MedChemComm

CONCISE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Med. Chem. Commun.*, 2015, **6**, 1173

Discovery of oxybisbenzoylamides as a new class of antimalarial agents†

A. Pancotti,[a] S. Parapini,[b] M. Dell'Agli,[b] L. Gambini,[a] C. Galli,[a] E. Sangiovanni,[b] N. Basilico,[c] E. Bosisio,[b] D. Taramelli[b] and S. Romeo*[a]

Received 24th March 2015,
Accepted 6th May 2015

DOI: 10.1039/c5md00115c

www.rsc.org/medchemcomm

We have previously described several potent dual inhibitors of *Plasmodium falciparum* (*Pf*) growth characterized by the presence of statine, a β-hydroxyl amino acid able to inhibit parasite's plasmepsins (PLMs). While investigating the mechanism of action of these inhibitors, new compounds derived from statine were synthesized, which lost the ability to inhibit PLMs, but retained a significant *Pf* growth inhibition. Further structural simplifications showed that the inhibition of *Pf* viability was ascribed to a new pharmacophore never described before as antimalarial.

Introduction

Malaria has a massive impact on human health, being one of the major infections in the world causing 219 million clinical cases and about 660 000 deaths in 2010, mainly children under the age of five.[1] The most deadly parasite among the five *Plasmodium* species that causes human malaria is *Plasmodium falciparum* (*Pf*).

Artemisinin-combination therapies (ACTs) represent the major forms of intervention against malaria; however, the spread of drug resistance, as recently reported for artemisinin derivatives[2,3] may become a major public health problem, hindering malaria treatment. Spreading of resistance to first line drugs over the past few decades has led to intensification of the monitoring of their efficacy, to ensure proper management of clinical cases and early detection of changing patterns of resistance in order to revise national malaria treatment policies. This issue, combined with few commercially available drugs and an increased focus on limiting malaria transmission, is the basis for the urgent need of new antimalarials with innovative mechanism of action to fight against the onset of cross-resistance and expand physician tools for malaria control and eventually elimination.

Plasmepsins (PLMs) are aspartic proteases that, in cooperation with other proteases, participate in the haemoglobin digestion in the parasitic food vacuole.[4] This process is crucial for the development of the parasite and its blockage leads to the *Plasmodium* death. Although the haemoglobin digestion process appears to be a good pharmacological target, molecules with a useful *in vivo* efficacy have not been obtained yet. During our studies interesting results were generated by applying the "double-drug" approach:[5–9] we synthesized molecules characterized by the presence of statine, a β-hydroxyl amino acid able to inhibit aspartic proteases, such as PLMs, joined with the 8-aminoquinolinic ring system (**1**) derived from primaquine (PQ) or a hepatic schizonticidal atovaquone (**2**) or the 4-aminoquinolinic ring system (**3**) derived from chloroquine (CQ) (Fig. 1).

These compounds were potent PLMs inhibitors and showed an antimalarial activity at concentrations significantly

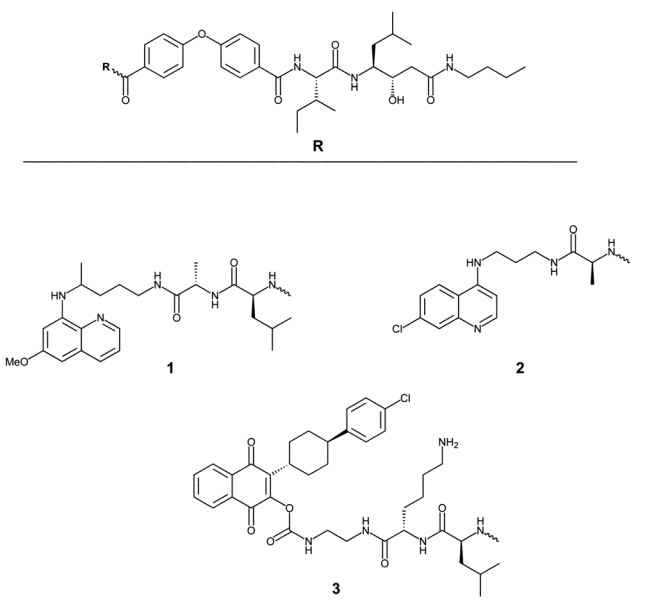


Fig. 1 Previously synthesized double drugs.

[a] *Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milan, Italy. E-mail: sergio.romeo@unimi.it*
[b] *Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, 20133 Milan, Italy*
[c] *Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Via Pascal 36, 20133 Milan, Italy*

† Electronic supplementary information (ESI) available: See DOI: 10.1039/c5md00115c

lower than any PLMs inhibitor previously reported. The antimalarial activity was also accompanied by low cytotoxicity and high selectivity towards human proteases.[6] The compounds shown in Fig. 1 are the most active representative for each class.

During our studies, discrepancies in the relationships between the inhibition of PLM2 and of *Pf* growth become evident. By comparing compounds **1**, **2** and **4** that present quinolinic substituents (Table 1), **2** and **4** showed very similar values of PLM2 inhibition. However compound **2**, having the quinolinic ring system derived from CQ, presented an antimalarial activity significantly higher (4–7 times) than compound **4** against the CQ-resistant, W2 strain. Furthermore, compound **4** was the best PLM2 inhibitor and the worst antimalarial.

To test the contribution of PLM2 inhibition to the antimalarial activity of the double drugs, we designed compounds **5**–**7** using compounds **1**, **2** and **4** as models, replacing statine, responsible for PLM2 inhibition, with leucine. (Table 1)

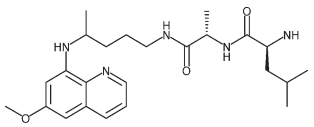
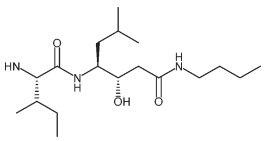
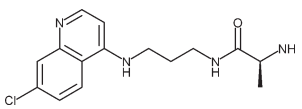
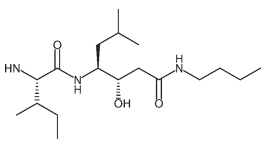
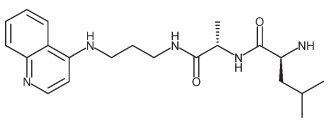
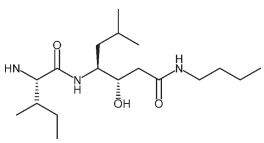
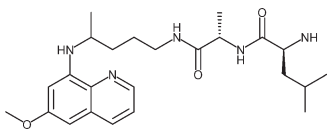
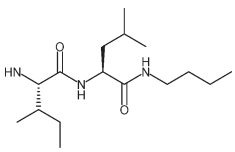
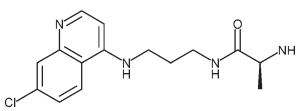
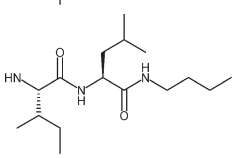
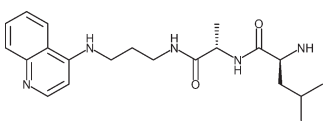
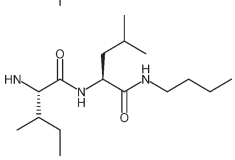
Results and discussion

Compounds **5**–**7** were prepared using the dipeptidic benzyl ester derivative of oxybisbenzoic acid **11** (Scheme 1) that was synthesized by coupling Boc-Leu with butylamine leading to amide **8**, which after acidic cleavage of Boc reacted with Boc-Ile generating dipeptide **9**. After deprotection, dipeptide **9** underwent a coupling reaction with 4-(4-((benzyloxy)carbonyl)phenoxy)benzoic acid **10** leading to compound **11** (Scheme 1).

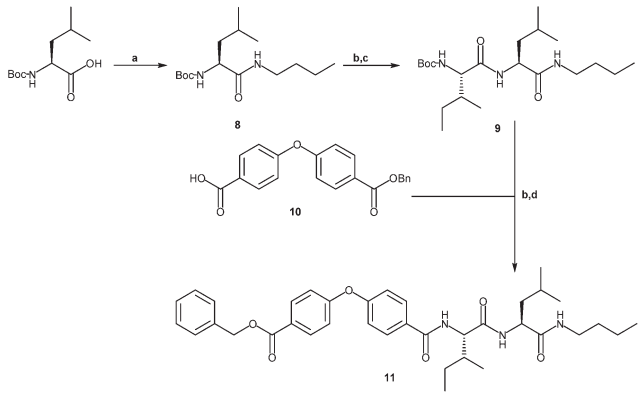
Compound **5** was obtained by coupling the dipeptide derivative of primaquine **13** with oxybisbenzoic ester **11**, after simultaneous deprotection by hydrogenolysis. (Scheme 2).

Compounds **6** and **7** were prepared according to Scheme 3. A nucleophilic substitution of chlorine in position 4 of 4,7-dichloroquinoline with a large excess of 1,3-diaminopropane followed by coupling with Boc-Ala led to intermediate **14**. The Boc-deprotected intermediate **14** underwent a further coupling reaction with Cbz-Leu to generate dipeptide **15** (Scheme 3).

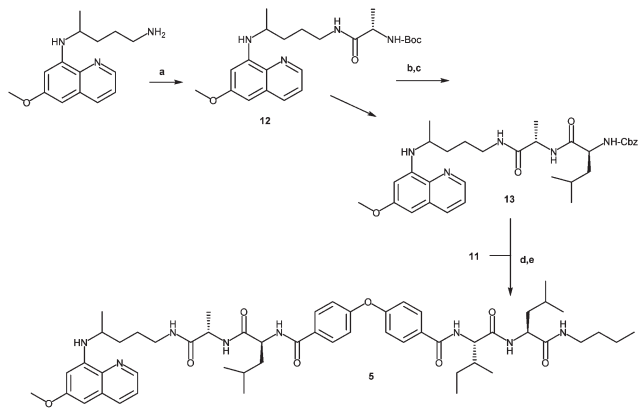
Table 1 Enzyme inhibition and antiplasmodial activity of compounds **1**–**2** and **4**–**7**

Entry	R	R′	PLM2 K_i (nM)	D10 IC_{50} (nM)[a]	W2 IC_{50} (nM)[a]
1[b]			26.3	200	300
2[c]			3.8	114	64
4[c]			2.6	161	474
5			n.a.	37	104
6			n.a.	43	39
7			n.a.	63	54
CQ			n.t.	23	412

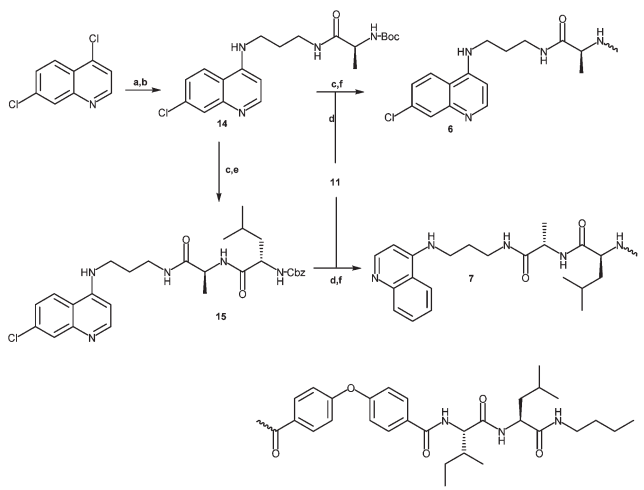
[a] Results are the mean of two experiments performed in duplicate. [b] From ref. 6. [c] From ref. 9. n.a.: no inhibition observed at 1 μM. n.t.: not tested.



Scheme 1 Reagents and conditions: (a) *n*-butylamine, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole(HOBt), 4-methylmorpholine (NMM); (b) HCl 4 N, dioxane; (c) BocIleOH, HBTU, HOBt, NMM; (d) HBTU, HOBt, NMM.



Scheme 2 Reagents and conditions: (a) BocAlaOH, HBTU, HOBt, NMM; (b) HCl 4 N, dioxane; (c) CbzLeuOH, HBTU, HOBt, NMM; (d) Pd/C, H_2, MeOH; (e) HBTU, HOBt, NMM.



Scheme 3 Reagents and conditions: (a) propane-1,3-diamine, 140 °C; (b) BocAlaOH, HBTU, HOBt, NMM; (c) HCl 4 N, dioxane; (d) Pd/C, H_2, MeOH (e) CbzLeuOH, HBTU, HOBt, NMM;. (f) HBTU, HOBt, NMM.

Compound **7** was obtained by a coupling reaction after simultaneous one-pot deprotection of benzyl ester **11** and dipeptide **15**. As expected, during catalytic hydrogenation, the chlorine atom in position 7 was removed[10] (Scheme 3).

Compound **6** was obtained by a coupling reaction between deprotected intermediates **14** and **11** (Scheme 3).

Compounds **5**–**7** were tested against W2, CQ-resistant and D10, CQ-sensitive *Pf* strains,[11] as well as for PLM2 inhibition and the results are shown in Table 1. As expected compounds **5**–**7** were unable to inhibit PLM2 proteolytic activity, but they showed an antimalarial activity significantly higher than their statine analogues against both *Pf* strains.

Interestingly, while compound **4** and **5** showed cross-resistance with CQ, compounds **6** and **7** demonstrated a similar activity on both D10 and W2 strains. Compound **6** being the most active was also tested for cytotoxicity against normal human fibroblast (FDH)[6] and against a macrophage cell line (HMEC-1), showing an effect only in the micromolar range (FDH, IC_{50}: 17.99 ± 8.7 μM; HMEC-1, IC_{50}: 17.58 ± 7.8 μM) with a good therapeutic index (approx. 450).

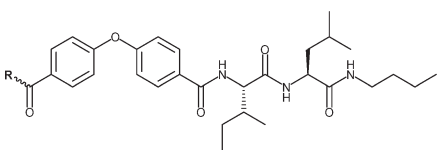
Therefore, compounds **5**–**7** are potent inhibitors of *Pf* growth, but they do not act through PLMs inhibition. To verify if these compounds share the same mechanism of action as CQ, we assessed the ability of compounds **5** and **6** to inhibit β-haematin formation using the BHIA method previously described.[12]

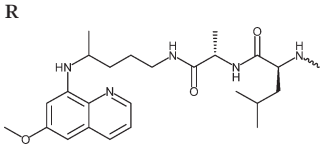
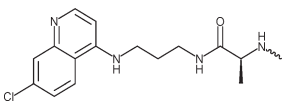
Table 2 shows that compound **6**, characterised by the 4-aminoquinoline ring system, inhibits β-haematin formation better than CQ (IC_{50} 0.79 *vs.* 1.36 of CQ), whereas compound **5** is inactive as PQ.

This is consistent with the previous data showing that the 8-aminoquinolinic ring system is not able to interfere with β-haematin formation.[12]

The lack of inhibition of β-haematin formation and the loss of PLM2 inhibition paired with the good antiplasmodial activity of compound **5**, led us to suppose that this new class of *Pf* inhibitors may owe its activity to a different mechanism of action.

Table 2 Inhibition of β-haematin formation[12]



Entry	R	BHIA[a]
5		>8
6		0.79
CQ		1.36
PQ		>10

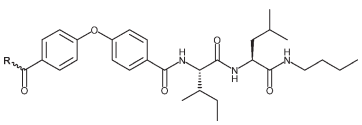
[a] The IC_{50} represents the molar equivalents of test compounds relative to haematin required to inhibit β-haematin formation by 50%.

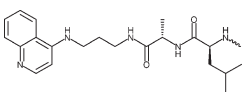
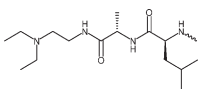
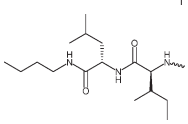
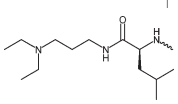
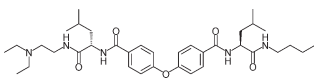
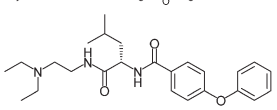
To verify this hypothesis, compounds **16–18** were synthesized replacing the quinolinic substructure with a short alkyl chain. The basic tertiary amine was introduced in compound **16** to improve aqueous solubility which is otherwise extremely low. Compound **17** shows the same substituent on both sides and compound **18** presents a one amino acid shortened peptide chain. The influence of the dipeptide IleLeu on the other side of the oxybisbenzoic scaffold was evaluated by removing Ile (**19**) and the entire substituent (**20**), thus achieving further reduction of the peptide character and molecular weight.

Compounds **16–18** were synthesized similarly to compound **5** (Scheme 2) using in the first step the appropriate amine (see the ESI†) while for the preparation of compounds **19** and **20**, a slightly different synthetic route was adopted (see the ESI†). Compounds **16–20** were tested against W2, CQ-resistant and D10, CQ-sensitive *Pf* strains and the results are shown in Table 3.

Compounds **16**, **18** and **19** showed an antiplasmodial activity in the nanomolar range while compound **17** resulted considerably less active compared to compound **7**, probably due to its limited aqueous solubility. Compound **20** showed very limited antiplasmodial activity. Compounds **18** and **19** were also tested for cytotoxicity against normal human fibroblast (FDH)[6] ((**18**) IC_{50}: 16.3 ± 4.9 μM; (**19**) IC_{50}: 23.0 ± 7.3 μM) showing an effect similar to compound **6**. Compound **18** showed a good therapeutic index (>100).

Table 3 Antiplasmodial activity of compounds **7**, **16–20**



Cmp	R	D10 IC_{50} (nM)[a]	W2 IC_{50} (nM)[a]
7		63	54
16		345	143
17		1981	1620
18		121	103
19		357	314
20		n.a.	n.a.

[a] Results are the mean of two experiments performed in duplicate. n.a. $IC_{50} > 5$ μg ml^{-1}.

Table 4 LLE[a] values for compounds **5–7**, **16**, **18**, and **19**

Entry	LLE (pH 7.4) D10	LLE (pH 7.4) W2
5	0.52	0.07
6	1.59	1.63
7	1.80	1.87
16	2.74	3.12
18	3.35	3.41
19	2.99	3.04

[a] $LLE^{13} = pIC_{50} - c\log D$ (pH 7.4).[14]

To assess the attractiveness of compounds **5–7**, **16**, **18**, and **19** as new hits, the values of ligand lipophilicity efficiency (LLE) were calculated.[13] Table 4 shows that the substitution of the quinolinic ring system with a tertiary amine resulted in a clear improvement of LLE. Compounds **18** and **19** are particularly attractive because they show very similar IC_{50} values against CQ-sensitive and CQ-resistant *Pf* strains and the highest LLE value.

Conclusions

On the basis of these results and the fact that **18** and **19** do not present any known antimalarial pharmacophoric group, it is likely that these molecules owe their activity to a new mechanism of action.

Considering that, any new antimalarial drug needs to be effective against drug-resistant strains, but also affordable for those in need in developing countries, it is essential to develop molecules with an inexpensive synthesis. The removal of the non-natural β-hydroxyl amino acid statine greatly facilitated the synthesis reducing, the inherent costs. Therefore, we believe that compounds **18** and **19** are good starting points for developing a new class of antimalarial agents.

Acknowledgements

Part of this manuscript was generated in the context of the AntiMal project, funded under the 6th Framework Programme of the European Community (contract no. IP-018834).

This work was supported by MIUR (PRIN2008, 20084MMXN: Leads ad Attività Antimalarica di Origine Naturale: Isolamento, Ottimizzazione e Valutazione Biologica).

Notes and references

1 World Health Organization, *World Malaria Report*, 2012.
2 A. M. Dondorp, R. M. Fairhurst, L. Slutsker, J. R. MacArthur, J. G. Breman, P. J. Guerin, T. E. Wellems, P. Ringwald, R. D. Newman and C. V. Plowe, *N. Engl. J. Med.*, 2011, **365**, 1073–1075.
3 O. Miotto, J. Almagro-Garcia, M. Manske, B. MacInnis, S. Campino, K. A. Rockett, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J. M. Anderson, S. Duong, C. Nguon, C. M. Chuor, D. Saunders, Y. Se, C. Lon, M. M. Fukuda, L. Amenga-Etego, A. V. O. Hodgson, V. Asoala, M. Imwong, S. Takala-Harrison, F. Nosten, X. Z. Su, P. Ringwald, F. Ariey, C. Dolecek, T. T.

Hien, M. F. Boni, C. Q. Thai, A. Amambua-Ngwa, D. J. Conway, A. A. Djimde, O. K. Doumbo, I. Zongo, J. B. Ouedraogo, D. Alcock, E. Drury, S. Auburn, O. Koch, M. Sanders, C. Hubbart, G. Maslen, V. Ruano-Rubio, D. Jyothi, A. Miles, J. O'Brien, C. Gamble, S. O. Oyola, J. C. Rayner, C. I. Newbold, M. Berriman, C. C. A. Spencer, G. McVean, N. P. Day, N. J. White, D. Bethell, A. M. Dondorp, C. V. Plowe, R. M. Fairhurst and D. P. Kwiatkowski, *Nat. Genet.*, 2013, **45**, 648–655.

4 K. Ersmark, B. Samuelsson and A. Hallberg, *Med. Res. Rev.*, 2006, **26**, 626–666.

5 S. Romeo, M. Dell'Agli, S. Parapini, L. Rizzi, G. Galli, M. Mondani, A. Sparatore, D. Taramelli and E. Bosisio, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2931–2934.

6 M. Dell'Agli, S. Parapini, G. Galli, N. Vaiana, D. Taramelli, A. Sparatore, P. Liu, B. M. Dunn, E. Bosisio and S. Romeo, *J. Med. Chem.*, 2006, **49**, 7440–7449.

7 S. Romeo, S. Parapini, M. Dell'Agli, N. Vaiana, P. Magrone, G. Galli, A. Sparatore, D. Taramelli and E. Bosisio, *ChemMedChem*, 2008, **3**, 418–420.

8 L. Janka, J. Clemente, N. Vaiana, A. Sparatore, S. Romeo and B. M. Dunn, *Protein Pept. Lett.*, 2008, **15**, 868–873.

9 N. Vaiana, M. Marzahn, S. Parapini, P. Liu, M. Dell'Agli, A. Pancotti, E. Sangiovanni, N. Basilico, E. Bosisio, B. M. Dunn, D. Taramelli and S. Romeo, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5915–5918.

10 M. V. N. de Souza, K. C. Pais, C. R. Kaiser, M. A. Peralta, M. D. L. Ferreira and M. C. S. Lourenco, *Bioorg. Med. Chem.*, 2009, **17**, 1474–1480.

11 M. T. Makler, J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbins and D. J. Hinrichs, *Am. J. Trop. Med. Hyg.*, 1993, **48**, 739.

12 S. Parapini, N. Basilico, E. Pasini, T. J. Egan, P. Olliaro, D. Taramelli and D. Monti, *Exp. Parasitol.*, 2000, **96**, 249–256.

13 P. D. Leeson and B. Springthorpe, *Nat. Rev. Drug Discovery*, 2007, **6**, 881–890.

14 Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 14.8.18, 2014, ChemAxon (http://www.chemaxon.com).