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DNA THREADING AGENTS: EFFECT OF SIDECHAIN BULK ON DNA BINDING AND CYTOTOXICITY OF 9-ANILINOACRIDINE-4-CARBOXAMIDES

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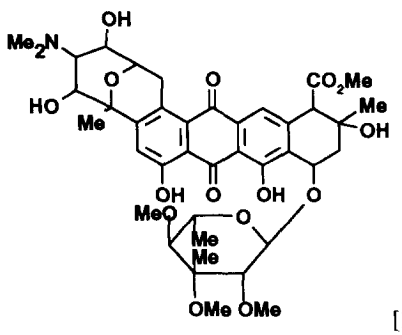
Abstract. A series of 9-anilinoacridine-4-carboxamides with cycloalkyl sidechains has been synthesised to study the effect of sidechain bulk on the DNA binding properties and biological activity of these potential threading intercalators. With sidechains larger than cyclohexane the pKa of the acridine is exceptionally low, so that DNA binding is restricted to pHs below 5. The compounds are cytotoxic to human colon carcinoma cells in the μM range irrespective of their ability to bind to DNA. Copyright © 1996 Elsevier Science Ltd

Compounds which bind to DNA by intercalation are of interest both as potential therapeutic agents and also as probes in the study of ligand-DNA interactions and investigation of nucleic acid structure. Nogalamycin [1] is an antitumour antibiotic which binds to DNA by a threading mode, in which the nogalose sugar is positioned in the minor groove and the fused bicyclic sidechain lies in the major groove[1]. A number of other compounds have also been shown to bind by the threading mechanism[2,3]. Nogalamycin is a potent inhibitor of transcription[4]. Its large lipophilic groups positioned in each groove lead to long residence times on DNA and to profound inhibition of the passage of RNA polymerase. It is therefore possible that synthetic analogues designed to bind in a similar fashion may also inhibit transcription and have potential as antitumour agents. 9-anilinoacridine-4-carboxamides have been shown[2] to bind to DNA via the threading mechanism, with the 9- and 4-substituents sited in different grooves and the acridine chromophore intercalated between the base pairs. Previous work[2] with hydrophilic and cationic carboxamide sidechains has shown that these compounds have DNA residence times much longer than those of 9-anilinoacridines unsubstituted at the 4-position. We have now synthesised a series of compounds in which the 4-carboxamide substituent is uncharged and varied systematically to explore the effect of hydrophobic bulk on DNA binding and cytotoxicity. Minimising the charge density of the compounds in this way will lower their affinity for DNA and may promote solid tumour activity through better tumour penetration and tissue distribution. In this communication we describe the synthesis of these compounds, initial DNA binding studies and the variation of biological activity with structure.



[1] Nogalamycin

Scheme. Synthetic route to 9-anilinoacridines and 9-aminoacridines

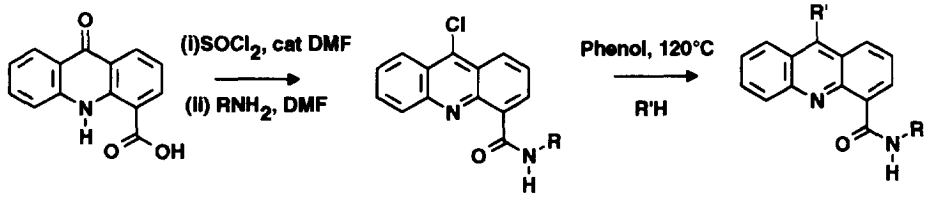


Table 1. 9-Anilinoacridines and 9-aminoacridines synthesised.

	R	R'	m.p. (°C)	%Yield
2	$(CH_3)_2NCH_2CH_2$	4-($MeSO_2NH$)-2-(CH_3O)PhNH	226-228	38
3	Cyclopropyl	4-($MeSO_2NH$)-2-(CH_3O)PhNH	106-109	47
4	Cyclohexyl	4-($MeSO_2NH$)-2-(CH_3O)PhNH	218-220	77
5	Cyclooctyl	4-($MeSO_2NH$)-2-(CH_3O)PhNH	130-132	52
6	Cyclododecyl	4-($MeSO_2NH$)-2-(CH_3O)PhNH	160-164	79
7	Adamantyl	4-($MeSO_2NH$)-2-(CH_3O)PhNH	174-175	50
8	$(CH_3)_2NCH_2CH_2$	NH_2	290-292	83
9	Cyclopropyl	NH_2	111-115	70
10	Cyclohexyl	NH_2	114-116	71
11	Cyclooctyl	NH_2	206-210	74
12	cyclododecyl	NH_2	189-193	52
13	adamantyl	NH_2	321-323	79

The compounds synthesised are shown in Table 1. The cycloalkane sidechains were varied in size from cyclopropane, which was expected to have a similar effect on DNA binding and cytotoxicity to the methyl carboxamide, up to cyclododecane and adamantane which might be expected to have large effects on these properties. The rigid adamantyl cage allows comparison with the flexible cyclic structures. The synthesis of the analogous 9-aminoacridine-4-carboxamides permits comparison of threading with non-threading derivatives. The N,N-dimethylaminoethyl-carboxamides **[2]** and **[8]** were included as examples of compounds which have a high affinity for DNA and which have been studied previously. All compounds were synthesised as shown in the Scheme. 9-Chloroacridine-4-carbonyl chloride was prepared by reaction of acridone-4-carboxylic acid[5] with thionyl chloride containing a few drops of DMF. The acid chlorides were then reacted in DMF with amine to give the required 4-carboxamide substituent[6]. Yields of the 9-chloro- derivatives were 40-70%. Reaction of these derivatives in phenol with either dry ammonia or 2-methoxy-4-methanesulphonamidoaniline gave the required acridines in 38-83% yield[6]. An interesting observation in the proton NMR (270MHz) of the 9-aminoacridine series is a significant downfield shift in the amide proton resonance compared to the 9-chloro-precursor (1-1.5ppm). This is consistent with significant hydrogen bonding between the pyridinic nitrogen N10

and the amide hydrogen, as described in the crystal structure of N-[2-(N,N-dimethylamino)ethyl]-9-aminoacridine-4-carboxamide[7].

The binding of intercalators to DNA may be studied by a number of spectroscopic means[8]. Acridines have been shown to undergo characteristic bathochromic and hypochromic changes to their UV/visible absorption spectra in the presence of DNA and while this is not a definitive test for intercalation, it indicates that DNA binding is occurring in a similar fashion to other, well studied, derivatives, such as amsacrine and 9-aminoacridine[2]. Values of extinction coefficients at λmax of the anilinoacridines **[2]**-**[7]** free in solution, and in the presence of DNA are shown in Table 2.

Table 2[9] UV/visible spectral characteristics of compounds free, in the presence of DNA and in SDS (Sodium dodecylsulphate)

	Free Drug pH 7		**Drug with DNA**		**Free Drug + SDS**	
Compound	λmax(nm)	ε x 10^{-3}	λmax(nm)	ε x 10^{-3}	λmax(nm)	ε x 10^{-3}
2	434	9.25	464	7.42	450	11.9
3	440	9.75	460	7.35	450	11.5
4	440	7.33	460	8.43	450	12.4
5	448	7.70	453	7.99	450	13.6
6	446	7.68	448	7.69	450	14.1
7	452	7.54	452	7.68	450	12.8
8	408	9.94	422	5.25	412	10.9
9	408	10.67	422	5.45	414	11.3
10	408	12.91	422	6.82	414	14.1
11	406	4.15	420	4.68	412	10.9
12	400	3.82	404	4.49	414	8.0
13	406	4.93	418	5.51	414	11.8

The known threading compound **[2]**[2], the N,N-dimethylaminoethyl derivative, displays bathochromic and hypochromic shifts consistant with intercalation. Amongst the compounds with uncharged sidechains the cyclopropyl derivative **[3]** also shows bathochromic and hypochromic changes in the presence of DNA suggesting it too binds by intercalation. By contrast, the compounds with bulky sidechains, **[5]**, **[6]** and **[7]**, appear not to be binding as judged by spectral measurements, since their absorption characteristics are little altered in the presence of DNA. The cyclohexyl compound **[4]** behaves somewhat enigmatically since λmax shifts 20nm in the presence of DNA to a value typical of that of bound amsacrine-4-carboxamides, but its extinction coefficient is enhanced. The bulky sidechain derivatives **[4-7]** share the characteristic that their free absorption spectra have unusually low extinction coefficients suggestive of aggregation[10]. To explore whether this is the case, absorption spectra were measured in the presence of SDS which is known to disaggregate stacked acridines. Table 2 shows a 18-30% enhancement in the extinction coefficient values for the charged compound **[2]** as well as for the smaller cycloaliphatic compound **[3]** in SDS solution. However the larger

neutral derivatives **[4-7]** have significantly greater increases in ε values (70-85%) in the presence of detergent. This supports the notion that these compounds are substantially aggregated when free in solution.

Table 2 also includes the wavelengths and extinction coefficients of the 9-aminoacridine-4-carboxamides when free and in the presence of DNA or SDS. The established intercalating N,N-dimethylaminoethyl derivative **[8]** again shows characteristic bathochromic and hypochromic shifts in the presence of DNA. The small-ring carboxamides [**9, 10**] show similar evidence of binding. However for the larger derivatives [**11**] and **[13]**, although increases in λmax are seen in the presence of DNA typical of binding, as with the anilino-cyclohexane carboxamide **[4]**, the extinction coefficient actually rises. The cyclododecyl derivative **[12]** shows no spectral evidence of binding. Again, as in the anilino series, the bulky compounds **[11-13]** have very low extinction coefficients suggestive of aggregation which was confirmed by the more than 2-fold increase in absorbance values obtained when measured in the presence of SDS.

Studies with CPK molecular models of the compounds that show little or no changes in λmax or ε values [**5,6,7,11,12,13**] in the presence of DNA, reveal that all appear to be capable of intercalation, there being no obvious steric restraint on fitting the bulkier sidechains into the minor or the major grooves. Thus, it seems likely that the apparent failure of these compounds to intercalate is a consequence of their propensity to aggregate, which may be related to the hydrophobicity of their sidechains and/or to the possibility that their acridine pKa's may be abnormally low. If the compounds are uncharged at pH 6-7, their affinity for DNA can be expected to be minimal and their potential for aggregation high. Examination of the CPK models of compounds **[5-7]** and **[11-13]** reveals that the acridine N_{10} would be sterically hindered by the carboxamide sidechain if there is free rotation about the C_4-C=O bond, suggesting that the pKa of these compounds may be lower than expected. Table 3 shows the spectral properties of the relevant ligands recorded over the pH range 4 to 7.

Table 3. Effect of pH on Extinction Coefficient of Compounds [5]-[7], [11]-[13]

No.	**ε @ pH 7.0**	**ε @ pH 6.0**	**ε @ pH 5.0**	**ε @ pH 4.0**
	(10^3)	(10^3)	(10^3)	(10^3)
5	7.6	8.2	9.9	13.6
6	7.6	8.1	8.1	8.4
7	7.5	7.4	8.3	12.5
11	4.2	4.4	8.9	8.5
12	3.8	3.8	4.6	5.6
13	4.9	7.0	8.3	12.1

As the pH is lowered, the extinction coefficients increase to values typical of protonated 9-aminoacridine- and 9-anilinoacridine-4-carboxamides. The only exceptions are the cyclododecyl derivatives [**6,12**] whose spectra are little perturbed. These data indicate that with the exception of the cyclododecyl derivatives, the compounds are charged and not aggregated at pH 4. Repeating the DNA-binding studies at this pH confirmed that, again with the exception of the cyclododecyl derivatives, all the ligands bind as judged by the distinctive bathochromic and hypochromic shifts seen in their absorption spectra in the presence of DNA (Table 4).

Table 4[11]. UV/visible Spectral Characteristics at pH 4.0 of compounds [5]-[7] and [11]-[13] free and in the presence of DNA

	Free Drug	Drug + DNA	Free Drug	Drug+DNA
Compound	**λmax/ε @ pH 7.0**	**λmax/ε @ pH 7.0**	**λmax/ε @ pH 4.0**	**λmax/ε @ pH 4.0**
5	448/7.7	453/8.0	442/13.5	454/9.4
6	446/7.6	448/7.7	448/8.4	452/8.0
7	452/7.5	452/7.7	442/12.5	452/9.4
11	406/4.2	420/4.2	406/8.5	420/4.7
12	400/3.8	404/4.5	404/5.6	416/4.8
13	406/4.9	418/5.5	406/12.1	420/5.7

The biological activity of the compounds was measured in the human colon carcinoma cell line HT29/219 and the results are shown in Table 5.

Table 5[12] Biological Activity of Compounds [2]-[13]

Compound	IC_{50} (μM)
2	0.061
3	3.6
4	4.7
5	5.8
6	3.3
7	9.8
8	0.14
9	5.8
10	5.6
11	5.2
12	7.7
13	0.57

In the threading series, the N,N-dimethylaminoethyl compound [**2**] has high activity, its IC_{50} being 2-fold lower than that found for its non-threading analogue [**8**], which is itself a known inhibitor of topoisomerase II[13].

Compounds [3] and [4], which bind to DNA at physiological pH, have much lower activity in this cell line, indicating that these two derivatives are poor topoisomerase II poisons. The inactivity of the cyclopropane derivative is somewhat surprising given that we find the threading N-methyl-4-carboxamide analogue (data not shown) and the N,N-dimethylaminoethyl compound [2] to have IC_{50} values in the range 0.06 to 0.09 μM. Clearly, the steric interactions between topoisomerase II, DNA and the ligand sidechain are subtle and unpredicatable for threading 9-anilinoacridine-4-carboxamides. Since compounds [5] to [7] do not bind to DNA at physiological pH their modest activity may be due to interactions with a different target, perhaps with the cell membrane. The activity of the DNA-binding 9-aminoacridine adamantyl derivative [13] is notable since it is 10-fold more active than its alkane homologues. The reasons for this are unclear.

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9)UV/visible spectra were obtained using a Hewlett Packard Vectra 286/12 at 20°C with 10mm path length length cuvettes. Concentrations of drug (10-20μM to minimise aggregation) were added to SHE buffer (I = 0.1) or to DNA (0.5mM) in SHE buffer (I = 0.1) or to 10 mM SDS in 0.1 SHE buffer. The spectra were run between 300 and 600 nm.
10)Denny, W.A, Wakelin, L.P.G ; *Cancer Res* , **1986** , *46* , 1717
11) These experiments were carried out as per the procedures outlined earlier for pH 7.0, except that in place of 0.1 SHE at pH 7.0, 0.1 sodium acetate buffer was used
12) For each drug treatment the test compound was added to cells at concentrations of 0.01,0.1,1.0 and 10μM. Cells were exposed continually to drug for 72 hours after which time they were trypsinized and counted using a Coulter Counter. IC_{50} is the concentration of drug required for 50% growth inhibition.
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