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Antitrypanosomal naphthylisoquinoline alkaloids and related compounds

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In view of the need to develop new drugs against human African trypanosomiasis, a series of naturally occurring naphthylisoquinoline alkaloids, axially chiral acetogenic products derived from tropical plants, have been investigated for their activity against *Trypanosoma brucei brucei* TC 221. Likewise compounds corresponding to the two molecular portions, the naphthalene and the isoquinoline parts were tested, as well as molecules related to the central biaryl core of the alkaloids. Among all compounds tested, the natural, genuine alkaloids themselves, in particular dioncophylline B with its biaryl system and a moderate number of free hydroxy functions, showed the highest activities. Our results demonstrate that naphthylisoquinoline alkaloids constitute an interesting novel class of antitrypanosomal compounds worth further optimization.

1. Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is one of Africa's old plagues [1]. While this disease was effectively controlled during late colonial times, the continent has seen a resurgence of HAT at an epidemic scale during the past three decades. Today some 500,000 people are considered to be infected with the parasite *Trypanosoma brucei,* and will head for a certain, gruesome death if left untreated [2, 3].

"Modern" medical treatment of HAT is based on only five drugs: pentamidine, suramin, melarsoprol, nifurtimox, and eflornithine [4]. None of these is younger than 20 years, and all have severe, often life-threatening side-effects [5]. Moreover, resistance of the parasite is spreading in Africa to an extent that in many places, patients with HAT can no longer be treated effectively [6, 7]. Consequently, there is an urgent need to broaden the therapeutic arsenal in the fight against this dreadful plague. New compounds with different modes of action against the parasite are required which are affordable to the health system of low income countries, better tolerated by the patients, and effective against resistant strains of the parasite [8].

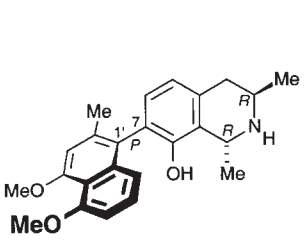
We have recently isolated new naphthylisoquinoline alkaloids from tropical lianas belonging to the Ancistrocladaceae and Dioncophyllaceae families [9, 10], some of which displayed quite promising *in vitro* activities against *Trypanosoma brucei* [11–14]. This prompted us to build up a library of related natural and synthetic analogs for antitrypanosomal testing in order to find a lead structure for further optimization. In this paper, we report, in a preliminary form, on the results of these investigations.

2. Investigations, results and discussion

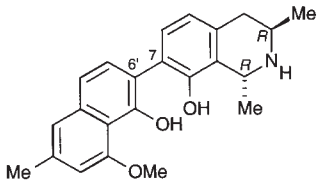
The results of this first screening are summarized in the Table 1. Dioncophylline A (**1**), one of the best-available naphthylisoquinoline alkaloids both, from Westafrican *Triphyophyllum peltatum* plants [15] and from total synthetic work [16], showed good activities, straightaway ($EC_{50} = 3.4$ nmol/ml), well in the range of those of established standards like suramine-Na (0.3 nmol/ml) and eflornithine-HCl (22.9 nmol/ml) (Table 1). Similar results were obtained with dioncophylline B (**2**) [17] (2.6 nmol/ml), and also with dioncophylline C (**3**) [18], which has a different molecular shape and a likewise good activity (4.2 nmol/ml). Of particular interest is the fact that dioncopeltine A (**4**) [19], with its additional oxygen function on the methyl group of the naphthalene portion, has a distinctly lower activity, nearly by an order of magnitude (22.2 nmol/ml).

In a first attempt to learn more about the required molecular prerequisites for good activities and to hopefully find even more active molecules with simplified structures, we have next tested compounds related to the molecular 'halves' of the alkaloids **1**–**4**. The natural tetraline *trans*-isoshinalolone (**5**) [20, 21], which is structurally and biosynthetically closely related to the naphthalene part of the above presented alkaloids, however, showed no activity (> 65 nmol/ml). Its oxidized form, the well-known [22, 23] naphthoquinone plumbagin (**6**), by contrast, again showed a very high activity (6.5 nmol/ml), but this might rather be the consequence of its quinoid character, by which it also differs from the naphthylisoquinoline alkaloids **1**–**4**, possibly hinting at a different mode of action.

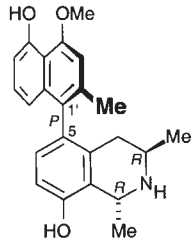
The naphthalene-free tetrahydroisoquinoline derivative **7**, which is closely related to the (likewise 1*R*,3*R*-configured)



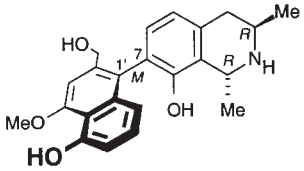
Dioncophylline A (**1**)



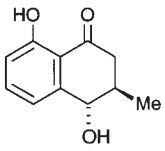
Dioncophylline B (**2**)



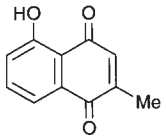
Dioncophylline C (**3**)



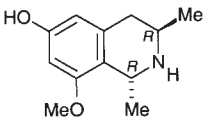
Dioncopeltine A (**4**)



rac-trans-Isoshinanolone (**5**)



Plumbagine (**6**)



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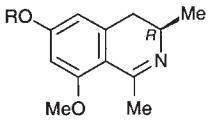
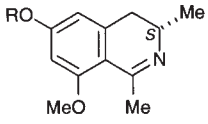
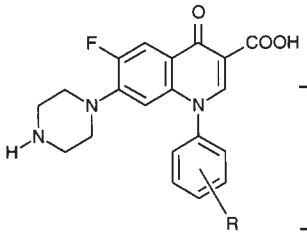


8	R
a	H
b	Me

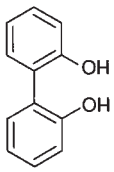


ent-**8**	R
a	H
b	Me



9	R
a	H
b	*p*-OMe
c	*p*-F
d	*m*-OH
e	*p*-OH

9a-e



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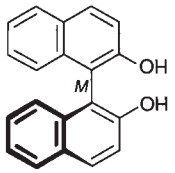
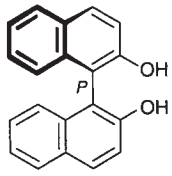
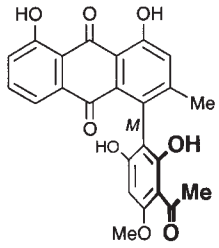


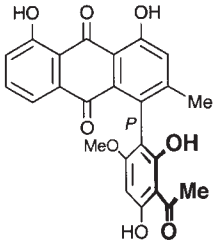
'(*R*)'-Binaphthol (*M*-**11**)



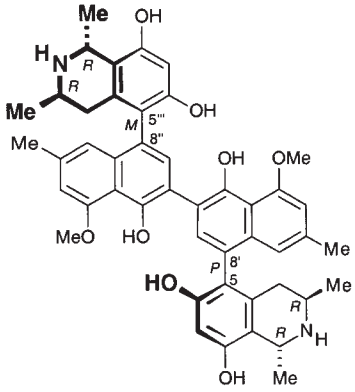
'(*S*)'-Binaphthol (*P*-**11**)



Knipholone (**12a**)



Bulbine-Knipholone (**12b**)



Michellamine B (**13**)

heterocyclic portions of the alkaloids **1**–**4** and available from synthetic work in our group [9, 24], was found to be inactive, as was the corresponding dihydroisoquinoline **8a**, while its *O*-methylated (and thus non-phenolic) analog **8b** exhibited a week, but significant activity (56.1 nmol/ml). In order to investigate the potential influence of the absolute configuration at the stereogenic center, we also investigated the enantiomers of the latter two compounds, which again revealed the influence of a phenolic OH group in that *ent*-**8a** was again inactive, while *ent*-**8b** showed some activity, this time even slightly better (21.7 nmol/ml) than for the *R*-enantiomer, **8b**. Thus, even naphthalene-free isoquinoline derivates like *ent*-**8b** – and hence clearly simplified 'analogs' of the naphthylisoquinoline alkaloids – can give significant antitrypanosomal activities, although somewhat lower as compared the authentic natural products **1**–**3**, but already similar to that of **4**. For this reason and in view of the close structural similarity of isoquinoline and quinoline derivatives and with respect to the high antitrypanosomal activity of the new 4-quinolone alkaloid antidesmone recently discovered [25], we likewise tested a small series of N1-phenyl substituted 4-quinolones **9a**–**e** [26]. Two of them, **9a** with the unsubstituted phenyl ring in position N1, and **9b,** bearing a *p*-methoxy group on that phenyl ring, exhibited good to moderate activities nearly comparable to those of the dioncophyllines **1**–**3.** (10.6 and 8.8 nmol/ml, respectively). However, they are significantly lower than, e.g., their tuberculostatic activities discovered earlier [27]. By contrast, 4-quinolones of high tuberculostatic activity, such as **9c**, **d**, and **e** exhibit only a moderate (**9c**: 30.4 nmol/l) or no antitrypanosomal potency. Of particular interest and significance for further structure-activity relationship investigations is the fact that the latter two compounds, **9d** and **9e,** both equipped with free phenolic OH groups, showed no activity at all (> 65 nmol/µl), which is in agreement with

Table: Results of the antitrypanosomal testing after 48 h

Compd.	Solvent	ED_{50} (nmol/ml)	MIC (nmol/ml)
Suramine-Na	H_2O	0.3	1
Eflornithine-HCl	H_2O	22.9	>65
1	DMSO	3.4	7.7
2	DMSO	2.6	7.6
3	DMSO	4.2	6.3
4	DMSO	22.2	62.2
5	DMSO	>65	>65
6	DMSO	6.5	>65
7	DMSO	>65	>65
8a	DMSO	>65	>65
8b	DMSO	56.1	>65
ent-**8a**	DMSO	>65	>65
ent-**8b**	DMSO	21.7	>65
9a	0.1 M NaOH	10.6	>65
9b	0.1 M NaOH	8.8	>65
9c	0.1 M NaOH	30.4	>65
9d	0.1 M NaOH	>65	>65
9e	0.1 M NaOH	>65	>65
10	DMSO	>65	>65
(P)-**11**	DMSO	33.1	>65
(M)-**11**	DMSO	8.1	>65
12a	DMSO	36.7	>65
12b	DMSO	23.8	>65
13	DMSO	>65	>65

the above stated decrease of activity with the presence of an additional OH group.

Since neither a naphthalene- nor an isoquinoline-related molecular half alone gave high activities (except for the quinone plumbagin, **6**) the questions was whether simplified active analogs might result from the inner biaryl core of the naphthylisoquinoline molecule. While, however, the central part of **2**, viz commercially available 2,2′-biphenol (**10**), was inactive (>65 nmol/ml), spatially larger analogs of this – indeed highly simplified – core molecule proved to be more active, such as e.g., the still simple (again commercially available) compound binaphthol (**11**), which gave quite high activities, most remarkably with distinctly different values for the two atropo-enantiomers (i.e. isomers arising from the hindered rotation at the central biaryl axis): thus, the moderate activity of *S*-binaphthol (*P*-**11**) (33.1 nmol/ml) is exceeded by that of the *R*-enantiomer (*M*-**11**) (8.1 nmol/ml), showing the importance of chirality not only for stereogenic centers, but also for chiral axes.

Moderate activities were found for (likewise axially chiral) phenylanthraquinones from African Asphodelaceae plants, like knipholone (**12a**) [28] (36.7 nmol/ml) and its regioisomer, bulbine-knipholone (**12b**) [29] (23.8 nmol/ml), again quinoid compounds.

Among the compounds tested so far, the most active ones, the naphthylisoquinoline alkaloids **1**–**3**, all bear joint structural elements like the free secondary amino function, one or two phenolic hydroxy groups – and the biaryl axis. It was thus of interest to test the activity of *dimeric* naphthylisoquinolines, among them the well-known anti-HIV michellamine B (**13**, tested as the diacetate [30], which consists of two identical naphthylisoquinoline portions and thus even possesses three such axes. This compound, however, which is likewise synthetically available [31], was found to be entirely inactive – possibly again a consequence of the now (due to the dimerization) very high number of even six free hydroxy functions, in agreement with the above presented experience (**4** vs. **1**–**3**, **9a**–**c** vs. **9d**, **e**) – and with Lipinski's 'Rule of the Five' [32].

Thus, the genuine, intact naphthylisoquinoline alkaloids **1**–**3**, in particular dioncophylline B (**2**), remain the most active compounds, so that it is now rewarding to investigate further representatives of this interesting class of natural products, to test their cytotoxicity towards mammalian cells, and, for the best compounds, to investigate their activity also *in vivo*. This work is in progress.

3. Experimental

3.1. Substances

Except for **10**, *P*-**11**, and *M*-**11**, which are commercially available (Aldrich), the compounds were prepared by isolation from natural sources or by chemical synthesis according to the references given in the text [9, 15–19, 21, 23, 24, 26, 28–31].

Suramine-Na was obtained from the commercial producer Bayer, eflornithine-HCl through the World Health Organisation.

3.2. Parasite culture

Trypomastigote forms of *Trypanosoma brucei brucei* laboratory strain TC 221 were cultured in Baltz medium according to standard conditions [33].

3.3. In vitro cytotoxicity assays

The test compounds were dissolved in DMSO or 0.1 M NaOH (Table). A defined number of parasites (10^4 trypanosomes per ml) were exposed in test chambers of 96-well plates to various concentrations of the test substances in a final volume of 200 µl. Positive (trypanosomes in culture medium) and negative controls (test substance without trypanosomes) were run with each plate.

The plates were then incubated at 37 °C in an atmosphere of 5% CO_2 for a total time period of 72 h. A reading was done at 48 and 72 h. The effect of test substances was quantified in ED_{50} values by linear interpolation [34] of three different measurements. The activity of the test substances was measured by light absorption in an MR 700 Microplate Reader at a wave length of 550 nm with a reference wave length of 630 nm, using the Alamar Blue® assay as previously described [35].

Because of small changes in the absorption, the minimum inhibitory concentration (MIC) values of the test substances were determined by microscopical inspection of the test chambers for the presence of trypanosomes after 48 and 72 h.

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