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Antiprotozoal activity of synthetic amino substituted 1-methyl-1*H*-α-carbolines

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Received June 21, 2013, accepted July 26, 2013

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Pharmazie 69: 83–85 (2014) *doi: 10.1691/ph.2014.3748*

The antiprotozoal properties of a series of amino substituted 1-methyl-1*H*-α-carbolines were investigated in a broad panel of parasites. Various substituents were systematically introduced at various positions on the carbocyclic ring of the parent 1-methyl-1*H*-α-carboline. Most compounds showed a potent antiprotozoal activity, although mostly accompanied by cytotoxicity on MRC-5 cells. One compound, containing the same amino-substitution as chloroquine, showed an IC_{50} against *Plasmodium falciparum* of 2.37 μM and was reasonably selective.

1. Introduction

Malaria is still a very common disease in many tropical and subtropical countries, especially in sub-Saharan Africa. According to the World Health Organization (WHO 2011), 3.3 billion people were at risk of malaria in 2010. Recrudescence of malaria is due to increasing resistance of the vector, the *Anopheles* mosquito, to insecticides, and of the parasite, mainly *Plasmodium falciparum*, to drugs (Gardella et al. 2008). Even resistance to artemisinin has recently been reported in Asia (Egan 2009). Therefore there is a continued need for new drugs against malaria. In the search for new antiprotozoal agent, the isolation of lead compounds from medicinal plants is one of the various strategies that can be followed (Xu et al. 2011). The indoloquinoline alkaloid neocryptolepine has been obtained from the African plant *Cryptolepis sanguinolenta*, the major alkaloid of which is cryptolepine, and synthetic derivatives have been investigated as antiplasmodial agent (Jonckers et al. 2002). The most promising compound obtained in this series was N^1,N^1-diethyl-N^4-(5-methyl-5*H*-indolo[2,3-*b*]quinolin-8-yl)pentane-1,4-diamine, i.e. neocryptolepine substituted in position 8 with the same substituent that is present in the well-known antimalarial compound chloroquine. This compound showed an IC_{50} of 0.01 μM against *Plasmodium falciparum* (Ghana strain) with a selectivity index (SI) of 1800 compared to MRC-5 cells (El Sayed et al. 2009). Also the tricyclic analogues of various indoloquinoline alkaloids have been investigated. In this series, 2-methyl-β-carboline or 2-methyl-2*H*-pyrido[3,4-*b*]indole showed the highest selectivity (IC_{50} 0.45 μM, SI > 1000 relative to L6 cells (Van Baelen et al. 2009). The α-carboline 1-methyl-1*H*-pyrido[2,3-*b*]indole displayed an IC_{50} of only 13.0 μM, with a SI of 18. Continuing our attempts to obtain highly selective antiplasmodial agents, and based on previous observations, it was expected that introduction of amino substituents on the C-ring of 1-methyl-1*H*-α-carboline could lead to potent antiplasmodial compounds with high selectivity and low cytotoxicity.

2. Investigations, results and discussion

The present study was undertaken to investigate the antiprotozoal properties of a series of amino substituted 1-methyl-1*H*-α-carbolines. The chloroquine-like diethylaminoalkyl side chain and various related substituents were systematically introduced at different positions on the carbocyclic ring of the parent 1-methyl-1*H*-α-carboline (Fig.) (Yadav et al. 2013).

The antiprotozoal activity in a broad panel of parasites of these amino substituted 1-methyl-1*H*-α-carbolines is shown in the Table. The most obvious observation is that the morpholino substituent had a dramatically negative effect on the biological activity: all three derivatives **1**, **2** and **3** were almost completely inactive. Focusing on the antiplasmodial activity, almost all other compounds are active against *P. falciparum*, although only one product (**8**), the 7-substituted dialkylamino-derivative, displayed an $IC_{50} < 1$ μM. Also the 6- and 7-substituted analogues (compounds **7** and **9**, respectively) had an $IC_{50} < 2$ μM. Nevertheless, the same compounds also exhibited activity in the same order of magnitude against the other parasites tested, and against the MRC-5 cell line, indicating a low selectivity. Compound **9** seemed to be the relatively most selective one in this group of three, with an SI of 11 (CC_{50} / IC_{50} *Pf*). In fact almost none of the compounds tested was selective active against any of the parasites of the screening panel. A notable exception was compound **15**, where the chloroquine-like side chain is present in position 8. It showed an IC_{50} against *P. falciparum* of 2.37 μM, which is rather moderate, but at least all other IC_{50}'s were > 10 μM, and the CC_{50} on MRC-5 cells was 48.30 μM, leading to an SI of 20 (CC_{50} / IC_{50} Pf).

Whereas the ß-carboline skeleton, biogenetically derived from tryptophan, is widely distributed in nature, α-carboline deriva-

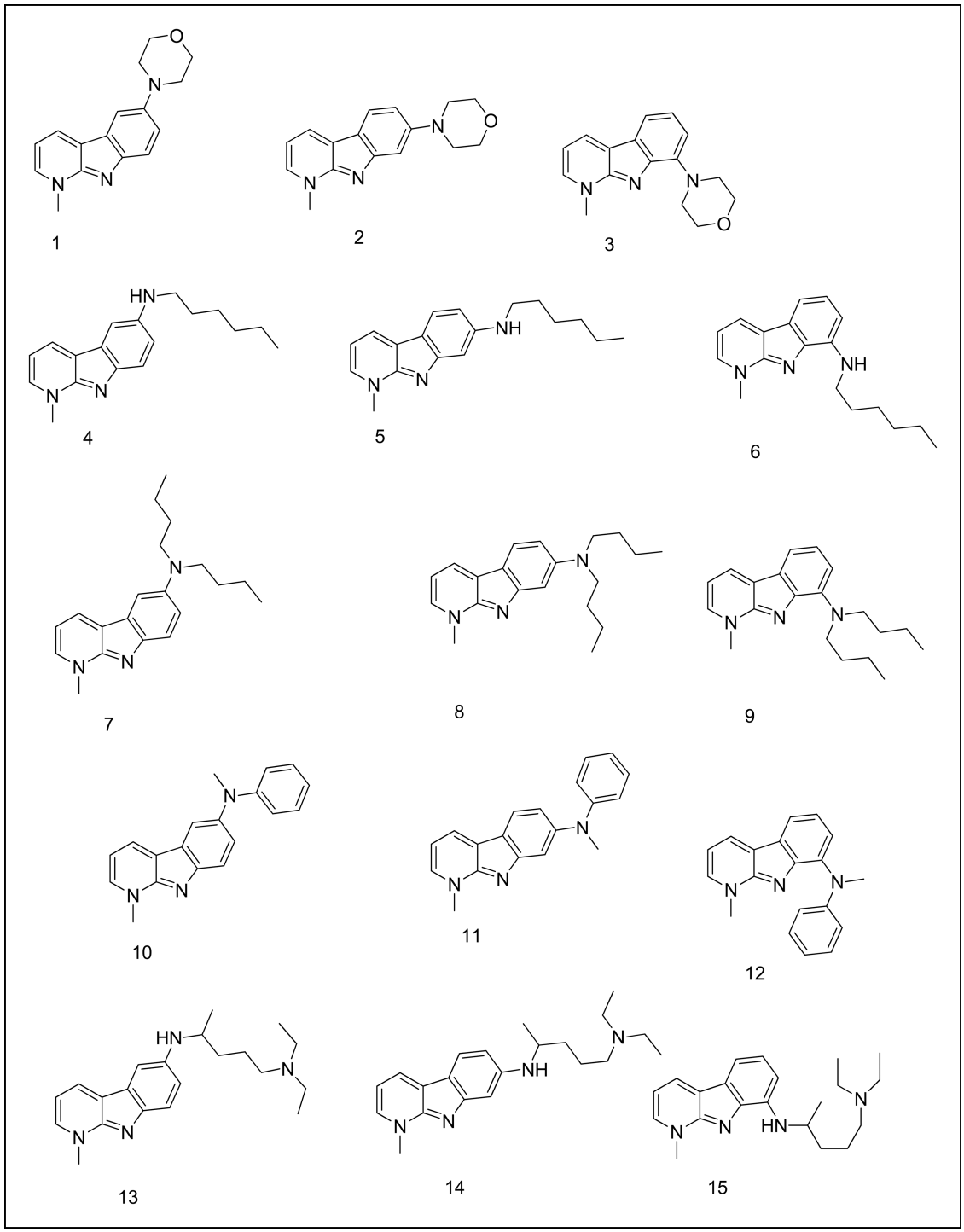

Fig.: Structure of the synthetic amino substituted 1-methyl-1*H*-α-carbolines **1**–**15**.

tives are relatively rare. Apart from neocryptolepine and related compounds, only few α-carboline alkaloids have been reported. Nevertheless, important biological activities appear to be related to this bicyclic ring system. Grossularine-1 and –2 were obtained from the tunicate *Dendrodoa grossularia*, and exhibited a cytotoxic activity on L1210 leukemia cells in culture, with IC_{50} values of 10 μg/ml and 3.5 μg/ml, respectively. Grossularine-2 exhibited DNA intercalative properties, which may be explained by its quasi-planar structure. Grossularine-1 on the other hand showed a non-intercalative DNA-binding, explained by the presence of the bulky indole chain (Helbecque 1987). *N,N*-Didemethyl grossularine-1 was isolated from the tunicate *Polycarpa aurata* (Abas 1996), and mescengricin, a substance of microbial origin, from *Streptomyces griseoflavus*. Mescengricin demonstrated neuronal cell protecting properties (Kim 1997). Isogranulatimide is an alkaloid from the ascidian *Didemnum granulatum*, and was found to be a G2 specific cell cycle checkpoint inhibitor (Roberge et al. 1998). Variolin B from the Antarctic sponge *Kirkpatrickia varialosa* was a cyclin-dependent kinase inhibitor and an antineoplastic agent (Perry et al. 1994; Simone et al. 2005).

In spite of these relatively modest results obtained for the synthetic α-carbolines in this study, the various tricyclic analogues of the different types of indoloquinoline alkaloids remain promising leads. Okanya et al. (2011) have obtained a series of pyrroloquinolines, i.e. marinoquinolines A - F, from the gliding bacterium *Ohtaekwangia kribbensis* (Bacteroidetes). The core alkaloid part of the marinoquinolines is 3*H*-pyrrolo[2,3-

Table: Antiprotozoal activity and cytotoxicity of compounds 1–15 and the reference compounds cryptolepine and neocryptolepine (IC_{50} and CC_{50}, respectively, μM, mean and standard deviation SD).

Compd.	MRC-5		T-cruz		L.inf		T.bruc		Pf-K1	
	IC_{50}	SD	IC_{50}	SD	IC_{50}	SD	IC_{50}	SD	IC_{50}	SD
1	> 64		> 64		> 64		> 64		> 64	
2	> 64		> 64		> 64		32.93	1.24	44.21	18.53
3	> 64		> 64		> 64		> 64		43.93	15.46
4	17.47	12.85	2.18	2.40	2.40	0.54	4.49	2.80	3.29	3.53
5	10.30	8.25	5.44	0.63	5.04	3.66	2.00	0.25	1.67	0.82
6	4.58	0.49	2.69	1.49	6.71	1.90	2.06	0.06	1.21	0.61
7	6.29	5.63	1.96	0.17	4.73	3.91	2.04	0.03	1.88	2.61
8	4.62	3.47	2.11	0.14	1.63	1.21	0.47	0.04	0.93	0.49
9	16.81	12.41	2.31	0.63	2.87	3.60	3.45	2.33	1.50	0.60
10	27.81	21.12	2.07	0.43	12.00	10.87	8.06	0.19	5.25	2.28
11	11.69	10.18	5.40	2.60	7.53	1.65	3.03	1.85	2.20	0.33
12	40.68	27.19	28.89	1.47	17.31	11.57	28.80	4.60	4.00	2.85
13	46.00	20.79	33.75	3.65	48.23	18.21	32.34	0.30	8.15	3.58
14	45.76	21.09	> 64		48.23	18.21	32.70	0.28	19.48	11.60
15	48.30	18.13	12.07	5.25	15.48	11.51	26.66	12.28	2.37	0.27
Neocryptolepine	7.36	2.86	5.73	2.03	7.79	0.65	2.07	0.04	7.73	3.22
Cryptolepine	3.30	0.68	1.96	0.70	1.84	0.38	2.06	0.02	1.66	0.81

T. cruz.: *Trypanosoma cruzi*; *L. inf.*: *Leishmania infantum*; *T. bruc.*: *Trypanosoma brucei*; Pf-K1: *Plasmodium falciparum* strain K1.

c]quinolone, the IC_{50} against *P. falciparum* of which was reported as 6.4 μM in our previous study (Van Baelen et al. 2009). Substitution of this core structure as in marinoquinolines B and F was able to reduce the IC_{50} down to 1.8 and 1.7 μM, respectively, although still accompanied by a relatively important cytotoxicity against various cell lines (CC_{50} values < 10 μM).

In conclusion, although the aim to prepare highly active and selective antiplasmodial compounds could not be achieved, the high potential of the 1-methyl-1*H*-α-carboline skeleton could be confirmed. The introduction of other substituents leading to more active and selective compounds should still be further investigated.

3. Experimental

A series of 15 amino substituted 1-methyl-1*H*-α-carbolines was prepared as reported before (Yadav et al. 2013). Structures (**1**–**15**) are shown in the Fig. Antiprotozoal activity was evaluated in a panel of parasites including *Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania infantum*, *Plasmodium falciparum* K1, and a cytotoxicity control consisting of MRC-5 cells, as described before (El Sayed et al. 2009). Benznidazol, suramine, miltefosin, chloroquine and tamoxifen, respectively, were included as positive controls. The original lead compounds neocryptolepine and cryptolepine were included for comparison. Antiprotozoal results are displayed in μM as $IC_{50} \pm SD$, and cytotoxicity as $CC_{50} \pm SD$. All values were calculated as the mean of 4 independent experiments.

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