were dissolved in 2% methanol in bidistilled water and injected under 0.1 mL per 10 g of body weight.

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Registry No. 16, 77327-05-0; 2, 117710-03-9; 3, 130406-04-1; 4, 130406-05-2; 5, 130406-06-3; 6, 130466-66-9; 7, 117733-99-0; 8a, 79-33-4; 8b, 611-71-2; 8c, 501-97-3; 8d, 112-67-4; 9, 117710-32-4; 10b, 130406-08-5; 10c, 130406-09-6; 10d, 130406-10-9; 11a, 117710-19-7; 11b, 130406-11-0; 11c, 130406-12-1; 11d, 130406-13-2; 12, 130406-07-4; 13a, 130466-67-0; 14a, 130466-68-1; BOC-Pro-Leu-OMe, 15136-16-0.

Potential Antitumor Agents. 62. Structure–Activity Relationships for Tricyclic Compounds Related to the Colon Tumor Active Drug 9-Oxo-9*H*-xanthene-4-acetic Acid

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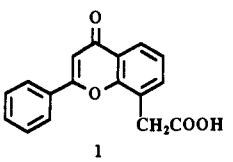
A series of tricyclic analogues of 9-oxo-9*H*-xanthene-4-acetic acid have been prepared and evaluated for their ability to cause hemorrhagic necrosis in subcutaneously implanted colon 38 tumors in mice, in an effort to extend the structure–activity relationships for this series. As was found previously with analogues of flavone-8-acetic acid (FAA) (Atwell et al. *Anti-Cancer Drug Des.* **1989**, *4*, 161), all electronic modifications of the XAA nucleus led to severe decreases or complete abolition of activity, suggesting narrow structure–activity relationships. Dipole moments for many of the compounds were computed, and the degree to which the molecular dipole moment lay out of the plane of the aromatic part of these molecules was found to be determined largely by the contributions from the acetic acid moiety relative to that from the tricyclic ring system. There did not appear to be any general relationship between the *magnitude* of the dipole moment and activity. However, for compounds containing the 9-carbonyl functionality, the *orientation* of the dipole vector may be of significance. In all compounds possessing an ether group peri to the acetic acid side chain, there was a close approach (ca. 2.4 Å) between this and the side chain OH.

Following the discovery of the selective solid tumor activity[1,2] and unusual biological effects[3–5] of the drug flavoneacetic acid (1), there have been a limited number



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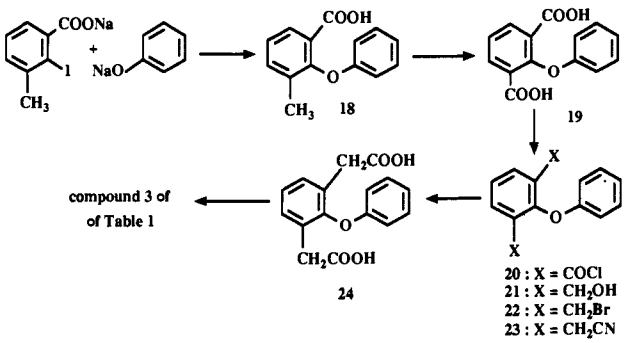
of reports[6–8] of analogue studies. The majority of these have been on compounds closely related to 1, and no clear structure–activity relationships (SAR) are yet apparent, although a limited study of chromophore variations[9] suggested that these are quite narrow. We have recently shown[10] that 9-oxo-9*H*-xanthene-4-acetic acid[11] (2, XAA) also has in vivo colon 38 activity and have demonstrated limited SAR for ring-substituted analogues,[12,13] some of which show much better dose-potency than FAA.

In order to further delineate the SAR for this novel type of antitumor agent, we have extended our studies to encompass a wider range of linear tricyclic chromophores and report this work here.

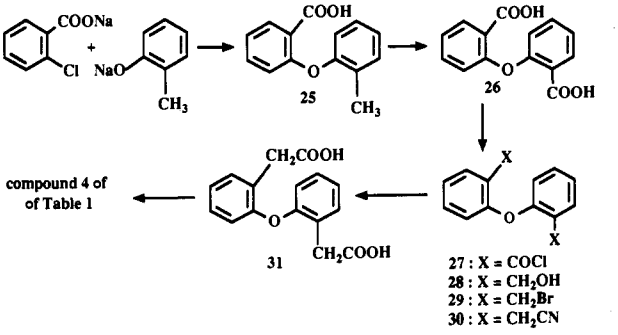
Chemistry

The 10,11-dihydro-10-oxodibenz[*b*,*f*]oxepin derivative (3) was synthesized by the method shown in Scheme I. Ullman condensation of 2-iodo-3-methylbenzoic acid and phenol using the phase-transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1)[14] gave a 42% yield of 3-methyl-2-phenoxybenzoic acid (18). A previous attempt to prepare this compound by the Ullmann route failed,[15] so the present work is a further indication of the usefulness of TDA-1 in such reactions.[12,14] Oxidation of 18 gave dicarboxylic acid 19, which was elaborated to the corresponding diacetic acid 24 via intermediates 20–23 in an

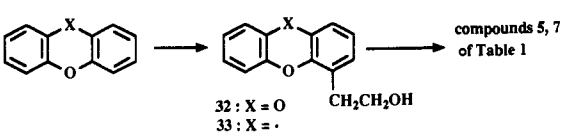
Scheme I



Scheme II

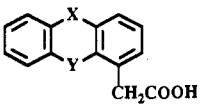


Scheme III



†University of Auckland.

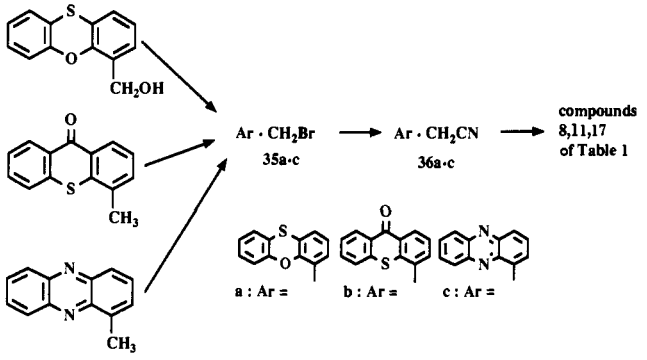
Table I. Physicochemical and Biological Properties of Tricyclic Acetic Acid Compounds



no.	X	Y	mp	formula	analyses	Rm[a]	colon 38 in vivo OD[b]	colon 38 in vivo activity[c]
2	CO	O				0.64	220[d]	++[d]
3	$COCH_2$	O	151	$C_{16}H_{12}O_4$	C, H	0.65	750	+
4	CH_2CO	O	200	$C_{16}H_{12}O_4 \cdot 1/4H_2O$	C, H	0.45	750	-
5	O	O	166-168	$C_{14}H_{10}O_4$	C, H	0.53	750	-
6	CH_2	O	136-140	$C_{15}H_{12}O_3$	C, H	0.70	500	-
7	*e*	O	213-214		217-218[f]	0.81	500	+
8	S	O	129	$C_{14}H_{10}O_3S$	C, H, S	0.60	330	-
9	SO	O	221-223	$C_{14}H_{10}O_4S$	C, H	0.50	750	-
10	SO_2	O	183	$C_{14}H_{10}O_5S$	C, H, S	0.80	750	-
11	CO	S	210-211	$C_{15}H_{10}O_3S$	C, H, S	0.60	330	-
12	CO	SO	213-214	$C_{15}H_{10}O_4S$	C, H, S	0.26	750	-
13	CO	SO_2	228-229	$C_{15}H_{10}O_5S$	C, H, S	0.47	750	-
14	CO	CO	232 (d)	$C_{16}H_{10}O_4$	C, H	0.63	750	+
15	O	*e*	170-171		171-172[f]	0.65	750	-
16	—CH=	—CH=	167-168		170-171[g]	0.84	750	+
17	—N=	—N=	196-198	$C_{14}H_{10}N_2O_2$	C, H, N	0.67	750	-

[a] Rm values determined by liquid-liquid chromatography in ref 19, using 4'-(9-acridinylamino)methanesulfonanilide (AMSA) as internal reference. [b] OD: optimal dose of drug in milligrams/kilogram, administered intraperitoneally as the sodium salt in 0.2 mL of water in a single dose. [c] Subcutaneous colon tumors were removed after 24 h, fixed in formalin, and stained in haematoxylin/eosin (ref 20). Sections were examined by histopathology and compared with those from control (untreated) tumors. [d] ++ = >90% hemorrhagic necrosis across entire section; + = 50-90% hemorrhagic necrosis; - = <50% hemorrhagic necrosis. [e] dibenzofuran chromophore. [f] Reference 33. [g] Reference 38.

Scheme IV



overall yield (from **18**) of 42%. Cyclodehydration of **24** with PPA at 120 °C (a method used previously for the preparation of other dibenzooxepines[16]) gave a good yield of the desired **3**.

A similar method (Scheme II) was used to prepare the isomeric 10,11-dihydro-11-oxodibenz[*b,f*]oxepin (**4**), via the known[17,18] symmetric 2,2'-oxybisbenzoic acid (**26**) and the corresponding bisacetic acid **31**. The dibenzodioxin and dibenzofuran analogues (**5** and **7**) were prepared from the parent tricyclic ethers by reaction of the lithio derivatives with oxirane and subsequent oxidation (Scheme III). Reduction of XAA (**2**) with $NaBH_4$ gave xanthene **6**.

Several other compounds (e.g. **8**, **11**, **17**) were prepared by the general method of Scheme IV, preparation of the

Table II. Molecular Dipole Moments[a] of Selected Tricyclic Acetic Acids of Table I

no.	μ,[b] D	θ,[c] deg	ϕ,[d] deg
2	3.87	51	112
3	3.38	*e*	*e*
4	3.38	*e*	*e*
5	5.08	41	144
6	5.86	32	151
7	5.58	37	166
14	5.76	50	120
15	4.06	50	152
16	5.15	30	153
17	4.74	46	131

[a] Calculated with AMPAC.[22] [b] Magnitude of molecular dipole moment (debye). [c] θ: the angle between the dipole vector and the plane defined by the atoms a, b, and c (see Figure 1). [d] ϕ: the angle the dipole vector makes with respect to carbon atoms a and b in this plane (see Figure 1). [e] Molecules nonplanar.

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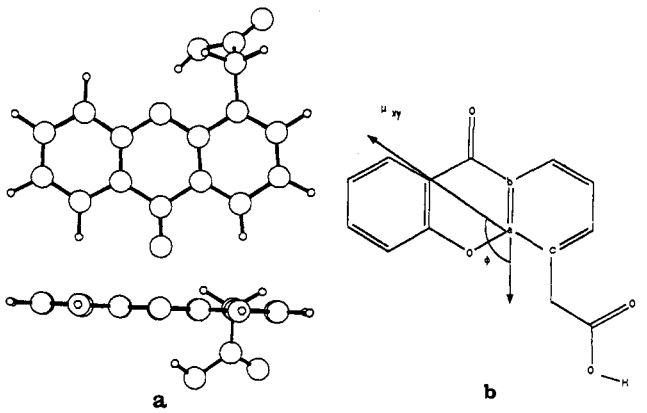

Figure 1. (a) Molecular structure of 9-oxo-9*H*-xanthene-4-acetic acid (**2**), computed by the AM1 semiempirical molecular orbital method with the program AMPAC.[22] (b) Orientation of the molecular dipole moment of **2**. The angle Θ is the angle between the molecular dipole vector and the plane defined by the atoms a, b, and c, and the angle ϕ is the angle the dipole vector makes with respect to carbon atoms a and b in this plane.

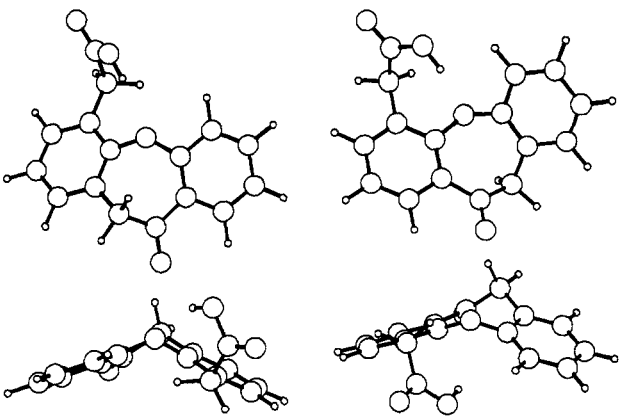

Figure 2. Conformations of the dibenzoxepins **3** (left) and **4** (right), computed by using the methods given in Figure 1.

bromomethyl derivatives (from either the methyl or hydroxmethyl compounds) and conversion of these via the acetonitriles, while compounds **14** and **16** were prepared by known methods, as discussed briefly in the Experimental Section.

Results and Discussion

Details of the physicochemical and biological properties of the compounds studied are given in Table I. Compound lipophilicity was measured by liquid–liquid thin-layer chromatography as described previously.[13,19] While most of the compounds appeared to be more lipophilic than XAA, there were no large variations across the series. Antitumor testing employed the subcutaneously implanted C38 colon tumor in mice, using the short-term histology assay[20] to measure in vivo antitumor effects. This correlates well with growth-delay assays (data not shown). Drugs were given as a single intraperitoneal injection of the sodium salt in water, with precautions taken to exclude light, given the known[21] photosensitivity of similar compounds. Each compound was tested over a range of doses increasing at 1.5-fold intervals up to the maximum tolerated dose, with at least three independent determinations at the optimal dose level.

In order to examine features of the electronic structure of these molecules that may be relevant to their activity, semiempirical molecular orbital calculations were carried out on molecules **2–7** and **14–17** using the AM1 semiempirical molecular orbital method with the program package AMPAC.[22] The derived property that was of interest from these calculations is the molecular dipole moment, μ. Both the magnitude (debye, D) and the orientations of the molecular dipole moment with respect to the acetic acid substituted benzene ring were computed (after full optimization of the molecular geometry with respect to all coordinates), and the data are given in Table II. The orientations of the molecular dipole moment are given by the angle Θ, a measure of the angle between the dipole vector and the plane defined by the atoms a, b, and c, and ϕ (the angle the dipole vector makes with respect to carbon atoms a and b in this plane). These orientations were calculated for each of the tricyclic rings. The calculated molecular structure of 9-oxo-9*H*-xanthene-4-acetic acid (**2**) itself shows a coplanar tricyclic ring system, in agreement with the crystal structures.[23,24] The carboxylic acid group is rotated out of the plane of the rings, with a torsion angle of about 90° about the CH_2–C(ring) bond (Figure 1), a feature which was found to be typical of all the systems calculated here. The ϕ angle of 112° places the positive pole of the dipole almost directly toward the 8-carbon of the xanthenone ring, although the Θ value of 51° indicates that it projects significantly out of the plane of the ring away from the acid side chain (Figure 1). In the planar parent xanthenone, the dipole moment is calculated to lie in the plane of the molecule (data not shown). Thus the nonplanarity of the dipole moment with the ring system in **2** is determined by the degree of rotation of the acetic acid group with respect to the ring, and the orientation of the carboxylic acid group around the CH_2–COOH bond.

The compounds in Table I are all analogues of 9-oxo-9*H*-xanthene-4-acetic acid (**2**), and can be divided into three groups.

(a) Compounds 3–10 (Variation of the Carbonyl Group of XAA). Compounds **3** and **4** explored the consequences of retaining the CO and O moieties of **2**, but in a nonplanar molecule. Expansion of the central ring to a seven-membered system leads to distinct folding of the molecule about the central ring. There appear to be two possible conformations of the molecules, which depend on the relative conformation of the $-CH_2CO-$ fragment, but in either case the angle between the terminal rings (dihedral angle) is 45°–48° (Figure 2). To the best of our knowledge there have been no crystal structures of this ring system determined. The compounds had dipole moments of lower magnitude than those of **2**. Compound **3** showed marginal activity at the highest practicable dose of 750 mg/kg, but the isomeric compound **4** was inactive.

Replacement of the 9-carbonyl group of **2** with an ether link to give dibenzo[1,4]dioxin derivative **5** resulted in complete loss of activity, despite retaining a planar ring system as shown by both the MO calculations and the literature.[25] The dipole moment of **5** (5.08 D) is consid-

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erably larger than that of **2** and is directed over the central ring (ϕ = 144°).

Replacement of the carbonyl by a methine gave xanthene **6**, which is also calculated to be planar. The dipole moment is even larger (5.86 D) and is again directed over the central ring (Table II). The compound was inactive at all doses. Direct bonding of the terminal rings gave dibenzofuran **7**. The MO calculations agreed with crystal structure data,[26] showing a rigid, coplanar system. This compound did show low levels of activity, albeit at a high dose.

Finally, phenoxathiin **8** and its oxide and dioxide derivatives (**9**, **10**) were also evaluated. Crystal structure studies[27] show this chromophore to be significantly bent, with a dihedral angle of ca. 140°, due to the longer C–S bond lengths. Both the parent compound **8** and the *S*-oxide and *S*-dioxides **9** and **10** were inactive.

(b) Compounds 11–14 (Variation of the Ether Group of XAA). These were designed to explore the consequences of retaining the 9-carbonyl group of **2** while varying the ether link group. Thioxanthenone **11** was of particular interest, since we have previously shown[9] that replacement of oxygen by sulfur in the analogous flavone-8-acetic acid system results in complete loss of biological activity. This was true in the present case also, with **11** proving inactive at the highest nontoxic dose. The corresponding *S*-oxide **12** and *S*-dioxide **13** were also inactive.

Replacing the ether link with a second carbonyl group gave anthraquinone **14**. This chromophore is known[28] to be coplanar, and the MO calculations confirmed that this was also the case for compound **14**. The compound has a larger dipole moment than XAA, but one of almost identical direction (Table II). The compound did show low but significant activity at the highest practicable dose, but is clearly inferior to XAA.

(c) Compounds 15–17 (Variation of Both Groups of XAA). Since no single change could be found which increased (or even retained) the level of activity of XAA, it seemed unlikely that alteration of both groups would be useful, but some compounds were explored. The isomeric dibenzofuran **15** had a relatively small dipole moment and was inactive at all doses. Of the two fully aromatic compounds, anthracene **16** did show significant activity, whereas phenazine **17** did not (Table II).

Conclusions

In the current absence of knowledge about the macromolecular receptor for FAA and the XAA analogues, drug development must necessarily proceed empirically. Previous structure–activity relationships with ring-substituted XAA derivatives[10,12] have not shown any correlation with substituent electronic properties and only a weak positive correlation with lipophilicity. While we as yet do not understand these SAR, this study of linear tricyclic acetic acids related to XAA shows that the xanthenone system appears to be optimal, with all alterations resulting in activity being severely decreased or abolished, a result similar to that found previously[9] for analogues of flavone-8-acetic acid (**1**).

We examined in particular the importance of compound dipole moments, since this property can be an important factor in the binding of ligands to macromolecules. The degree to which the molecular dipole moment was found to lie out of the plane of the aromatic part of these molecules was determined largely by the contributions from the acetic acid moiety relative to that from the tricyclic ring system. In all cases it was found that the acetic acid group was rotated out of the plane of the rings (ca. 90°). There did not appear to be any general relationship between the *magnitude* of the dipole moment and activity. However, for compounds containing the 9-carbonyl functionality (i.e., **2**, **3**, **4**, **14**) the *orientation* of the dipole vector may be of significance. In this subset, biological activity is confined to those molecules with $\theta \lesssim 50°$ and $\phi \gtrsim 130°$, with XAA itself having the lowest ϕ value of 112°. Similar calculations of the dipole moments of the isomeric 9-oxo-9*H*-xanthene-1-, -2-, and -3-acetic acid substituted xanthenones (all of which are inactive)[10] give very different values of the angle ϕ.

The calculations reported here show that in all compounds containing an ether, aromatic nitrogen or carbonyl oxygen peri to the acetic acid side chain, there is an interaction between the carboxyl OH group and this atom (Y), with an OH···Y distance of 2.2–2.4 Å (data not shown). This suggests a degree of hydrogen bonding, an interaction which might significantly lower the acid pK_a, and account for the apparent necessity for this functionality. Wherever this phenomenon occurred, the torsion angle of the carboxylic acid carbon about the CH_2–COOH was twisted from 90° to ca. 65–70°, which was the lowest-energy conformer in each case. This angle was important in determining the in-plane orientation of the molecular dipole moment. When the torsion angle was 90°, the dipole was always oriented approximately along the a–b bond (ϕ = 100°), with the extent it was out of the plane being determined by the size of the ring dipole moment contribution.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand, and were within ±0.4% of the theoretical values unless indicated. Melting points were determined on an Electrothermal apparatus, using the supplied stem-corrected thermometer, and are as read. All compounds had ^{1}H NMR spectra in accord with the assigned structures. Column chromatography was performed by the method of Still et al.[29] using Merck silica gel 60 (200–400 mesh). Compound **15** was kindly provided by Dr. L. M. Werbel of the Warner-Lambert Co.

9-Oxo-9*H*-xanthene-4-acetic Acid (2). This was prepared by the literature method:[10] mp 214–215 °C; ^{1}H NMR (CD_3COCD_3) δ 4.06 (s, CH_2), 7.44 (dd, H-2, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 7.3 Hz), 7.48 (ddd, H-7, $J_{7,5}$ = 1.0 Hz, $J_{7,6}$ = 7.1 Hz, $J_{7,8}$ = 8.0 Hz), 7.64 (ddd, H-5, $J_{5,6}$ = 8.5 Hz, $J_{5,7}$ = 1.0 Hz, $J_{5,8}$ = 0.4 Hz), 7.83 (m, H-3, $J_{3,1}$ = 1.7 Hz, $J_{3,2}$ = 7.3 Hz, J_{3,CH_2} = 1.5 Hz), 7.87 (ddd, H-6, $J_{6,5}$ = 8.5 Hz, $J_{6,7}$ = 7.1 Hz, $J_{6,8}$ = 1.7 Hz), 8.20 (dd, H-1, $J_{1,2}$ = 8.0 Hz, $J_{1,3}$ = 1.7 Hz), 8.26 (ddd, H-8, $J_{8,5}$ = 0.4 Hz, $J_{8,6}$ = 1.7 Hz, $J_{8,7}$ = 8.0 Hz); ^{13}C NMR (CD_3COCD_3) δ 35.47 (C14), 119.05 (C5), 122.38, 122.57 (C2, C7), 124.54, 125.15 (C2, C7), 126.00, 127.04 (C1, C8), 136.04, 137.47 (C3, C6), 155.30, 156.75 (C11, C12), 172.05 (C9), 177.05 (C15).

10,11-Dihydro-10-oxodibenz[*b*,*f*]oxepin-4-acetic Acid (3). A stirred suspension of potassium 2-iodo-3-methylbenzoate (33 g, 0.116 mol), sodium phenoxide (1.62 g, 0.14 mol), and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1;[14] 7.5 g, 20 mol %) in dry dioxane (250 mL) and benzene (50 mL) was heated to boiling, with the benzene being allowed to azeotrope off any moisture. CuCl (1.2 g, 10 mol %) was then added and the mixture was heated under reflux for 18 h. The solvent was removed under reduced pressure, and the residue was extracted into 1 N aqueous NaOH. After filtration to remove insoluble copper salts, the

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solution was poured slowly into 2 N HCl to precipitate 3-methyl-2-phenoxybenzoic acid (18; 11.2 g, 42%): mp (benzene–petroleum ether) 120–121 °C (lit.[13] mp 126–127 °C); ^{1}H NMR ($(CD_3)_2SO$) δ 7.77–6.37 (m, 8 H, ArH), 2.06 (s, 3 H, Me).

Oxidation of the above acid with $KMnO_4$ in water containing a slight excess of NaOH gave a 71% yield of 2-phenoxybenzene-1,3-dicarboxylic acid (19): mp (benzene–Me_2CO) 205–206 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.98 (d, J = 7.7 Hz, 2 H, H-4,6), 7.63–6.53 (m, 6 H, ArH). Anal. ($C_{14}H_{10}O_5$) C, H. This diacid (8.6 g, 33 mmol) was converted to the corresponding acid chloride (20) by heating in $SOCl_2$ with a trace of DMF. All volatiles were removed under reduced pressure, and the crude acid chloride was then added to a solution of $NaBH_4$ (3 g, 79 mmol) in diglyme (50 mL) at room temperature. After a further 5 min the diglyme was removed under reduced pressure, and the residue was worked up to give crude 2-phenoxybenzene-1,3-dimethanol (21) as an oil (7.34 g, 97%). ^{1}H NMR ($CDCl_3$) δ 7.50–6.45 (m, 8 H, ArH), 4.46 (m, 4 H, CH_2), 2.31 (m, 2 H, exch with D_2O, OH).

A solution of crude diol 21 in benzene was treated with a slight excess of PBr_3, and after washing with 2 N NaOH, solution the benzene was dried and removed to give 1,3-bis(bromomethyl)-2-phenoxybenzene (22) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.60–6.53 (m, 8 H, ArH), 4.28 (s, 4 H, CH_2). Crude dibromide 22 in CH_2Cl_2 was reacted with an excess of aqueous NaCN solution in a two-phase system, using tetrabutylammonium bromide as the phase-transfer catalyst. Workup gave 2-phenoxybenzene-1,3-diacetonitrile (23) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.60–6.50 (m, 8 H, ArH), 3.59 (s, 4 H, CH_2). Crude dinitrile 23 was heated under reflux in a mixture of EtOH and 2 N aqueous KOH solution for 16 h, the EtOH was boiled off, and the remaining aqueous solution was filtered through Celite. Acidification with 2 N HCl then gave 2-phenoxybenzene-1,3-diacetic acid (24; 4.0 g, 42% overall yield from 2-phenoxy-1,3-benzenedicarboxylic acid, 19). Crystallization from aqueous EtOH gave needles: mp 182–183 °C; ^{1}H NMR ($CDCl_3$–$(CD_3)_2SO$) δ 7.41–6.65 (m, 8 H, ArH), 3.41 (s, 4 H, CH_2). Anal. ($C_{16}H_{14}O_5$) C, H.

The above diacid 24 (3.65 g, 12.7 mmol) was added with stirring to 50 g of PPA (preheated to 120 °C). After 5 min at 120 °C the mixture was poured into hot water, and after cooling the precipitated solid was collected and washed well with water. The solid was dissolved in 2 N NaOH solution, the mixture was filtered through Celite and then acidified with 2 N HCl to give 10,11-dihydro-10-oxodibenz[b,f]oxepin-4-acetic acid (3; 2.62 g, 77% yield): mp (MeOH) 200 °C; ^{1}H NMR ($CDCl_3$–$(CD_3)_2SO$) δ 8.09–6.90 (m, 7 H, ArH), 4.03 (s, 2 H, 11-CH_2), 3.75 (s, 2 H, CH_2CO). Anal. in Table I.

10,11-Dihydro-11-oxodibenz[b,f]oxepin-4-acetic acid (4). With a sequence of reactions similar to the above, 2-methylphenol was condensed with 2-chlorobenzoic acid to give 2-(2-methylphenoxy)benzoic acid (25).[16,30] Oxidation with $KMnO_4$ then gave 2,2′-oxybisbenzoic acid (26),[15,16] which was reduced by using the $SOCl_2$/$NaBH_4$ method to give 2,2′-oxybisbenzenemethanol (28) in 84% overall yield. Recrystallization from aqueous EtOH gave needles: mp 88–90 °C (lit.[31] 96–98 °C); ^{1}H NMR ($CDCl_3$) δ 7.45–6.67 (m, 8 H, ArH), 4.59 (s, 4 H, CH_2), 3.44 (m, 2 H, exchangeable on deuteration, OH).

The above dialcohol was reacted with PBr_3 as before to give a 90% yield of 2,2′-bis(bromomethyl)-1,1′-oxybisbenzene (29): mp (hexane) 87–89 °C (lit.[31] mp 86.5–87.5 °C); ^{1}H NMR ($CDCl_3$) δ 7.53–6.69 (m, 8 H, ArH), 4.60 (s, 4 H, CH_2). Reaction of 29 with NaCN in a two-phase system gave a 96% yield of 2,2′-oxybisbenzeneacetonitrile (30): mp (aqueous MeOH) 73 °C; ^{1}H NMR ($CDCl_3$) δ 7.61–6.68 (m, 8 H, ArH), 3.78 (s, 4 H, CH_2). Anal. ($C_{16}H_{12}N_2O$) C, H, N. Hydrolysis of dinitrile 30 with KOH in aqueous EtOH gave a 94% yield of 2,2′-oxybisbenzeneacetic acid (31): mp (aqueous EtOH) 202 °C; ^{1}H NMR ($CDCl_3$–$(CD_3)_2SO$) δ 8.69 (m, 2 H, CO_2H), 7.55–6.64 (m, 8 H, ArH), 3.62 (s, 4 H, CH_2). Anal. ($C_{16}H_{14}O_5$) C, H.

Ring closure of diacid 31 with PPA at 120 °C gave 10,11-dihydro-11-oxodibenz[b,f]oxepin-4-acetic acid (4), which crystallized from benzene–petroleum ether) as needles: mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ 9.40 (m, 2 H, COOH), 8.12–7.83 (m, 1 H, H-1), 7.59–6.87 (m, 6 H, ArH), 4.02 and 4.00 (2 s, 4 H, CH_2). Anal. in Table I.

Dibenzo[1,4]dioxin-1-acetic Acid (5). n-Butyllithium (9.3 mL of a 1.40 N solution in hexane, 13.0 mmol) was added dropwise at −15 °C under N_2 to a stirred solution of dibenzo[1,4]dioxin (2.00 g, 10.8 mmol) in THF (30 mL). After 5 h, oxirane (2.00 mL, 40.7 mmol) was added, and the mixture was allowed to come to 20 °C over 1 h. The mixture was poured into brine, extracted with EtOAc, and worked up, and the residue was chromatographed on silica gel. Elution with EtOAc–petroleum ether (2:5) gave 1-(2-hydroxyethyl)dibenzo[1,4]dioxin (32; 1.03 g, 42% yield), which crystallized from petroleum ether (bp 50–60 °C) as cubes: mp 103 °C. Anal. ($C_{14}H_{12}O_3$) C, H. A solution of this alcohol (32; 1.02 g, 4.51 mmol) in CH_2Cl_2 (10 mL) was added in one portion to a vigorously stirred suspension of pyridinium chlorochromate (2.43 g, 11.28 mmol) in CH_2Cl_2 (30 mL). After 3 h at 20 °C, Et_2O (30 mL) was added, and the mixture was filtered through a short column of SiO_2, eluting with CH_2Cl_2 to give the crude aldehyde (1.01 g). This was treated with Jones' reagent in Me_2CO (15 mL), and the crude product was chromatographed on silica gel. Elution with EtOAc–MeOH (1:20) gave dibenzo[1,4]dioxin-1-acetic acid (5; 0.76 g, 70% yield from alcohol 32), which crystallized from petroleum ether (bp 60–80 °C) as needles: mp 166–168 °C; ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 2 H, CH_2COOH), 6.77–6.89 (m, 7 H); ^{13}C NMR ($CDCl_3$) δ 34.52 (C13), 115.78, 116.29, 116.46 (C4, C5, C8), 121.54 (C1), 123.28, 123.79, 124.02, 125.47 (C2, C3, C6, C7), 140.52, 141.94, 142.07, 142.26 (C9, C10, C11, C12), 177.07 (C14). Anal. in Table I.

Xanthene-4-acetic Acid (6). Reduction of 9-oxo-9H-xanthene-4-acetic acid (2) with excess $NaBH_4$ in refluxing EtOH gave a 54% yield of xanthene-4-acetic acid (6): mp (aqueous MeOH) 136–140 °C; ^{1}H NMR ($CDCl_3$) δ 7.10 (m, 4 H, H-2,3,6,7), 6.98 (m, 3 H, H-1,5,8), 4.00 (s, 2 H, 9-CH_2), 3.81 (s, 2 H, CH_2CO); ^{13}C NMR ($CDCl_3$) δ 27.94 (C9), 35.57 (C14), 116.46 (C8), 120.64, 120.79 (C10, C13), 121.36 (C1), 122.65, 123.16 (C4, C5), 127.58, 128.33, 128.71, 129.17 (C2, C3, C6, C7), 150.06, 151.68 (C11, C12), 178.16 (C15). Anal. in Table I.

Dibenzofuran-4-acetic Acid (7). A solution of dibenzofuran (12.6 g, 75 mmol) in dry THF (75 mL) was cooled to −10 °C under an atmosphere of N_2 and treated with 2.5 M n-BuLi (45 mL, 1.5 equiv). The mixture was allowed to warm to room temperature and stirred for a further 3 h. The solution was then cooled to −10 °C and treated with an excess of gaseous oxirane, stirred for a further 2 h, and worked up. A sample of the crude product (11.2 g) was purified from some unreacted starting material by chromatography on silica. Elution with CH_2Cl_2–hexane (3:2) gave dibenzofuran-4-ethanol (33), which was recrystallized from aqueous MeOH: mp 69 °C (lit.[32] mp 70–71 °C). ^{1}H NMR ($CDCl_3$) δ 8.04–7.08 (m, 7 H, ArH), 4.00 (t, J = 7 Hz, 2 H, CH_2O), 3.20 (t, J = 7 Hz, 2 H, CH_2Ar), 1.81 (s, 1 H, exch with D_2O, OH).

Oxidation of the above crude alcohol with Jones' reagent in Me_2CO gave dibenzofuran-4-acetic acid (7): mp (aqueous MeOH) 213–214 °C (lit.[33] mp 217–218 °C); ^{1}H NMR ($(CD_3)_2SO$) 8.24–7.13 (m, 7 H, ArH), 3.96 (s, 2 H, CH_2).

Phenoxathiin-4-acetic Acid (8). A solution of n-butyllithium (2.5 M in hexane, 37 mL) was added slowly under N_2 to a stirred solution of phenoxathiin (18.7 g, 93 mmol) in dry THF (300 mL) at −78 °C. The mixture was allowed to warm to −35 °C, kept there for 2 h, then cooled again to −78 °C and treated with excess CO_2 gas. Solvent was then removed at room temperature, and the residue was dissolved in water and washed with EtOAc. The aqueous solution was acidified with 2 M HCl to give a solid which was dried and recrystallized from benzene to give crude phenoxathiin-4-carboxylic acid.[34] This was converted to the acid chloride with $SOCl_2$ and then reduced with $NaBH_4$ in diglyme at 20 °C. The resulting crude product (13 g) was chromatographed on SiO_2, elution with CH_2Cl_2–hexane (1:1) giving 4-(hydroxymethyl)phenoxathiin (34; 12.3 g, 57% based on phenoxathiin): mp (MeOH) 77 °C; ^{1}H NMR ($CDCl_3$) δ 6.99 (m, 7 H, aromatic

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protons), 4.75 (s, 2 H, CH_2), 2.46 (m, 1 H, OH). Anal. ($C_{13}H_{10}O_2S$) C, H.

The above alcohol **34** (11.7 g) was treated with HBr in AcOH at 20 °C, the solvent was removed, and the residue was dissolved in EtOAc and washed with aqueous $NaHCO_3$. Removal of solvent gave crude 4-(bromomethyl)phenoxathiin (**35**; 14.2 g, 96%), which was recrystallized from MeOH: mp 102–103.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.10 (m, 7 H, aromatic protons), 4.59 (s, 2 H, CH_2Br). Anal. ($C_{13}H_9BrOS$) C, H, Br.

The above bromo compound (9.2 g) was dissolved in CH_2Cl_2 and treated with excess aqueous NaCN under phase-transfer conditions, using tetrabutylammonium bromide as catalyst. The product was recrystallized from MeOH to give phenoxathiin-4-acetonitrile (**36**; 6.55 g, 85%): mp 50–51 °C; ^{1}H NMR ($CDCl_3$) δ 7.07 (m, 7 H, aromatic protons), 3.78 (s, 2 H, CH_2CN). Anal. ($C_{14}H_9NOS$) C, H, N.

Hydrolysis of the above acetonitrile with KOH in aqueous EtOH gave a 91% yield of phenoxathiin-4-acetic acid (**8**): mp (benzene–petroleum ether) 129 °C; ^{1}H NMR $CDCl_3$) δ 9.50 (m, 1 H, COOH), 7.00 (m, 7 H, aromatic protons), 3.74 (CH_2CO). Anal. in Table I.

Phenoxathiin-4-acetic Acid 10-Oxide (9). Oxidation of phenoxathiin-4-acetic acid (**8**) with excess 27% aqueous H_2O_2 in EtOH under reflux[35] gave a 70% yield of phenoxathiin-4-acetic acid 10-oxide (**9**): mp (MeOH) 193–194 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 8.10–7.22 (m, 7 H, aromatic protons), 3.91 (s, 2 H, CH_2CO). Anal. in Table I.

Phenoxathiin-4-acetic Acid 10,10-Dioxide (10). Oxidation of phenoxathiin-4-acetic acid (**8**) with excess aqueous 27% H_2O_2 in glacial AcOH[35] gave an 83% yield of phenoxathiin-4-acetic acid 10,10-dioxide (**10**): mp (MeOH) 183 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 8.21–7.32 (m, 7 H, aromatic protons), 3.96 (s, 2 H, CH_2CO). Anal. in Table I.

9-Oxo-9*H*-thioxanthene-4-acetic Acid (11). A mixture of 4-methyl-9*H*-thioxanthen-9-one[36] (2.94 g, 0.013 mol), powdered *N*-bromosuccinimide (2.49 g, 0.014 mol), and benzoyl peroxide (40 mg) in CCl_4 (60 mL) was stirred under reflux for 3 h. The mixture was filtered hot, and the residue left after removal of solvent was dissolved in CH_2Cl_2, washed with 1 N NaOH, and dried. Removal of solvent and two recrystallizations from benzene–petroleum ether gave 4-(bromomethyl)thioxanthenone (**37**; 2.91 g, 73%): mp 172–173 °C. Anal. ($C_{14}H_9BrOS$) C, H, Br.

Solutions of the above bromomethyl compound (**37**; 3.05 g, 0.01 mol) and tetrabutylammonium bromide (10 mol %) in CH_2Cl_2 (25 mL) and KCN (1.95 g, 0.03 mol) in water (25 mL) were stirred vigorously at 20 °C for 8 h. Evaporation of solvents gave a residue which was washed well with water and dissolved in boiling EtOH. Slow cooling to 20 °C precipitated impurities which were removed by filtration. Concentration of the mother liquor and prolonged cooling gave a solid which was recrystallized from EtOH to give 9-oxo-9*H*-thioxanthene-4-acetonitrile (**38**; 1.66 g, 66%), mp 194–196 °C. Anal. ($C_{15}H_9NOS$) C, H, N.

The acetonitrile (2.36 g) was heated under reflux for 3 h in a 1:1:1 mixture of AcOH, conc. H_2SO_4, and water (45 mL). Dilution with water gave a crude product which was purified by dissolving in dilute aqueous $KHCO_3$, filtration, and precipitation with acid. Crystallization from MeOH gave pure 9-oxo-9*H*-thioxanthene-4-acetic acid (**11**; 74%), mp 210–211 °C. Anal. in Table I.

9-Oxo-9*H*-thioxanthene-4-acetic Acid 10-Oxide (12). Treatment of 9-oxo-9*H*-thioxanthene-4-acetic acid (**11**) with MeOH/HCl gave the methyl 9-oxo-9*H*-thioxanthene-4-acetate (**39**), mp (MeOH) 143–144 °C. Anal. ($C_{16}H_{12}O_3S$) C, H. This ester (2.27 g, 8 mmol) was dissolved in CH_2Cl_2 (25 mL) and the stirred solution was treated portionwise at 20 °C with 3-chloroperoxybenzoic acid (85%, 1.83 g, 9 mmol). The mixture was stirred for a further 2 h and then shaken with 10% aqueous $KHCO_3$. The residue from workup of the organic layer was extracted exhaustively with boiling water, and the hot extracts were clarified by filtration through a Celite pad. Cooling gave a mixture of the desired oxide ester contaminated with both **39** and the dioxide ester. This mixture was saponified, and the product recrystallized twice from MeOH to give pure 9-oxo-9*H*-thioxanthene-4-acetic acid 10-oxide (**12**) as colorless prisms (0.71 g, 31%), mp 213–214 °C. Anal. in Table I.

9-Oxo-9*H*-thioxanthene-4-acetic Acid 10,10-Dioxide (13). Treatment of the above methyl ester (**39**) with an excess of 3-chloroperoxybenzoic acid for 4 h at 20 °C and workup followed by crystallization of the product from MeOH gave the crude dioxide ester. Saponification and two recrystallizations from MeOH gave pure 9-oxo-9*H*-thioxanthene-4-acetic acid (**13**) in 63% yield, mp 228–229 °C. Anal. in Table I.

Anthraquinone-1-acetic Acid (14). 1-Aminoanthraquinone was diazotized and reacted with 1,1-dichloroethene in MeOH according to the literature procedure[37] to give methyl anthraquinone-1-acetate (**40**): mp (MeOH) 188–190 °C; ^{1}H NMR ($CDCl_3$) 8.43–8.14 (m, 3 H, H-1,5,8), 7.92–7.50 (m, 4 H, H-2,3,6,7), 4.20 (s, 2 H, CH_2), 3.73 (s, 3 H, Me). Hydrolysis of this with 2 N aqueous NaOH gave anthraquinone-1-acetic acid (**14**), mp 232 °C dec. Anal. in Table I.

Anthracene-1-acetic Acid (16). A mixture of anthraquinone-1-acetic acid (2.66 g, 10 mmol), concentrated NH_4OH (50 mL), water (50 mL), excess Zn powder, and a trace of $CuSO_4$ was heated under reflux for 30 min, the solids were filtered off, and the solution was worked up to give anthracene-1-acetic acid (**16**; 2.35 g, 95%): mp (aqueous MeOH) 167–168 °C (lit.[38] mp 170–171 °C); ^{1}H NMR ($(CD_3)_2SO$) δ 8.54 (m, 2 H, H-9,10), 8.00 (m, 3 H, H-4,5,8), 7.43 (m, 4 H, H-2,3,6,7), and 4.18 (s, 2 H, CH_2).

Phenazine-1-acetic Acid (17). A solution of 1-(bromomethyl)phenazine[39] (2 g, 7.3 mmol) in CH_2Cl_2 (50 mL) was reacted under phase-transfer conditions (rapid stirring, using 10 mol % tetrabutylammonium bromide) with excess NaCN (0.72 g, 14 mmol) in water (50 mL) for 4 h. The organic layer was separated and washed with water, yielding phenazine-1-acetonitrile (**41**; 0.72 g, 45%): mp (MeOH) 174–175 °C; ^{1}H NMR ($CDCl_3$) δ 8.47–7.71 (m, 7 H, aromatic H) and 4.50 (s, 2 H, CH_2CN). Anal. ($C_{14}H_9N_3$) C, H, N. Hydrolysis of the acetonitrile (NaOH–EtOH–water) as usual gave phenazine-1-acetic acid (**17**) in 55% yield: mp (MeOH) 196–198 °C; ^{1}H NMR ($CDCl_3$) δ 7.81–8.37 (m, 7 H, aromatics), 4.42 (s, 2 H, CH_2). Anal. in Table I.

MO Computations. Dipole moments were calculated for the xanthenone acetic acids and related compounds (Table II) by using the AM1 semiempirical molecular orbital method with the program package AMPAC[22] implemented on the University of Auckland Centre for Information Science IBM 3081 computer. The dipole moments were calculated after a full geometry optimization for each molecule.

Biological Testing. This was carried out essentially as noted previously.[12] 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 (defined as the highest dose in the above protocol which did not cause death in 24 h) 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 when given at a dose of 330 mg/kg caused necrosis across 90–100% of the tumor section (scored as ++). Compounds showing lesser but still extensive necrosis (50–90%) were scored as +, and those showing less than 50% necrosis were scored as negative (-).

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