Articles

Potential Antitumor Agents. 54. Chromophore Requirements for in Vivo Antitumor Activity among the General Class of Linear Tricyclic Carboxamides

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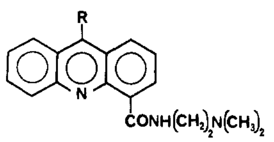
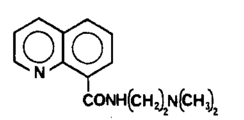
Structure–antitumor activity relationships are reported for a number of different examples (acridine, phenazine, anthracene, acridone, xanthenone, thioxanthenone, anthraquinone, pyridoquinazoline, dibenzodioxin, thianthrene, phenothiazine, phenoxazine, dibenzofuran, carbazole, and pyridoindole) of the general class of *N*-[2-(dimethylamino)ethyl] linear tricyclic carboxamides. Only the compounds containing coplanar chromophores intercalated DNA. There is an absolute requirement for an oxygen or aromatic nitrogen (possibly as hydrogen-bond acceptors) peri to the carboxamide, together with a planar ring geometry for biological activity. In addition to further delineating the nature of the pharmacophore for this class of compounds, the work has also identified dibenzo[1,4]dioxin as a novel DNA-intercalating chromophore with in vivo antitumor activity.

The majority of DNA monointercalating antitumor drugs have a common general structure, comprising a tri- or tetracyclic chromophore to which is attached one or two flexible side chains bearing cationic charges.[1,2] We recently described[3] further examples of this broad class, based on the compound *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (**2a**). These derivatives



2a R=NH$_2$
2b R=H



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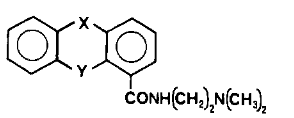

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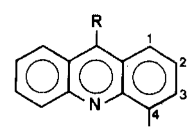
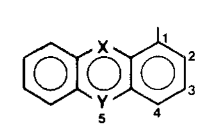
show good in vivo activity against leukemia models, but are not effective against remotely implanted solid tumors, which impose transport barriers and thus model the clinical problem more realistically. However, drastic reduction in the pK_a of the acridine chromophore, either by the attachment of electron-withdrawing groups[4] or by removal of the 9-amino function,[5] provides compounds (e.g. **2b**) with a broad-spectrum in vivo activity against both leukemia and solid tumor models.

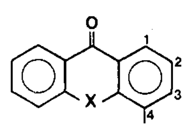
In both the acridine and 9-aminoacridine series (exemplified by **2a** and **2b**), the nature and positioning of the side chain was found to be critical, with only a *N,N*-(dialkylamino)ethylcarboxamide linked peri to the acridine nitrogen, proving acceptable. The excellent solid tumor activity shown by members of these series and the clearly defined structure–activity relationships for the side chain prompted a more general study of the nature of the chromophore. One fundamental constraint is the requirement for it to bind efficiently to DNA by intercalation. Thus, the two-ring quinolinecarboxamide (**1**) binds strongly to DNA (log *K* value for binding to poly[d(A-T)] of 5.12), but does not intercalate, and is completely inactive both in vitro and in vivo. These results together with other work[6] indicate that a linear tricyclic chromophore is the minimum required for efficient intercalative binding. We therefore report in this paper structure–activity relationships for the chromophore within the class of linear tricyclic carboxamides of general formula I. A total of 22 compounds (**1–9b**), encompassing a wide variety of different tricyclic structures were synthesized, and relationships between their molecular structure, DNA binding properties, and antitumor activities were examined.



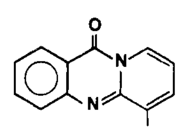
2a R=NH$_2$
2b R=H
2c R=CH$_3$
2d R=Ph



	X	Y
3a	N	CH
3b	N	N
3c	CH	CH



4a X= NH
4b X= CO
4c X= S
4d X= O



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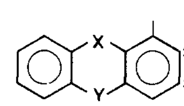
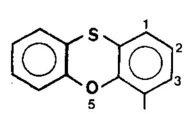


	X	Y
6a	O	O
6b	S	S
6c	S	O
6d	NH	S
6e	NH	O



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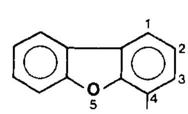

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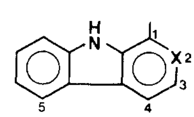

9a X=CH
9b X=N

Chemistry

Since efficient and general methods are available[5,7] for attachment of the *N,N*-dimethylethylenediamine side

(1) Wakelin, L. P. G.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 855.
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Table I. Physiochemical and Biological Data for Tricyclic Carboxamides

$ArCONH(CH_2)_2N(CH_3)_2$

				log K[d]				P388		LL	
no.	Θ[a]	R_m[b]	pK_a[c]	AT	GC	φ[e]	IC_{50}[f]	OD[g]	ILS_{max}[h]	OD	ILS_{max}
1		−0.38	3.07	5.12	5.35	0	>25000	150	NA		
2a	179[i]	−1.11	8.30	6.65	6.95	17	15	4.5	98 (1)[j]	5	NA[k]
2b	179[l]	−0.20	3.54	5.41	5.90	21	98	66	91	100	(6)[m]
2c	(180)[n]	−0.33	4.34	6.48	5.97	16	150	45	NA		
2d	(180)	0.26	3.49	5.84	5.98	17	1370	65	NA		
3a	179[l]	−0.57	4.24	6.04	6.43	12	17000	150	NA	150	NA
3b	180[o]	−0.29	0.84	5.74	6.04	18	1715	150	88	150	57
3c	180[p]	−0.02		5.78	5.25	8	5300	150	NA	150	NA
4a	ca. 180[q]	−0.28		5.04	5.40	14	1700	150	NA		
4b	180[r]	−0.35		5.28	5.34	8	4200	100	NA		
4c	179[s]	−0.16		5.51	5.18	12	6300	225	NA		
4d	175[t]	−0.26		5.29	5.05	16	6500	150	NA		
5	(180)	−0.55		6.14	6.23	18	1600	45	NA		
6a	180[u]	0.01		5.83	6.10	20	14	150	76	150	NA
6b	131[v]	0.01		4.97	4.81	0	>22000	225	NA		
6c	138[w]	0.02		4.85	4.50	0	>22000	100	NA		
6d	158[x]	0.13		5.78	5.83	0	4400	45	37	45	NA
6e	180[y]	0.07		5.20	5.39	10	4000	100	NA		
7	138[w]	0.00		5.09	4.93	0	4500	100	NA		
8	179[z]	−0.06		5.67	5.37	12	72	100	NA		
9a	178[aa]	−0.20		5.46	5.39	0	5100	65	NA		
9b	(178)[ab]	−0.29		5.34	5.26	19	6600	225	NA		

[a] Θ: Angle (in degrees) between the planes of the A and C rings of the parent chromophores, as measured by X-ray crystallography. An angle of 180° implies a coplanar system. [b] R_m values were determined as in ref 3, with 4′-(9-acridinylamino)methanesulfonanilide as a standard. [c] pK_a values for those compounds with ionizable chromophores were determined spectrophotometrically in aqueous solution as detailed in ref 45. [d] log K: binding constant to poly[d(A-T)] and poly[d(G-C)], determined by ethidium displacement, see ref 46. [e] φ: DNA unwinding angle (degrees) measured using closed *E. coli* plasmid pNZ 116, relative to ethidium as 26°, determined as in ref 3. [f] IC_{50}: concentration of drug in nanomolar to inhibit growth of murine leukemia (L1210) cells in culture by 50%, following a 40 h exposure. See ref 54. [g] OD: optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol/water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells or on days 5, 9, and 13 after intravenous inoculation of 10^6 Lewis lung carcinoma cells. See ref 55. [h] ILS_{max}: the percentage increase in lifespan of drug-treated tumor-bearing animals compared to nontreated tumor-bearing controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [i] Reference 29. [j] Numbers in parentheses indicate the number of animals in a group of six that were long-term survivors (50 days for P388, 60 days for Lewis lung); such animals are normally considered cured. [k] Compound inactive at all dose levels up to toxic ones. [l] Reference 30. [m] All animals long-term survivors. [n] Θ values in parentheses are assumed; no crystallographic data available. [o] Reference 28. [p] Reference 31. [q] Reference 32. [r] Reference 33. [s] Reference 34. [t] Reference 35. [u] Reference 36. [v] Reference 37. [w] Reference 38. [x] Reference 39. [y] Reference 40. [z] Reference 42. [aa] Reference 43. [ab] Reference 44.

chain to an aromatic carboxylic acid to give the desired carboxamides (**1**–**9b**) recorded in Tables I and II, the chemistry was concerned with the preparation of the corresponding linear tricyclic carboxylic acids. Compounds **2a**–**c** of Table I have been described previously.[3,5]

9-Phenylacridine-4-carboxylic acid for compound **2d** was obtained from reaction of 2-aminobenzophenone and diphenyliodonium-2-carboxylate,[8] followed by selective cyclization of the resulting *N*-(2-benzoylphenyl)anthranilic acid in mild acid.

Acridine-1-carboxylic acid was most efficiently prepared via the mixture of 9-oxoacridancarboxylic acids obtained in quantitative yield by H_2SO_4-induced cyclization of *N*-(3-carboxyphenyl)anthranilic acid. This mixture (74% 1-acid and 26% 3-acid) proved difficult to separate, but reduction with Al/Hg amalgam[5] followed by fractional crystallization gave pure acridine-1-carboxylic acid.

Carboxylic acids for the preparation of compounds (**3b**, **3c**, **4a**, and **4b**) have been reported and were prepared by the published methods. Reductive ($NaBH_4$) ring closure[9] of 3-nitro-*N*-phenylanthranilic acid gave phenazine-1-carboxylic acid. Oxidation[10] of benzanthrone (CrO_3/H_2SO_4) gave 9,10-dioxoanthracene-1-carboxylic acid, and Cu-catalyzed reduction[11] of this with Zn/NH_4OH gave anthracene-1-carboxylic acid. 9-Oxoacridan-4-carboxylic acid was prepared in quantitative yield by H_2SO_4-induced cyclodehydration of diphenylamine-2,2′-dicarboxylic acid.[3]

Oxothioxanthene-4-carboxylic acid was prepared by Zn/Cu/NaOH-induced condensation of 2-iodobenzoic acid

Table II. Physiochemical Properties for the New Compounds of Table I

no.	mp, °C	formula	analyses
1	66–67	$C_{14}H_{17}N_3O{\cdot}2HCl$	C, H, N, Cl
2d	205–208	$C_{24}H_{23}N_3O{\cdot}2HCl$	C, H, N, Cl
3a	214–216	$C_{18}H_{19}N_3O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl
3c	220–222	$C_{19}H_{20}N_2O{\cdot}HCl$	C, H, N, Cl
4a	284–286	$C_{18}H_{19}N_3O_2{\cdot}HCl$	C, H, N, Cl[a]
4b	241–243	$C_{19}H_{18}N_2O_3{\cdot}HCl$	C, H, N, Cl
4c	237–239	$C_{18}H_{18}N_2O_2S{\cdot}HCl$	C, H, N
4d	217–229	$C_{18}H_{18}N_2O_3{\cdot}HCl$	C, H, N, Cl
5	133–135	$C_{17}H_{18}N_4O_2$	C, H, N
6a	178–182	$C_{17}H_{18}N_2O_3{\cdot}HCl{\cdot}H_2O$	C, H, N, Cl
6b	182–183	$C_{17}H_{18}N_2S_2O{\cdot}HCl$	C, H, N, S
6c	127–130	$C_{17}H_{18}N_2O_2S{\cdot}HCl{\cdot}MeOH$	HRMS[c]
6d	205–208	$C_{17}H_{19}N_3OS{\cdot}HCl$	C, H, N, S
6e	201–202	$C_{17}H_{19}N_3O_2{\cdot}HCl$	C,[b] H, N, Cl
7	165–166	$C_{17}H_{18}N_2O_2S{\cdot}HCl{\cdot}MeOH$	C, H, N, Cl
8	184–188	$C_{17}H_{18}N_2O_2{\cdot}HCl$	C, H, N
9a	236–238	$C_{17}H_{19}N_3O{\cdot}HCl$	C, H, N, Cl
9b	242–244	$C_{16}H_{18}N_4O{\cdot}2HCl$	C,[b] H, N, Cl

[a] Cl out by 0.5%. [b] C out by 0.5%. [c] High-resolution mass spectral determination of free base.

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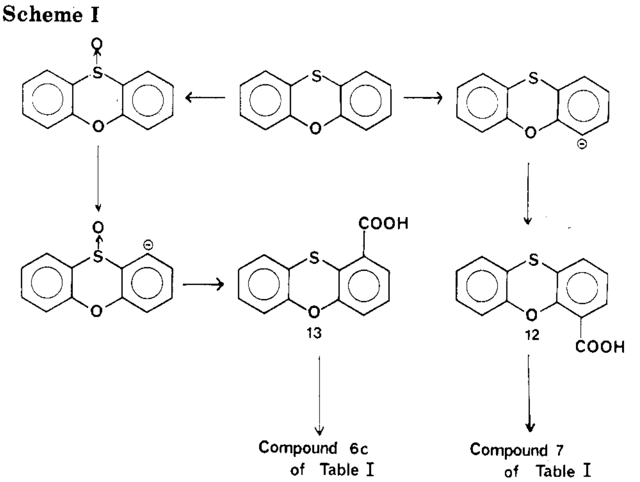
Table III. Metalation Reactions

	metalation conditions[a]							
substrate	mole ratio of *n*-BuLi	solvent	temp, °C	time, h	electrophile	product	yield, %	ref
dibenzodioxin	1:1.5	THF	25	1	DMF	**10**	64[c]	50
thianthrene	1:1.2	Et_2O	20	18	CO_2	**11**	34	51
phenoxathiin	1:1.05	Et_2O	reflux	1.5	CO_2	**12**	37	16
phenoxathiin 10-oxide	1:3.1	Et_2O	−25	24	CO_2	**13**	32	18
phenothiazine	1:4	DME	20	24	CO_2	**14**	43	52
dibenzofuran	1:1.5	THF	40	1	DMF	**16**	62[d]	53

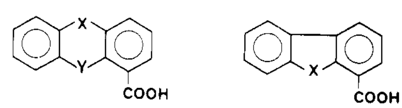
[a] See the Experimental Section for representative procedures. [b] Commercially available solutions of *n*-BuLi in hexane (ca. 1.5 N) were used and were standardized by titration using 2,5-dimethoxybenzyl alcohol as an indicator. [c] Yield of intermediate aldehyde was 83%. [d] Yield of intermediate aldehyde was 77%.

Scheme I



and thiosalicyclic acid,[12] followed by cyclization with PPA. Oxoxanthene-4-carboxylic acid was obtained by cyclodehydration of bis(2-carboxyphenyl) ether,[13] obtained by reaction of diphenyliodonium-2-carboxylate with 2-methylphenol[14] and oxidation of the resulting 2-methylphenyl 2-carboxyphenyl ether. The pyrido[2,1-*b*]quinazoline acid for **5** was made as reported[15] by Ullmann condensation of 2-chloronicotinic acid and anthranilic acid.

The acids required for compounds (**6a–e**, **7**, and **8**) were obtained by metalation of the parent heterocycles with *n*-butyllithium by using modifications of published procedures, followed by quenching of the resulting aryllithium compound with solid CO_2. In the cases of the dibenzodioxin and dibenzofuran acids (**10** and **16**), higher yields



10 X=Y=O
11 X=Y=S
14 X=S, Y=NH
15 X=O, Y=NH

16 X=O
17 X=NH

were obtained by treatment of the aryllithium compound with DMF to give the aldehyde, followed by $KMnO_4$ oxidation to the required acid. Table III gives the metalation conditions found to give acceptable yields of the carboxylic acids. Controlled metalation of phenoxathiin (Scheme I) gave the 4-carboxylic acid **12**.[16] For preparation of the isomeric 1-acid **13**, it was necessary to metalate phenoxathiin 10-oxide,[17] which has been shown[18] to proceed at the 1-position with concomitant reduction of the sulfoxide group. The resulting 1-acid was the major component of a complex mixture, from which it was separated by chromatography of the methyl esters. Although both carbazole[19] and phenoxazine[20] have been metalated with *n*-butyllithium, the reported yields of carboxylic acids were extremely low and, like others,[21] we were unable to obtain synthetically useful quantities of material via the metalation route. Phenoxazine-1-carboxylic acid (**15**) was therefore prepared by the four-step cyclization sequence reported by Blank and Baxter,[21] while carbazole-1-carboxylic acid (**17**) was obtained by oxidation of the methyl ester of 5,6,7,8-tetrahydro-9*H*-carbazole-1-carboxylic acid[22] with DDQ.[23] After completion of this work, improved lithiation procedures were developed for the synthesis of the 1-carboxylic acid derivatives of carbazole,[24] phenothiazine,[25] and phenoxazine.[26]

9*H*-Pyrido[3,4-*b*]indole-1-carboxylic acid for compound **9b** was conveniently prepared by $KMnO_4$ oxidation of the corresponding benzal derivative, which was obtained from the naturally occurring β-carboline harman as reported.[27]

Results and Discussion

Chromophore Geometry. Table I records physicochemical and biological data for 21 carboxamides representing a number of different tricyclic ring systems, together with the quinoline derivative **1**. To provide general information about the chromophore geometry, the dihedral angles (Θ) between the A and C rings of the parent chromophores for each system are given, where they are available from published X-ray crystallographic studies. While the fully aromatic ring systems (anthracene, acridine, phenazine) used in compounds **2a–d** and **3a–c** of Table I are known to be coplanar,[28-31] less information is

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available on the structures of the parent compounds of compounds **4a–d**. However, the existing data suggests that these compounds too will be essentially coplanar. Studies on acridone and *N*-alkylacridones,[32] anthraquinone,[33] and thioxanthenone[34] show they all have a coplanar conformation with measured dihedral angles of essentially 180°. However, xanthenone, which lacks the ability of thioxanthenone to form delocalized zwitterionic structures,[34] has been shown[35] to have a slight butterfly conformation, with a dihedral angle of 175°. The pyridoquinazoline **5** is expected to be coplanar, but no crystallographic information is available.

The ring systems of the parent chromophores of compounds **6a–e** and **7** of Table I show more variable geometry. While the dibenzodioxin system of compound **6a** is completely coplanar,[36] thianthrene (compound **6b**) is severely distorted, with a dihedral angle of 131° between the A and C rings,[37] and phenoxathiin (compounds **6c** and **7**) is almost equally bent,[38] with an angle of 138°. This distortion is due to a combination of the much longer C–S bonds (1.75–1.77 Å) compared with the C–O and C–N bonds (1.37–1.40 Å) in these compounds and the much more acute C–S–C bond angles (98–104°) compared with C–O–C (116–119°) and C–N–C (123–124°) bond angles. Thus phenothiazine, with one sulfur atom, is also butterfly shaped (angle Θ of 158°),[39] while the limited crystal structure data[40] for phenoxazine (compound **6e**) suggests a planar structure, although a bent conformation has been proposed[41] on the basis of NMR evidence. The dibenzofuran and carbazole nuclei (compounds **8**, **9a**, and **9b**), with a central five-membered ring, have less conformational flexibility, and all have coplanar structures.[42-44]

Physicochemical Properties. pK_a values for the ionizable chromophores were determined by spectrophotometry in aqueous solution as before[45] and are recorded in Table I. Only the 9-aminoacridine derivative **2a** with a pK_a of 8.3 is likely to be ionized at physiological pH.

Drug lipophilicity was determined by liquid–liquid chromatography in the presence of 0.3% methanesulfonic acid.[45] The 9-aminoacridine derivative **2a** runs as the dication, with the chromophore charged, which accounts for its low measured lipophilicity (R_m −1.11). The much lower pK_as of the acridine derivatives (**2b–d**, **3a**) makes it probable that they run predominantly with uncharged chromophores. This is certainly the case for the phenazine derivative **3b**, with a pK_a of only 0.84, which is observed to run as a yellow spot quite unlike the bright red dicationic species observed in strong acid. With the exception of the more lipophilic 9-phenylacridine (**2d**) and the anthracene **3c**, the planar chromophores have R_m values (−0.2 to −0.5) similar to that of the acridinecarboxamide **2b**. In contrast, the predominantly nonplanar compounds (**6a–e**, **7**) are more lipophilic, with R_m values from 0.0 to 0.10.

DNA Binding. Binding of the compounds to DNA was determined as previously described by the ethidium displacement assay, with a correction for any quenching of ethidium fluorescence caused by bound drug.[46] As noted previously,[5] loss of the 9-amino group from **2a** to give **2b** results in 1 order of magnitude decrease in DNA binding, and the isomeric acridine **3a** shows a similar binding level. The phenazine **3b** has a DNA affinity between those of the two acridine isomers as does the anthracene derivative **3c**, despite its lack of polar functionality. The fully aromatic compounds generally show a slight preference for binding to GC sites and all intercalate, as shown by unwinding angles of 12–20°.

The carbonyl-containing chromophores (**4a–d**), although planar, bind significantly less tightly, but they all appear to intercalate DNA. However, the binding mode of compounds (**6a–e** and **7**) shows a dependence on conformation. The planar dibenzodioxin **6a** binds by intercalation as tightly as the fully aromatic phenazine but the thianthrene **6b** binds 10-fold less strongly, with no evidence for chromophore intercalation since the compound fails to unwind and rewind closed circular DNA (Table I). The remainder of the nonplanar compounds (**6c**, **6d**, and **7**) also bind less strongly and also do not show evidence for intercalation. It is particularly interesting that only compounds **6a** and **6e**, containing the planar dibenzodioxin and phenoxazine chromophores, show evidence of intercalative binding to DNA. The dibenzofuran and carbazole compounds (**8**, **9a**, and **9b**) bind as tightly as the acridinecarboxamide **2b**, but the failure of the carbazole **9a** to intercalate is puzzling.

In Vitro Cytotoxicity. The compounds show great variation in cytotoxic potency. Among the fully aromatic derivatives, the 9-aminoacridine (**2a**) was by far the most potent, with an IC_{50} of 15 nM, while the acridine-1-carboxamide (**3a**) was the least potent. The 200-fold difference in potencies between these two compounds is striking, given the similarity of their DNA equilibrium binding properties. While the pyridoquinazoline **5** and the acridone **4a** showed potentially interesting levels of cytotoxicity (IC_{50} = ca. 1500 nM), the other carbonyl-containing compounds (**4b–d**) were much less toxic. The dibenzodioxin **6a** proved to be exceptionally toxic, with an IC_{50} of 14 nM, while the nonintercalating thianthrene **6b** was more than 2000-fold less potent. Although more potent than the thianthrene, the remaining six-membered ring compounds were not exceptional, with IC_{50} values of ca. 4000 nM.

Interesting contrasts were also seen with the five-membered ring compounds; the dibenzofuran **8** proved very cytotoxic, while the carbazole derivatives **9a** and **9b** were unexceptional.

In Vivo Activity. For DNA-intercalating agents, generally, there is a broad but inexact correlation between high

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cytotoxic potency and in vivo antileukemic activity, and this is true of the present compounds. Of the five compounds of Table I with IC_{50} values below 200 nM, the known[3,5] acridines **2a** and **2b** and the dibenzodioxin **6a** show good in vivo activity, although the equally potent dibenzofuran (**8**) and the 9-methylacridine (**2c**) do not. Four compounds showed intermediate levels of cytotoxicity (ca. 1500 nM), with one (**3b**) being active in vivo and three (**2b**, **4a**, and **5**) being inactive. However, 11 of the remaining 12 compounds with IC_{50} values above 4000 nM are all inactive in vivo. The exception is the phenothiazine **6d**, which does not intercalate DNA and has an IC_{50} of 4400 nM, yet shows low but positive in vivo activity. The related compound chlorpromazine is also reported to have low antitumor activity, possibly via a free radical oxidation mechanism.

Thus, cytotoxic potency broadly relates to in vivo antileukemic activity even among this diverse set of DNA-intercalating agents. IC_{50} values below ca. 200 nM denote active chromophores (acridine, dibenzodioxin), while IC_{50} values of ca. 1000–2000 nM are also of interest, since either the parent molecule itself (phenazine) or a substituted compound may be active (in the case of the acridone chromophore, a number of substituted derivatives show in vivo activity[47]). However, parent chromophores that show IC_{50} values greater than ca. 2000 nM seem unlikely to be worth pursuing.

Conclusions

The aim of this study was to examine chromophore structure–activity relationships for the broad class of linear, tricyclic carboxamides, in order to further delineate the nature of the allowed pharmacophore, initially formulated as I. Among the fully conjugated compounds, the inactivity of the acridine-1-carboxamide **3a** and the anthracene **3c** showed the necessity for a nitrogen atom peri to the carboxamide side chain. This is not a simple requirement for a positive charge, since the active phenazine (**3b**) is unlikely to be protonated at physiological pH. The planar acridone **4a** and phenoxazine (**6e**), where the peri NH is a H-bond donor, are also inactive. It seems more likely that the peri nitrogen is required to act as an H-bond acceptor, given the activity of the dibenzodioxin **6a**, where the peri oxygen can act equally well. None of the compounds containing a bent chromophore intercalated DNA. The inactivity of all these compounds, including the phenoxathiin-4-carboxamide (**6c**), which has the correctly placed peri oxygen, suggests that chromophore planarity (thus allowing intercalation) is an additional requirement. These results imply that the "essential pharmacophore" can be further restricted to formula I, where the ring system is planar (but not necessarily fully conjugated), and Y is an H-bond acceptor.

In addition to these general conclusions, which may allow the design of further DNA-intercalating agents of this general class, the present work has also identified dibenzodioxin as a novel chromophore, with in vivo activity. Despite the fact that **6a** is inactive against the LL solid tumor, the high cytotoxicity and good antileukemic activity of the compound make it worthy of further development.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, and were within ±0.4% of the theoretical value unless indicated. Melting points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer and are reported as read. Et_2O, THF, and DME were distilled under nitrogen from sodium benzophenone ketyl and used immediately. All metalation reactions were performed in an oven-dried flask and maintained under a positive pressure of nitrogen (balloon). Column chromatography was performed by the method of Still et al.[48] with Merck silica gel 60 (230–400 mesh). Petroleum ether refers to the fraction with bp 40–60 °C.

9-Phenylacridine-4-carboxylic Acid. A mixture of 2-aminobenzophenone (5.92 g, 0.03 mol), diphenyliodonium-2-carboxylate (10.7 g, 0.033 mol), and cupric acetate (0.5 g) in DMF (20 mL) was heated with stirring at 100 °C for 2 h and concentrated under reduced pressure, and the residue was shaken with water. The resulting solid was extracted with hot 2N aqueous Na_2CO_3, and the solution was treated with charcoal, extracted with benzene, and then acidified. The precipitate was crystallized from benzene/petroleum ether and then aqueous EtOH to give *N*-(2-benzoylphenyl)anthranilic acid as yellow prisms (2.47 g, 26%), mp 183–184 °C. Anal. ($C_{20}H_{15}NO_3$) C, H, N.

The above acid (1.75 g) was dissolved in AcOH (16 mL) and concentrated H_2SO_4 (4 mL), and the mixture was stirred at 95 °C for 30 min, cooled, and diluted with water. The solution was just neutralized with NH_4OH, and the precipitate was extracted with hot 1 N NH_4OH. This solution was clarified and acidified (AcOH), and the resulting precipitate was crystallized from aqueous EtOH, giving pure 9-phenylacridine-4-carboxylic acid as yellow needles (83% yield), mp 229–230 °C. Anal. ($C_{20}H_{13}NO_2$) C, H, N.

Acridine-1-carboxylic Acid. *N*-(3-Carboxyphenyl)anthranilic acid (58 g) was dissolved in concentrated H_2SO_4 (100 mL) and kept at 100 °C for 1 h. The hot mixture was poured slowly into water, and the resulting solid was collected, dissolved in dilute NH_4OH/EtOH, and precipitated with AcOH to give a granular mixture of 9-oxoacridan-1-carboxylic acid and 9-oxoacridan-3-carboxylic acid in a 74:26 ratio (determined by HPLC, using pure 3-acid as a standard). A solution of the above mixture (5 g, 21 mmol) and NaOH (1 g, 25 mmol) in water was treated at the boil with Al/Hg amalgam[5] (3 g) over 90 min. After filtration, the filtrate was acidified with HCl and treated with a solution of $FeCl_3$ (5 g) in water (150 mL). The initial precipitate redissolved after 10 min at reflux, and NaOH was then added to precipitate iron species. The filtrate was acidified to pH 5, and the resulting crude solid (two spots on TLC) was recrystallized from EtOH to give pure acridine-1-carboxylic acid (1.5 g, 32%), mp >325 °C. Anal. ($C_{14}H_{19}NO_2$) C, H, N.

Oxoxanthene-4-carboxylic Acid. 2-Methylphenol (33 g, 0.30 mol) was added to a solution of Na (2.1 g, 90 mmol) in MeOH (100 mL), and the MeOH was then removed under vacuum. Diphenyliodonium-2-carboxylate (19.6 g, 60 mmol) and cupric acetate (0.5 g) were added, and the mixture was heated at 100 °C for 5 h before being diluted with 2 N NaOH solution and filtered through Celite. The clear solution was acidified with concentrated HCl, and NH_4OH was then added slowly until the cloudy white precipitate just redissolved (pH ca. 8). Excess 2-methylphenol was removed by two extractions with EtOAc, and the aqueous solution was poured slowly into 2 N HCl to give 2-(2-methylphenoxy)benzoic acid as a white solid (8.85 g, 64%), mp (benzene) 138–139 °C (lit.[49] mp 133.5 °C). Oxidation of the above compound with $KMnO_4$[13] gave bis(2-carboxyphenyl) ether in 89% yield, mp 230 °C (lit.[13] mp 230 °C). Ring closure of this diacid with polyphosphate ester gave oxoxanthene-4-carboxylic acid in 84% yield, mp 289 °C (lit.[13] mp 289 °C).

11-Oxo-11*H*-pyrido[2,1-*b*]quinazoline-6-carboxylic Acid. 2-Chloronicotinic acid (7.3 g, 46 mmol) and anthranilic acid (6.5 g, 47 mmol) were suspended in dry *N*-methylpyrrolidone (20 mL) in an open beaker. K_2CO_3 (9.8 g, 70 mmol) was added, and the mixture was stirred until gas evolution ceased. After addition of Cu/CuO (1:1; 0.2 g), the mixture was heated with stirring at 150 °C until it solidified (15 min), and the cooled solid was dissolved in water (300 mL). The solution was just neutralized with AcOH, filtered to remove a small amount of black solid, and then acidified with AcOH to recover the acid as a yellow solid

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(6.25 g, 57%). A sample was recrystallized from water as a yellow powder, mp 233–235 °C (lit.[15] mp 221 °C). Anal. ($C_{13}H_8N_2O_3$) C, H, N.

Dibenzo[1,4]dioxin-1-carboxylic Acid (10). *n*-Butyllithium (5.43 mL of a 1.50 N solution in hexane, 8.14 mmol, 1.5 equiv) was added dropwise at 25 °C under N_2 to a stirred solution of dibenzo[1,4]dioxin (1.00 g, 5.43 mmol) in THF (15 mL). After 1 h, DMF (0.63 mL, 8.14 mmol) was added in one portion, and after a further 10 min, the mixture was poured into brine, extracted with EtOAc, and worked up to give crude dibenzo[1,4]-dioxin-1-carboxaldehyde as an oily yellow solid (0.96 g, 83%). This was dissolved in a mixture of Me_2CO (20 mL) and 1 N H_2SO_4 (10 mL), and powdered $KMnO_4$ (2 g, 13 mmol) was added in portions with vigorous stirring. After 1 h, the mixture was poured into brine and extracted with Et_2O. The extract was washed with 10% aqueous sodium bisulfite until colorless and then extracted with 2 N KOH solution. Acidification of this basic extract followed by extraction with EtOAc gave dibenzo[1,4]dioxin-1-carboxylic acid (0.80 g, 64% overall), which crystallized from glacial AcOH as cubes, mp 205–207 °C (lit.[50] mp 210 °C).

Phenoxathiin-1-carboxylic Acid (13). Concentrated HNO_3 (d 1.42) (143 mL) was added dropwise to a stirred solution of phenoxathiin (14.3 g, 0.07 mol) in AcOH (290 mL), and the solution was warmed at 35–40 °C for 30 min and then poured into brine (1 L). The mixture was extracted with EtOAc and worked up to give phenoxathiin 10-oxide, which was crystallized from $CHCl_3$/petroleum ether as needles (8.36 g, 54%), mp 157–158 °C (lit.[17] mp 151–154 °C).

A suspension of phenoxathiin 10-oxide (24.6 g, 0.114 mol) in dry Et_2O (500 mL) was cooled to −25 °C under an atmosphere of dry N_2 and treated dropwise over 1 h with a solution of *n*-butyllithium in hexane (233.4 mL of a 1.51 N solution, 0.352 mol, 3.1 equiv). The solution was stirred for a further 6 h at −25 °C, kept at −25 °C overnight, and then poured in a steady stream onto solid CO_2. The product was partitioned between Et_2O and water, and the organic layer was washed once with 2 N NaOH. The combined aqueous solutions were washed with Et_2O, acidified with HCl, and extracted with EtOAc to give a mixture of acids as a yellow oil. This was dissolved in dry MeOH (250 mL) with concentrated H_2SO_4 (5 mL), and the solution was heated under reflux for 5 h and poured into saturated $NaHCO_3$ solution. Extraction with EtOAc afforded a mixture of methyl esters (30.1 g), which was chromatographed on SiO_2. Elution with petroleum ether gave methyl pentanoate, while petroleum ether/EtOAc (95:5) gave methyl phenoxathiin-1-carboxylate, which was distilled at 90–94 °C (0.35 mmHg) to give a colorless oil (9.46 g, 32%).

Hydrolysis of this ester with 5 N aqueous NaOH (20 mL) in MeOH (100 mL) at reflux for 2 h gave a quantitative yield of phenoxathiin-1-carboxylic acid, which crystallized from EtOAc as a cream-colored powder, mp 220–221 °C (lit.[18] mp 221–222 °C).

Preparation of the Carboxamides of Table I. A Typical Example: *N*-[2-(Dimethylamino)ethyl]phenoxathiin-1-carboxamide (6c). 1,1′-Carbonyldiimidazole (6.05 g, 0.037 mol) was added to a solution of phenoxathiin-1-carboxylic acid (**13**, 4.56 g, 0.019 mol) in dry DMF (30 mL), and the solution was warmed at 40 °C for 20 min. *N,N*-Dimethylethylenediamine (3.07 g, 0.028 mol) was then added, and after a further 30 min, the solution was concentrated at reduced pressure. The residue was dissolved in EtOAc, washed with water, and extracted with 3 N HCl. This extract was basified with NH_4OH, extracted with EtOAc, and worked up to give an oil, which was chromatographed on a short SiO_2 column. Elution with EtOAc/MeOH/Et_3N (94:5:1) gave the carboxamide **6c** as an oil (3.64 g, 62%). This was dissolved in MeOH and treated with a solution of dry HCl gas in Et_2O to give the hydrochloride salt, which was crystallized from Me_2CO/Et_2O as white rosettes, mp 127–130 °C. Anal. (Table II).

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Registry No. 1, 112022-03-4; 1·2HCl, 112022-18-1; **2a**, 89459-43-8; **2b**, 89459-25-6; **2c**, 89459-33-6; **2d**, 112022-04-5; **2d**·2HCl, 112022-19-2; **3a**·2HCl, 89458-99-1; **3b**, 103942-97-8; **3c**, 112022-05-6; **3c**·HCl, 112022-20-5; **4a**, 103554-58-1; **4a**·HCl, 112022-21-6; **4b**, 112022-06-7; **4b**·HCl, 112022-22-7; **4c**, 112022-07-8; **4c**·HCl, 112041-58-4; **4d**, 112022-08-9; **4d**·HCl, 112022-23-8; **5**, 112022-09-0; **6a**, 112022-10-3; **6a**·HCl, 112022-24-9; **6b**, 112022-11-4; **6b**·HCl, 112022-25-0; **6c**, 112041-57-3; **6c**·HCl, 112022-26-1; **6d**, 112022-12-5; **6d**·HCl, 112022-27-2; **6e**, 112022-13-6; **6e**·HCl, 112022-28-3; **7**, 112022-14-7; **8**, 112022-15-8; **8**·HCl, 112022-29-4; **9a**, 112022-16-9; **9a**·HCl, 112041-59-5; **9b**, 112022-17-0; **9b**·2HCl, 112022-30-7; **10**, 51689-36-2; 9-phenylacridine-4-carboxylic acid, 112022-31-8; 2-aminobenzophenone, 2835-77-0; diphenyliodonium-2-carboxylate, 1488-42-2; *N*-(2-benzoylphenyl)-anthranilic acid, 18964-23-3; acridine-1-carboxylic acid, 106626-85-1; *N*-(3-carboxyphenyl)anthranilic acid, 27693-67-0; oxoxanthene-4-carboxylic acid, 42073-77-8; 2-methylphenol, 95-48-7; 2-(2-methylphenoxy)benzoic acid, 6325-68-4; 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-6-carboxylic acid, 4393-98-0; 2-chloronicotinic acid, 2942-59-8; anthranilic acid, 118-92-3; dibenzo-[1,4]dioxin, 262-12-4; bis(2-carboxyphenyl) ether, 37424-29-6; dibenzo[1,4]dioxin-1-carboxaldehyde, 51689-41-9; phenoxthiin-1-carboxylic acid, 99420-27-6; phenoxathiin, 262-20-4; phenoxathiin 10-oxide, 948-44-7; methyl phenoxathiin-1-carboxylate, 112022-32-9; *N,N*-dimethylenediamine, 108-00-9; trianthrene, 92-85-3; phenothiazine, 92-84-2; dibenzofuran, 132-64-9.

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