Bioorganic & Medicinal Chemistry 24 (2016) 3636–3642

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Bistacrine derivatives as new potent antimalarials

Ines Schmidt [a], Gabriele Pradel [b], Ludmilla Sologub [b], Alexandra Golzmann [b], Che J. Ngwa [b], Anna Kucharski [a], Tanja Schirmeister [c], Ulrike Holzgrabe [a,*]

[a] Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
[b] Institute of Molecular Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
[c] Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55099 Mainz, Germany

ARTICLE INFO

Article history:
Received 15 February 2016
Revised 30 May 2016
Accepted 2 June 2016
Available online 4 June 2016

Keywords:
Bistacrines
Antimalarials
Falcipain-2 inhibitors

ABSTRACT

Linking two tacrine molecules results in a tremendous increase of activity against *Plasmodia* in comparison to the monomer. This finding prompted the synthesis of a library of monomeric and dimeric tacrine derivatives in order to derive structure–activity relationships. The most active compounds towards chloroquine sensitive *Plasmodium* strain 3D7 and chloroquine resistant strain Dd2 show IC_{50} values in the nanomolar range of concentration, low cytotoxicity and target the cysteine protease falcipain-2, which is essential for parasite growth.

1. Introduction

In 2013 the World Health Organization estimated 198 million cases of malaria, whereof 584,000 were fatal.[1] The causative pathogens of this disease are four species of the genus *Plasmodium*, i.e., *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, all of them being sporozoic parasites. Malaria—still remaining one of the most deadly diseases—occurs mainly in Africa, where 78% of the deaths apply to children under the age of five.[2] However, the disease also appears in South-East Asia and South America. Nearly 50% of the world population lives in an area of risk. Due to the small number of efficient therapeutics and the increasing rate of resistance the search for new drugs against malaria is urgently needed.

Tacrine **1a** (1,2,3,4-tetrahydroacridine, THA, Fig. 1), which is known as a reversible acetylcholinesterase inhibitor, was in clinical use for the treatment of Alzheimer's disease. It is a common approach to expand the application of drugs that have already been evaluated as drug leads for other diseases.[3–5] Interestingly, in an antiprotozoal screening tacrine was found to exhibit antimalarial activity (IC_{50} = 12.5 μM against chloroquine sensitive strain 3D7). Furthermore, the linking of two tacrine moieties with an alkyl chain of varying length resulted in a tremendous increase of the activity.

These preliminary results prompted the synthesis of a library of monomeric and dimeric tacrine derivatives with different substitution pattern and tether length, and evaluation of the antimalarial activity. Inhibition of β-hematin formation, which is the assumed mechanism of action of chloroquine, was tested because of the similarity of the aminoquinoline moiety of chloroquine and tacrine.[6] Additionally, the inhibition of the cysteine protease falcipain-2 (FP2), which is essential for parasite survival by playing a key role in host hemoglobin degradation, was investigated.

2. Results and discussion

2.1. Chemistry

Monomeric tacrine derivatives were prepared as reported for the unsubstituted compound by means of condensation and subsequent cyclization of 2-aminobenzonitriles and cyclohexanones in toluene with catalytic amounts of *p*-toluene sulfonic acid (Scheme 1).[7] All compounds **1a**–**1f** were obtained in moderate yields after recrystallisation from acetone.

The synthesis of dimers using tacrine derivatives and 1,ω-dibromoalkanes as alkylating reagents failed due to side reactions in addition to low reproducibility. Thus, the dimerisation reaction started off from 9-chloro-1,2,3,4-tetrahydroacridine derivatives, which were obtained by condensation of equimolar amounts of the appropriate 2-aminobenzoic acids with cyclohexanones using an excess of $POCl_3$ (Scheme 2).[8] The excess of $POCl_3$ and high temperatures are necessary to prevent the formation of 4-quinolinones.

The achieved 9-chloro-intermediates (**2a**–**2j**) were heated under reflux with 0.5 equiv of 1,ω-diaminoalkanes for several

* Corresponding author. Tel.: +49 (0) 931/31 85461; fax: +49 (0) 931/31 85494.
E-mail address: ulrike.holzgrabe@uni-wuerzburg.de (U. Holzgrabe).

http://dx.doi.org/10.1016/j.bmc.2016.06.003
0968-0896/

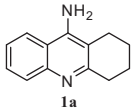


Figure 1. Tacrine (1,2,3,4-tetrahydroacridine, THA).

hours in phenol under an argon atmosphere. Dimeric tacrine compounds **3a**–**3i**, **4a**–**4g**, **5a** and **5b** were obtained in mostly good yields.

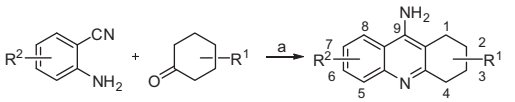
2.2. Biology

2.2.1. In vitro activity against *Plasmodium falciparum*

The antimalarial activity and cytotoxicity of monomeric compounds **1a**–**1f**, unsubstituted dimeric compounds **3a**–**3i** and substituted dimeric compounds **4a**–**4g**, **5a** and **5b** were determined in vitro against *Plasmodium falciparum* chloroquine sensitive strain 3D7, and against J774.1 macrophages by means of the Malstat[9] and the AlamarBlue® assay,[10] respectively. Additionally, the unsubstituted dimeric compounds **3a**–**3i** as well as **5a** and **b** were tested against the chloroquine resistant strain Dd2.

For the monomeric compounds **1a**–**1f**, the following structure–activity relationships could be derived: Introduction of a weak electron-donating propyl group in position 2 leads to an increase of activity to a low, single-digit micromolar IC_{50} value (Table 1).

Besides, exchanging the aromatic proton in position 7 by a halogen substituent as chlorine results in a further significant increase of activity. Of note, electron-withdrawing substituents such as a nitro group as well as electron-donating groups, e.g., methyl groups, in these positions show similar effects. Therefore, a single substitution in position 7 increases activity independently of the electronical structure of the introduced substituent, whereas the combination of chlorine in position 7 and propyl in position 2 is superior. Thus, 7-chloro-2-propyl-1,2,3,4-tetrahydroacridine **1d** exhibited the highest antimalarial activity (IC_{50} = 0.21 μM) (Table 1).



1a: R^1 = H, R^2 = H
1b: R^1 = 2-propyl, R^2 = H
1c: R^1 = H, R^2 = 7-Cl
1d: R^1 = 2-propyl, R^2 = 7-Cl
1e: R^1 = H, R^2 = 7-NO_2
1f: R^1 = H, R^2 = 7-Me

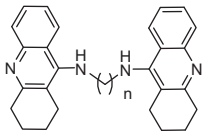
Scheme 1. Reagents and conditions: (a) *p*-TsOH, toluene, 30–192 h, reflux. The description of positions for R^1 and R^2 is outlined.

Table 1
Increasing activities and cytotoxicities in relation to substitution

Compound	R^1	R^2	Yield [%]	IC_{50} (3D7) [μM]	J774.1 [μM]
1a	H	H	68	12.50 ± 4.65	>100
1b	2-Propyl	H	40	1.52 ± 0.28	43.0
1c	H	7-Cl	50	4.02 ± 1.87	43.2
1d	2-Propyl	7-Cl	24	0.21 ± 0.06	8.40
1e	H	7-NO_2	10	3.23 ± 2.73	44.6
1f	H	7-Me	31	2.04 ± 0.03	44.3

Chloroquine: IC_{50} (3D7) = 20 nM, IC_{50} (J774.1) = 18.4 μM.

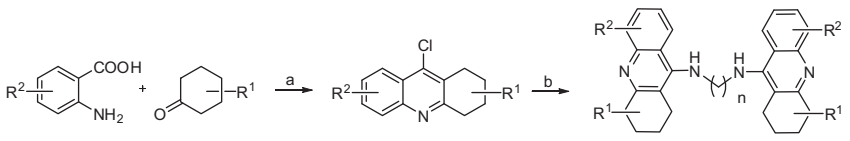
Table 2
In vitro activities against *Plasmodium falciparum* strains 3D7 and Dd2



Compound	*n*	IC_{50} (3D7) [μM]	IC_{50} (Dd2) [μM]	J774.1 [μM]	SI[a/b]
3a	2	0.40 ± 0.07	6.11 ± 2.90	45.2	113 (7.40)
3b	3	0.49 ± 0.16	3.99 ± 3.30	42.6	86.9 (10.7)
3c	4	0.17 ± 0.02	1.39 ± 0.38	8.30	48.8 (5.97)
3d	5	0.63 ± 0.18	0.55 ± 0.09	1.80	2.86 (3.27)
3e	6	0.10 ± 0.03	n.d.	2.97	29.7
3f	7	0.07 ± 0.01	0.22 ± 0.16	1.90	27.1 (8.64)
3g	8	0.11 ± 0.01	0.30 ± 0.02	1.90	17.3 (6.33)
3h	9	0.05 ± 0.02	0.15 ± 0.03	1.80	36.0 (12.0)
3i	10	0.11 ± 0.01	0.14 ± 0.2	1.80	16.4 (12.9)

n.d.: not determined.
[a] IC_{50} (J774.1)/IC_{50}(3D7).
[b] IC_{50} (J774.1)/IC_{50}(Dd2).

The dimerization leads to a substantial increase in activity, depending on the length of the linker (compounds **3a**–**3i**) (Table 2). Interestingly, even a short linker of two methylene units displays a 30-fold enhancement of activity in comparison to THA, which could be increased up to IC_{50} = 50 nM for a chain length of six to nine methylene groups.



2a: R^1 = H, R^2 = H
2b: R^1 = 3,3-dimethyl, R^2 = H
2c: R^1 = 3-methyl, R^2 = 6-Cl
2d: R^1 = 3,3-dimethyl, R^2 = 6-Cl
2e: R^1 = H, R^2 = 6-Cl
2f: R^1 = 2-methyl, R^2 = 6-Cl
2g: R^1 = 3-methyl, R^2 = 7-NO_2
2h: R^1 = 3,3-dimethyl, R^2 = 7-NO_2
2i: R^1 = H, R^2 = 7-OMe
2j: R^1 = 2-OEt, R^2 = 6-Cl

3a-i: R^1 = H, R^2 = H, n = 2 - 10
4a: R^1 = 3,3-dimethyl, R^2 = H, n = 6
4b: R^1 = 3-methyl, R^2 = 6-Cl, n = 6
4c: R^1 = 3,3-dimethyl, R^2 = 6-Cl, n = 6
4d: R^1 = H, R^2 = 6-Cl, n = 6
4e: R^1 = 2-methyl, R^2 = 6-Cl, n = 6
4f: R^1 = 3-methyl, R^2 = 7-NO_2, n = 6
4g: R^1 = 3,3-dimethyl, R^2 = 7-NO_2, n = 6
5a: R^1 = H, R^2 = 7-OMe, n = 9
5b: R^1 = 2-OEt, R^2 = 6-Cl, n = 9

Scheme 2. Reagents and conditions: (a) excess $POCl_3$, 20 min–4 h, reflux; (b) 1,ω-diaminoalkane, NaI, phenol, 2–4 h, reflux.