Potential Antitumor Agents. 58. Synthesis and Structure–Activity Relationships of Substituted Xanthenone-4-acetic Acids Active against the Colon 38 Tumor in Vivo

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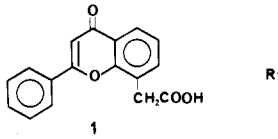
In a search for compounds related to flavoneacetic acid with activity against solid tumors, a series of methyl-, methoxy-, chloro-, nitro-, and hydroxy-substituted xanthenone-4-acetic acids have been synthesized and evaluated against subcutaneously implanted colon adenocarcinoma 38 in vivo, using a short-term histology assay as a primary screening system. A major goal of this work was to identify compounds with similar profiles of activity to that of flavoneacetic acid but of higher potency. The level of activity of the compounds appeared to depend more on the nature of the substituent than its positioning, in the order Cl > Me, OMe > NO_2, OH. However, the potency of the compounds was related much more to the position rather than the nature of the substitution, with 5-substituted compounds being clearly the most dose potent. 5-Methylxanthenone-4-acetic acid has a similar level of activity to that of flavoneacetic acid in the test systems employed but is more than 7-fold as dose potent.

There has recently been much interest in the drug flavoneacetic acid (FAA: NSC 347512) (1) following initial reports[1,2] of its unusual spectrum of anticancer activity. While possessing very limited in vivo activity against P388 and other leukemia models, it is curative against the colon 38 adenocarcinoma and is active in a variety of other colon and pancreatic tumors that are very resistant to most chemotherapeutic agents.[3,4] Additionally, 1 has a different toxicity profile to most anticancer drugs, with no significant myelosuppression observed.[5] The unique therapeutic and toxicity profiles of 1 suggest it has a novel mechanism of action, but this has not yet been elucidated. At high doses it inhibits RNA and DNA synthesis, but does not appear to induce DNA breakage.[5] The drug is known to rapidly induce hemorrhagic necrosis in both colon 26 and colon 38 tumors in mice,[6] reminiscent of the action of tumor necrosis factor (TNF). It has also been shown[7] to act as a biological response modifier, inducing natural killer cell activity.



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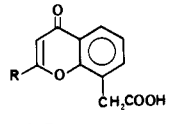
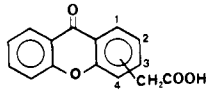


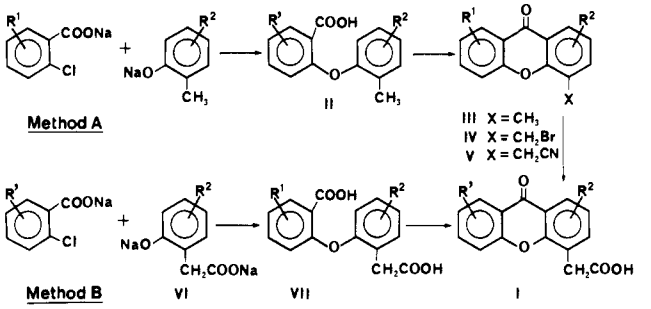
2 R = 2-naphthyl
3 R = benzyl
4 R = 2-furyl



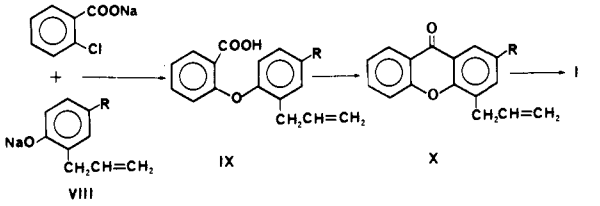
5 1-CH_2COOH
6 2-CH_2COOH
7 3-CH_2COOH
8 4-CH_2COOH

The drug is not very potent, with a recent phase I clinical study[8] suggesting optimal doses of 4.8–8.6 g/m^2. Such high doses, in conjunction with the insolubility of the compound at neutral and acidic pH, have led to concerns about potential nephrotoxicity due to drug precipitation in the kidney[8,9] and require patients to be hydrated and alkalinized during drug infusion.

Scheme I



Scheme II



Method C

In spite of the novel biological profile of flavoneacetic acid, there has been little work on related compounds, with the exception of a limited structure–activity study of substituted flavoneacetic acids themselves.[2] We have initiated a search for compounds of similar structure that might retain the same selective activity against solid tumors but have improved potency. In this paper we detail the synthesis and structure–activity relationships for a new class of compounds, xanthenone-4-acetic acids (I), which fulfill these goals to some extent.

Chemistry

Although many syntheses of substituted xanthenones have been reported,[10,11] the most versatile are those in-

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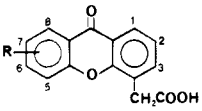
Table I. Physicochemical and Biological Properties of Substituted Xanthenone-4-acetic Acids



			colon 38 histology assay			P388 leukemia	
no.	R	synth[a]	R_m[b]	OD[c]	act.[d]	OD[e]	ILS[f]
1	FAA		0.06	330	++	150	NA[g]
5	1-acetic acid		0.47	500	-	500	NA
6	2-acetic acid		0.57	330	-	225	21
7	3-acetic acid		0.60	500	-	225	NA
8	H	A, B	0.64	220	++	150	NA
9	1-Me	B	0.47	150	+	100	NA
10	1-OMe	D[h]	0.41	500	-		
11	1-Cl	A	0.53	330	++	220	NA
12	1-OH	E	0.32	750	-		
13	2-Me	C	0.55	500	+	330	NA
14	2-OMe	C	0.47	750	+	225	NA
15	2-Cl	C	0.63	330	+	150	NA
16	2-OH	E	0.40	750	-		
17	3-Me	B	0.50	150	+	100	26
18	3-OMe	D	0.42	220	-	220	NA
19	3-Cl	A	0.53	500	++	150	NA
20	3-OH	E	0.35	500	-	500	NA
21	5-Me	A, B	0.62	45	++	30	27
22	5-OMe	B	0.40	150	+	150	42
23	5-Cl	A	0.60	150	++	65	34
24	5-NO_2	B	0.27	220	+	150	NA
25	5-OH	E	0.36	750	-	150	NA
26	6-Me	B	0.59	220	+	100	24
27	6-OMe	A, D	0.46	150	+	65	28
28	6-Cl	A	0.67	150	+	65	NA
29	6-OH	E	0.34	750	±	225	NA
30	7-Me	B	0.65	500	+	500	NA
31	7-OMe	A	0.46	500	+	150	NA
32	7-Cl	A	0.65	500	+	150	NA
33	7-NO_2	B	0.28	500	-	225	NA
34	7-OH	E	0.38	750	-		
35	8-Me	B	0.55	330	-	150	NA
36	8-OMe	B	0.44	750	-	330	NA
37	8-Cl	B	0.65	750	+	330	NA

[a] See the Experimental Section for details. [b] R_m values determined by liquid–liquid chromatography as in ref 19, using 4'-(9-acridinylamino)methanesulfonanilide (AMSA) as internal standard. [c] OD: optimal dose of drug in milligrams/kilogram, administered intraperitoneally as the sodium salt in 0.2 mL of water in a single dose. [d] Subcutaneous colon 38 tumors (4–8 mm in diameter) were treated with the optimal dose of drug (at least three mice per dose; 1.5-fold dose intervals). Tumors were removed after 24 h, fixed in formalin, and stained in hematoxylin/eosin (ref 18). Sections were examined by histopathology and compared to those from control tumors. + = 50–90% hemorrhagic necrosis across section; ++ = >90% hemorrhagic necrosis across section. [e] OD: optimal in P388 assays in milligrams/kilogram per day, given as above on days 1, 5, and 9 after intraperitoneal injection of 10^6 P388 leukemia cells, as in ref 27. [f] ILS: the average percentage increase in lifespan of treated animals over that of control groups of untreated tumor-bearing animals. Values of ≥20% are considered statistically significant. [g] NA: no activity at all dose levels up to toxic ones. [h] Method D for preparation of certain methoxyxanthenones from the corresponding chloro compounds followed that of ref 23.

volving initial formation of a substituted 2-phenoxybenzoic acid by an Ullmann-type reaction, followed by cyclodehydration, and variants of this basic route have been used to prepare the xanthenone-4-acetic acids listed in Table I (see Schemes I and II).

Formation of substituted 2-phenoxybenzoic acids II by copper-assisted condensation of 2-halobenzoic acids and phenols (method A of Scheme I) is a widely used reaction.[12] Although the reaction is hindered by ortho substituents on the phenol, 2-(2-methylphenoxy)benzoic acid can be prepared in moderate yield from 2-chlorobenzoic acid and 2-methylphenol.[13] However, greatly improved yields can be achieved by use of the phase-transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1).[14] As an example, reaction of 2-chlorobenzoic acid and 2-methylphenol in dioxane using TDA-1 gives an 80% yield of 2-(2-methylphenoxy)benzoic acid, compared with a 30–40% yield under classical Ullmann conditions (K_2CO_3/Cu/DMSO/120–130 °C/4–6 h).

Cyclodehydration of the 2-(2-methylphenoxy)benzoic acids II with polyphosphate ester or 80% H_2SO_4 gives good yields of the corresponding 4-methylxanthenones III. Monobromination with *N*-bromosuccinimide/benzoyl peroxide in CCl_4 gave the bromomethyl compounds IV, which were converted to the cyanides V and hydrolyzed to the desired xanthenone-4-acetic acids I. However, the nitro compounds proved too insoluble for this method to be used, and it was also not generally suitable for preparation of the methyl-substituted derivatives.

A shorter route to many substituted xanthenone-4-acetic acids (I) was achieved by the direct TDA-1 assisted coupling of 2-halobenzoic salts with 2-hydroxyphenylacetic salts VI, which gave the 2-[(2-carboxymethyl)phenoxy]-benzoic acids VII in generally good yields (method B of Scheme I). Reaction with 80% H_2SO_4 gave the xanthe-

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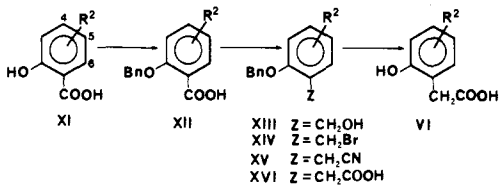
Scheme III



none-4-acetic acids I exclusively (the product of benzoic acid cyclodehydration) with no sign of the alternative product of acetic acid cyclodehydration. The lowest yields of compounds VII were recorded with 3-substituted halobenzoic acids, especially with 2-iodo-3-methylbenzoic acid, due to steric shielding of the reaction center. An alternative preparation of 5-methylxanthenone-4-acetic acid (**19**) was also developed, beginning with the known[15,16] 4,5-dimethylxanthenone, which was monobrominated with NBS in 30% yield and further elaborated following method A.

The method was not successful for the preparation of 6-nitroxanthenone-4-acetic acid, since the only compound isolated from the condensation of 2-chloro-4-nitrobenzoic acid and 2-hydroxyphenylacetic acid was the dimerization product 5,5′-dinitrobiphenyl-2,2′-dicarboxylic acid. This method was also unsuccessful for the preparation of 1-nitroxanthenone-4-acetic acid since 2-(2-hydroxy-4-nitrophenyl)acetic acid failed to condense with 2-chlorobenzoic acid.

Several of the substituted 2-hydroxyphenylacetic acids VI needed for syntheses by method B were prepared from the corresponding salicylic acids XI by the method of Scheme III. Treatment of the benzyl ether derivatives XII with $SOCl_2$ followed by $NaBH_4$ gave alcohols XIII, which were converted to the bromides XIV in nearly quantitative yield with PBr_3. Conversion to the acetonitriles XV with NaCN and the phase-transfer catalyst tetrabutylammonium bromide was also virtually quantitative. Hydrolysis to the acetic acids XVI and deprotection by hydrogenolysis then gave the required substituted hydroxyphenylacetic acids VI in excellent overall yield.

Where suitably substituted 2-hydroxyphenylacetic acids were not available by this route, the corresponding ring C substituted xanthenone-4-acetic acids (I: R^1 = H) were prepared by method C, outlined in Scheme II. The substituted allylphenols VIII were prepared by the Claisen rearrangement,[17] and condensed with 2-chlorobenzoic acid in anisole with TDA-1, to give the 2-(2-allylphenoxy)-benzoic acids IX in good yield. Cyclodehydration with polyphosphate ester gave the 4-allylxanthenones X, which were oxidized with $KMnO_4$ in aqueous acetic acid to the xanthenone-4-acetic acids I. This method failed to work for 2-nitroxanthenone-4-acetic acid, since the initial condensation between 2-chlorobenzoic acid and 2-allyl-4-nitrophenol could not be effected.

Several of the methoxyxanthenones of Table I were prepared from the corresponding chloro compounds by displacement with methoxide (method D in Table I). The hydroxy compounds were prepared by demethylation of the corresponding methoxy derivatives in HBr/AcOH (method E in Table I).

Biological Evaluation

The striking activity of flavoneacetic acid (1) against the subcutaneous (sc) colon adenocarcinoma C38 tumor in mice has been shown due to its ability to rapidly induce hemorrhagic necrosis in the tumor.[6] Accordingly, the xanthenoneacetic acids were evaluated in this tumor model as the primary screen.[18] Colon 38 tumor fragments were implanted subcutaneously and allowed to grow to a diameter of 4–8 mm, when drug was given as a single dose intraperitoneally. The tumor was removed surgically 24 h later, stained, and examined histologically for evidence of necrosis. The standard used was flavoneacetic acid given at 330 mg/kg, which caused significant levels of tumor necrosis, scored as ++ (>90% across the whole of the tumor). Compounds showing lesser but still extensive necrosis (50–90%) were scored as + (see Table I). This assay thus provides a fairly stringent criterion of activity, since compounds that clearly showed effects of <50% were scored as negative.

Table II. Activity of Selected Xanthenone-4-acetic Acids against Sc Colon 38 Tumors in Vivo

compound	dose, mg/kg	schedule	growth delay,[a] days
5-fluorouracil	65	q4d × 3[b]	12.5
cyclophosphamide	220	single dose	6.5
FAA (1)	330	single dose	17
8	220	q7d × 2[c]	11
21	45	q7d × 2[c]	13
23	100	single dose	5.2
27	100	single dose	0

[a] Mice with palpable tumors were randomized in groups of five and treated as indicated. Tumor volumes were estimated by use of the formula $0.52x^2y$ where x and y are the minor and major tumor axes, respectively. Conditions are similar to that described in ref 18. [b] Three equal doses 4 days apart. [c] Two equal doses 7 days apart.

Selected compounds were tested for their ability to induce a delay in the growth of subcutaneous colon 38 tumors in mice. The tumor was grown as above, and compounds were administered intraperitoneally in aqueous solution. Tumor diameters (major and minor axes) were measured twice weekly after the beginning of drug treatment, and calculated tumor volumes were compared with those of untreated control animals. Under these conditions, the clinical drugs 5-fluorouracil and cyclophosphamide showed mean tumor growth delays of 12.5 and 6.5 days, respectively, but provided no complete regressions (see Table II).

In order to determine the selectivity of antitumor effects, many of the active compounds were also assayed in the P388 leukemia model using standard protocols (see Table I).

Results and Discussion

Published structure–activity relationships for the colon 38 activity of analogues of flavoneacetic acid (1) show that, while there is some latitude for substitution at the 2-position (the 2-phenyl, 2-benzyl, and 2-furyl compounds **2**–**4** are all active), there seemed to be a definite requirement for placement of the acetic acid side chain, with the 8-isomer being active but the 6-isomer inactive.[2] Thus we first studied the set of isomeric xanthenoneacetic acids (**5**–**8**), in which the 2-phenyl ring of flavoneacetic acid is fused to the chromone ring rather than pendant to it. The 1-, 2-, and 4-acetic acid derivatives (**5**, **6**, and **8**) have been prepared previously and evaluated as antiinflammatory agents.[10] These compounds proved considerably more lipophilic than flavoneacetic acid (lipophilicity was mea-

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(16) Kanaoka, Y.; Tanizawa, K.; Sato, E.; Yonemitsu, O.; Ban, Y. *Chem. Pharm. Bull.* **1967**, *15*, 593.
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sured by thin-layer liquid–liquid chromatography, as described previously[19]), with R_m values of 0.5–0.6, compared to that of 0.06 for 1. While the 1-, 2-, and 3-acetic acids (5–7) proved inactive in the colon 38 histology assay at all doses up to the toxic limits, the 4-acetic acid (8) had activity comparable to that of FAA (1), with a single dose of 220 mg/kg causing extensive (>90%) hemorrhagic necrosis across the entire tumor. Like FAA, it showed a steep dose–response curve and at a dose of 150 mg/kg only limited tumor necrosis was evident. Compound 8 was also the only isomer to show in vivo activity against the P388 leukemia, albeit at a low level (ILS 56% when given at 225 mg/kg per day on days 1, 5, and 9).

A systematic study of monosubstituted analogues of the 4-acetic acid 8 was then undertaken. Previous work with substituted FAA compounds was too limited to provide structure–activity relationships (SAR), but did show that methoxy substitution on the phenyl ring was acceptable.[2] Xanthenone-4-acetic acid has seven different positions available for substitution, and in the complete absence of any SAR for this type of biological activity, we planned to evaluate methyl, methoxy, chloro, nitro, and hydroxy groups at all seven possible positions. These groups provide a reasonable variation of electronic and hydrophobic properties, while being of a similar size. Thus a study of the effect of these substituents at a particular position would provide information about electronic and hydrophobic effects, while comparison of the sets of substituents at different positions would provide data on steric acceptability of this size of group. The groups were not expected to alter the pK_a of the acid function, due to its isolation by the methylene group. The effects of group lipophilicity were as expected from their known[20] π values, with the Cl compounds being the most lipophilic (average R_m 0.60 ± 0.06), followed by the Me (0.56 ± 0.06), OMe (0.44 ± 0.03), NO_2 (0.28), and OH compounds (0.36 ± 0.03). There was little variation in group lipophilicity contribution with position, although substituents did appear to be slightly less lipophilic at the 1- and 8-positions than at other positions (see Table I).

All the drugs were evaluated in the colon 38 histology assay. Single intraperitoneal doses were given at 1.5× dose intervals up to a maximum of 750 mg/kg, or to the toxic limit if this was less, and the results are given in Table I. The optimal dose (OD) is defined as the highest dose in the above dose escalation where no deaths due to acute toxicity (within 24 h) were seen. Analysis of the results by position shows a clear preference for the 5- and 6-positions, with substitution at most of the other positions being roughly equivalent. Substitution in the 7- and 8-positions (compounds **30–37**) generally gave active compounds, but activity was lower than that of the parent, with the compounds also being less potent. Substitution in the 1-, 2-, and 3-positions (compounds **9–19**) was generally neutral, resulting in compounds of similar or slightly lower activity than the parent and of similar potency (with the exception of the poorly potent 1- and 2-OMe compounds). The best of these compounds was the 1-Cl derivative (**11**), which was equiactive and nearly equipotent to 8. However, substitution in the 5- and 6-positions was clearly beneficial, with all of these compounds (**21–29**) showing activity in the colon 38 assay and some proving to be considerably more potent than the unsubstituted parent. In particular, the 5-Me derivative (**21**) proved as active as FAA (1) (>90% hemorrhagic necrosis) at a dose of 45 mg/kg. A dose of 65 mg/kg was toxic, while a dose of 30 mg/kg induced only slight hemorrhagic necrosis. This compound is thus as active as the clinical drug FAA against colon 38 in vivo, but is more than 7 times as potent.

Overall, the activity of these compounds was determined more by the nature of the substituent than the position of substitution. The Cl substituent gave active compounds (four ++ and three +) in every position. The Me and OMe substituents were less effective overall, and the NO_2 and OH groups were the least effective (in fact the poor showing of the latter two groups caused us to terminate preparation of these compounds before the full sets of seven isomers were made). However, the potency of the derivatives was related much more to the position rather than the nature of substitution. Thus, the seven methyl analogues had an average optimal dose (OD) of 270 mg/kg, compared to 430 mg/kg for the OMe compounds and 390 mg/kg for the set of Cl derivatives: a range of 1.6-fold. In contrast, the range of average ODs for the set of Me-, OMe-, and Cl-substituted compounds at each position was much wider (4.6-fold), from 115 mg/kg for all compounds at the 5-position to 530 mg/kg for all compounds at positions 2 and 8. All of the OH compounds were inactive at the highest dose tested.

Selected compounds were also evaluated against the sc colon 38 tumor in a longer term assay, to measure their ability to induce tumor-growth delays and regressions. This is a very refractory tumor system, since the two drugs most widely used in the clinic against colon cancer, 5-fluorouracil and cyclophosphamide, give only moderate growth delays and no regressions in this model (Table II). FAA (1) at 330 mg/kg produced a substantial growth delay of 17 days, with XAA (8) being slightly less active. However, the 5-methyl derivative (**21**) showed activity equal to that of FAA but at 45 mg/kg, an improvement in dose potency of 7-fold.

Many of the xanthenone-4-acetic acids were also evaluated against the P388 leukemia, using a standard protocol (Table I) but most proved inactive, as did FAA. A few compounds showed low antileukemic activity (ILS values of 20–50%), but there was no apparent correlation between this and colon 38 activity.

Conclusions

The aim of this work was to study substituent effects in the new class of xanthenone-4-acetic acids with in vivo colon 38 activity similar to that of the drug FAA (1) now in clinical trial. A major goal was the identification of compounds with comparable activity but much higher potency, and this has been achieved to some extent with the discovery of the 5-methyl compound **21**, which has activity in this test system similar to that of FAA but is more than 7-fold more potent. Overall it is clear that substitution in the xanthenone ring has significant effects on the activity of these compounds, with lipophilic derivatives appearing most effective. There is no evidence that group electronic properties are important, since the Cl and Me groups have both provided analogues of equivalent activity to that of the parent compound. The

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Table III. Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	anal. or lit. mp, °C
5	252–254	$C_{15}H_{10}O_4$	245[a]
6	224–226	$C_{15}H_{10}O_4$	217–219[a]
7	227.5–228.5	$C_{15}H_{10}O_4$	C, H
8	214–215	$C_{15}H_{10}O_4$	205–207[a]
9	206–209	$C_{16}H_{12}O_4$	C, H
10	196–198	$C_{16}H_{12}O_5$	C, H
11	208–211	$C_{15}H_9ClO_4$	C, H, Cl
12	223–227	$C_{15}H_{10}O_5$	C, H
13	243–245	$C_{16}H_{12}O_4$	C, H
14	229–231	$C_{16}H_{12}O_5$	C, H
15	272–273	$C_{15}H_9ClO_4$	C, H, Cl
16	269–271	$C_{15}H_{10}O_5$	C, H
17	235–237	$C_{16}H_{12}O_4$	C, H
18	274	$C_{16}H_{12}O_5$	C, H
19	214–218	$C_{15}H_9ClO_4$	C, H, Cl
20	265–266	$C_{15}H_{10}O_5 \cdot H_2O$	C, H
21	206–208	$C_{16}H_{12}O_4$	C, H
22	223–224	$C_{16}H_{12}O_5$	C, H
23	238.5–239.5	$C_{15}H_9ClO_4$	C, H, Cl
24	244–249	$C_{15}H_9NO_6$	C, H, N
25	269–270	$C_{15}H_{10}O_5$	C, H
26	224–225	$C_{16}H_{12}O_4$	C, H
27	205–207	$C_{16}H_{12}O_5$	C, H
28	248–249	$C_{15}H_9ClO_4$	C, H, Cl
29	303–305	$C_{15}H_{10}O_5$	C, H
30	209–212	$C_{16}H_{12}O_4$	C, H
31	220–221	$C_{16}H_{12}O_5$	C, H
32	235–236	$C_{15}H_9ClO_4$	C, H, Cl
33	274–276	$C_{15}H_9NO_6$	C, H, N
34	233–235	$C_{15}H_{10}O_5$	C, H
35	198–201	$C_{16}H_{12}O_4$	C, H
36	223–225	$C_{16}H_{12}O_5 \cdot H_2O$	C, H
37	205–207	$C_{15}H_9ClO_4$	C, H

[a] Reference 11.

poor activity of the nitro and hydroxy compounds may therefore be due to hydrophilicity alone. It is also clear that, in terms of drug potency, there are preferable positions for substitution, with the 5-substituted compounds as a class being the most potent derivatives. We are currently studying xanthenone-4-acetic acids bearing a wider range of substituents at this position, in order to further delineate SAR.

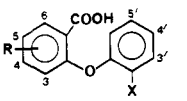
Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, 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. Column chromatography was performed by the method of Still et al.,[21] using Merck silica gel 60 (230–400 mesh). Petroleum ether refers to the fraction bp 40–60 °C. All key compounds had ^1H NMR spectra in accord with the assigned structures.

Preparation of 6-Chloroxanthenone-4-acetic Acid (28). Example of Method A of Scheme I. 4-Chloro-2-(2-methylphenoxy)benzoic Acid (49). A mixture of the sodium salts of 2,4-dichlorobenzoic acid (27.7 g, 130 mmol) and 2-methylphenol (18.9 g, 145 mmol) were suspended in dry dioxane (300 mL). CuCl (1.3 g, 13 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (4.2 g, 13 mmol) were added, and the mixture was heated at reflux under N_2 for 8 h. Excess solvent was evaporated under reduced pressure, and the residue was diluted with water and filtered. The filtrate was acidified with 2 N HCl, and the resulting precipitate was collected, washed well with water, and dried to yield 4-chloro-2-(2-methylphenoxy)benzoic acid (49) (27 g, 79%), which was suitable for the next step. A sample was crystallized from aqueous MeOH as prisms, mp 158–159 °C. Anal. Table III.

6-Chloro-4-methylxanthenone (80). The above acid (8.85 g, 34 mmol) was dissolved in polyphosphate ester and heated at 100 °C until all volatiles were removed and for a further 30 min. The mixture was diluted with an equal volume of MeOH and basified with Na_2CO_3. Addition of water then precipitated 6-chloro-4-methylxanthenone (80), which was dried and crystallized from ligroin/ether to give needles (7.6 g, 93%), mp 145–146 °C. Anal. Table III.

Table IV. Substituted 2-Phenoxybenzoic Acids II



no.	R	X	mp, °C	formula	anal. or lit. mp, °C
38	5′-CH_3	CH_2COOH	183–186	$C_{16}H_{14}O_5$	C, H
39	5′-Cl	CH_3	116–118	$C_{14}H_{11}ClO_3$	117–118[a]
40	4′-OCH_3	$CH_2CH{=}CH_2$	168–170	$C_{17}H_{16}O_4$	C, H
41	4′-Cl	$CH_2CH{=}CH_2$	133–135	$C_{16}H_{13}ClO_3$	C, H
42	3′-CH_3	CH_2COOH	205	$C_{16}H_{14}O_5$	C, H
43	3′-Cl	CH_3	167–168	$C_{14}H_{11}ClO_3$	168–169
44	3-OCH_3	CH_2COOH	186–187	$C_{16}H_{14}O_6$	C, H
45	3-Cl	CH_3	125–126	$C_{14}H_{11}ClO_3$	C, H, Cl
46	3-NO_2	CH_2COOH	196–197	$C_{15}H_{11}NO_7$	C, H, N
47	4-CH_3	CH_2COOH	209–211	$C_{16}H_{14}O_5$	C, H
48	4-OCH_3	CH_3	163.5–164.5	$C_{15}H_{14}O_4$	C, H
49	4-Cl	CH_3	158–159	$C_{14}H_{11}ClO_3$	C, H, Cl
50	5-CH_3	CH_2COOH	226–227	$C_{16}H_{14}O_5$	C, H
51	5-OCH_3	CH_3	132–133.5	$C_{15}H_{14}O_4$	C, H
52	5-Cl	CH_3	126–127	$C_{14}H_{11}ClO_3$	C, H
53	5-NO_2	CH_2COOH	244–246	$C_{15}H_{11}NO_7$	C, H, N

[a] Reference 23.

6-Chloro-4-(bromomethyl)xanthenone (81). A well-stirred mixture of the above 6-chloro-4-methylxanthenone (80) (7.6 g, 31 mmol), *N*-bromosuccinimide (5.5 g, 31 mmol), and benzoyl peroxide (30 mg) in dry CCl_4 (250 mL) was heated at reflux under intense illumination for 3 h. The hot mixture was filtered, the filtrate was evaporated, and the residue was crystallized from the minimum volume of boiling petroleum ether (ca. 1800 mL) to give 6-chloro-4-(bromomethyl)xanthenone (81) (7.4 g, 71%) as colorless needles, mp 217–218 °C. Anal. Table IV.

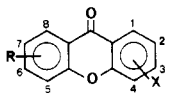
6-Chloroxanthenone-4-acetonitrile (82). The above 6-chloro-4-(bromomethyl)xanthenone (81) (6.47 g, 20 mmol) was finely powdered and suspended in EtOH (150 mL). A hot solution of KCN (2.6 g, 40 mmol) in water (25 mL) was added, and the mixture was heated under reflux for 1 h. A limited amount of hot water was added to precipitate impurities, which were removed by filtration. Further dilution with water then gave a crude product, which was dried and crystallized from benzene/petroleum ether to give 6-chloroxanthenone-4-acetonitrile (82) (3.25 g, 62%). A sample recrystallized from MeOH gave prisms, mp 200–202 °C. Anal. Table V.

6-Chloroxanthenone-4-acetic Acid (28). The above acetonitrile (82) (2 g) was dissolved in a mixture of AcOH (8 mL), concentrated H_2SO_4 (8 mL), and water (8 mL) and heated under reflux for 90 min. Slow dilution with water gave a crystalline product, which was dissolved in warm aqueous $KHCO_3$. The solution was filtered and acidified with 2 N HCl, and the resulting solid was crystallized from EtOH to give 6-chloroxanthenone-4-acetic acid (28) (1.6 g, 74%), mp 248–249 °C. Anal. Table III. The water-soluble sodium salt was crystallized from MeOH/EtOAc.

Similar reactions on other substituted xanthenones provided substituted xanthenone-4-acetic acids via the intermediates recorded in Tables III and IV, while the same sequence applied to unsubstituted 1-, 2-, 3-, and 4-methylxanthenones provided the isomeric xanthenoneacetic acids (5–8) via the intermediates recorded in Table V.

Preparation of 7-Methylxanthenone-4-acetic Acid (30). Examples of Method B of Scheme I. 2-[2-(Carboxymethyl)phenoxy]-5-methylbenzoic Acid (50). A mixture of the potassium salt of 2-iodo-5-methylbenzoic acid (10 g, 33 mmol), the disodium salt of 2-hydroxyphenylacetic acid (7.8 g, 40 mmol), CuCl (0.4 g, 4 mmol), and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (1.3 g, 4 mmol) in dry dioxane (150 mL) was heated under reflux with stirring for 5 h. The dioxane was removed under reduced pressure, and the residue was dissolved in 0.1 N NaOH (100 mL). After filtration to remove insoluble copper salts, the solution was acidified with dilute HCl and extracted with EtOAc.

Table V. Substituted Xanthenones



no.	R	X	mp, °C	formula	anal. or lit. mp, °C
54	H	1-CH_2Br	159.5–160	$C_{14}H_9BrO_2$	C, H, Br
55	H	1-CH_2CN	189–190	$C_{15}H_9NO_2$	C, H, N
56	H	2-CH_2Br	177–179	$C_{14}H_9BrO_2 \cdot H_2O$	C, H
57	H	2-CH_2CN	190–191	$C_{15}H_9NO_2$	C, H, N
58	H	3-CH_2Br	161–162	$C_{14}H_9BrO_2$	C, H, Br
59	H	3-CH_2CN	149–150	$C_{15}H_9NO_2$	C, H, N
60	H	4-CH_2Br	191–192	$C_{14}H_9BrO_2$	C, H, Br
61	H	4-CH_2CN	177–178	$C_{15}H_9NO_2$	C, H, N
62	1-Cl	4-CH_3	130–133	$C_{14}H_9ClO_2$	133–134[a]
63	1-Cl	4-CH_2Br	200–203	$C_{14}H_9BrClO_2$	C, H, Br
64	1-Cl	4-CH_2CN	182–185	$C_{15}H_8ClNO_2$	C, H, N
65	2-CH_3	4-$CH_2CH{=}CH_2$	97–98	$C_{17}H_{14}O_2$	C, H
66	2-OCH_3	4-$CH_2CH{=}CH_2$	115–118	$C_{17}H_{14}O_3$	C, H
67	2-Cl	4-$CH_2CH{=}CH_2$	110–111	$C_{16}H_{11}ClO_2$	C, H
68	3-Cl	4-CH_3	164–166	$C_{14}H_9ClO_2$	163–164[b]
69	3-Cl	4-CH_2Br	175–178	$C_{14}H_8BrClO_2$	186–188[b]
70	3-Cl	4-CH_2CN	214–217	$C_{15}H_8ClNO_2$	C, H, N
71	5-OCH_3	4-CH_3	205–206	$C_{15}H_{12}O_3$	C, H
72	5-OCH_3	4-CH_2Br	205–206	$C_{15}H_{11}BrO_3$	C, H, Br
73	5-OCH_3	4-CH_2CN	202–203	$C_{16}H_{11}NO_3$	C, H, N
74	5-Cl	4-CH_3	176–176.5	$C_{14}H_9ClO_2$	C, H
75	5-Cl	4-CH_2Br	181–182	$C_{14}H_8BrClO_2$	C, H, Br
76	5-Cl	4-CH_2CN	162–163	$C_{15}H_8ClNO_2$	C, H, N
77	5-CH_3	4-CH_2Br	171–172	$C_{15}H_{11}BrO_2$	C, H, Br
78	5-CH_3	4-CH_2CN	177–178	$C_{16}H_{11}NO_2$	C, H
79	6-OCH_3	4-CH_3	151–152[c]	$C_{15}H_{12}O_3$	C, H
80	6-Cl	4-CH_3	145–146	$C_{14}H_9ClO_2$	C, H, Cl
81	6-Cl	4-CH_2Br	217–218	$C_{14}H_8BrClO_2$	C, H, Br
82	6-Cl	4-CH_2CN	200–202	$C_{15}H_8ClNO_2$	C, H, N
83	7-OCH_3	4-CH_3	123–124	$C_{15}H_{12}O_3$	C, H
84	7-OCH_3	4-CH_2Br	183–185	$C_{15}H_{11}BrO_3$	C, H, Br
85	7-OCH_3	4-CH_2CN	203–205	$C_{16}H_{11}NO_3$	C, H, N
86	7-Cl	4-CH_3	143–145	$C_{14}H_9ClO_2$	C, H, Cl
87	7-Cl	4-CH_2Br	200.5–201	$C_{14}H_8BrClO_2$	C, H, Br
88	7-Cl	4-CH_2CN	198–199	$C_{15}H_8ClNO_2$	C, H, N

[a] Reference 24. [b] Reference 25. [c] Reference 26 gives mp 146 °C.

The organic layer was extracted with dilute aqueous NH_3, and the resulting aqueous solution was added slowly with stirring to excess dilute HCl. The resulting precipitate was collected and dried to give 2-[2-(carboxymethyl)phenoxy]-5-methylbenzoic acid (**50**) (8.72 g, 91%), mp (EtOAc) 226–227 °C. Anal. Table IV.

The above compound (6 g) was dissolved in 80% H_2SO_4 (20 mL) and kept at 80 °C for 10 min after dissolution. Dilution with water then gave 7-methylxanthenone-4-acetic acid (**30**) (4.9 g, 87%), mp 209–212 °C. Anal. Table III. Similar reactions using substituted 2-halobenzoic acids gave other ring A substituted xanthenone-4-acetic acids via the intermediates recorded in Table IV. However, reaction of 2-chloro-4-nitrobenzoic acid yielded only the dimerization product 5,5′-dinitrobiphenyl-2,2′-dicarboxylic acid, mp (EtOAc) 283–285 °C. Anal. ($C_{14}H_8N_2O_8$) C, H, N.

Preparation of 2-Methylxanthenone-4-acetic Acid (13). Example of Method C of Scheme II. 4-Allyl-2-methylxanthenone (65). A mixture of the potassium salt of 2-chlorobenzoic acid (13.6 g, 70 mmol), the sodium salt of 2-allyl-4-methylphenol (14.3 g, 84 mmol) (prepared by Claisen rearrangement[17]), CuCl (0.8 g, 8 mmol), and tris[2-(2-methoxyethoxy)ethyl]amine (2.6 g, 8 mmol) in anisole (200 mL) was heated under reflux with stirring for 3 h. Excess solvent was removed under reduced pressure, and the residue was extracted with dilute aqueous NH_3. After filtration to remove insoluble copper salts, the aqueous solution was washed twice with EtOAc and acidified with concentrated HCl. Extraction with EtOAc gave crude 2-(2-allyl-4-methylphenoxy)benzoic acid (10.6 g, 47%), which was used without further purification. The total crude acid was dissolved in polyphosphate ester and the mixture was heated at 100 °C for 30 min, allowing solvent to boil off. The residue was basified with 2 N aqueous NaOH and extracted with petroleum ether to give 4-allyl-2-methylxanthenone (**65**) (6.5 g, 66%), mp (MeOH) 97–98 °C. Anal. Table V.

2-Methylxanthenone-4-acetic Acid (13). A solution of 4-allyl-2-methylxanthenone (**65**) (5 g, 20 mmol) in a mixture of AcOH (75 mL), Me_2CO (75 mL), and water (50 mL) was cooled to below 5 °C, and $KMnO_4$ (15.8 g, 5 equiv) was added in small portions over 6 h. After being stirred for a further 1 h, the mixture was poured into water (1 L) containing $Na_2S_2O_8$ to remove MnO_2. The remaining solid was collected by filtration, dissolved in dilute aqueous NH_3, clarified with charcoal, and acidified with concentrated HCl to give 2-methylxanthenone-4-acetic acid (**13**) (3.2 g, 60%), mp (EtOH) 243–245 °C. Anal. Table III.

Example of the Synthesis of Substituted 2-Hydroxyphenylacetic Acids by the Method of Scheme III. 2-(2-Hydroxy-4-methylphenyl)acetic Acid. A two-phase mixture of 4-methylsalicylic acid (45.6 g, 0.3 mol), NaOH (36 g, 0.9 mol), benzyl bromide (154 g, 0.9 mol), and tetrabutylammonium bromide (10 g, 30 mmol) in water (200 mL) and CH_2Cl_2 (200 mL) was stirred at 20 °C for 3 h. The layers were separated, and the residue from the organic layer was dissolved in a mixture of EtOH (250 mL) and 2 N NaOH (50 mL) and heated under reflux for 30 min. Most of the EtOH was removed under reduced pressure, and the residue was diluted with water and washed with EtOAc. Acidification of the aqueous layer (2 N HCl) gave 4-methyl-2-(phenylmethoxy)benzoic acid (XII: R = 4-CH_3) (65.3 g, 90%), suitable for use in the next step. A sample crystallized from aqueous MeOH, mp 105–107 °C. Anal. ($C_{15}H_{14}O_3$) C, H. Similar reactions with the appropriate salicylic acid gave 6-methyl-2-(phenylmethoxy)benzoic acid (79% yield), mp (aqueous MeOH) 89 °C. Anal. ($C_{15}H_{14}O_3$) C, H.

A solution of the above compound (XII: R = 4-CH_3) (30.3 g, 0.125 mol) in $SOCl_2$ (100 mL) and DMF (0.1 mL) was heated under reflux for 30 min. Excess reagent was removed under reduced pressure, and the residue was azeotroped with benzene to remove residual $SOCl_2$. The resulting crude oily acid chloride was added slowly to a mixture of $NaBH_4$ (10 g) in dry diglyme (200 mL) at 10–20 °C. The mixture was stirred at 20 °C for 30 min, and the solvent was then removed under reduced pressure. Water (100 mL) was added to the solid residue, followed by AcOH (10 mL) to ensure $NaBH_4$ decomposition, and the mixture was then basified (NH_4OH) and extracted with EtOAc to give crude 4-methyl-2-(phenylmethoxy)benzenemethanol (XIII: R = 4-CH_3) as an oil (26.6 g, 93%).

The above crude product (20 g, 87.6 mmol) was dissolved in dry benzene (100 mL) and treated with PBr_3 (9.1 mL, 96 mmol). After stirring for 10 min at 20 °C, the mixture was treated with 2 N NaOH (50 mL), and the organic layer was separated and dried to give crude 1-(bromomethyl)-4-methyl-2-(phenylmethoxy)-benzene (XIV: R = 4-CH_3) as an oil (23.7 g, 93%).

A two-phase mixture of the above compound (21.8 g, 75 mmol), NaCN (11 g, 0.22 mol), and tetrabutylammonium bromide (2.4 g, 7.5 mmol) in water (25 mL) and CH_2Cl_2 (50 mL) was stirred at 20 °C for 1 h. The organic layer was separated and washed well with water to remove tetrabutylammonium salts, and the solvent was removed to give crude [4-methyl-2-(phenylmethoxy)phenyl]acetonitrile (XV: R = 4-CH_3) as an oil (17.8 g, 100%).

A solution of the above acetonitrile (15 g, 63 mmol) in EtOH (200 mL) and water (50 mL) containing NaOH (10 g, 0.25 mol) was heated under reflux for 15 h, and the EtOH was removed under reduced pressure. The residue was diluted with water and washed with benzene, and the aqueous layer was acidified to give [4-methyl-2-(phenylmethoxy)phenyl]acetic acid (XVI: R = 4-CH_3) as a solid (14.2 g, 88%), suitable for use in the next step. A sample was crystallized from aqueous MeOH, mp 77–80 °C. Anal. ($C_{16}H_{16}O_3$) C, H.

A solution of the above acid in EtOH was hydrogenated over Pd/C to give a quantitative yield of the desired (2-hydroxy-4-methylphenyl)acetic acid (VI: R = 4-CH_3), mp (benzene) 117–120 °C (lit.[22] mp 124 °C). Anal. ($C_9H_{10}O_3$) C, H.

A similar sequence using the protected 6-methylsalicylic acid noted above gave [6-methyl-2-(phenylmethoxy)phenyl]acetic acid, mp (aqueous MeOH) 100 °C. Anal. ($C_{16}H_{16}O_3$) C, H. Hydrogenolysis of this gave (2-hydroxy-6-methylphenyl)acetic acid, mp (benzene) 111 °C (lit.[22] mp 110.5 °C). Anal. ($C_9H_{10}O_3$) C, H.

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2′-Fluorinated Isonucleosides. 1. Synthesis and Biological Activity of Some Methyl 2′-Deoxy-2′-fluoro-2′-pyrimidinyl-D-arabinopyranosides

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New reactions of methyl 2,2-difluoro glycosides are described that were utilized for synthesis of some novel nucleoside derivatives. Thus, treatment of methyl 2-deoxy-2,2-difluoro-3,4-*O*-isopropylidene-α(β)-D-*erythro*-pyranoside (**2**) with anhydrous HCl resulted in selective displacement of one fluorine atom with chlorine to give a 2-deoxy-2-chloro-2-fluoro glycoside **3**. Reaction of **3** with silylated uracil in the presence of $SnCl_4$ provided a 2-deoxy-2-fluoro-2-uracil-substituted glycoside **4**. 2-Fluoro-2-deoxy glycosides substituted with other pyrimidines at C-2 were prepared similarly by the reaction of acylated 2,2-difluoro or 2-fluoro-2-bromo derivatives (**5** and **6**, respectively) with silylated pyrimidines. The resulting 2′-fluorinated isonucleosides were evaluated for their antitumor and antiviral activities. Compounds **7a,b**, **8a,b**, and **10a,b** demonstrated 50% tumor cell growth inhibition in vitro (IC_{50}) at 10^{-4}–10^{-5} M. At similar concentrations no antiviral activity was observed in vitro. Therapeutic activity was obtained with **7a,b** and **8a,b** in DBA/2 mice with L1210 leukemia. Administration of **7a,b** at 500 mg/kg, ip daily, for 5 consecutive days, resulted in a 55% increase in life span (% ILS) while administration of **8a,b** in the same manner at 200 mg/kg caused a 29% ILS. Treatment with **7a,b** to mice with drug-resistant L1210 sublines (5-FU and araC) resulted in 22 and 57% increases in life span, respectively. Lewis lung carcinoma and M5076 sarcoma in mice also responded to the administration of **7a,b** with reductions in tumor growth for both tumors and significant increases in life span in mice with Lewis lung carcinoma. Although the mechanism of action of **7a,b** is not known, it has been found to be a relatively fast-acting, cell-cycle nonspecific cytotoxic agent that decreases [^{3}H]deoxyuridine incorporation, blocks L1210 cells at the G_2 phase of the cell cycle, and is not reversed by exogenous thymidine. These 2′-fluorinated isonucleosides have demonstrated biological activity and may have potential as antitumor drugs.

As part of our investigation of *gem*-difluoro monosaccharides,[1] we recently reported a selective nucleophilic displacement of fluorine in methyl 2,2-difluoro glycosides.[2,3] Various nucleophiles, including C-substituents and heterocyclic bases can be introduced at C-2 of methyl glycosides, the products of this reaction being novel carbohydrate derivatives substituted, at C-2, with fluorine and the entering substituent. In this paper, we describe the synthesis and the results of initial antiviral and antitumor evaluation of some 2′-fluoro analogues of methyl 2′-pyrimidinylarabinopyranosides.

Chemistry. Methyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside[4,5] was oxidized by a chromium trioxide–pyridine–acetic anhydride complex[6] to give methyl 3,4-*O*-isopropylidene-β-D-*erythro*-pentopyranosid-2-ulose (**1**, Scheme I) in 84% yield. While compound **1** was previously prepared by similar oxidation using different reagents,[6-8] the present method gave improved yields and was found better suitable for large-scale preparations. Fluorination

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