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Synthesis and biological studies of 1-amino β-carbolines

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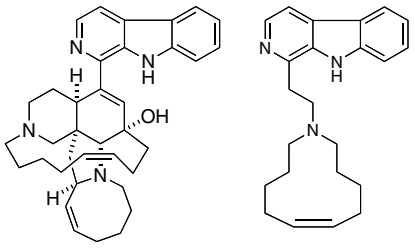
Abstract—A selection of 1-amino-substituted β-carbolines have been prepared by amination of 1-chloro-β-carboline as simple mimics of manzamine A and chloroquine and their intercalating ability, anticancer and antimalarial activity were studied.

There are a considerable number of biologically active alkaloids that contain the β-carboline (pyrido[3,4-*b*]indole) ring system. One such class of compounds are the manzamine alkaloids, first isolated in 1986.[1,2] Manzamine A, **1** (Fig. 1), was found to exhibit significant anticancer activity against P-388 mouse leukaemia (IC_{50} 0.07 μg/mL).[1] More recently, manzamine A has been found to have potent antimalarial activity.[3] Interest in this natural product has continued with reports of its activity as an anti-inflammatory agent,[4] and as an insecticidal and fungicidal compound.[5] Despite the growing number of reports of the activities of the manzamines, and approaches to their synthesis,[6] it is not clear how these compounds exert their biological activity. The structurally related, but simpler manzamine C, **2**, and some analogues have been tested as anticancer agents and the β-carboline unit was shown to play a key role in the cytotoxicity.[7,8] One possibility, at least for simple β-carbolines, is that such compounds intercalate DNA, since it is known that β-carbolines can interact with DNA by GC-selective intercalation.[9] A recent report indicates that cytotoxicity could be due to delayed cell cycle progression.[10]

As part of our interest in manzamine A,[11] we set out to prepare analogues for biological tests. Various routes can be used to access the β-carboline ring system, including the Pictet–Spengler and Bischler–Napieralski type cyclizations. However, we decided to explore the direct amination of the intact 1-chloro-β-carboline **4**, itself prepared readily from tryptamine (**3**) in three steps according to a known procedure (Scheme 1).[12] Amination of 2-chloro-pyridines[13] provides a method to access 2-amino-pyridines, although this chemistry has not, as far as we are aware, been extended to the β-carboline ring system.



1 manzamine A **2** manzamine C

Figure 1. Manzamine A and C.

The chloride **4** was heated with various amines to give the desired products **5**. Successful aminations were achieved by heating the chloride **4** with an excess of the amine without solvent (or with some *N*-methyl pyrrolidin-2-one) for several hours at 170 °C; alternatively, where studied, improved results (higher yields and slightly shorter reaction times) were obtained by microwave-assisted heating (200 W, 170 °C). The products **5a**–**l** were isolated in reasonable to high yield (Fig. 2). The secondary amines required for the formation of the products **5i** and **5l** were prepared by reductive amination of *N*-Boc-piperidin-4-one [using $Ti(O^iPr)_4$ and the amines diethylamine or azacyclooctane then $NaBH_3CN$] and hydrolysis (CF_3CO_2H) of the *N*-Boc group.

Keywords: β-Carboline; Manzamine; Anticancer; Antimalarial.
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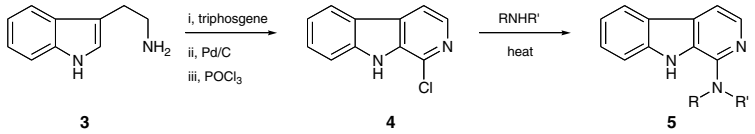

Scheme 1. Preparation of the 1-amino-β-carbolines.

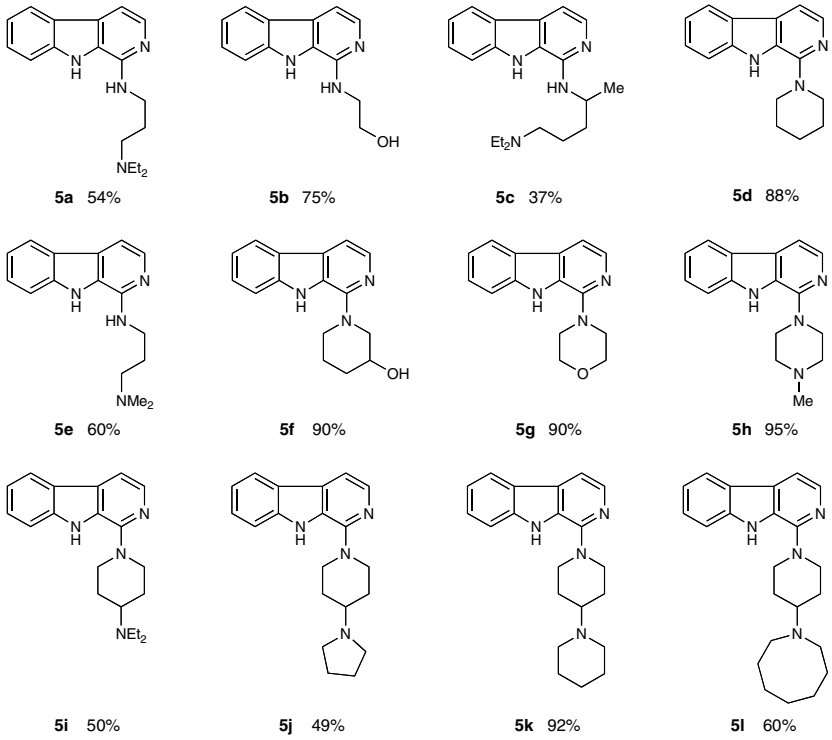

Figure 2. β-Carbolines prepared following Scheme 1 with yields for the final amination step.

The compounds **5a**–**l** (except **5e**, **5j**) were screened for their anticancer activities by the National Cancer Institute (NCI). Three compounds, **5a**, **5c** and **5h** were selected for the NCI 60 cell line panel and showed moderate activities against various cell lines (Table 1). Of these compounds, the β-carboline **5a** showed the best activities, with, for example, 50% growth inhibition, GI_{50} 0.38 μM against HOP-92 nonsmall cell lung cancer. The NCI has screened the natural product manzamine A **1** (NSC 670851) and this compound has an average GI_{50} 0.33 μM over all cell lines (with GI_{50} 0.25 μM against HOP-92 nonsmall cell lung cancer). These data indicate, therefore, that the complex polycyclic ring system in manzamine A can be substituted with simpler analogues to provide active compounds.

The β-carbolines **5a**–**l** were screened against the parasites *T. cruzi* (tulahuen C4 strain), *P. falciparum* (K1 strain), *L. donovani* (MHOM-ET-67/L84 strain) and *T.b. rhodesiense* (STIB 900 strain) by the World Health Organization (WHO) and the results are given in Table 2. Significant activity was obtained against all these parasites except *L. donovani*, although the compounds showed evidence of cytotoxicity (as determined by comparison with the standard podophyllotoxin against rat skeletal myoblast L6 cell line).

To probe one possible mechanism of action of these compounds, viscosity studies with the β-carboline **5h** and the standard agents norharman and harmol were carried out using calf thymus DNA (CT-DNA).[14] A plot

Table 1. Selected cell lines and anticancer activities of compounds **5a**, **5c** and **5h**

	GI_{50} (μM)						
	Leukaemia K-562	Nonsmall cell lung cancer HOP-92	Colon HT29	Melanoma M14	Melanoma UACC-62	Ovarian OVCAR-3	Breast MDA-MB-231/ATCC
5a	3.2	0.38	2.7	2.0	2.3	1.6	5.2
5c	12.2	4.5	22.9	16.4	24.0	22.8	—
5h	22.5	18.6	—	14.9	17.8	19.9	3.9

Table 2. Antiparasitic activities of compounds **5a–l**

Compound	IC_{50} μg/mL			
	T. cruzi	*P. falciparum*	*L. donovani*	*T.b. rhodesiense*
5a	1.62	0.64	>90	0.18
5b	4.25	2.4	>90	1.8
5c	3	0.42	>90	0.13
5d	>30	1.2	>90	28.1
5e	4.2	0.45	>90	0.09
5f	19.35	3.4	>90	8.9
5g	25.1	4.59	>90	18.2
5h	4.3	0.62	>90	0.26
5i	5.8	0.41	>90	0.63
5j	6.1	0.45	>90	0.52
5k	2.35	0.39	>90	1.8
5l	6.5	0.35	>90	0.61

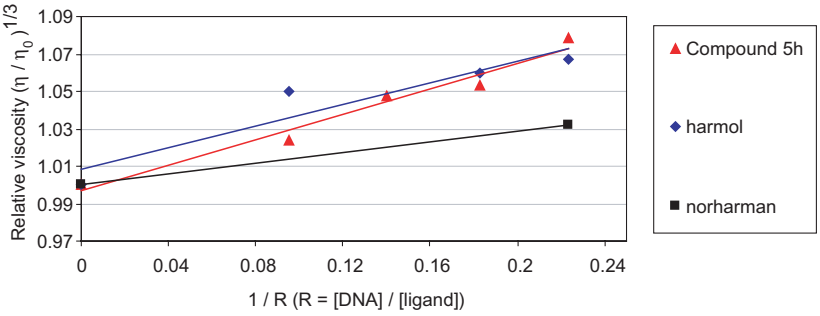

Figure 3. Relative viscosity of CT-DNA on increasing molar ratio of ligand/CT-DNA at 27 °C.

of the relative viscosity for the CT-DNA with each of these compounds is shown in Figure 3. Relative viscosity increases with increasing molar ratio of ligand to CT-DNA, indicating that the DNA helix lengthens as base pairs are separated to accommodate the ligand. These results suggest that β-carboline **5h** (which shows similar results to the known intercalator harmol) binds to CT-DNA by intercalation.[9]

As a further study, the β-carboline **5h** was evaluated for its ability to affect the thermal denaturation of DNA.[15] The denaturation temperature of DNA is dependent on any interaction with drugs and provides a simple means to give evidence for DNA binding and its relative strength. A plot of the change in the relative DNA melting temperature with increasing concentration of the β-carboline **5h** is given in Figure 4. The measurements were performed in buffered solution at pH 6 using 50 μM CT-DNA; the β-carboline **5h** was dissolved in DMSO and the thermal stabilities are in comparison with CT-DNA with the same relative proportion of DMSO (CT-DNA T_m 67.7 °C with no **5h**, 0.076% DMSO). The data show that the melting temperature increases with increasing concentration of **5h**, showing that this compound stabilizes the double helix of CT-DNA.

In summary, we have described an efficient synthesis of a range of 1-amino-β-carbolines, which are simple analogues of manzamine A and chloroquine. These compounds show significant activity as anticancer and antiparasitic agents. There is evidence that such compounds can intercalate DNA. It can be envisaged that the β-carboline ring system, having a planar aromatic

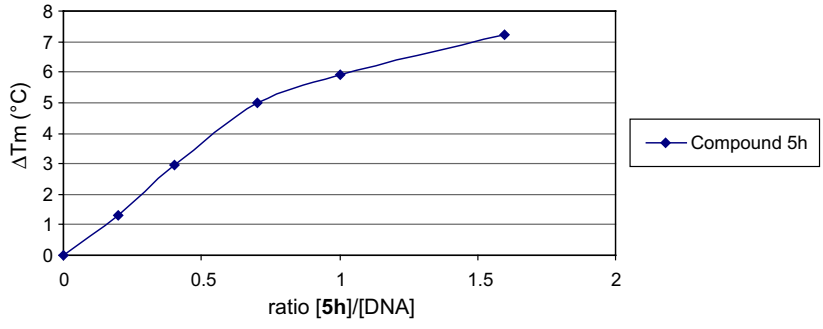

Figure 4. Change in melting temperature of CT-DNA on increasing molar ratio of **5h**/CT-DNA.

structure, could stack in the base pairs of DNA and such intercalation could contribute to the biological activity, particularly as an anticancer agent. Further experiments to quantify the binding are in progress.

Acknowledgements

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