Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3783–3787

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antimalarial Evaluation of New 1,4-bis(3-aminopropyl)piperazine Derivatives

Adina Ryckebusch,[a] Rébecca Deprez-Poulain,[a] Marie-Ange Debreu-Fontaine,[a] Richard Vandaele,[a] Elisabeth Mouray,[b] Philippe Grellier[b] and Christian Sergheraert[a,*]

[a]*Institut de Biologie et Institut Pasteur de Lille, UMR 8525 CNRS, Université de Lille II, 1 rue du Professeur Calmette, B.P. 447, 59021 Lille, France*

[b]*USM 0504 'Biologie fonctionnelle des protozoaires' Département 'Régulations, Développement, Diversité Chimique', Muséum National d'Histoire Naturelle, 61 rue Buffon, 75005 Paris, France*

Received 31 March 2003; revised 14 July 2003; accepted 30 July 2003

Abstract—Synthesis and evaluation of the activity of a new family of 1,4-bis(3-aminopropyl)piperazine derivatives against a chloroquine-resistant strain of *Plasmodium falciparum,* and as inhibitors of β-hematin formation, are described. The highest antimalarial activities were obtained for compounds displaying the highest predicted vacuolar accumulation ratios and the best potencies as inhibitors of β-hematin formation. The most potent compound displayed an activity 3-fold better than chloroquine for a comparable selectivity index upon MRC-5 cells. Therefore, in this series, the replacement of the 7-chloroquinoline group can constitute a strong rationale for further investigation.

Introduction

Among antimalarial standard therapies, chloroquine (CQ) is believed to exert its antimalarial activity mainly by inhibiting hemozoin formation in the food vacuole of the parasite where it accumulates by pH gradient.[1–3] However, CQ uptake has been demonstrated to be saturable and energy-dependent, suggesting thereby an additional concentration mechanism.[4] CQ readily forms a complex with free Fe (III)FPIX in vitro[5] and the idea that such an interaction could contribute to the specific accumulation of CQ is now well-accepted.[6]

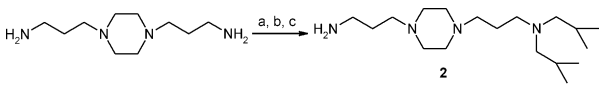
Biochemical studies have indicated that CQ-resistant isolates accumulate less drug than their more sensitive counterparts.[7] However opinion remains divided upon the mechanistic explanation for this lower accumulation.[8,9] Several works suggest that an enhanced CQ efflux by a multidrug-resistance mechanism may be involved.[10,11] It has been recently reported that CQ-resistance could result from mutations in a new vacuolar transporter, PfCRT.[12]

The antimalarial activities of 4-amino-quinoline-type drugs are supposed to be a function of both the ability of the drug to accumulate relevant pharmacological concentrations in the acidic vacuole and the ability to interfere with the haemozoin formation.[13,14] The 7-chloroquinoline moiety may contribute both to the accumulation by its basic nitrogen atom and by the inhibition of hemozoin formation involving a π–π stacking interaction with the porphyrin ring system. In this respect, the influence of an electron-donating or withdrawing group at the 7-position as well as the position of the amino group have been recently analyzed.[15,16] However, despite the development of these SAR for CQ, an important question, particularly in terms of resistance phenomenon, remains unanswered: the effect of replacing the quinoline moiety as a whole.

We have previously reported SAR of a series of 60 compounds with a common N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl) piperazine moiety displaying both variable vacuolar accumulation and efficiency to inhibit β-hematin formation.[17] Amongst them, compound **1** (Fig. 1) containing a diisobutyl group displayed the highest activity against the CQ-resistant strain FcB1 (0.9 nM) and the highest selectivity index towards MRC-5 cells.

*Corresponding author. Tel.: +33-3-2087-1210; fax: +33-3-2087-1233; e-mail: christian.sergheraert@ibl.fr

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.07.008



Scheme 1. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, rt, 16 h, 45%; (b) $(CH_3)_2CH$–CHO, $NaHB(OAc)_3$, CH_2Cl_2, rt, 3 h, 80%; (c) HCl, ethanol, reflux, 1.5 h, 100%.

Therefore, with the aim of analyzing the role and the importance of the quinoline moiety, a family of analogues corresponding to a common N^1,N^1-diisobutyl-1,4-bis(3-aminopropyl) piperazine motif linked to a variety of aromatic entities, was designed. Three series of compounds: arylamines (Series 1), benzylamines (Series 2) and amides (Series 3) (Fig. 2) were synthesized in order to study simultaneously, influence of the nature of the chemical link upon inhibition of β-hematin formation, vacuolar accumulation ratio (VAR) and antimalarial activity against the CQ-resistant strain FcB1. This preliminary work on the replacement of the quinoline moiety in our series, was aimed at finding new leads for design and synthesis of new antimalarials.

Chemistry

Synthesis

Compound **2** obtained by Boc monoprotection of 1,4-bis(3-aminopropyl)-piperazine followed by bis-alkylation and Boc deprotection (Scheme 1), was used as a precursor for synthesis of all compounds. Compounds **3–8** were obtained by nucleophilic aryl substitution (Scheme 2). Synthesis of benzylamines **9–13** was achieved by reductive amination from appropriate aldehyde and amine **2** using sodium borohydride as reducing agent. Amides **14–17** were prepared by reaction of amine **2** with various carboxylic acids in CH_2Cl_2, using HBTU as coupling agent and DIEA as a base.

Biological and physico-chemical assays

Antimalarial activity and cytotoxicity. The antimalarial activities of the three series of compounds were determined by their inhibition of parasite growth using the CQ-resistant strain FcB1 (IC_{50} chloroquine = 126 nM).[18,19] Results are given in Table 1.

In parallel, all compounds were tested for cytotoxicity upon a human diploid embryonic lung cell line (MRC5 cells) using the colorimetric MTT assay.[20]

In vitro inhibition of β-hematin formation

Compounds were tested for their ability to inhibit formation of β-hematin (synthetic equivalent of hemozoin) induced by 1-monooleoyl glycerol (MOG).[21,22]

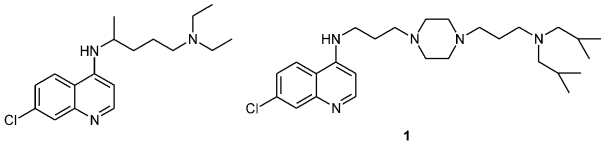
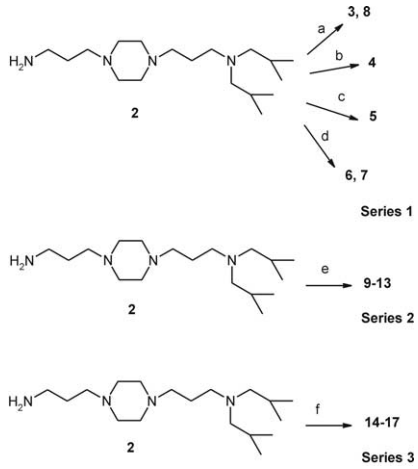


Figure 1. Chloroquine and compound **1**.



Scheme 2. Reagents and conditions: (a) appropriate aromatic chloride, *n*-pentanol, DIEA, reflux, 16–48 h, 11–19%; (b) propan-2-ol, TEA, reflux, 16–48 h, 10%; (c) *n*-butanol, TEA, reflux, 48 h, 8%; (d) EtOH, TEA, reflux, 48 h, 47–57%; (e) appropriate aldehyde, TEA, CH_3OH, rt, 4 h then $NaBH_4$, 12–96%; (f) appropriate carboxylic acid, DIEA, HBTU, HOBT, CH_2Cl_2, rt, 4 h, 37–99%.

Results and Discussion

The following SAR model for CQ has recently been proposed:[16] (i) the 4-aminoquinoline nucleus alone provides an Fe(III)PPIX complexing template but is not sufficient for inhibition of hemozoin formation; (ii) introduction of a 7-chloro group is responsible for inhibition of hemozoin formation but has probably little influence on the strength of association with Fe(III)-PPIX; and (iii) the aminoalkyl side chain is required for strong antimalarial activity by facilitating drug accumulation in the food vacuole. Further study with a family of derivatives of CQ[15] showed a linear dependence of the IC_{50} normalized by vacuolar accumulation ratio (VAR) on β-hematin inhibitory activity. This study supports that both pH trapping and β-hematin inhibition are the basis of antiplasmodial activity of aminoquinolines.

Evaluation of the three parameters: inhibition of β-hematin formation, vacuolar accumulation ratio (VAR)[23] and antiplasmodial activity could allow an analysis of this SAR in our three series of compounds.

Antimalarial activities against the FcB1 strain range from 0.9 nM to 5.27 μM (Table 1). In Series 1, quinoline **3** prepared to analyze the influence of the chloro substituent, and the benzimidazole derivative **8** were the only compounds to display better activities than CQ. Replacement of the chlorine atom in **1** with a hydrogen

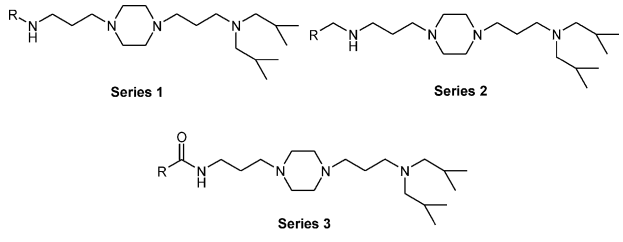
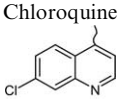
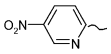
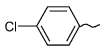
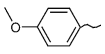
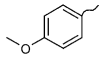


Figure 2.

atom in **3** induced a 20-fold decrease of activity while it plays a minor role for inhibition of hemozoin formation. Replacement of a 2-benzimidazole moiety (compound **8**) with a benzoxazole (compound **7**) lead to a large increase of the IC_{50} value (1.33 μM). The large reduction in the inhibition of β-hematin formation is also noteworthy.

In Series 2, replacement of the phenyl ring of compound **11** (IC_{50} = 454 nM) by a naphtyl moiety (compound **9**)

Table 1.

No.	R	IC_{50} of parasite growth (nM)[a]	IC_{50} of β-hematin formation (μM)[a]	VAR×10[3b]	CC_{50} (μM)[c]	Selectivity index (CC_{50}/IC_{50})
	Chloroquine	126 (±26)	76.5	5.3	50	397
Series 1						
1		0.9 (±0.1)	43.4	597.2	8.5	9444
3		18.9 (±0.6)	62.3	882.2	30	1587
4		531 (±93)	>200	3.6	80	151
5		1233 (±58)	53%[d]	6.2	82	67
6		758 (±68)	53%[d]	3.8	49	65
7		1333 (±57)	>200	298.1	>100	>75
8		87.3 (±12.8)	63.6	387.8	16	183
Series 2						
9		121 (±10)	98%[d]	475.0	6.5	54
10		842 (±97)	>200	504.0	30	36
11		454 (±130)	Nd[e]	476.3	21	46
12		40.6 (±4.7)	94.7	475.0	13	320
13		111 (±19)	170	475.8	12	108
Series 3						
14		672 (±38)	54%[d]	3.3	44	>65
15		1550 (±50)	0%[d]	3.2	>100	65
16		4433 (±153)	>200	3.1	57.7	13
17		5267 (±472)	0%[d]	3.4	93	18

[a]IC_{50} values were obtained from triplicate experiments performed on the FcB1 strain. Standard error is given in parentheses.
[b]Vacuolar accumulation ratio.
[c]MRC-5 cells.
[d]Percentage of inhibition of β-hematin formation at 200 μM of compound.
[e]Nd, not determined.

and especially its substitution by a chloro and a methoxy group (compounds **12** and **13**), were favourable, resulting in a 4- and 10-fold increase in activity for derivatives **9** and **12**, respectively. Conversely, compound **10** with a heteroatom in the ring was found to be 2-fold less active than the phenyl compound **11**.

In Series 3, introduction of an amide link provided compounds with heavily diminished antimalarial activity. For instance, activities of the chlorophenyl and methoxyphenyl compounds **16** and **17** were found to be, respectively, 130- and 40-fold inferior to those of their counterparts amines **12** and **13** belonging to Series 2.

The highest antimalarial activities were obtained for compounds displaying both the highest predicted VAR values and the highest potencies as inhibitors of β-hematin formation (compounds **1**, **3**, **8**, **9**, **12**, **13**). Compounds **12** and **13** were found to be weaker inhibitors of β-hematin formation than CQ but displayed a better inhibition of parasite growth, which could be explained by their better accumulation in the food vacuole. Conversely, for the benzimidazole compound **8** (in contrast to its benzoxazole analogue **7**), the efficiency towards inhibition of β-hematin formation counterbalanced a moderate VAR.

The weak antimalarial activities of Series 3 can be attributed both to low VAR values and weak potencies as inhibitors of β-hematin formation.

The cytotoxicity of compounds upon MRC-5 cells ranges from 6.5 μM to more than 100 μM (Table 1). Benzimidazole compound **8** and especially chlorophenyl compound **12** displayed a selectivity index close to that of CQ. The encouraging result obtained for compound **8** was the starting point for the synthesis of a new series of benzimidazole derivatives with the aim of improving antimalarial activity, that will be reported in due course.

Conclusion

Recent works suggest that resistance to CQ could be a structure-based process that could be overcome by structural modifications.[16,22] In this regard, compounds which do not display any quinoline-based structure could provide an alternative solution for therapy on chloroquine-resistant strains. Even if we cannot rule out the possibility that additional targets might be involved in the mechanism of action, the results show that a simple benzimidazole moiety can advantageously replace the 7-chloroquinoline against a CQ-resistant strain. They also provide consistent elements of correlation since in this family of N^1,N^1-diisobutyl-1,4-bis(3-aminopropyl) piperazine derivatives, both a good predicted VAR value and a good efficiency to inhibit β-hematin formation lead to good antimalarial activities. In this respect, the rational replacement of the 7-chloroquinoline group can be envisaged particularly in the most chemically accessible benzylamine series, especially since that a more convenient quantitative assay of β-hematin inhibitory activity has been recently described.[15,25]

Acknowledgements

These works are supported by CNRS (GDR 1077, FR CNRS 63, UMR CNRS 8525) and Université de Lille II-Droit et Santé. Adina Ryckebusch was on scholarship granted by CNRS/ Région Nord-Pas de Calais, France.

References and Notes

1. Geary, T. G.; Jensen, J. B.; Ginsburg, H. *Biochem. Pharmacol.* **1986**, *35*, 3805.
2. Yayon, A.; Cabantchik, Z. I.; Ginsburg, H. *EMBO J.* **1984**, *3*, 2695.
3. Hawley, S. R.; Bray, P. G.; O'Neill, P. M.; Park, B. K.; Ward, S. A. *Mol. Biochem. Parasitol.* **1996**, *80*, 15.
4. Fitch, C. D. *Science* **1970**, *169*, 289.
5. Chou, A. C.; Chevli, R.; Fitch, C. D. *Biochemistry* **1980**, *19*, 1543.
6. Francis, S. E.; Sullivan, D. J.; Goldberg, D. E. *Annu. Rev. Biochem.* **1997**, *51*, 97.
7. Bray, P. G.; Ward, S. A. *Pharmacol. Ther.* **1998**, *77*, 1.
8. Ginsburg, H.; Stein, W. D. *Biochem. Pharmacol.* **1991**, *41*, 1463.
9. Wünsch, S.; Sanchez, C. P.; Gekle, M.; Grosse-Wortmann, L.; Wiesner, J.; Lanzer, M. *J. Cell. Biol.* **1998**, *140*, 335.
10. Krogstad, D. J.; Gluzman, I. Y.; Kyle, D. E.; Oduola, A. M.; Martin, S. K.; Milhous, W. K.; Schlesinger, P. H. *Science* **1987**, *238*, 1283.
11. Reed, M. B.; Saliba, K. J.; Caruana, S. R.; Kirk, K.; Cowman, A. F. *Nature* **2000**, *403*, 906.
12. Carlton, J. M.; Fidock, D. A.; Djimde, A.; Plowe, C. V.; Wellems, T. E. *Curr. Opin. Microbiol.* **2001**, *4*, 415.
13. Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. *J. Med. Chem.* **2002**, *45*, 4975.
14. Hawley, S. R.; Bray, P. G.; Mungthin, M.; Atkinson, J. D.; O'Neill, P. M.; Ward, S. A. *Antimicrob. Agents. Chemother.* **1998**, *42*, 682.
15. Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, R.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531.
16. Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283.
17. Ryckebusch, A.; Deprez-Poulain, R.; Maes, L.; Debreu-Fontaine, M.-A.; Mouray, E.; Grellier, P.; Sergheraert, C. *J. Med. Chem.* **2002**, *46*, 542.
18. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
19. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
20. Mossman, T. *J. Immunol. Methods* **1983**, *65*, 55.
21. Fitch, C. D.; Cai, G.; Chen, Y.-F.; Shoemaker, J. D. *Biochim. Biophys. Acta* **1999**, *1454*, 31.
22. Ayad, F.; Tilley, L.; Deady, L.-W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2075.
23. Vacuolar accumulation ratios (VARs) were calculated mathematically from the equation below:

$$\mathrm{VAR} = \frac{1 + \sum_{n=1}^{4} 10^{-n\mathrm{pH_v} + \sum_{i=1}^{n} \mathrm{p}K_a i}}{1 + \sum_{n=1}^{4} 10^{-n\mathrm{pH_o} + \sum_{i=1}^{n} \mathrm{p}K_a i}}$$

where: $\mathrm{pH_v}$ = pH inside the vacuole (assumed to be pH 5.0)
$\mathrm{pH_o}$ = pH externally (assumed to be pH 7.4)
n is the number of protonation sites on the compound

This equation proceeds from a derivation of the Henderson–Hasselbach equation, based on predicted values of drug pK_a according to previous works of Hawley et al.[24]

Values of pK_a were calculated using ACD/pKa DB software from Avanced Chemistry Development Inc., Toronto, Canada.

24. Hawley, S.; Bray, P. C.; O'Neill, P. M.; Park, B. K.; Ward, S. A. *Biochem. Pharmacol.* **1996**, *52*, 723.
25. Parapini, S.; Basilico, N.; Pasini, E.; Egan, T. J.; Olliaro, P.; Taramelli, D.; Monti, D. *Exp. Parasitol.* **2000**, *96*, 249.