four-parameter logistic equation as described above. In some experiments, for example those in Figure 3, there was no preincubation with the test compounds, rather they were added to the cells with the degranulatory stimuli. Results were the same with or without the preincubation.

Registry No. 1, 3811-56-1; 2, 137872-78-7; 3, 137872-79-8; 4, 137895-36-4; 5, 137872-80-1; 6, 137872-81-2; 7, 137872-82-3; 8, 5443-31-2; 9, 137872-83-4; 10, 101890-98-6; 11, 137872-84-5; 12, 6269-68-7; 13, 137872-85-6; 14, 6954-99-0; 15, 109094-06-6; 16, 103270-77-5; 17, 137872-86-7; 18, 137872-87-8; 19, 137872-88-9; 20, 137872-89-0; 21, 137872-90-3; 22, 137872-91-4; 23, 137872-92-5; 24, 137872-93-6; 25, 137872-94-7; 26, 137872-95-8; 27, 137872-96-9; 28, 137872-97-0; oxalyl chloride, 79-37-8; malonyl chloride, 1663-67-8; adipoyl chloride, 111-50-2; sebacoyl chloride, 111-19-3; benzylamine hydrochloride, 3287-99-8; *n*-octylamine hydrochloride, 142-95-0; *n*-octylamine, 111-86-4; 6-acetamido-4-methoxy-2-methylquinoline, 100795-23-1; 6-acetamido-4-(benzylamino)-2-methylquinoline hydrochloride, 137872-98-1; 6-amino-4-(benzylamino)-2-methylquinoline, 137872-91-4; 2-chlorocinnamoyl chloride, 35086-82-9; 6-amino-4-methoxy-2-methylquinoline, 84264-27-7; 4-hydroxy-2-methylquinoline-6-carboxylic acid *n*-octylamide, 137872-99-2; 4-hydroxy-2-methylquinoline-6-carboxylic acid, 103853-88-9; 4-methoxy-2-methylquinoline-6-carboxylic acid *n*-octylamine, 137873-00-8; anaphylatoxin C5a, 80295-54-1.

Potential Antitumor Agents. 64. Synthesis and Antitumor Evaluation of Dibenzo[1,4]dioxin-1-carboxamides: A New Class of Weakly Binding DNA-Intercalating Agents

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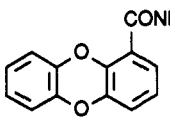
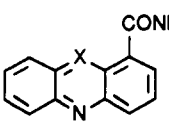
Received May 7, 1991

A series of substituted dibenzo[1,4]dioxin-1-carboxamides has been synthesized and evaluated for in vitro and in vivo antitumor activity. The required substituted dibenzo[1,4]dioxin-1-carboxylic acids were prepared by a variety of methods. No regiospecific syntheses were available for many of these, and separation of the mixtures of regioisomers obtained was sometimes difficult. The dibenzo[1,4]dioxin-1-carboxamides are active against wild-type P388 leukemia in vitro and in vivo, with structure–activity relationships resembling those for both the acridine-4-carboxamide and phenazine-1-carboxamide series of DNA-intercalating antitumor agents. In all three series, substituents placed peri to the carboxamide sidechain (the 5-position in the acridines, and the 9-position in the phenazines and dibenzo-[1,4]dioxins) enhance activity and potency. The 9-chlorodibenzodioxin-1-carboxamide was also curative against the remotely sited Lewis lung carcinoma. Several of the compounds showed much lower levels of cross-resistance to the P388/AMSA line than classical DNA-intercalating agents, which suggests that their primary mechanism of action may not be via interference with topoisomerase IIα. This is of interest with regard to the development of drugs to combat resistance mechanisms which arise by the expression of the topo IIβ isozyme.

In a general study of the antitumor properties of linear tricyclic carboxamides, we recently[1] noted the in vivo antileukemic activity of the DNA-intercalating dibenzo-[1,4]dioxin-1-carboxamide (1). While DNA-intercalating



1



2 : X = CH
3 : X = N

agents form an important class of anticancer drugs, a common limitation of such compounds is their poor extravascular distributive properties.[2-4] This is particularly true for compounds where a cationic charge is located on the DNA-binding chromophore, for example acridine-based compounds such as 2. A previous study[5] of analogues of 2 showed that activity against remotely sited Lewis lung tumors was exhibited only by those analogues where the acridine chromophores were uncharged at physiological pH.

Structures such as the parent dioxin 1, with small neutral chromophores, are therefore of particular interest, since they are likely to have better ability to diffuse efficiently into solid tumor tissue. While this compound has only modest antitumor activity,[1] recent work[6] with similar phenazinecarboxamides (3) has demonstrated that dramatic improvements in activity can be achieved by suitable substitution of the chromophore. A recent survey[7] of tricyclic carboxamides showed that 1 was virtually inactive as a frameshift mutagen, unlike many acridine-based derivatives. In the present work we therefore outline the

(1) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 54. Chromophore requirements for in vivo antitumor activity among the general class of tricyclic carboxamides. *J. Med. Chem.* 1988, *31*, 707–712.
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(7) Denny, W. A.; Turner, P. M.; Atwell, G. J.; Rewcastle, G. W.; Ferguson, L. R. Structure–activity relationships for the mutagenic activity of tricyclic intercalating agents in *Salmonella typhimurium*. *Mutat. Res.* 1990, *232*, 233–241.

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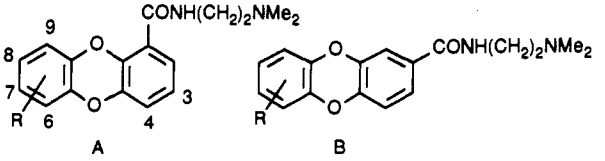
Table I. Physicochemical and Biological Properties of Dibenzo[1,4]dioxincarboxamides



no.	formula	R	mp (°C)[a]	formula	analyses	Rm[b]	log K[c]	cytotoxicity: IC_{50} (μM)[d] P388/W[e]	cytotoxicity: IC_{50} (μM)[d] P388/AMSA[f]	P388/W in vivo OD[g]	P388/W in vivo ILS[h]
1	A	H	178–182	$C_{17}H_{18O}N_2O_2$·HCl	*i*	0.01	5.05	3.0	1.8	150	76
4	A[j]	H[j]	157–160	$C_{19}H_{22}N_2O_3$·HCl	C,H,N,Cl	0.13	4.90	>20		150	NA[k]
5	A	3-NO_2	229–231	$C_{17}H_{17}N_3O_5$	C,H,N	−0.16	5.37	1.7		225	NA
6	A	6-Cl	203–205	$C_{17}H_{17}ClN_2O_3$·HCl	C,H,N,Cl	0.21	5.15	1.3		100	NA
7	A	6-Me	103–105	$C_{18}H_{20}N_2O_3$	C,H,N	0.14	5.26	2.1		45	NA
8	A	6-OMe	136–137	$C_{18}H_{20}N_2O_4$	C,H,N	−0.19	5.18	1.6		100	NA
9	A	6-NO_2	128–130	$C_{17}H_{17}N_3O_5$	C,H,N	−0.20	5.06	2.5		150	NA
10	A	6-aza	124–126	$C_{16}H_{17}N_3O_3$	C,H,N	−0.49	4.73	8.0		100	NA
11	A	7-Cl[l]	134–140	$C_{17}H_{17}ClN_2O_3$	C,H,N,Cl	0.10	5.11	1.6		100	NA
12	A	8-Cl									
13	A	7-Br	140	$C_7H_{17}BrN_2O_2$	C,H,N,Br	0.18	5.34	1.9		100	NA
14	A	7-Me[l]	194	$C_{18}H_{20}N_2O_3$·HCl	C,H,N	0.20	5.11	3.3		45	NA
15	A	8-Me									
16	A	7-NO_2	211[m]	$C_{17}H_{19}N_3O_5$	C,H,N[m]	−0.14	5.64[m]	6.0		150[m]	96 (1)[m]
17	A	8-Br	168	$C_{17}H_{17}BrN_2O_2$·HCl·H_2O	C,H,N,Cl	0.18	5.35	3.0		150	NA
18	A	8-NO_2	211[m]	$C_{17}H_{17}N_3O_5$	C,H,N[m]	−0.14[m]	5.64[m]	3.7		150[m]	96 (1)[m]
19	A	9-Cl	172–175	$C_{17}H_{17}ClN_2O_3$·HCl·2.5H_2O	C,H,N,Cl	−0.01	5.48	0.23	2.6	100	106 (1)[n]
20	A	9-Br	164	$C_{17}H_{17}BrN_2O_3$	C,H,N,Br	−0.01	5.23	0.21	0.73	100	154
21	A	9-Me	197–200	$C_{18}H_{20}N_2O_3$·HCl·0.5H_2O	C,H,N,Cl	0.09	5.28	0.25	7.7	100	144
22	A	9-OMe	140–140.5	$C_{18}H_{20}N_4O_2$	C,H,N	−0.02	5.55	0.38	0.52	45	45
23	A	9-aza	79–81	$C_{16}H_{17}N_3O_3$	C,H	−0.38		0.83	0.93	65	NA
24	A	9-NO_2	146–148	$C_{17}H_{17}N_3O_5$	C,H,N	−0.28	5.11	0.032	0.45	20	NA
25	A	9-X[o]	250	$C_{22}H_{28}N_4O_4$·2HCl	C,H,N,Cl	−0.68	4.48	0.72		100	NA
26	A	7,8-diCl	130–132	$C_{17}H_{16}Cl_2N_2O_3$	C,H,N,Cl	0.25		5.13	0.83	150	NA
27	B	H	140	$C_{17}H_{18}N_2O_3$·HCl	C[p],H,N	0.11	4.84	5.8		150	NA
28	B	7-X[o]	274–275	$C_{20}H_{28}N_4O_4$·2HCl·0.5H_2O	C,H,N,Cl	−1.21	6.81	>10		225	NA

[a] Melting point of the form (free base or salt) indicated by the formula. [b] Rm: relative measure of lipophilicity, determined as detailed in ref 28, using 4′-(9-acridinylamino)methanesulfonanilide (AMSA) as internal standard. [c] log K: binding constant to poly[d(A-T)], determined by ethidium bromide displacement; see ref 15. [d] IC_{50}: concentration of drug in μM to inhibit cell growth in culture to 50% of controls, using the protocol detailed in ref 17; values are means of three determinations. [e] P388(W): wild-type P388 murine leukemia. [f] P388(AMSA): mutant P388 line characterized by an altered topoisomerase II enzyme and resistant to classical topo II inhibitors. [g] OD: optimal dose of drug in milligrams/kilogram per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v EtOH/water on days 1, 5 and 9, 24 h after intraperitoneal inoculation of 10^6 tumor cells. [h] ILS: percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose. Values of ILS >20% are considered statistically significant. All tests are carried out in groups of 6 mice and are duplicate. [i] See ref 1. [j] Compound contains the $(CH_2)_4NMe_2$ side chain. [k] NA: compound not active (ILS <20%) at all dose levels. [l] Inseparable mixture of isomers: data obtained for the mixture. [m] Marked data obtained for the mixture of 7-NO_2 and 8-NO_2 isomers 16 and 18: small amounts were separated to measure the individual IC_{50} values. [n] Values in parentheses give the average number of animals (out of a group of six which were long-term (60-day) survivors (not examined for presence of tumor at sacrifice). [o] X = $CONH(CH_2)_2NMe_2$. [p] C out by 0.5%.

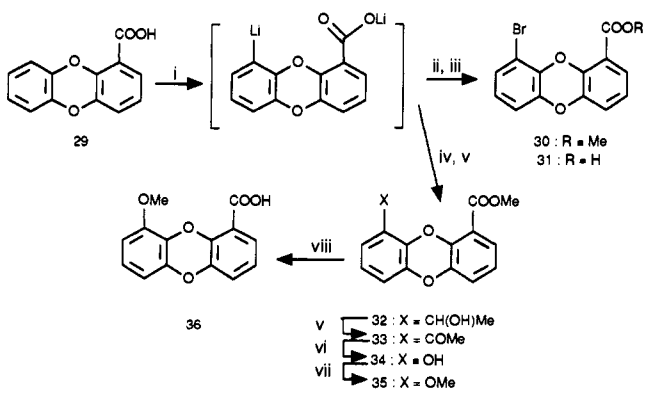
synthesis of a number of ring-substituted dibenzo[1,4]-dioxin-1-carboxamides and study structure–activity relationships for their activity against both wild-type and resistant P388 murine leukemia cell lines in vitro and in vivo.

Chemistry

The substituted dibenzo[1,4]dioxin-1-carboxylic acids required for this work were prepared by a variety of methods. No regioselective syntheses are available for the preparation of 6-, 7-, and 8-substituted compounds, and separation of the mixtures of regioisomers obtained was sometimes difficult. The carboxamides of Table I were prepared by activation of the corresponding acids with 1,1′-carbonyldiimidazole[8] and reaction with *N*,*N*-dimethylethylenediamine.

9-(and 6-)Substituted Dibenzo[1,4]dioxin-1-carboxylic Acids. Several of the required 9-substituted acids (9-Me, 9-Cl, 9-COOH) had been prepared previously

Scheme I[a]



[a] (i) *t*-BuLi/TMEDA, (ii) Br_2, (iii) MeOH/H^+, (iv) CH_3CHO, (v) PCC, (vi) *m*-CPBA/*p*-TsOH, (vii) Me_2SO_4/K_2CO_3, (viii) aqueous KOH.

via our recently described procedures for the efficient preparation of dibenzo[1,4]dioxin-1-carboxylic acid (**29**) and its regioselective metalation at the C-9 position.[9] This

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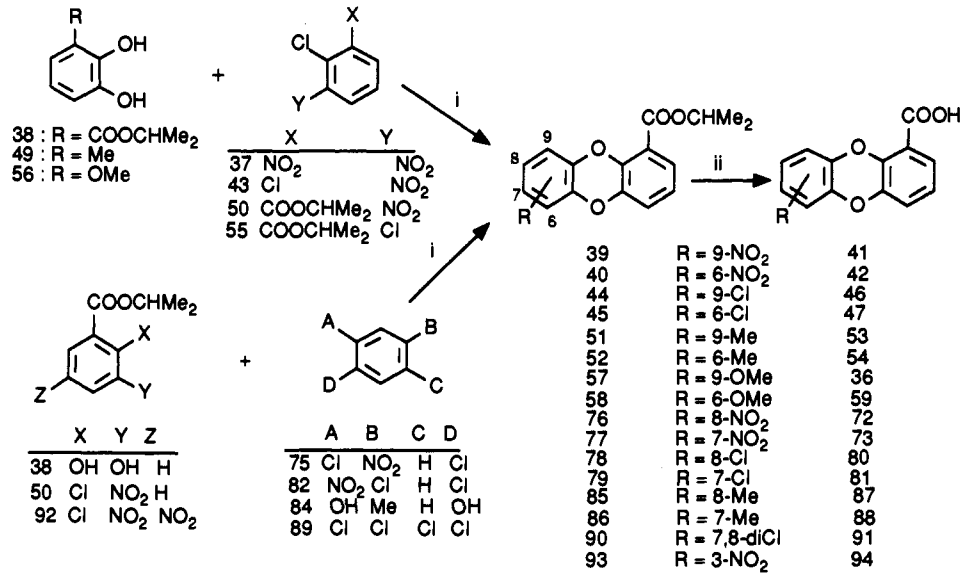
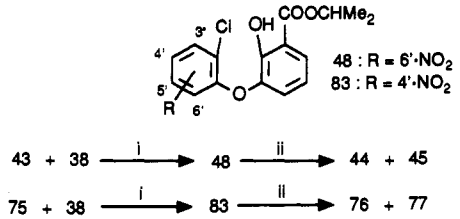
Scheme II[a]



[a] (i) NaH or K/THF/HMPA (80–110 °C/10 min–22 h), (ii) aqueous 2 N KOH/(dioxane or MeOH)/20 °C/20 h or aqueous H_2SO_4/dioxane/reflux/24 h.

Scheme III[a]



[a] (i) NaH/THF/HMPA (20 °C/20–40 h), (ii) K/HMPA (110 °C/2.5 h).

procedure was also used in the present work to prepare the 9-Br and (indirectly) the 9-OMe substituted acids (Scheme I). For the latter compound, metalation of **29** and quenching with acetaldehyde, followed by esterification, gave the 9-(2'-hydroxyethyl) compound **32**, which was oxidized with pyridinium chlorochromate to the ketone **33**. Baeyer–Villiger oxidation of this using 3-chloroperoxybenzoic acid gave the 9-acetoxy derivative, which spontaneously hydrolyzed during chromatographic purification to the corresponding phenol **34**. Methylation with Me_2SO_4 then gave the required 9-methoxy compound **35**, which was hydrolyzed in base to give the desired acid **36**. Other seemingly more direct methods for introduction of a phenol functionality from the 9-lithio compound[10,11] gave lower yields of impure material.

The remainder of the 9-substituted acids (and the corresponding 6-isomers) were prepared either by cyclocondensation of the dianion of isopropyl 2,3-dihydroxybenzoate with 3-substituted 1,2-dichloro- or *o*-chloronitrobenzenes (Scheme II), or the dianion of methylcatechols with 1,2-nitrochloro- or 1,2-dichlorobenzoates (Scheme III). We have recently demonstrated the utility of these methods for the synthesis of substituted dibenzo[1,4]dioxin-1-carboxylic acids,[12] while noting the potential disadvantages of the unavoidable formation of mixtures of regioisomers.

Thus reaction of 1-chloro-2,6-dinitrobenzene (**37**) and isopropyl 2,3-dihydroxybenzoate (**38**) (Scheme II) gave a 1:2.2 mixture of the 9- and 6-nitro-substituted dibenzo[1,4]dioxin-1-carboxylates (**39** and **40**), which could be separated by chromatography. The ester of lower R_f value (mp 139–141 °C) was shown to be the 6-isomer **40** by X-ray crystallography. Similar reaction of 1,2-dichloro-3-nitrobenzene (**43**) gave the pair of 9- and 6-chloro-substituted compounds (**44** and **45**). While this reaction could not be used to prepare the corresponding methyl compounds (**51** and **52**) because of the unfavorable electronic effects of the methyl group,[12] these were obtained in good yield from the reaction of 3-methylcatechol (**49**) and isopropyl 2-chloro-3-nitrobenzoate (**50**) (Scheme III). Similarly, 3-methoxycatechol (**56**) and isopropyl 2-chloro-3-nitrobenzoate (**50**) gave a 1:1.2 mixture of the 9- and 6-methoxy isopropyl esters (**57** and **58**). The ester of higher R_f (mp 55–57 °C) was identified as the 9-isomer by comparison with the product of the unequivocal route above (Scheme I).

Study of the ^1H NMR spectra of the four pairs of 6/9 isomers available (**39** and **40**; **44** and **45**; **51** and **52**; **57** and **58**) showed systematic differences which were of value in assigning structures. While the 6-proton of the 9-substituted compounds and the 9-proton of the 6-substituted compounds are in very similar local environments, the 1-ester group has a sufficient deshielding influence to ensure that the 9-proton in the latter compounds always resonated at lower field. The 4-protons of the 6-substituted isomers always resonated at lower field than did those of the 9-compounds, reflecting long-range through-space shielding effects. The ester C*H*Me_2 protons in the 9-isomers were always at lower field than in the corresponding 6-isomers. These differences, together with the definitive X-ray structure for **40**, allowed confident assignment of structures to all 6- and 9-substituted compounds which were not prepared unequivocally by the lithiation procedure.

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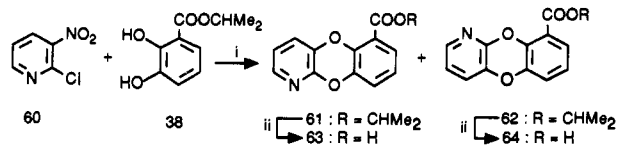
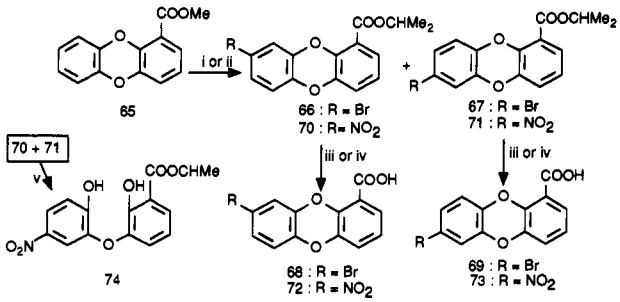
(11) Taddei, M.; Ricci, A. Electrophilic hydroxylation with bis(trimethylsilyl)peroxide. A synthon for the hydroxyl cation. *Synthesis* **1986**, 633–635.
(12) Lee, H. H.; Denny, W. A. An improved synthesis of substituted dibenzo[1,4]dioxins. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1071–1074.

Scheme IV[a]



[a] (i) NaH/HMPA (110 °C/4–22 h), (ii) aqueous 2 N KOH/dioxane or MeOH (20 °C/20 h).

Scheme V[a]



[a] (i) $KBrO_3/KBr$, (ii) $HNO_3/Ac_2O/AcOH$, (iii) aqueous KOH, (iv) aqueous H_2SO_4/dioxane, (v) NaH/THF/HMPA (20 °C/20–40 h).

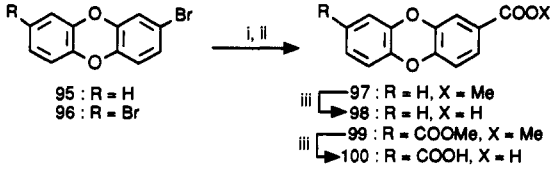
Since these cyclization reactions proceed in a two-step fashion,[12] it was of interest to see if only one intermediate condensation product was produced. Thus, reaction of 1,2-dichloro-3-nitrobenzene (**43**) with the dianion of isopropyl 2,3-dihydroxybenzoate (**38**) under mild conditions gave a 77% yield of the single noncyclized product **48** (Scheme III), the structure of which was determined by ^1H NMR spectroscopy. This is the expected product, since the 3-O^- of **38** should be a better nucleophile than the 2-O^-, due to conjugation with the ester, and the 2-Cl group of **43** (ortho to the nitro) should be the more reactive toward nucleophiles. Steric effects may also play a role in the observed regioselectivity.

However, cyclization of **48** in base still gave a mixture of 9- and 6-chloro isomers (**44** and **45**, respectively) in the same ratio (ca. 1:2.3) as that seen in the one-step reaction, even at the lowest temperature for appreciable reaction (ca. 80 °C) as a result of a rapid Smiles rearrangement, a phenomenon which has been noted previously in similar reactions.[13] Finally, cyclization of 2-chloro-3-nitropyridine (**60**) with **38** gave a good yield of the corresponding 6- and 9-aza compounds (**61** and **62**) in a ratio of 1:0.3 (Scheme IV).

8-(and 7-)Substituted Dibenzo[1,4]dioxin-1-carboxylic Acids. Synthesis of the 7- and 8-substituted compounds could be achieved either by cyclocondensation reactions similar to the above, or by electrophilic aromatic substitution reactions on the parent dibenzo[1,4]dioxin-1-carboxylic acid chromophore, but each method gave mixtures of the regioisomers. These proved much more difficult to separate than the 6/9 isomers, and in some cases this could not be achieved.

Bromination of methyl dibenzo[1,4]dioxin-1-carboxylate (**65**) with $KBrO_3/KBr$ in aqueous acetic acid gave a 1:1 mixture of the methyl esters of the 8- and 7-bromo isomers (**66** and **67**), which could be separated by careful flash chromatography (Scheme V). The less polar compound (mp 103 °C) was assigned the 8-bromo structure **66** and that of mp 106.5 °C the 7-bromo isomer **67** from consideration of their high-field ^1H NMR spectra. 2D ^1H–^1H correlation spectroscopy was used to unambiguously assign the coupled partners in each of the aromatic rings of the isomers. The bromo-substituted ring in each isomer contained only one resonance showing meta coupling (i.e., H-9 of the 8-bromo- and H-6 of the 7-bromodibenzo[1,4]dioxins **66** and **67**). Assignment of regiochemistry was based on the expectation that this signal in the 8-bromo isomer (δ 7.11 ppm) would be deshielded by its proximity to the 1-ester group, compared with that of the 7-bromo isomer (δ 7.00 ppm). The H-9 proton of the 7-bromo isomer also resonates downfield (δ 6.83 ppm) of H-6 of the 8-bromo compound (δ 6.71 ppm). Similarly, the H-9 proton of the parent methyl dibenzo[1,4]dioxin-1-carboxylate (**65**) resonates 0.11 ppm downfield (δ 6.94 ppm) from H-6 (δ 6.83 ppm).

Scheme VI[a]



[a] (i) *n*-BuLi/CO_2, (ii) $MeOH/H_2SO_4$, (iii) aqueous KOH.

Nitration of **65** with acetyl nitrate gave a 1:1.6 mixture of the 7- and 8-nitro isomers (**71** and **70**), but these could not be separated chromatographically. A similar mixture of nitro isomers, in the ratio 1:2, was also obtained by the reaction of the dianion of isopropyl 2,3-dihydroxybenzoate (**38**) with 1,2-dichloro-4-nitrobenzene (**75**) (Scheme V). As before, reaction under mild conditions (20 °C for 40 h) gave an 88% yield of a single noncyclized product presumed to be **83**, but cyclization of this under the mildest possible conditions still gave the same mixture of 7- and 8-nitro isomers. While basic hydrolysis of this ester mixture gave the ring-opened diphenyl ethers (**74** and isomer), acid hydrolysis gave the desired acids (**72** and **73**). There also could not be separated, but preparative-scale resolution of the corresponding carboxamides (**18** and **16**) was achieved using reversed-phase HPLC.

Assignment of regiochemistry to the separated carboxamides (**16** and **18**) and their precursors was based on similar ^1H NMR considerations as for the 7- and 8-bromo isomers. Thus, H-9 of the 8-nitro isomer **18** resonates downfield (δ 7.90 ppm) of H-6 of the 7-nitro isomer **16** (δ 7.78 ppm), while H-9 of **16** is downfield (δ 7.09 ppm) of H-6 the 8-nitro compound **18** (δ 7.00 ppm).

The corresponding mixtures (approximately 1:1) of the 8- and 7-chloro isomers (**80** and **81**) and of the 8- and 7-methyl isomers (**87** and **88**) were prepared as shown in Scheme II, respectively, but in these cases the mixtures were inseparable as either the esters or the acids. Some resolution of the corresponding carboxamides (**11**/**12** and **14**/**15**) could be achieved analytically by HPLC, but preparative separation was not attempted, since both isomer mixtures proved inactive in vivo (Table I).

Other Substituted Dibenzo[1,4]dioxin Carboxylic Acids. The 7,8-dichloro acid **91** and the 3-nitro acid **94** were both obtained in moderate yields from cyclocondensation reactions of the appropriate starting materials (Scheme II). The 2-acid **98** and the 2,8-diacid **100** were obtained by halogen–metal exchange on the known 2-bromo- and 2,8-dibromodibenzodioxins (**95** and **96**), followed by carbonation with CO_2 (Scheme VI).[14]

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Results and Discussion

A total of 22 substituted dibenzo[1,4]dioxin-1-carboxamides were prepared in order to obtain an overall view of structure–activity relationships (SAR) in this series. In view of the SAR observed for the topologically similar phenazinecarboxamides,[6] the majority of the compounds studied had substituents in the ring not containing the carboxamide group. Overall drug lipophilicity was estimated by thin-layer chromatography. The results were as expected, showing that substituents in the 9-position exhibited lesser effects on overall drug lipophilicity than did the same substituents in more exposed positions. The aza derivatives **10** and **23** were exceptionally hydrophilic. Estimates of DNA binding were obtained by the ethidium displacement assay.[15] The parent compound **1** binds relatively weakly to DNA (log K = 5.03) compared to similar compounds with charged chromophores.[16] With the exception of the aza derivatives, which did show significantly lower binding, substitution of the chromophore had little effect on DNA binding.

The compounds were all evaluated against wild-type P388 leukemia (P388/W), using a 96-well plate assay.[17] Selected compounds were also evaluated against a mutant line (P388/AMSA), which has a structurally altered topoisomerase II enzyme,[18] and is highly resistant in culture (IC_{50} ratios of 40–70 fold) to DNA-intercalating agents which act by inhibition of topoisomerase II.[19] Full dose–response curves were determined for each compound. These (for a given compound) had similar slopes in both cell lines, and IC_{50} levels were used as a representative measure of cytotoxicity. The parent compound showed only moderate cytotoxicity against wild-type P388 leukemia (3 μM, Table I),[20] and lengthening the side chain (compound **4**) proved very dystherapeutic, as repeatedly seen in the tricyclic carboxamides.[1,6] Substitution in the 3-, 6-, 7-, and 8-positions had little effect on cytotoxicity, but 9-substituted compounds were generally about 10-fold more cytotoxic (Table I). Both the parent compound and several of the more potent 9-substituted derivatives had much lower IC_{50} ratios (ca. 2–10 fold) than typical DNA-intercalating agents, suggesting that they may not act exclusively via topoisomerase IIα, which is thought to mediate the cytotoxicity of most DNA-intercalating agents.[21,22]

The parent dibenzodioxincarboxamide **1** showed only moderate in vivo activity against P333/W, but the majority of the 9-substituted compounds (with the exception of the 9-nitrodibenzodioxin **24**) showed superior activity. This is a similar pattern to the SAR of the phenazine-1-carboxamides, where the 9-substituted compounds showed the highest activity.[6] The 9-chloro derivative **19** also proved to be very active against the remotely sited Lewis lung carcinoma (using the same protocol and dose) with an ILS of 114% and 4/6 long-term (60-day) survivors.

Conclusions

Our primary interest in development of the dibenzo[1,4]dioxin class was their physicochemical properties (a noncharged chromophore and weak intercalative DNA binding), which appeared to favor efficient extravascular distribution. However, an analysis of the SAR of this class is interesting, in that it appears similar to that of both the acridine-4-carboxamides and the phenazine-1-carboxamides. All these compounds would be expected to be similarly charged under physiological conditions, with a moderately basic side chain (pK_a ca. 9.0) and very weakly basic or nonbasic chromophores. In all three series, substituents placed peri to the carboxamide side chain (the 5-position in the acridines;[23] the 9-position in the phenazines[6] and dibenzo[1,4]dioxins) enhance activity and potency. Thus, although the parent dibenzo[1,4]dioxin **1** was not active in the Lewis lung carcinoma,[1] the 9-chlorodibenzodioxin compound **19** shows curative activity comparable to that of the acridine-4-carboxamides[23] and 9-substituted phenazine-1-carboxamides.[6]

In view of this broad-spectrum activity, the lesser cross-resistance of the P388/AMSA line to several of these compounds (a property not shared by the acridine-4-carboxamides[19]) is noteworthy. One form of resistance to current clinical topoisomerase II directed drugs (which target topo IIα) appears to be a change in the expression of topo II isozymes from IIα to IIβ. The development of DNA-intercalating agents which do not act via topo IIα is a topic of current interest.[24,25] Recent work with derivatives of amsacrine has suggested that topo IIβ selective compounds can be prepared by suitable modification of the side chain, which is thought to contact the enzyme.[25] The dibenzodioxins studied here, being reasonably potent DNA-intercalating agents which may not act primarily via topo IIα, are also of interest in this regard.

Experimental Section

Analyses indicated by symbols of the elements were within ±0.4% of theoretical. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal apparatus with a stem-corrected thermometer and are recorded as read. NMR spectra were obtained on a Bruker AM-400 spectrometer and are referenced to Me_4Si. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}). Column

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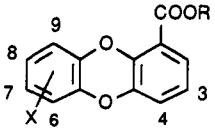
Table II. Physicochemical Data for Substituted Dibenzo[1,4]dioxin Acids and Esters and Their Aza Analogues



no.	X	R	mp, °C	formula	anal.
30	9-Br	Me	107–110	$C_{14}H_9BrO_4$	C,H,Br
33	9-COMe	Me	141–143	$C_{16}H_{12}O_5$	C,H
35	9-OMe	Me	79	$C_{15}H_{12}O_5$	C,H
36	9-OMe	H	209–211	$C_{14}H_{10}O_5$	C,H
39	9-NO_2	$CHMe_2$	109–110	$C_{16}H_{13}NO_6$	C,H,N
40	6-NO_2	$CHMe_2$	139–141	$C_{16}H_{13}NO_6$	C,H,N
41	9-NO_2	H	243–245	$C_{13}H_7NO_6$	C,H,N
42	6-NO_2	H	275–277	$C_{13}H_7NO_6$	C,H,N
47	6-Cl	H	289–291	$C_{13}H_7ClO_4$	C,H,Cl
52	6-OMe	$CHMe_2$	82–84	$C_{17}H_{16}O_3$	C,H
54	6-Me	H	251–253	$C_{14}H_{10}O_4$	C,H
59	6-OMe	H	227–229	$C_{14}H_{10}O_5$	C,H
61	9-aza	$CHMe_2$	85–86	$C_{15}H_{13}NO_4$	C,H,N
63	6-aza	H	313–315	$C_{12}H_7NO_4$	C,H,N
64	9-aza	H	255–257	$C_{12}H_7NO_4$	C,H,N
66	8-Br	Me	103	$C_{14}H_9BrO_4$	C,H
67	7-Br	Me	106.5	$C_{14}H_9BrO_4$	C,N
70, 71	8- and 7-NO_2	Me	176–178	$C_{14}H_9NO_6$	C,H,N
72, 73	8- and 7-NO_2	H	266–268	$C_{13}H_7NO_6$	C,H,N
76, 77	8- and 7-NO_2	$CHMe_2$	113	$C_{16}H_{13}NO_6$	C,H,N
80, 81	8- and 7-Cl	H	219	$C_{13}H_7ClO_4$	C,H,Cl
87, 88	8- and 7-Me	H	190	$C_{14}H_{10}O_4$	C,H
90	7,8-diCl	$CHMe_2$	88–89	$C_{16}H_{12}Cl_2O_4$	C,H,Cl
91	7,8-diCl	H	298–301	$C_{13}H_6Cl_2O_4 \cdot 0.5H_2O$	C,H
93	3-NO_2	$CHMe_2$	135–137	$C_{16}H_{13}NO_6$	C,H,N
94	3-NO_2	H	251–253	$C_{13}H_7NO_6$	C,H,N

chromatography was carried out on silica gel (230–400 mesh), eluting with mixtures of EtOAc/petroleum ether.

9-Bromodibenzo[1,4]dioxin-1-carboxylic Acid (31). *tert*-Butyllithium (17.1 mL of a 1.6 N solution in pentane, 0.027 mol) was added dropwise under N_2 to a solution of dibenzo[1,4]dioxin-1-carboxylic acid[9,12] (**29**) (3.04 g, 0.013 mol) and TMEDA (2.21 mL, 0.015 mol) in THF (100 mL) at −78 °C. After 1 h at this temperature, Br_2 (1.36 mL, 0.027 mol) was added, and the temperature was allowed to rise to 20 °C over 1 h. The mixture was then poured into 3 N HCl, extracted with EtOAc, washed with aqueous sodium sulfite, worked up, and esterified (concentrated H_2SO_4/MeOH). Chromatography (elution with EtOAc/petroleum ether (1:9)) gave **methyl 9-bromodibenzo[1,4]dioxin-1-carboxylate (30)** (2.6 g, 62%): mp (petroleum ether) 107–110 °C; ^1H NMR ($CDCl_3$) δ 7.48 (dd, 1 H, *J* = 7.66, 1.90 Hz, H-2), 7.14 (dd, 1 H, *J* = 8.10, 6.13 Hz, H-7), 7.00 (dd, 1 H, *J* = 8.02, 1.90 Hz, H-4), 6.95 (dd, 1 H, *J* = 8.02, 7.66 Hz, H-3), 6.79 (m, 2 H, H-6,8), 3.89 (s, COOMe). Anal. Table II. Hydrolysis with 3 N KOH in MeOH at reflux for 3 h gave 9-bromodibenzo-[1,4]dioxin-1-carboxylic acid (**31**) (100%).

9-Methoxydibenzo[1,4]dioxin-1-carboxylic Acid (36) and the 6-Methoxy Isomer (59). A. The 9-Methoxy Isomer by Lithiation. A solution of dibenzo[1,4]dioxin-1-carboxylic acid[9,12] (**29**) (5.39 g, 0.024 mol) and TMEDA (3.92 mL, 0.026 mol) in THF (150 mL) was treated with *tert*-butyllithium (2.05 equiv) at −78 °C as described above. After 1 h, acetaldehyde (2.64 mL, 0.047 mol) was added, and the solution was allowed to rise to room temperature over 1 h. Workup followed by esterification (diazomethane) and chromatography (elution with EtOAc/petroleum ether, 1:9) gave methyl dibenzo[1,4]dioxin-1-carboxylate (**65**) (0.96 g). Elution with EtOAc/petroleum ether (2:3) gave methyl 9-(1′-hydroxyethyl)dibenzo[1,4]dioxin-1-carboxylate (**32**) (3.70 g, 56%), which was used directly. The alcohol **32** (3.70 g, 0.013 mol) in CH_2Cl_2 (10 mL) was added in one portion to a vigorously stirred suspension of pyridinium chlorochromate (5.57 g, 0.026 mol) and finely ground 4A molecular sieves (1 g) in CH_2Cl_2 (50 mL). After 24 h the mixture was poured directly onto a dry column of silica gel and eluted with CH_2Cl_2 to give **methyl 9-acetyldibenzo-[1,4]dioxin-1-carboxylate (33)** (2.16 g, 58%): mp ($CHCl_3$/petroleum ether) 141–143 °C; ^1H NMR δ ($CDCl_3$) 7.44 (dd, 1 H, *J* = 7.60, 1.97 Hz, H-2), 7.33 (dd, 1 H, *J* = 6.76, 2.82 Hz, H-8), 7.02 (dd, 1 H, *J* = 8.02, 1.97 Hz, H-4), 7.00–6.94 (m, 3 H, H-3,6,7), 3.90 (s, 3 H, COOMe), 2.79 (s, 3 H, COMe). Anal. Table II.

A solution of the ketone **33** (1.51 g, 5.31 mmol), 4-toluenesulfonic acid (0.13 g, 0.70 mmol), and 3-chloroperoxybenzoic acid (1.37 g of 80% material, 6.37 mmol) in CH_2Cl_2 (50 mL) was allowed to stand at 20 °C for 60 h. The solution was washed with aqueous $NaHWSO_3$, followed by aqueous $NaHCO_3$, and chromatography was performed. Elution with EtOAc/petroleum ether (1:9) gave foreruns, while EtOAc/petroleum ether (1:5) gave crude methyl 9-hydroxydibenzo[1,4]dioxin-1-carboxylate (**34**) (0.97 g, 70%). This (0.70 g, 2.70 mmol) was suspended in dimethyl sulfate (0.28 mL, 2.96 mmol), K_2CO_3 (0.56 g), and Me_2CO (70 mL) and heated under reflux for 3 h. Chromatography (elution with EtOAc/petroleum ether, 1:5 gave **methyl 9-methoxydibenzo[1,4]dioxin-1-carboxylate (35)** (0.61 g, 83%): mp (petroleum ether) 79 °C; ^1H NMR δ ($CDCl_3$) 7.48 (dd, 1 H, *J* = 7.87, 1.73 Hz, H-2), 7.01 (dd, 1 H, *J* = 8.02, 1.73 Hz, H-4), 6.85 (dd, *J* = 8.02, 7.87 Hz, H-3), 6.74 (m, 1 H, H-7), 6.58 (dd, 1 H, *J* = 8.40, 1.39 Hz, H-6), 6.49 (dd, 1 H, *J* = 8.28, 1.39 Hz, H-8), 3.94 (s, 3 H, COOMe), 3.91 (s, 3 H, OMe). Anal. Table II. Basic hydrolysis as above gave **9-methoxydibenzo[1,4]dioxin-1-carboxylic acid (36)** (100%): mp (EtOAc/MeOH) 209–211 °C; ^1H NMR (CD_3SOCD_3) δ 13.60 (br s, 1 H, exchangeable with D_2O, COO*H*), 7.31 (dd, 1 H, *J* = 7.8, 1.6 Hz, H-2), 7.13 (dd, 1 H, *J* = 8.0, 1.6 Hz, H-4), 7.02 (t, 1 H, *J* = 7.9 Hz, H-3), 6.94 (t, 1 H, *J* = 8.3 Hz, H-7, 6.76 (dd, 1 H, *J* = 8.4, 1.2 Hz, H-6), 6.59 (dd, 1 H, *J* = 8.2, 1.2 Hz, H-8), 3.82 (s, 3 H, Me). Anal. Table II.

B. Both Isomers by Cyclocondensation. (Example of General K/HMPA Reaction: Scheme II). A mixture of 3-methoxycatechol (**56**) (1.82 g, 13.0 mmol) and NaH (0.62 g, 26.0 mmol) in anhydrous THF (8 mL) was stirred at room temperature. When gas evolution had ceased, isopropyl 2-chloro-3-nitrobenzoate (**50**) (2.44 g, 10.0 mmol) in HMPA (20 mL) was added, and the mixture was stirred at 110 °C (bath temperature) for 10 min, cooled, and partitioned between EtOAc and water and worked up. Chromatography (elution with petroleum ether/EtOAc, 30:1) gave **isopropyl 9-methoxydibenzo[1,4]dioxin-1-carboxylate (57)** (1.23 g, 41%): mp (petroleum ether/EtOAc) 55–57 °C; ^1H NMR ($CDCl_3$) δ 7.43 (dd, 1 H, *J* = 7.9 and 1.8 Hz, H-2), 6.96 (dd, 1 H, *J* = 7.9, 1.8 Hz, H-4), 6.90 (t, 1 H, *J* = 7.9 Hz, H-3), 6.84 (t, 1 H, *J* = 8.3 Hz, H-7), 6.57 (dd, 1 H, *J* = 8.3, 1.3 Hz, H-6), 6.48 (dd, 1 H, *J* = 8.3, 1.3 Hz, H-8), 5.29 (sp, 1 H, *J* = 6.2 Hz, C*H*-(CH_3)$_2$), 3.88 (s, 3 H, OMe), 1.41 (d, 6 H, *J* = 6.2 Hz, 2 × Me). Further elution with the same solvent mixture gave **isopropyl 6-methoxydibenzo[1,4]dioxin-1-carboxylate (58)** (1.48 g, 49%): mp (petroleum ether/EtOAc) 82–84 °C; ^1H NMR ($CDCl_3$) δ 7.39 (dd, 1 H, *J* = 8.0, 1.6 Hz, H-2), 7.09 (dd, 1 H, *J* = 8.0, 1.6 Hz, H-4), 6.91 (t, 1 H, *J* = 8.0 Hz, H-3), 6.86 (t, 1 H, *J* = 8.3 Hz, H-8), 6.59 (dd, 1 H, *J* = 8.3, 1.3 Hz, H-9), 6.57 (dd, 1 H, *J* = 8.3, 1.3 Hz, H-7), 5.27 (sp, 1 H, *J* = 6.2 Hz, C*H*(CH_3)$_2$), 3.89 (s, 3 H, OMe), 1.39 (d, 6 H, *J* = 6.2 Hz, 2 × Me). Anal. Table II. Basic hydrolysis (KOH/aqueous dioxane/21 h/20 °C) gave 9-methoxydibenzo-[1,4]dioxin-1-carboxylic acid (**36**), mp and mixed mp 209–211 °C, and **6-methoxydibenzo[1,4]dioxin-1-carboxylic acid (59)**: mp (EtOAc/MeOH) 227–229 °C; ^1H NMR (CD_3SOCD_3) δ 13.12 (br s, 1 H, exchangeable with D_2O, COO*H*), 7.35 (dd, 1 H, *J* = 7.8, 1.6 Hz, H-2), 7.18 (dd, 1 H, *J* = 8.0, 1.6 Hz, H-4), 7.02 (t, 1 H, *J* = 7.9 Hz, H-3), 6.94 (t, 1 H, *J* = 8.3 Hz, H-8), 6.79 (dd, 1 H, *J* = 8.4, 1.3 Hz, H-9), 6.56 (dd, 1 H, *J* = 8.2, 1.3 Hz, H-7), 3.83 (s, 3 H, Me). Anal. Table II.

9-Nitrodibenzo[1,4]dioxin-1-carboxylic Acid (41) and the 6-Nitro Isomer (42). Cyclocondensation of **38** and **37** as above (molar ratio 1:0.75/110 °C/10 min), followed by chromatography (elution with EtOAc/petroleum ether 1:50) gave **isopropyl 9-nitrodibenzo[1,4]dioxin-1-carboxylate (39)** (140 mg, 22%): mp (EtOAc/petroleum ether) 109–110 °C; ^1H NMR ($CDCl_3$) δ 7.48 (m, 2 H, H-2, H-8), 7.08 (dd, *J* = 8.2, 1.7 Hz, 1 H, H-6), 7.01 (m, 3 H, H-3, H-4, H-7), 5.29 (sp, *J* = 6.3 Hz, 1 H, C*H*(CH_3)$_2$), 1.41 (d, *J* = 6.3 Hz, 6 H, 2 × Me). Anal. Table II. Later eluates gave **isopropyl 6-nitrodibenzo[1,4]dioxin-1-carboxylate (40)** (310 mg, 49%): mp (EtOAc/petroleum ether) 139–141 °C; ^1H NMR ($CDCl_3$) δ 7.58 (dd, *J* = 8.3, 1.6 Hz, 1 H, H-7), 7.51 (dd, *J* = 8.0, 1.7 Hz, 1 H, H-2), 7.16 (dd, *J* = 8.2, 1.6 Hz, 1 H, H-9), 7.14 (dd, *J* = 8.0, 1.7 Hz, 1 H, H-4), 7.01 (t, *J* = 8.3 Hz, 1 H, H-8), 7.00 (t,

J = 8.0 Hz, 1 H, H-3), 5.27 (sp, J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 1.40 (d, J = 6.3 Hz, 6 H, 2 × Me). Anal. Table II.

Basic hydrolysis of **39** and **40** gave the respective acids **9-nitrobenzo[1,4]dioxin-1-carboxylic acid (41) and 6-nitrodibenzo[1,4]dioxin-1-carboxylic acid (42)**. **41**: mp (MeOH) 243–245 °C; ^{1}H NMR (CD_3SOCD_3) δ 13.2 (s, 1 H, exchangeable with D_2O, COOH), 7.61 (dd, J = 8.3, 1.5 Hz, 1 H, H-8), 7.39 (dd, J = 7.9, 1.6 Hz, 1 H, H-2), 7.34 (dd, J = 8.3, 1.5 Hz, 1 H, H-6), 7.22 (dd, J = 7.9, 1.6 Hz, 1 H, H-4), 7.18 (t, J = 8.3 Hz, 1 H, H-7), 7.12 (t, J = 7.9 Hz, 1 H, H-3). Anal. Table II. **42**: mp (MeOH/EtOAc) 275–277 °C; ^{1}H NMR (CD_3SOCD_3) δ 13.3 (br, s, 1 H, exchangeable with D_2O, COOH), 7.67 (dd, J = 8.3, 1.2 Hz, 1 H, H-7), 7.46 (dd, J = 7.9, 1.3 Hz, 1 H, H-2), 7.32 (dd, J = 8.3, 1.2 Hz, 1 H, H-9), 7.24 (dd, J = 7.9, 1.3 Hz, 1 H, H-4), 7.16 (t, J = 8.3 Hz, 1 H, H-8), 7.09 (t, J = 7.9 Hz, 1 H, H-3). Anal. Table II.

9-Chlorodibenzo[1,4]dioxin-1-carboxylic Acid (46) and the 6-Chloro Isomer (47). Cyclocondensation of **38** and **43** as above (1:0.67 molar ratio/100 °C/90 min), followed by chromatography (elution with CH_2Cl_2/petroleum ether 1:10) gave isopropyl 9-chlorodibenzo[1,4]dioxin-1-carboxylate (**44**) (180 mg, 20%) as an oil and isopropyl 6-chlorodibenzo[1,4]dioxin-1-carboxylate (**45**) (400 mg, 44%) as an oil. **44**: ^{1}H NMR ($CDCl_3$) δ 7.46 (dd, J = 7.4, 2.2 Hz, 1 H, H-2), 6.98 (m, 2 H, H-4, H-8), 6.95 (t, J = 7.4 Hz, 1 H, H-3), 6.84 (t, J = 8.2 Hz, 1 H, H-7), 6.74 (dd, J = 8.2, 1.5 Hz, H-6), 5.31 (sp, J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 1.42 (d, J = 6.3 Hz, 6 H, 2 × Me); ^{13}C NMR ($CDCl_3$) δ 164.6 (C=O), 142.9, 142.2, 141.5 and 138.7 (dioxin ring C's), 126.3, 125.0, 123.9, 123.5, 121.7, 120.8, 119.8, 114.6, 69.2 [CH(CH_3)$_2$], and 22.0 (2 × CH_3); **45** ^{1}H NMR ($CDCl_3$) δ 7.44 (dd, J = 8.0, 1.6 Hz, 1 H, H-2), 7.09 (dd, J = 8.0, 1.6 Hz, 1 H, H-4), 6.98 (dd, J = 7.0, 2.7 Hz, 1 H, H-7), 6.95 (t, J = 8.0 Hz, 1 H, H-3), 6.83 (m, 2 H, H-8, H-9), 5.27 (septet (sp), J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 1.39 (d, J = 6.3 Hz, 6 H, 2 × Me); ^{13}C NMR ($CDCl_3$) δ: 164.1 (C=O), 142.8, 142.3, 141.9 and 138.7 (dioxin ring C's), 126.3, 125.2, 123.6, 123.1, 121.1, 120.3, 120.0, 115.2, 68.9 (CH(CH_3)$_2$) and 21.9 (2 × CH_3).

If the reaction was carried out at 20 °C for 20 h, **isopropyl 3-(2′-chloro-6′-nitrophenoxy)-2-hydroxybenzoate (48)**, mp (EtOAc/petroleum ether) 96–98 °C, was isolated in 77% yield: ^{1}H NMR ($CDCl_3$) δ 11.26 (s, 1 H, exchangeable with D_2O, OH), 7.90 (dd, J = 8.1, 1.6 Hz, 1 H, H-9), 7.72 (dd, J = 8.1, 1.6 Hz, 1 H, H-7), 7.60 (dd, J = 8.0, 2.3 Hz, 1 H, H-2), 7.34 (t, J = 8.1 Hz, 1 H, H-8), 6.77 (dd, J = 8.0, 2.3 Hz, 1 H, H-4), 6.74 (t, J = 8.0 Hz, 1 H, H-3), 5.31 (sp, J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 1.41 (d, J = 6.3 Hz, 6 H, 2 × Me); ^{13}C NMR ($CDCl_3$) δ 169.6 (C=O), 151.6, 145.3, 144.9, 135.2, 130.5, 125.7, 124.2, 124.0, 120.2, 118.1, 114.4, 69.7 [CH(CH_3)$_2$], and 21.8 (2 × Me). Anal. ($C_{16}H_{14}ClNO_6$) C, H, N, Cl. Further treatment of this compound with K in dry HMPA at 110 °C gave **44** and **45** in a similar ratio to the initial reaction.

Hydrolysis of the esters (**44** and **45**) with KOH/MeOH as above gave respectively 9-chlorodibenzo[1,4]dioxin-1-carboxylic acid (**46**) and 6-chlorodibenzo[1,4]dioxin-1-carboxylic acid (**47**). **46**: mp (MeOH) 223–225 °C (lit.[9] mp 221–222 °C); ^{1}H NMR (CD_3SOCD_3) δ 7.39 (dd, J = 7.9, 1.3 Hz, 1 H, H-2), 7.19 (dd, J = 7.9, 1.3 Hz, 1 H, H-4), 7.15 (dd, J = 7.9, 1.6 Hz, 1 H, H-8), 7.08 (t, J = 7.9 Hz, 1 H, H-3), 7.01 (t, J = 7.9 Hz, 1 H, H-7), 6.97 (dd, J = 7.9, 1.6 Hz, 1 H, H-6); ^{13}C NMR (DMSO-d_6) δ 165.4 (C=O) 142.4, 141.6, 140.3, and 137.9 (dioxin ring C's), 125.8, 125.1, 124.7, 124.2, 121.3, 120.4, 119.7, 115.2, **47**: mp (MeOH/EtOAc) 289–291 °C; ^{1}H NMR (CD_3SOCD_3) δ 7.41 (dd, J = 7.9, 1.5 Hz, 1 H, H-2), 7.24 (dd, J = 7.9, 1.5 Hz, 1 H, H-4), 7.16 (dd, J = 8.1, 1.6 Hz, 1 H, H-7), 7.07 (t, J = 7.9 Hz, 1 H, H-3), 7.01 (t, J = 8.1 Hz, 1 H, H-8), 6.96 (dd, J = 8.1, 1.6 Hz, 1 H, H-9); ^{13}C NMR (CD_3SOCD_3) δ 165.4 (C=O), 142.2, 141.4, 140.6 and 137.9 (dioxin ring C's), 126.3, 125.3, 124.6, 123.9, 120.9, 120.0, and 115.6. Anal. Table II.

9-Methyldibenzo[1,4]dioxin-1-carboxylic Acid (53) and the 6-Methyl Isomer (54). Cyclocondensation of **49** and **50** as above (molar ratio 1:1/110 °C/20 h) followed by chromatography (elution with petroleum ether) gave isopropyl 9-methyldibenzo[1,4]dioxin-1-carboxylate (**51**) (250 mg, 22%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.43 (dd, J = 7.8, 1.8 Hz, 1 H, H-2), 6.96 (dd, J = 7.8, 1.8 Hz, 1 H, H-4), 6.90 (t, J = 7.8 Hz, 1 H, H-3), 6.81 (br t, J = 7.6 Hz, 1 H, H-7), 6.77 (ddq, J = 7.6, 2.1, and 0.7 Hz, 1 H, H-8), 6.67 (br dd, J = 7.6, 2.1 Hz, 1 H, H-6), 6.30 (sp, J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 2.31 (br s, 3 H, Ar-Me), 1.40 (d, J = 6.3 Hz, 6 H, 2 × Me); ^{13}C NMR ($CDCl_3$) δ 164.7 (C=O), 142.8, 142.5, 141.8, and 140.4 (dioxin ring C's), 126.5, 125.9, 125.6, 123.4, 122.7, 120.2, 119.8, 113.6, 68.7 ($CH(CH_3)_2$), 22.0 (2 × Me), 15.4 (Ar-Me). Elution with EtOAc/petroleum ether (1:100) gave isopropyl 6-methyldibenzo[1,4]dioxin-1-carboxylate (**52**) (400 mg, 35%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.39 (dd, J = 8.0, 1.7 Hz, 1 H, H-2), 7.00 (dd, J = 8.0, 1.7 Hz, 1 H, H-4), 6.89 (t, J = 8.0 Hz, 1 H, H-3), 6.80 (t, J = 7.5 Hz, 1 H, H-8), 6.76 (dd, J = 7.5, 2.2 Hz, 1 H, H-7), 6.74 (dd, J = 7.5, 2.2 Hz, 1 H, H-9), 5.27 (sp, J = 6.3 Hz, 1 H, CH(CH_3)$_2$), 2.23 (s, 3 H, Ar-Me), 1.39 (d, J = 6.3 Hz, 6 H, 2 × Me); ^{13}C NMR ($CDCl_3$) δ 164.5 (C=O), 143.0, 142.5, 141.6, and 140.0 (dioxin ring C's), 125.9, 125.5, 123.1, 122.6, 120.1, 119.9, 114.2, 68.7 [$CH(CH_3)_2$] 22.0 (2 × Me), 14.9 (Ar-Me).

Hydrolysis of the esters (**51** and **52**) with KOH in MeOH gave respectively 9-methyldibenzo[1,4]dioxin-1-carboxylic acid (**53**) and **6-methyldibenzo[1,4]dioxin-1-carboxylic acid (54)**. **53**: mp (MeOH/$CHCl_3$) 224–226 °C (lit.[9] mp 220–222 °C); ^{1}H NMR (CD_3SOCD_3) δ 13.10 (br s, 1 H, exchangeable with D_2O, COOH), 7.39 (dd, J = 7.9, 1.5 Hz, 1 H, H-2), 7.15 (dd, J = 7.9, 1.5 Hz, 1 H, H-4), 7.02 (t, J = 7.9 Hz, 1 H, H-3), 6.92–6.86 (m, 2 H, H-6, H-7), 6.83–6.79 (m, 1 H, H-8), 2.23 (s, 3 H, Me); ^{13}C NMR (CD_3SOCD_3) δ 165.6 (C=O), 142.2, 141.5, 141.3 and 139.4 (dioxin ring C's), 126.0, 125.8, 125.7, 123.8, 123.4, 120.7, 119.6, 113.6, 14.4 (Me). **54**: mp (MeOH/CH_2Cl_2) 251–253 °C; ^{1}H NMR (CD_3SOCD_3) δ 13.15 (s, 1 H, exchangeable with D_2O, COOH), 7.36 (dd, J = 7.9, 1.6 Hz, 1 H, H-2), 7.17 (dd, J = 7.9, 1.6 Hz, 1 H, H-4), 7.02 (t, J = 7.9 Hz, 1 H, H-3), 6.91–6.84 (m, 2 H, H-8, H-9), 6.81–6.75 (m, 1 H, H-7), 2.21 (s, 3 H, Me); ^{13}C NMR (CD_3SOCD_3) δ 165.5 (C=O), 142.1, 141.1, 140.9 and 139.3 (dioxin-ring C's), 126.0, 125.5, 125.4, 123.5, 123.2, 120.6, 119.7, 114.0, 14.5 (Me). Anal. Table II.

6-Azadibenzo[1,4]dioxin-1-carboxylic Acid (63) and the 9-Aza Isomer (64). Cyclocondensation of **38** and **60** (molar ratio 1:1.2/110 °C/4 h) followed by chromatography (elution with petroleum ether/CH_2Cl_2, 2:1) gave **isopropyl 6-azadibenzo[1,4]dioxin-1-carboxylate (61)** (1.25 g, 49%): mp (petroleum ether/EtOAc) 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (dd, 1 H, J = 4.8, 1.5 Hz, H-7), 7.45 (dd, 1 H, J = 8.0, 1.6 Hz, H-2), 7.24 (dd, 1 H, J = 7.8, 1.5 Hz, H-9), 7.12 (dd, 1 H, J = 8.0, 1.6 Hz, H-4), 6.98 (t, 1 H, J = 8.0 Hz, H-3), 6.95 (dd, 1 H, J = 7.8, 4.8 Hz, H-8), 5.27 (sp, 1 H, J = 6.2 Hz, CH(CH_3)2), 1.40 (d, 6 H, J = 6.2 Hz, 2 × Me). Anal. Table II. Later eluates gave **isopropyl 9-azadibenzo[1,4]dioxin-1-carboxylate (62)** (370 mg, 15%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.85 (dd, 1 H, J = 4.8, 1.3 Hz, H-8), 7.41 (dd, 1 H, J = 7.7, 2.0 Hz, H-2), 7.15 (dd, 1 H, J = 7.8, 1.3 Hz, H-6), 6.99 (dd, 1 H, J = 7.7, 2.0 Hz, H-4), 6.96 (t, 1 H, J = 7.7 Hz, H-3), 6.93 (dd, 1 H, J = 7.8, 4.8 Hz, H-7), 5.28 (sp, 1 H, J = 6.2 Hz, CH(CH_3)$_2$), 1.41 (d, 6 H, J = 6.2 Hz, 2 × Me).

Hydrolysis of the esters (**61** and **62**) with KOH in dioxane gave respectively **6-azadibenzo[1,4]dioxin-1-carboxylic acid (63)** and **9-azadibenzo[1,4]dioxin-1-carboxylic acid (64)**. **63**: mp (MeOH/CH_2Cl_2) 313–315 °C; ^{1}H NMR (CD_3SOCD_3) δ 7.85 (dd, 1 H, J = 4.8, 1.3 Hz, H-7), 7.43 (dd, 1 H, J = 8.0, 1.3 Hz, H-2), 7.41 (dd, 1 H, J = 7.8, 1.3 Hz, H-9), 7.24 (dd, 1 H, J = 8.0, 1.3 Hz, H-4), 7.10 (dd, 1 H, J = 7.8, 1.3 Hz, H-8), 7.08 (t, 1 H, J = 8.0 Hz, 1 H, H-3). Anal. Table II. **64**: mp (MeOH) 255–257 °C; ^{1}H NMR (CD_3SOCD_3) δ 13.20 (br s, 1 H, exchangeable with D_2O, COOH), 7.84 (dd, 1 H, J = 4.8, 1.5 Hz, H-8), 7.44 (dd, 1 H, J = 8.0, 1.5 Hz, H-2), 7.38 (dd, 1 H, J = 7.9, 1.5 Hz, H-6), 7.19 (dd, 1 H, J = 8.0, 1.5 Hz, H-4), 7.11 (dd, 1 H, J = 7.9, 4.8 Hz, H-7), 7.07 (t, 1 H, J = 8.0 Hz, H-3). Anal. Table II.

8-Bromodibenzo[1,4]dioxin-1-carboxylic Acid (68) and the 7-Isomer (69). A mixture of methyl dibenzo[1,4]dioxin-1-carboxylate[9] (**65**) (1.40 g, 5.78 mmol), $KBrO_3$ (0.96 g, 5.78 mmol), and KBr (2.10 g, 0.017 mol) in AcOH (100 mL) and water (10 mL) was refluxed for 2 h and then poured into EtOAc. The mixture was washed well with aqueous $NaHCO_3$ until all of the AcOH was removed and worked up to give a residue which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (3:97) gave the 8-bromo isomer **66** (0.58 g, 31%) which crystallized from petroleum ether as rosettes: mp 103 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (dd, 1 H, J = 7.6, 1.9 Hz, H-2), 7.11 (d, 1 H, J = 2.3 Hz, H-9), 7.03 (dd, 1 H, J = 8.6, 2.3 Hz, H-7), 6.98 (dd, 1H, J = 6.2, 1.9 Hz, H-4), 6.92 (dd, 1 H, J = 7.6, 6.2 Hz, H-3), 6.71 (d, 1 H, J = 8.6 Hz, H-6), 3.92 (s, 3 H, COOMe); ^{13}C NMR δ 164.97, 142.40, 142.41, 141.09, 127.16 (CH), 126.10 (CH), 123.26 (CH), 120.24 (CH), 120.03

(CH), 119.58, 117.37 (CH), 115.53, 52.31 (COO*Me*). Anal. Table II.

Further elution with EtOAc/petroleum ether (3:97) gave the 7-bromo isomer **67** (0.61 g, 33%) which crystallized from Me_2CO as needles: mp 106.5 °C; ^1H NMR ($CDCl_3$) δ 7.44 (dd, 1 H, J = 7.7, 1.8 Hz, H-2), 7.03 (dd, 1 H, J = 8.5, 1.8 Hz, H-8), 7.00 (d, 1 H, J = 1.8 Hz, H-6), 6.99 (dd, 1 H, J = 6.2, 1.8 Hz, H-4), 6.93 (dd, 1 H, J = 7.7, 1.8 Hz, H-3), 6.83 (d, 1 H, J = 8.5 Hz, H-9), 3.92 (s, 3 H, COOMe); ^{13}C NMR δ 164.94, 142.45, 142.23, 142.11, 140.91, 126.88 (CH), 126.24 (CH), 123.12 (CH), 120.30 (CH), 119.47, 119.35 (CH), 118.09 (CH), 115.90, 52.30 (COO*Me*). Anal. Table II. Basic hydrolysis of the esters gave the respective acids (**68**, **69**), which were used directly.

8-Nitrodibenzo[1,4]dioxin-1-carboxylic Acid (72) and the 7-Nitro Isomer (73). A. By Nitration of Methyl Dibenzo-[1,4]dioxin-1-carboxylate: Scheme IV. A solution of HNO_3 (0.40 mL of d 1.42, 6.19 mmol) in Ac_2O (10 mL) was added to a solution of the ester **65** (1.00 g, 4.13 mmol) in AcOH (50 mL), and the solution was stirred at 60 °C for 1 h. The cooled solution was poured into water, and the precipitate was collected to give a 1:1.6 mixture of methyl 7- and 8-nitrodibenzo[1,4]dioxin-1-carboxylates (**71** and **70**) (1.13 g, 96%): mp ($CHCl_3$/petroleum ether) 176–178 °C; ^1H NMR δ ($CDCl_3$) 7.84 (m, 1 H, H-7 (H-8 values in parentheses refer to the minor component of the mixture), 7.71 (d, 1 H, J = 2.6 Hz, H-9 (H-6)), 7.49 (2 × dd, 1 H, J = 7.48, J^1 = 1.92 Hz, H-2), 7.02 (2 × dd, J = 8.84, J^1 = 1.92 Hz, H-4), 6.99 (dd, 1 H, J = 8.84, J^1 = 7.48 Hz, H-3), 6.97 and 6.92 (2 × d, 1 H, J = 8.72 Hz, H-6 (H-9)), (3.95), and 3.93 (2 × s, 3 H, COOMe). Anal. Table II.

B. By Cyclization: Scheme II. Cyclocondensation of **38** and **75** (molar ratio 1:1.5/110 °C/4 h), followed by chromatography gave a nonseparable mixture of isopropyl 8- and 7-nitrodibenzo[1,4]dioxin-1-carboxylates (**76** and **77**) in the ratio ca. 2:1, respectively: mp (MeOH) 113 °C; ^1H NMR ($CDCl_3$) δ 7.86 (dd, J = 8.8, 2.6 Hz, H-7 of **76**), 7.85 (dd, J = 8.8, 2.6 Hz, H-8 of **77**), 7.79 (d, J = 2.6 Hz, H-9 of **76**), 7.73 (d, J = 2.6 Hz, H-6 of **77**), 7.47 (dd, J = 7.6, 1.9 Hz, H-2 of **76**), 7.46 (dd, J = 7.3, 2.2 Hz, H-2 of **76**), 7.04–6.96 (m, H-9 of **77**, H-3 and H-4 of **76** and **77**), 6.94 (d, J = 8.8 Hz, H-6 of **76**), 5.29 (sp, J = 6.3 Hz, C*H*$(CH_3)_2$ of **76**), 5.28 (sp, J = 6.3 Hz, C*H*$(CH_3)_2$ of **77**), 1.41 (d, J = 6.3 Hz, methyls of **76**), 1.40 (d, J = 6.3 Hz, methyls of **77**); ^{13}C NMR ($CDCl_3$) δ 163.8 and 163.7 (C=O), 147.1, 147.0, 144.0, 143.8, 141.8, 141.6, 141.5, and 140.9 (dioxin ring C's), 126.9, 126.6, 124.1, 123.7, 120.7, 120.6, 120.3, 120.1, 116.8, 116.3, 112.7, 112.2, 69.2, and 69.1 (*C*H$(CH_3)_2$), 21.9 (methyls). Anal. Table II.

When the above reaction of **38** and **75** was carried out at 20 °C for 40 h, the intermediate **isopropyl 3-(2′-chloro-4′-nitrophenoxy)-2-hydroxybenzoate (83)**: mp (EtOAc/petroleum ether) 111–111.5 °C was isolated in 88% yield; ^1H NMR ($CDCl_3$) δ 11.2 (s, 1 H, exchangeable with D_2O, OH), 8.38 (d, J = 2.6 Hz, 1 H, H-3′), 8.20 (dd, J = 9.2, 2.6 Hz, 1 H, H-5′), 7.83 (dd, J = 7.9, 1.5 Hz, 1 H, H-6), 7.36 (dd, J = 7.9, 1.5 Hz, 1 H, H-4), 6.96 (t, J = 7.9 Hz, 1 H, H-5), 6.74 (d, J = 9.2 Hz, 1 H, H-6′), 5.31 (sp, J = 6.3 Hz, 1 H, C*H*$(CH_3)_2$), 1.42 (d, J = 6.3 Hz, 6 H, 2 × Me; ^{13}C NMR ($CDCl_3$) δ 169.3 (C=O), 158.7, 153.7, 142.4, 141.6, 127.9, 127.7, 126.4, 123.5, 123.4, 119.1, 115.2, 114.9, 70.1 (*C*H$(CH_3)_2$), 21.8 (2 × Me). Anal. ($C_{16}H_{13}ClNO_6$) C, H, N. This underwent cyclization of with potassium metal in HMPA at 110 °C to give a similar mixture of **76** and **77**.

Hydrolysis of the Nitrodibenzo[1,4]dioxin Ester Mixture (70 and 71). Basic Hydrolysis. Hydrolysis with KOH/MeOH gave, after chromatography on silica gel and elution with EtOAc, methyl 3-(2′-hydroxy-4′-nitrophenoxy)-2-hydroxybenzoate (**74**) (34% yield) as a yellow oil: ^1H NMR ($CDCl_3$) δ 8.00 (dd, 1 H, J = 9.01, 2.64 Hz, H-4′), 7.67 (dd, 1 H, J = 7.49, 2.08 Hz, H-3), 7.41 (d, 1 H, J = 2.64 Hz, H-4), 7.34 (dd, 1 H, J = 7.28, 2.08 Hz, H-6), (dd, 1 H, J = 7.49, 7.28 Hz, H-5), 7.05 (d, 1 H, J = 9.01 Hz, H-3′), 4.10 (s, 3 H, COOMe); MS m/z 305 (M^+, 100) 287 (17), 273 (26), 256 (25), 180 (25), 137 (92), 133 (97).

Acidic Hydrolysis. Hydrolysis with dioxane/50% aqueous H_2SO_4 (1:1) (reflux/24 h) gave an inseparable mixture of 8-nitro- and 7-nitrodibenzo[1,4]dioxin-1-carboxylic acids (**72** and **73**): mp (EtOAc/MeOH) 266–268 °C; ^1H NMR (CD_3SOCD_3) δ 8.44 (br s, 1 H, COOH), 7.84 (m, 1 H, H-7 (H-8 values in parentheses refer to the minor component of the mixture, the 7-nitro isomer)), 7.73 (dd, 1 H, J = 6.60, 2.64 Hz, H-2), 7.15 (m, 2 H, H-9 (H-6), H-4), 6.87 (m, 2 H, H-3,6 (H-9)). Anal. Table II.

8-Chlorodibenzo[1,4]dioxin-1-carboxylic Acid (80) and the 7-Chloro Isomer (81). Cyclocondensation of **38** and **82** (molar ratio 1:1.2/110 °C/4 h) gave a nonseparable mixture of isopropyl 8- and 7-chlorodibenzo[1,4]dioxin-1-carboxylates (**78** and **79**) (750 mg, 82%) in the ratio ca. 1.3:1 as an oil: ^1H NMR ($CDCl_3$) δ 7.41 (dd, J = 7.7, 1.9 Hz, H-2 of **79**), 7.40 (dd, J = 7.6, 2.0 Hz, H-2 of **78**), 6.99–6.85 (m, H-3, H-4, H-7, and H-9 of **78**; H-3, H-4, H-6, H-8 of **79**); 6.84 (d, J = 8.3 Hz, H-9 of **79**), 6.77 (d, J = 8.5 Hz, H-6 of **78**), 5.27 (sp, J = 6.3 Hz, C*H*$(CH_3)_2$ of **78**), 5.26 (sp, J = 6.3 Hz, C*H*$(CH_3)_2$ of **79**), 1.40 (d, J = 6.3 Hz, methyls of **78**), 1.39 (d, J = 6.3 Hz, methyls of **79**). Basic hydrolysis of the ester mixture as above gave an inseparable mixture of the 8-chloro- and 7-chlorodibenzo[1,4]dioxin-1-carboxylic acids (**80** and **81**) (ratio ca. 1.3:1): mp (EtOAc) 219 °C; ^1H NMR ($CDCl_3$) δ 7.63 (dd, J = 7.9, 1.7 Hz, H-2 of **81**), 7.61 (dd, J = 7.9, 1.7 Hz, H-2 of **80**), 7.08 (dd, J = 7.9, 1.7 Hz, H-4 of **81**), 7.07 (dd, J = 7.9, 1.7 Hz, H-4 of **80**), 7.03 (d, J = 2.4 Hz, H-9 of **80**), 7.0 (t, J = 7.9 Hz, H-3 of **80** and **81**), 6.93 (dd, J = 8.5, 2.4 Hz, H-7 of **80**), 6.92–6.89 (m, H-6, H-8, H-9 of **81**), 6.81 (d, J = 8.5 Hz, H-6 of **80**). Anal. Table II.

8-Methyldibenzo[1,4]dioxin-1-carboxylic Acid (87) and the 7-Methyl Isomer (88): Scheme VI. Cyclocondensation of **84** and **50** (molar ratio 1:1/110 °C/20 min) gave a nonseparable mixture of the isopropyl 8- and 7-methyldibenzo[1,4]dioxin-1-carboxylates (**85** and **86**) (260 mg, 92%) in the ratio 1:1.2 as an oil: ^1H NMR ($CDCl_3$) δ 7.38 (br dd, J = 7.8, 1.6 Hz, 0.55 H, H-2 of **86**), 7.37 (br dd, J = 7.8, 1.6 Hz, 0.45 H, H-2 of **85**), 6.96 (br dd, J = 8.0, 1.6 Hz, 0.55 H, H-4 of **86**), 6.95 (br dd, J = 8.0, 1.6 Hz, 0.45 H, H-4 of **85**), 6.89 (br t, J = 7.9 Hz, 0.45 H, H-3 of **85**), 6.88 (br t, J = 7.9 Hz, 0.55 H, H-3 of **86**), 6.79 (br d, J = 8.2 Hz, 0.55 H, H-9 of **86**), 6.73–6.68 (m, 1.9 H, H-6, H-7, and H-9 of **85**; H-8 of **86**), 6.65 (m, $W_{1/2}$ = 4.0 Hz, 0.55 H, H-6 of **86**), 5.27 (sp, J = 6.3 Hz, 0.45 H, C*H*$(CH_3)_2$ of **85**), 5.26 (sp, J = 6.3 Hz, 0.55 H, C*H*$(CH_3)_2$ of **86**), 2.24 (s, 1 H, Ar-CH_3), 1.40 (d, J = 6.3 Hz, 0.45 H, methyls of **85**), 1.39 (d, J = 6.3, Hz, 0.55 H, methyls of **86**); ^{13}C NMR ($CDCl_3$) δ 164.5 (C=O), 142.9, 142.8, 141.4, 141.3, 139.5, 139.4, 134.2, 133.9 (dioxin ring C's), 125.5, 125.4, 124.5, 124.3, 122.7, 122.6, 120.3, 120.2, 119.8, 119.7, 117.2, 116.6, 116.3, 115.7, 68.7 ($CH(CH_3)_2$), 21.96 and 21.95 (methyls), 20.75 and 20.69 (Ar-CH_3).

Basic hydrolysis of the ester mixture as above gave an inseparable mixture of 8-methyl- and 7-methyldibenzo[1,4]dioxin-1-carboxylic acids (**87** and **88**) (ratio ca. 1:1.2): mp (EtOH) 190 °C; ^1H NMR ($CDCl_3$) δ 7.60 (m, 1 H, H-2 of **87** and **88**), 7.05 (m, 1 H, H-4 of **87** and **88**), 6.95 (t, J = 7.9 Hz, 1 H, H-3 of **87** and **88**), 6.87 (d, J = 8.2 Hz, 0.55 H, H-9 of **88**), 6.82 (br s, 0.45 H, H-9 of **87**), 6.73 (m, 1.45 H, H-6 and H-7 of **88**; H-8 of **87**), 6.68 (br s, 0.55 H, H-6 of 8a), 2.26 (s, 3 H, CH_3 of **87** and **88**). Anal. Table II.

7,8-Dichlorodibenzo[1,4]dioxin-1-carboxylic Acid (91). Cyclocondensation of **38** and **89** (molar ratio 1:2.4/110 °C/22 h), followed by chromatography (elution with petroleum ether followed by EtOAc/petroleum ether), gave **isopropyl 7,8-dichlorodibenzo[1,4]dioxin-1-carboxylate (90)** (320 mg, 63%); mp (MeOH) 88–89 °C; ^1H NMR ($CDCl_3$) δ 7.43 (dd, J = 8.0, 2.1 Hz, 1 H, H-2), 7.02 (s, 1 H, H-9), 6.98 (dd, J = 8.0, 2.1 Hz, 1 H, H-4), 6.96 (s, 1 H, H-6), 6.94 (t, J = 8.0 Hz, 1 H, H-3), 5.26 (sp, J = 6.3 Hz, C*H*$(CH_3)_2$), 1.40 (d, J = 6.3 Hz, 6 H, 2 × Me). Anal. Table II. Hydrolysis of **90** with aqueous KOH/*p*-dioxane gave **7,8-dichlorodibenzo[1,4]dioxin-1-carboxylic acid (91)**: mp ($MeOH/CH_2Cl_2$) 298–301 °C dec; ^1H NMR (CD_3SOCD_3) δ 7.36 (s, 1 H, H-9), 7.32 (dd, J = 7.8, 1.7 Hz, 1 H, H-2), 7.27 (s, 1 H, H-6), 7.11 (d, J = 7.8, 1.7 Hz, 1 H, H-4), 7.03 (t, J = 7.8 Hz, 1 H, H-3). Anal. Table II.

3-Nitrodibenzo[1,4]dioxin-1-carboxylic Acid (94). Cyclocondensation of catechol and **92** (molar ratio 1:1/80–90 °C/15 min), followed by chromatography (elution with EtOAc/petroleum ether, 50:1, then 15:1), gave **isopropyl 3-nitrodibenzo[1,4]dioxin-1-carboxylate (93)** (310 mg, 49%): mp (MeOH/EtOAc) 135–137 °C; ^1H NMR ($CDCl_3$) δ 8.31 (d, J = 2.8 Hz, 1 H, H-2), 7.82 (d, J = 2.8 Hz, 1 H, H-4), 7.03–6.87 (m, 4 H, H-6, H-7, H-8, H-9), 5.30 (sp, J = 6.2 Hz, 1 H, C*H*$(CH_3)_2$), 1.42 (d, J = 6.2 Hz, 6 H, 2 × Me). Anal. Table II. Hydrolysis of **93** as above gave **3-nitrodibenzo[1,4]dioxin-1-carboxylic acid (94)**: mp (MeOH/EtOAc) 251–253 °C; ^1H NMR ($CDCl_3$) δ 8.49 (d, J = 2.7

Hz, 1 H, H-2), 7.89 (d, J = 2.7 Hz, 1 H, H-4), 7.08–6.91 (m, 4 H, H-6, H-7, H-8, H-9). Anal. Table II.

Dibenzo[1,4]dioxin-2-carboxylic Acid (98). A solution of 2-bromodibenzo[1,4]dioxin (**95**)[14] (1.08 g, 4.56 mmol) and TMEDA (0.69 mL, 4.56 mmol) in THF (30 mL) was treated with n-butyllithium (1.05 equiv) at −78 °C. After 5 min, a stream of dry CO_2 gas was passed through the solution as it was allowed to warm to room temperature. The mixture was partitioned between Et_2O and water, the aqueous layer was acidified with 3 N HCl and extracted into EtOAc and worked up, and the residue was esterified (concentrated H_2SO_4/MeOH) to give **methyl dibenzo[1,4]dioxin-2-carboxylate (97)** (0.72 g, 69%): mp (petroleum ether) 94 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (dd, J = 8.4, 2.0 Hz, 1 H, H-3), 7.48 (d, J = 2.0 Hz, 1 H, H-1), 6.92–6.87 (m, 2 H, H-7,8), 6.85–6.80 (m, 3 H, H-4,6,9), 3.87 (s, 3 H, COOMe). Basic hydrolysis gave dibenzo[1,4]dioxin-2-carboxylic acid (**98**): mp 230–232 °C (lit.[26] mp 236 °C).

Dibenzo[1,4]dioxin-2,8-dicarboxylic Acid (100). Reaction of 2,8-dibromodibenzo[1,4]dioxin (**96**)[14] with n-butyllithium followed by quenching with CO_2 and esterification as above gave dimethyl dibenzo[1,4]dioxin-2,8-dicarboxylate (**99**) (47%): mp ($CHCl_3$/petroleum ether) 169–170 °C (lit.[14] mp 168–171 °C); ^{1}H NMR ($CDCl_3$) δ 7.60 (dd, J = 8.47, 2.05 Hz, 2 H, H-3,7), 7.49 (d, J = 2.05 Hz, 2 H, H-1,9), 6.86 (d, J = 8.47 Hz, 2 H, H-4,6), 3.89 (s, 6 H, COOMe). Basic hydrolysis, followed by trituration of the residue into MeOH/acetone (1:5) and concentration of the supernatant, gave dibenzo[1,4]dioxin-2,8-dicarboxylic acid (**100**): mp >300 °C (lit.[14] mp >300 °C).

Preparation of *N*-[2-(Dimethylamino)ethyl]-6-nitrodibenzo[1,4]dioxin-1-carboxamide (9) of Table I. Example of General Method. A mixture of 6-nitrodibenzo[1,4]dioxin-1-carboxylic acid (**42**) (590 mg, 2.16 mmol) and 1,1′-carbonyldiimidazole (530 mg, 3.24 mmol) in dry DMF (5 mL) was stirred at 40–45 °C until gas evolution ceased (10 min). The mixture was cooled, *N*,*N*-dimethylethylenediamine (0.71 ml, 6.48 mmol) was added, and after stirring at 20 °C for 30 min, the mixture was poured into excess 0.5 N aqueous Na_2CO_3. The resulting pale yellow precipitate was collected, washed with water, and dried to give pure **9** (680 mg, 92%): mp (MeOH/EtOAc) 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (br s, 1 H, exchangeable with D_2O, NH), 7.79 (dd, J = 7.8, 1.9 Hz, 1 H, H-7), 7.63 (dd, J = 8.2, 1.6 Hz, 1 H, H-2), 7.17 (dd, J = 8.2, 1.6 Hz, 1 H, H-4), 7.13 (dd, J = 7.8, 1.9 Hz, 1 H, H-9), 7.07 (t, J = 7.8 Hz, 1 H, H-8), 7.05 (t, J = 8.2 Hz, 1 H, H-3), 3.58 (q, J = 5.9 Hz, 1 H, collapsing into t after D_2O, $CONHC_{H2}$), 2.57 (t, J = 5.9 Hz, 1 H, $CONHCH_2CH_2$), 2.35 (s, 6 H, NMe_2). Anal. Table I.

The other compounds of Table I were prepared similarly (some compounds were extracted from the aqueous layer with EtOAc), and all were characterized by ^{1}H NMR (data not shown). The free bases were converted into the hydrochloride salts by dissolution in MeOH saturated with HCl gas, followed by addition of EtOAc or Et_2O to precipitate the salt.

HPLC Separation of Nitro Isomers 16 and 18. This was performed on a 30 × 2.5 cm C-18 ODS steel column, using MeOH/water (2:3) containing 10 mM triethylammonium phosphate buffer as mobile phase. The flow rate was 1.8 mL/min, with detection being by UV absorbance at 254 nm. A 2-mg sample of the mixed isomers was separated by repeated runs and followed by pooling of appropriate fractions, evaporation to dryness, and removal of salts by chromatography on silica gel and elution with Et_3N/MeOH/EtOAc (0.1:1:9). This gave pure 8-nitro isomer **18** (1.1 mg, eluted first on HPLC) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.15 (br, 1 H, NH), 7.92 (dd, 1 H, J = 8.7, 2.6 Hz, H-7), 7.90 (d, 1 H, J = 2.6 Hz, H-9), 7.82 (dd, 1 H, J = 7.4, 2.6 Hz, H-2), 7.06 (dd, 1 H, J = 7.5, 2.6 Hz, H-4), 7.04 (dd, 1 H, J = 7.5, 7.4 Hz, H-3), 7.00 (d, 1 H, J = 8.7 Hz, H-6), 3.59 (dt, 2 H, J = 5.9, 5.6 Hz, $CONHCH_2$), 2.62 (t, 2 H, J = 5.9 Hz, CH_2NMe_2), 2.44 (s, 6 H, NMe_2). This was followed by the 7-nitro isomer **16** (0.7 mg) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.88 (dd, 1 H, J = 8.8, 2.6 Hz, H-8), 7.78 (d, 1 H, J = 2.6 Hz, H-6), 7.73 (dd, 1 H, J = 7.5, 2.5 Hz, H-2), 7.09 (d, 1 H, J = 8.5 Hz, H-9), 7.07–7.00 (m, 2 H, H-3,4), 3.62 (dt, 2 H, J = 5.8, 5.6 Hz, $CONHCH_2$), 2.64 (t, 2 H, J = 5.8 Hz, CH_2NMe_2), 2.39 (s, 6 H, NMe_2), 1.70 (br, 1 H, NH).

Crystallographic Determination of Compound 42. 6-Nitrodibenzo[1,4]dioxin-1-carboxylic acid (**42**) crystallized from MeOH/EtOAc as yellow crystals, space group $C_{2/c}$; cell constants a = 21.722 (3) b = 7.084 (2) c = 16.500 (5) Å, β = 120.34 (2)°; z = 8; V = 2191.3 (3) Å^3. Lattice constants and intensity data were measured using graphite monochromated Mo Kα radiation, λ = 0.71069 Å, on a Nonius CAD-4 diffractometer. The data set consisted of 2242 unique reflections, of which 1192 were considered observed ($I > 3\sigma > (I)$). The structure was solved by direct methods and refined using SHELX-76.[27] The largest shift/esd values for non-hydrogen atoms during the final refinement were <0.05. Maximum and minimum peaks in the final difference map were +0.31 and −0.44 e Å^{-3}, respectively. At convergence, R and R_w were 0.0655 and 0.0672, respectively.

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Supplementary Material Available: X-ray crystallographic data for 6-nitrodibenzo[1,4]dioxin-1-carboxylic acid (**42**) (5 pages). Ordering information is given on any current masthead page.

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