 (m, 4 H), 1.70 (m, 4 H), and 1.35 (m, 8 H); IR (KBr) 3420, 2940, 1910, and 1450 cm^{-1}; MS (CI/CH_4) 363 (M + H). Anal. ($C_{22}H_{42}N_4$·4HCl) C, H, N, Cl.

***N,N'*-Bis(3-aminopropyl)-*N,N'*-dimethyl-1,8-octanediamine Tetrahydrochloride (46).** A solution of compound **16** (1 g, 4 mmol), sodium hydroxide (0.32 g, 8 mmol), and acrylonitrile (0.43 g, 8 mmol) in ethanol (16 mL) was stirred for 72 h at ambient temperature. The mixture was filtered and the filtrate was evaporated. The residue was stirred with ether (50 mL) and filtered. The filtrate was evaporated to give compound **45** (1 g, 90%) as a clear oil: NMR ($CDCl_3$) δ 2.8–2.35 (m, 12 H), 2.2 (s, 6 H), and 1.30 (m, 12 H). Compound **45** was taken up in a mixture of acetic acid (26 mL) and concentrated HCl (1.3 mL); the solution was hydrogenated in a Parr apparatus in the presence of PtO_2 (0.2 g). The catalyst was removed and the solution was evaporated. The residue was recrystallized from 2-propanol to give **46** (130 mg, 7.4%) as a white solid: mp >300 °C; IR (KBr) 2950, 1610, and 1460 cm^{-1}; NMR (Me_2SO-d_6/D_2O, 1/1) δ 3.05 (m, 8 H), 2.88 (t, J = 7.0 Hz, 4 H), 2.65 (s, 6 H), 1.95 (m, 4 H), 1.6 (m, 4 H), and 1.3 (m, 8 H); MS (EI) 286 (M). Anal. ($C_{16}H_{38}N_4$·4HCl·$^1/_2H_2O$) C, H, N, Cl.

Similarly prepared was **21**: NMR (Me_2SO-d_6/D_2O) 3.50 (t, J = 6 Hz, 4 H), 2.90 (m, 12 H), and 2.00 (m, 8 H); IR (KBr) 3480, 2520, 1600, and 1525, cm^{-1}; MS (CI/CH_4) 247 (M + H). Anal. ($C_{12}H_{30}N_4O$·4HCl·$^1/_2H_2O$) C, H, N; Cl: calcd, 35.45; found, 34.04.

Registry No. 8, 39801-32-6; 9, 125763-67-9; 10·2HCl, 51920-08-2; 10 (free base), 13643-20-4; 11·2HCl, 51920-09-3; 11 (free base), 102203-35-0; 13·2HCl, 125763-68-0; 13 (free base), 86108-46-5; 14·2HCl, 89990-48-7; 14 (free base), 2157-24-6; 15, 105-83-9; 16·2HCl, 63869-19-2; 16 (free base), 33563-54-1; 17·3HCl, 125763-69-1; 17 (free base), 75403-53-1; 18·3HCl, 82958-56-3; 18 (free base), 6711-48-4; 19·3HCl, 82958-51-8; 19 (free base), 53774-74-6; 20, 54443-83-3; 21·4HCl, 102203-40-7; 21 (free base), 102203-41-8; 22, 117654-82-7; 23b, 122560-29-6; 25b·4HCl, 122560-26-3; 25b (free base), 122560-20-7; 26a·4HCl, 122560-24-1; 26a (free base), 122560-19-4; 26b·4HCl, 122560-25-2; 26b (free base), 122560-21-8; 26c·4HCl, 125763-79-3; 26c (free base), 122560-23-0; 26d·4HCl, 117654-75-8; 26d (free base), 125763-86-2; 26e·4HCl, 117654-74-7; 26e (free base), 117654-73-6; 27·4HCl, 125763-70-4; 27 (free base), 125763-82-8; 28, 125763-71-5; 29·4HCl, 125763-72-6; 29 (free base), 125763-83-9; 31, 122560-30-9; 32, 122560-31-0; 33, 122560-32-1; 34·4HCl, 125763-73-7; 34 (free base), 122560-22-9; 35, 92136-43-1; 36, 125763-74-8; 37, 125781-08-0; 38·3HCl, 125763-75-9; 38 (free base), 125763-81-7; 39, 117654-97-4; 40, 117654-99-6; 41, 117655-01-3; 42·4HCl, 117681-74-0; 43 (free base), 125763-84-0; 43, 82409-00-5; 44, 125763-76-0; 45, 125763-77-1; 46·4HCl, 125781-09-1; 46 (free base), 125763-85-1; 47, 99207-33-7; 48, 63344-92-3; 49, 125763-78-2; $PhCH_2NH(CH_2)_8NHCH_2Ph$, 39624-13-0; $H_2N(CH_2)_3NH(CH_2)_8NH(CH_2)_3NH_2$, 54443-83-3; $Me_2NCH_2CH_2CN$, 1738-25-6; $H_2N(CH_2)_8NH_2$, 373-44-4; $H_3CC{-}H{=}CHCN$, 4786-20-3; $MeCOCH{=}CH_2$, 78-94-4; $(BOC)N[(C{-}H_2)_3OH]_2$, 125763-80-6; $HN[(CH_2)_3OH]_2$, 14002-33-6; $HO(C{-}H_2)_3NH(CH_2)_8NH(CH_2)_3OH$, 117654-98-5; $MeSO_3(CH_2)_3N{-}(BOC)(CH_2)_8N(BOC)(CH_2)_3OSO_2Me$, 117655-00-2; $H_2C{=}CHCN$, 107-13-1; $Cl(CH_2)_3OH$, 627-30-5; *N*-(3-bromopropyl)phthalimide, 5460-29-7.

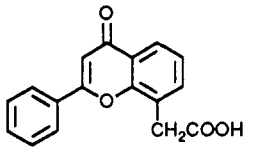
Potential Antitumor Agents. 60. Relationships between Structure and in Vivo Colon 38 Activity for 5-Substituted 9-Oxoxanthene-4-acetic Acids

Graham J. Atwell, Gordon W. Rewcastle, Bruce C. Baguley, and William A. Denny*

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.
Received June 29, 1989

9-Oxoxanthene-4-acetic acids are a class of antitumor agents effective against the mouse colon adenocarcinoma 38 in vivo. Within this class, 5-substituents on the xanthenone are known to enhance potency. To extend structure–activity relationships for the class, a series of derivatives bearing a wide variety of substituents at the 5-position have been prepared and evaluated. The results suggest that activity correlates better with the lipophilic properties of substituents rather than with their electronic properties. Generally, lipophilic substituents result in more active compounds, but there may be a size limitation on such substituents. The 5-methyl derivative is the most dose-potent of the analogues studied.

The recent discovery[1-5] of the unusual antitumor profile of flavoneacetic acid (**1**, FAA, NSC 347512) has sparked interest in the development of related compounds of similar activity. While FAA has not yet shown clinical activity,[6,7] its unique effects against experimental colon tumors make it an important lead. Although it has been shown to act as a biological response modifier, inducing natural killer cell activity,[4] and to have marked effects on tumor blood flow,[8,9] its mode of action is not yet known. In the absence of this information new drug development is of necessity slow and relies on the determination of structure–activity relationships (SAR) among related compounds.



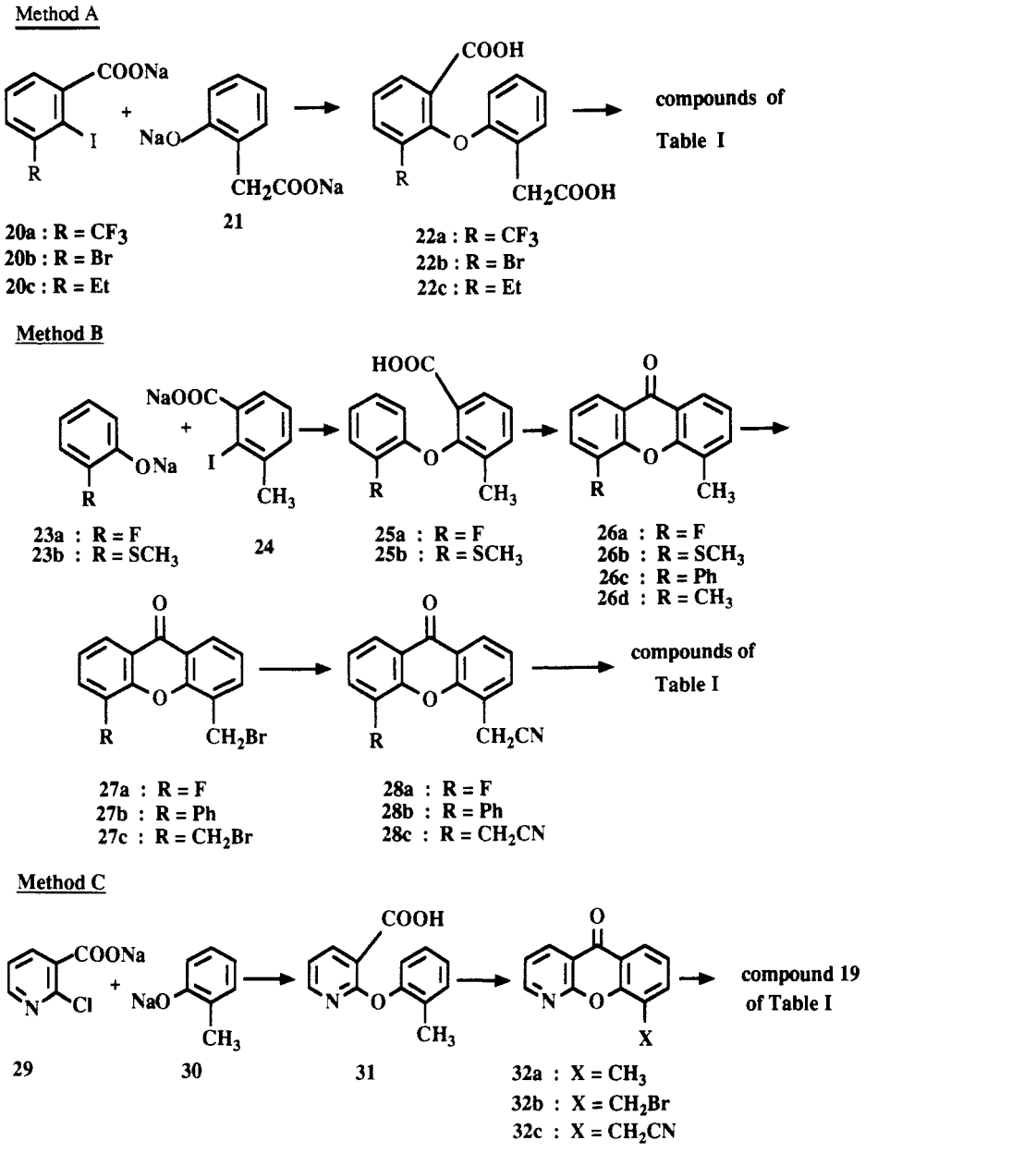
1

We have recently reported SAR for in vivo colon 38 activity among analogues of FAA itself[10] and also[11] for a

(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.
(4) Ching, L-M.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1047.
(5) Capolongo, L. S.; Balconi, G.; Ubezio, P.; Giavazzi, R.; Taraboletti, G.; Regonesi, A.; Yoder, O. C.; D'Incalci, M. *Eur. J. Clin. Oncol.* **1987**, *23*, 1529.
(6) Kerr, D. J.; Kaye, S. B.; Cassidy, J.; Bradley, C.; Rankin, E. M.; Adams, L.; Setanoians, A.; Young, T.; Forrest, S.; Soukop, M.; Calvel, M. *Cancer Res.* **1987**, *47*, 6776.
(7) Weiss, R. B.; Greene, R. F.; Knight, R. D.; Collins, J. M.; Pelosi, J. J.; Sulkes, A.; Curt, G. A. *Cancer Res.* **1988**, *48*, 5878.
(8) Bibby, M. C.; Double, J. A.; Loadman, P. M.; Duke, C. V. *JNCI, J. Natl. Cancer Inst.* **1989**, *81*, 216.
(9) Zwi, L. J.; Baguley, B. C.; Gavin, J. B.; Wilson, W. R. *JNCI, J. Natl. Cancer Instit.* **1989**, *81*, 1005.
(10) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Des.* **1989**, *4*, 161.
(11) Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Calveley, S. B.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 793.

0022-2623/90/1833-1375$02.50/0

Scheme I



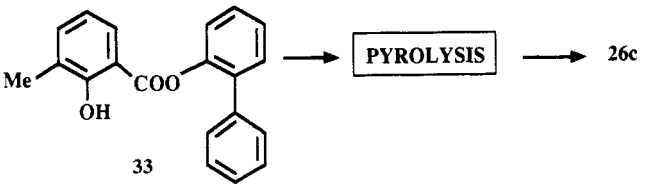
series of methyl-, methoxy-, chloro-, nitro-, and hydroxy-substituted 9-oxoxanthene-4-acetic acids (XAA analogues), as part of a program to develop compounds with a similar profile of activity to FAA but with greater potency. SAR for the xanthenones using this limited set of substituent groups showed that substitution at the 5-position generally provided the most active analogues, with both the 5-chloro and the 5-methyl derivatives showing high activity and improved dose potency over the parent unsubstituted compound.

We now report the synthesis and evaluation of a larger series of 5-substituted 9-oxoxanthene-4-acetic acids, designed to enable a more detailed study of structure–activity relationships among this class of compound. The substituents cover a wide range of electronic and hydrophobic parameters, and the influence of these properties on activity is explored.

Chemistry

The most direct route[11] to the desired substituted 9-oxoxanthene-4-acetic acids was by direct coupling of 3-substituted-2-halobenzoic acids (**20**) and 2-hydroxyphenylacetic acid (**21**) using the phase-transfer catalyst

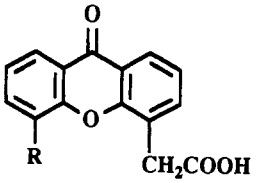
Scheme II



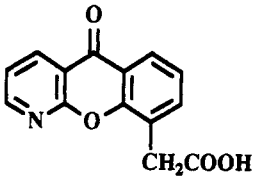
tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1)[12] to give diacids **22** in good yields. Acid-catalyzed cyclodehydration then occurs selectively to give the desired 9-oxoxanthene-4-acetic acids (method A of Scheme I). Some analogues (e.g. **6**, **7**, **16**, **19**) were derived from the precursor 5-substituted-4-methylxanthenones (**26**), elaborating the methyl group via the bromomethyl (**27**) and acetonitrile (**28**) intermediates by using standard methods.[11] The requisite 4-methylxanthenones (**26** and **32a**) were prepared

(12) Soula, G. *J. Org. Chem.* **1986**, *50*, 3717.

Table I. Physicochemical and Biological Properties of 5-Substituted 9-Oxoxanthene-4-acetic Acids



2 - 18



19

no.	R	mp, °C	formula	analyses	π^a	σ^b	MR[c]	method[d]	colon 38 OD[e]	colon 38 activity[f]
2	H[g]				0.0	0.0	1.03	g	220	++
3	CF_3	208–209	$C_{16}H_9F_3O_4$	C, H, F	0.88	0.54	5.02	A	220	+
4	Br	254–255	$C_{15}H_9BrO_4$	C, H	0.86	0.23	8.88	A	100	–
5	Cl[g]				0.71	0.23	6.03	g	150	++
6	F	189	$C_{15}H_9FO_4$	C, H, F	0.14	0.09	0.92	B	330	
7	Ph	248–249	$C_{21}H_{14}O_4$	C, H	1.96	–0.01	25.36	D	750	–
8	Et	210–211	$C_{17}H_{14}O_4$	C, H	1.02	–0.15	10.30	A	100	++
9	Me[g]				0.56	–0.17	5.65	g	45	++
10	OEt	278–279	$C_{17}H_{14}O_5$	C, H	0.38	–0.24	12.47	C	330	++
11	OPr	257–257.5	$C_{18}H_{16}O_5$	C, H	1.05	–0.25	17.06	C	150	+
12	OMe[g]				–0.02	–0.27	7.87	g	150	+
13	OBu	205.5–206.5	$C_{19}H_{18}O_5$	C, H	1.55	–0.32	21.66	C	220	+
14	NO_2[g]				–0.28	0.78	7.36	g	330	+
15	NHCOMe	301–303	$C_{17}H_{13}NO_5$	C, H, N	–0.97	0.00	14.93		330	–
16	CH_2COOH	303–305	$C_{17}H_{12}O_6$	C, H	–0.32	0.00	13.0	B	330	–
17	OH[g]				–0.67	–0.37	2.85	g	330	–
18	NH_2	266–267	$C_{15}H_{11}NO_4$	C, H, N	–1.23	–0.66	5.42		1125	+
19	=N—	229–230	$C_{14}H_9NO_4$	C, H, N	–1.51	0.78[h]	0.5	B	750	–

[a,b] π and σ substituent group values from ref 20. [c,d] Method of synthesis, see the Experimental Section. [e] OD: optimal dose of drug in milligrams/kilogram, administered intraperitoneally as the sodium salt in 0.2 mL of water in a single dose. [f] Subcutaneous colon 38 tumors (5–10 mm in diameter) were treated with the optimal dose of drug. Tumors were removed after 24 h, fixed in formalin, and stained in hematoxylin/eosin (ref 15). Sections were examined by histopathology and compared with those from control tumors. + = 50–90% hemorrhagic necrosis cross section; ++ = >90% hemorrhagic necrosis across section (average of three independent experiments). [g] Data taken from ref 11. [h] Assumed.

either by coupling of 2-chloronicotinic acid (**29**) with 2-methylphenol (**30**) (method C of Scheme I) or 2-iodo-3-methylbenzoic acid (**24**) with 2-substituted phenols **23** (method B of Scheme I). 4-Methyl-5-phenylxanthenone (**26c**) for the preparation of **7** was made from aryl ester **33**[13] by pyrolysis[14] (method D, Scheme II). Ethers (**10**, **11**, and **13**) were prepared by direct alkylation of the known[11] 5-hydroxy derivative **17**.

Results and Discussion

The compounds were evaluated against the subcutaneous C38 colon tumor in vivo, using the short term (24 h) histology assay[15] and a single-dose protocol, and the results are given in Table I. Previous studies[11] with substituted 9-oxoxanthene-4-acetic acids using the same assay suggested that dose potency depended largely on the position of the substituent, with 5-substituted compounds proving the most potent. In contrast, levels of activity were less dependent on the positioning of the substituent than on its nature, but too few substituents were available to properly explore this. The 5-substituted compounds studied here constitute a larger set, where the lipophilic and electronic properties of the substituents (determined by π and σ parameters) vary orthogonally over a wide range. The coefficient for the π/σ correlation is 0.103, indicating essentially no interrelationship. To aid in discerning possible SAR, the compounds are listed in Table

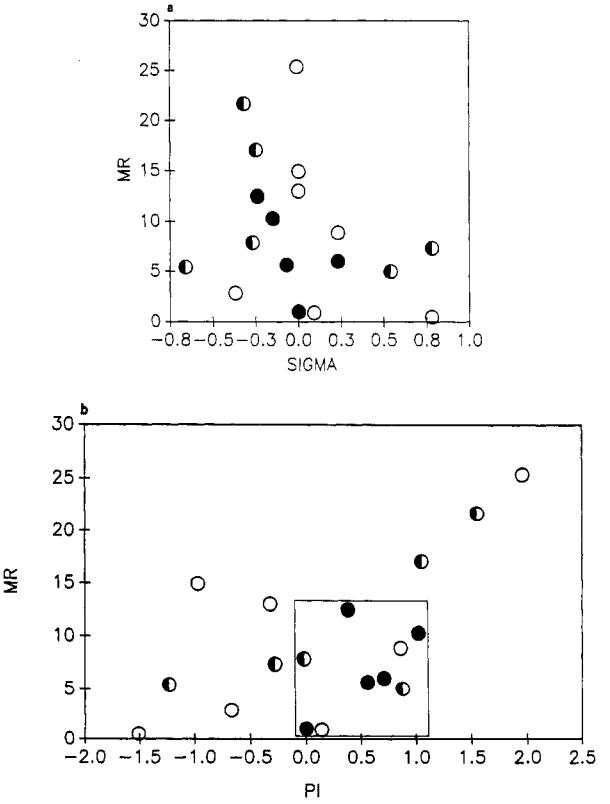


Figure 1. Relationship between biological activity and various substituent properties for 5-substituted 9-oxoxanthene-4-acetic acids. Compounds are designated ● for high activity, ◐ for low activity, and ○ for inactive: (a) plot of substituent size (MR) versus electronic property (δ), (b) plot of substituent size (MR) versus lipophilicity (π).

(13) Kanaoka, Y.; Tanizawa, K.; Sato, E.; Yonemitsu, O.; Ban, Y. *Chem. Pharm. Bull.* **1967**, *15*, 593.
(14) Schopff, M. *Chem. Ber.* **1892**, *25*, 3642.
(15) Baguley, B. C.; Calveley, S. B.; Crowe, K. K.; Fray, L. M.; O'Rourke, S. A.; Smith, G. P. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 263.

I in two groups: those (3–13) with lipophilic substituents and those (14–19) with hydrophilic substituents. Within each group, the compounds are arranged in order of increasing substituent electron-donating ability.

Table I suggests that activity in the colon 38 assay broadly correlates more with the lipophilic rather than the electronic properties of substituents. This can be seen more easily in Figure 1, where compounds are designated ● for ++ activity, ◐ for + activity, and ○ for inactive and plotted two-dimensionally in substituent size/electronic parameter space (Figure 1a) and subtituent size/lipophilic parameter space (Figure 1b), using the MR parameter for substituent size, σ for electronic character, and π for lipophilicity.[16] In Figure 1a there is no obvious grouping of the active compounds. However Figure 1b suggests that activity correlates broadly with substituent lipophilicity, with 9/11 active compounds having substituent π values >0. The two exceptions are the NO_2 and NH_2 derivatives (14 and 18). While the amino compound is very hydrophilic it is also very nonpotent, showing activity only above 1000 mg/kg. Figure 1b also suggests that, even for the lipophilic substituents, there may be a size limitation. OEt compound 8 exhibits ++ activity, but the more lipophilic OPr and OBu compounds 11 and 13 are less active while the very lipophilic phenyl derivative 7 is inactive. In fact, all five of the compounds with ++ activity have substituents which fit into a relatively small area of the size/lipophilicity parameter space explored (boxed area in Figure 1b). While this is a very simple analysis, it agrees with the tentative conclusions of the earlier study[11] and suggests that to retain high activity in the series while seeking higher potency requires small, lipophilic substituents.

Conclusions

While retention of high levels of activity is a necessary first requirement in the development of XAA analogues, high potency is also very important, since a real clinical problem with FAA (1) is the high doses which must be given.[6] Table I shows the striking effect of the methyl substituent on potency, making compound 9 7-fold more dose-potent than the parent 2 and 3-fold more potent than 5-ethyl analogue 8. The results suggest that, while activity may be governed largely by substituent lipophilicity, there may also be an upper size limitation for lipophilic substituents. This leads to the conclusion that polysubstitution with small, lipophilic groups is worth exploring, and this is being done.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical, and analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus using the supplied, stem-corrected thermometer and are as read. All compounds had NMR spectra (measured on a Bruker WP-60 (Me_4Si) consistent with their assigned structures.

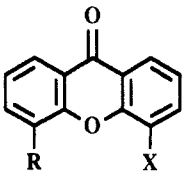
9-Oxo-5-(trifluoromethyl)xanthene-4-acetic Acid (3) by Method A of Scheme I. 3-(Trifluoromethyl)-2-iodobenzoic acid (**20a**) [mp (petroleum ether, bp 40–60 °C) 134 °C. Anal. ($C_8H_4F_3IO_2$) C, H, F.] was prepared from the corresponding anthranilic acid[17] by the Sandmeyer reaction. A mixture of the sodium salt of **20a** (5.41 g, 16 mmol), the disodium salt of 2-hydroxyphenylacetic acid (**21** 3.76 g, 19 mmol), CuCl (0.16 g, 1.6 mmol), and TDA-1 (0.78 g, 2.4 mmol) in a mixture of dry 1,4-dioxane (30 mL) and dry DMSO (15 mL) was heated at 110 °C with stirring for 12 h. The solvents were removed under reduced pressure, and the residue was dissolved in excess 0.1 N NaOH and filtered to remove insoluble copper salts. The filtrate was acidified with 2 N HCl and extracted with EtOAc, and the organic layer was then extracted with dilute aqueous ammonia. The aqueous layer was added slowly to a stirred excess of 2 N HCl to give a crude product. Crystallization from benzene gave 2-[2-(carboxymethyl)phenoxy]-3-(trifluoromethyl)benzoic acid (**22a**, 2.23 g, 41%) suitable for use in the next step. A sample further crystallized from benzene had mp 202–203 °C. Anal. ($C_{16}H_{11}F_3O_5$) C, H, F.

Similar reactions using 3-bromo-2-iodobenzoic acid [**20b**; prepared by Sandmeyer from 3-bromoanthranilic acid, mp (EtOAc/petroleum ether, bp 40–60 °C) 125–128 °C (lit.[18] mp 148.5–151 °C by subl). Anal. $C_7H_4BrIO_2$) C, H, Br] and 3-ethyl-2-iodobenzoic acid [**20c**; prepared similary from 3-ethylanthranilic acid,[19] mp (petroleum ether, bp 40–60 °C) 109.5–110 °C. Anal. ($C_9H_9IO_2$) C, H, I] gave respectively 2-[2-(carboxymethyl)phenoxy]-3-bromobenzoic acid **22b**; 61% yield, mp (benzene–petroleum ether, bp 40–60 °C) 185–186 °C. Anal. ($C_{15}H_{11}BrO_5$) C, H, Br] and 2-[2-(carboxymethyl)phenoxy]-3-ethylbenzoic acid [**22c**; 69% yield, mp (CH_2Cl_2) 151–153 °C. Anal. ($C_{17}H_{16}O_5$) C, H].

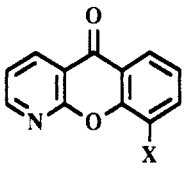
Crude 2-[2-(carboxymethyl)phenoxy]-3-(trifluoromethyl)benzoic acid (**22a**, 2.0 g, 5.88 mmol) was dissolved in a mixture of concentrated H_2SO_4 (27 mL) and water (3 mL) and heated at 80 °C for 10 min and then cooled and diluted with water. The precipitate was collected, washed with water, and extracted with dilute aqueous $KHCO_3$. The extract was filtered and acidified with HCl, and the resulting precipitate was crystallized from MeOH and then benzene to give oxo-5-(trifluoromethyl)-xanthene-4-acetic acid (**3**, 1.38 g, 73%), mp 254–255 °C. Anal. in Table I. The sodium salt was crystallized from MeOH/EtOAc. Similar reactions gave compounds 4 and 8 of Table I.

9-Oxo-5-azaxanthene-4-acetic Acid (19) by Method B of Scheme I. A mixture of sodium 2-chloronicotinate (**29**, 17.9 g, 0.1 mol), sodium 2-methylphenate (**24**, 13.0 g, 0.1 mol), TDA-1 (3.2 g, 10 mmol), and CuCl (1 g, 10 mmol in dry 1,4-dioxane (200 mL) was heated under reflux for 2 h. Excess solvent was removed under reduced pressure and the residue was diluted with water, filtered, and acidified with HCl. Extraction with EtOAc gave crude 2-(2-methylphenoxy)nicotinic acid (**31**, 17.2 g), which was dissolved in polyphosphoric acid (250 g) and heated at 120 °C for 90 min. The cooled mixture was diluted with water and brought to neutral pH with 40% aqueous NaOH (cooling). The resulting precipitate was collected and extracted into CH_2Cl_2 to

Table II. Analytical Data for New 4,5-Disubstituted Xanthenones



26 - 28



32

no.	R	X	mp, °C	formula	anal.
26a	F	CH_3	152–153	$C_{14}H_9FO_2$	C,H,N
26b	SMe	Me	176–178	$C_{15}H_{12}O_2S$	C,H,S
26c	Ph	Me	140–141	$C_{20}H_{14}O_2$	C,H
27a	F	CH_2Br	156	$C_{15}H_8BrFO_2$	C,H
27b	Ph	CH_2Br	184–185	$C_{20}H_{13}BrO_2$	C,H,Br
27c	CH_2Br	CH_2Br	257–258	$C_{15}H_{10}Br_2O_2$	C,H,Br
28a	F	CH_2CN	140	$C_{15}H_8FNO_2$	C,H,N
28b	Ph	CH_2CN	178–179	$C_{21}H_{13}NO_2$	C,H,N
28c	CH_2CN	CH_2CN	257–259	$C_{17}H_{10}N_2O_2$	C,H,N
32a	—N=	CH_3	153–155	$C_{13}H_9NO_2$	C,H,N
32b	—N=	CH_2Br	214–215	$C_{13}H_8BrNO_2$	C,H,N,Br
32c	—N=	CH_2CN	200–201[a]	$C_{14}H_8N_2O_2$	C,H,N

[a] Reference 21 gives mp 196–198 °C.

(16) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.
(17) Fuller, R. W.; Molloy, B. B.; Day, W. A.; Roush, B. W.; Marsh, M. M. *J. Med. Chem.* **1973**, *16*, 101.
(18) Twiss, D.; Heinzelmann, R. V. *J. Org. Chem.* **1950**, *15*, 496.
(19) Piozzi, F.; Langella, M. R. *Gazz. Chim. Ital.* **1963**, *93*, 1392.

give 5-aza-4-methylxanthenone (**32a**, 14.1 g, 72% overall). A sample was crystallized from MeOH as needles, mp 153–155 °C. Anal. in Table II.

A solution of 5-aza-4-methylxanthenone (**32a**, 5 g, 25 mmol), *N*-bromosuccinimide (4.4 g, 25 mmol), and benzoyl peroxide (30 mg) in CCl_4 (200 mL) was stirred under reflux under illumination from a 100-W tungsten lamp for 3 h. The mixture was filtered hot, the filtrate was evaporated, and the residue was crystallized from petroleum ether (bp 80–100 °C) to give 5-aza-4-(bromomethyl)xanthenone (**30a**, 4.9 g, 70% yield), mp 214–215 °C. Anal. in Table II. A suspension of this compound (4.7 g, 17 mmol) in EtOH (100 mL) was treated with a solution of KCN (2.28 g, 35 mmol) in water (20 mL) under reflux for 1 h. Dilution with water and crystallization of the resultant dried precipitate from benzene/petroleum ether (bp 80–100 °C) gave 5-aza-9-oxoxanthene-4-acetonitrile (**32b**, 2.5 g, 65% yield), mp 200–201 °C. Anal. in Table II. This acetonitrile (3 g) was hydrolyzed in AcOH/H_2SO_4/H_2O (1:1:1) under reflux for 90 min. Dilution with water followed by crystallization of the resulting precipitate from EtOH gave the desired 9-oxo-5-azaxanthene-4-acetic acid (**19**, 2.4 g, 73% yield), mp 229–230 °C. Anal. in Table I.

5-Fluoro-9-oxoxanthene-4-acetic Acid (6) by Method C of Scheme I. Reaction of 3-methyl-2-iodobenzoic acid (**24**) and 2-fluorophenoil (**23a**) gave 2-(2-fluorophenoxy)-3-methylbenzoic acid [**25**, mp (benzene/petroleum ether, bp 40–60 °C) 154 °C. Anal. ($C_{14}H_9FO_2 \cdot 0.5H_2O$) C, H], which was similarly cyclized to 4-fluoro-5-methylxanthenone [**26a**; mp (MeOH) 154 °C. Anal. in Table II.] This was converted to **6** via the intermediates **27a** and **28a** as detailed above.

A similar reaction using 2-(methylthio)phenol (**23b**) gave 2-[2-(methylthio)phenyl]-3-methylbenzoic acid [25b, mp (benzene–petroleum ether, bp 40–60 °C) 135–137 °C. Anal. ($C_{15}H_{14}O_3S$) C, H], which was cyclized to 4-methyl-5-(thiomethyl)xanthenone (**26b**). However, this compound could not be converted to the corresponding bromomethyl compound with *N*-bromosuccinimide.

4-Methyl-5-phenylxanthenone (26c) by Method D of Scheme II. A mixture of 2-hydroxy-3-methylbenzoic acid (60.8 g, 0.4 mol), 2-hydroxybiphenyl (68 g, 0.4 mol), and polyphosphate ester (180 mL of the solution prepared by the method of ref 20) was heated on a water bath with occasional swirling for 3 h. Volatiles were removed under reduced pressure, and the residue was poured onto crushed ice. Excess powdered $NaHCO_3$ was added, and the mixture was kept at 20 °C for 12 h and extracted with CH_2Cl_2. Removal of solvent gave an oil, which was extracted with hot petroleum ether (bp 40–60 °C) to give crude biphenyl ester **33** (89 g, 73%) as an oil of ca. 85% purity (TLC), suitable for use in the next stage.

Crude ester **33** (78 g, ca. 0.25 mol of pure compound) was heated to 280–300 °C in a flask connected to a short distillation pathway. A vigorous exotherm occurred, resulting in rapid distillation of the pyrolysate. The highest boiling point fraction (340–370 °C, ca. 26 g) was heated under reflux for 5 min in a mixture of EtOH (300 mL) and 5 N aqueous NaOH (60 mL). The resulting hot solution was diluted with a limited volume of water and cooled well, and the precipitated crude product was collected and washed with water. TLC of the mother liquors indicated the presence of 4,5-dimethylxanthenone (as a byproduct of the pyrolysis). Two crystallizations of the precipitate from petroleum ether (bp 80–100 °C) gave pure 4-methyl-5-phenylxanthenone (**26c**, 8.8 g, 14% based on pure starting material) as colorless needles, mp 140–141 °C. Anal. ($C_{20}H_{14}O_2$) C, H. The compound was then elaborated by method B to give compound **7** of Table I.

Compound **18** was prepared by catalytic hydrogenation of **14**, and acylation of **18** gave **15**.

9-Oxo-5-propoxyxanthene-4-acetic Acid (11) from 17. A mixture of powdered 9-oxo-5-hydroxyxanthene-4-acetic acid (**17**, 1.00 g, 3.7 mmol), powdered K_2CO_3 (1.02 g, 7.4 mmol) and *n*-propyl 4-toluenesulfonate (2.38 g, 11.1 mmol) in dry *N*,*N*-dimethylacetamide (8 mL) was stirred at 80 °C for 2 h and then cooled and diluted with excess water. The resulting solid was collected, washed with 0.5 N NaOH and water, and then saponified (1 N NaOH/aqueous EtOH/heat). Acidification followed by crystallization from aqueous DMF gave pure 9-oxo-5-propoxyxanthene-4-acetic acid (**11**, 0.99 g, 86% yield) as colorless needles, mp 257–257.5 °C. Anal. in Table I.

Similar reactions employing the requisite alkyl 4-toluenesulfonates gave compounds **10** [82% yield, mp (MeOH) 278–279 °C. Anal. in Table I] and **13** [74% yield, mp (MeOH) 205.5–206.5 °C. Anal. in Table I].

Biological Testing. This was carried out by using published protocols.[15] Briefly, 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 at a range of doses escalating by 1.5-fold up to a maximum dose of 750 mg/kg, or at the maximum tolerated dose if this was lower. The maximum tolerated dose was defined as the highest dose in the above protocol which did not cause death in 24 h. 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 when given at a dose of 330 mg/kg caused necrosis across the whole of the tumor section (scored as ++).[15] Compounds showing lesser but still extensive necrosis (50–90%) were scored as +. The assay provides a stringent criterion of activity, since compounds showing less than 50% necrosis were scored as negative (–).

Acknowledgment. We thank Stephen Calveley and Li Zhuang for histology assessment, Kym Crowe and Wayne Joseph for animal testing, and Lynden Hull for preparation of the manuscript. This work was supported by the Auckland Division of the Cancer Society of New Zealand and the Medical Research Council of New Zealand.

(20) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. 1, p 892.
(21) Nakanishi, M.; Oe, T.; Tsuruda, M.; Matsuo, H.; Sakuragi, S.; Maruyama, Y. *Yakukaku Zasshi* **1976**, *96*, 99; *Chem. Abstr.* **1976**, *84*, 135515*b*.