Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 345–348
Bioorganic & Medicinal Chemistry Letters

Potent 4-aminopiperidine based antimalarial agents

Kristin M. Brinner,[a,†] Mary Ann Powles,[b] Dennis M. Schmatz[b] and Jonathan A. Ellman[a,*]

[a]*Center for New Directions in Organic Synthesis, Chemistry Department, University of California, Berkeley, CA 94720, USA*
[b]*Merck and Co., Rahway, NJ 07065, USA*

Received 17 July 2004; revised 20 October 2004; accepted 23 October 2004
Available online 13 November 2004

Abstract—A series of compounds with potent activity against a multi-drug-resistant strain of *Plasmodium falciparum*, the causative agent of the deadliest strain of malaria, is described. These compounds were also tested for cytotoxicity in human foreskin fibroblast assays, evaluated to determine their log *D*, and assayed for metabolism by human and murine hepatocytes. This work resulted in the development of compounds **9e** and **10d**, which showed good potency (IC_{50} = 75 nM and <60 nM, respectively, against Dd2), acceptable log *D* values, and reasonable metabolic stability.

Malaria is a disease of worldwide implications. It is estimated that 40% of the world's population resides in malaria-endemic regions of the world. Over 300 million cases are reported annually, resulting in 1–3 million deaths.[1] The clinical symptoms and the majority of deaths caused by malaria occur during the intra-erythrocytic phase of the parasite's complex life cycle within its human host. While the parasites develop within human red blood cells, fever, chills, anemia, and more severe clinical complications are all observed.[2] During this phase of the parasite's life cycle, hemoglobin is consumed as a primary source of amino acids to fuel the explosive growth of the parasite. Upon catabolism of hemoglobin, heme is released, which would be cytotoxic to the parasite in its soluble form. However, *Plasmodium* have evolved a unique heme detoxification pathway, where free heme is dimerized whereupon hydrogen bonding between heme dimers results in polymeric non-toxic hemozoin formation.[3] There is considerable evidence that the aminoquinoline based antimalarials, including chloroquine, quinine, and mefloquine, act through inhibiting this heme detoxification pathway.[4] Unfortunately, increasing drug resistance to these agents complicates the treatment of malaria. Additionally, resistance to these common antimalarial medications cannot be overcome by increasing their dosages, as they have extremely narrow therapeutic windows.

We previously reported the synthesis and evaluation of a novel series of amino-piperidine based compounds that showed promising activity against both chloroquine-sensitive (3D7) and resistant (W2) strains of *P. falciparum*, the deadliest strain of the malarial parasite.[5] While the mechanism of action of these compounds was not known, there was evidence that they acted by inhibiting heme polymerization. When comparing the structure of the best compound from this series to that of chloroquine, we observed that the basic nitrogens are arrayed in a similar fashion (Fig. 1).

By combining structural features of both compound **1** and chloroquine, we hoped to further improve the activity of the compounds. This would allow us not only to capitalize on a drug intervention pathway already well established by the aminoquinoline based drugs, but also to bypass established mechanisms of resistance development by avoiding the aminoquinoline scaffold itself. Upon synthesis and evaluation of compound **2**, we observed that the compound maintained potent activity against a chloroquine sensitive strain of *P. falciparum*, but had significantly less activity against a multi-drug-resistant strain of the parasite (Dd2). However, we were encouraged to optimize the activity of additional compounds of this structural type, because **2** had low levels of toxicity.

Here we report the synthesis of analogs of compound **2**, their activity against a multi-drug-resistant strain of

Keywords: Antimalarial; *Plasmodium falciparum*; Multi-drug resistant; Metabolism; Log *D*.
* Corresponding author. Tel.: +1 510 642 4488; fax: +1 510 642 8369; e-mail: ellman@cchem.berkeley.edu
† Present address: Chiron Corporation, Emeryville, CA 94608, USA.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.069

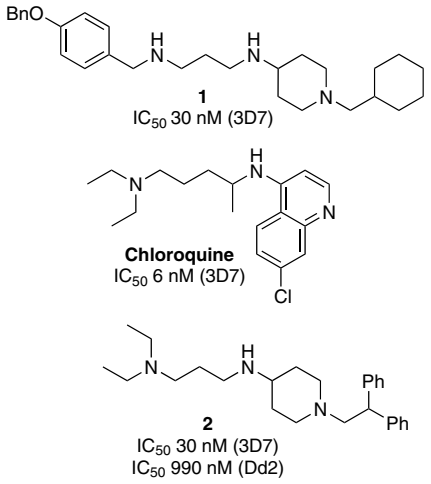
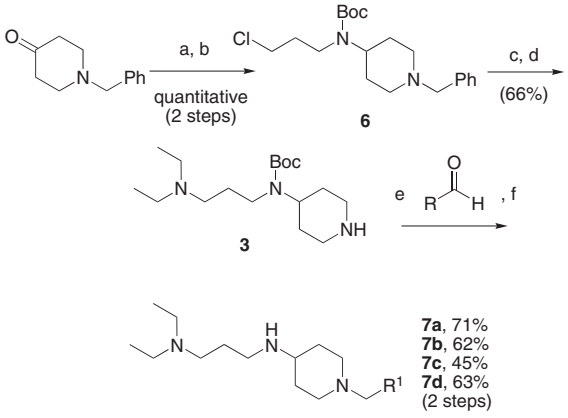


Figure 1. Merging of structures of lead compounds and chloroquine.

P. falciparum, and their cytotoxicity. These compounds showed promising activity as well as low levels of toxicity, and were further characterized for their drug-like properties and metabolic lability.

Three regions of compound **2** could easily be modified to rapidly assess structure–activity relationships (SAR) (Fig. 2). The R^1 substitution could be varied through reductive alkylation of amino piperidine **3**. Additionally, R^2 and R^3 could be varied through alkylation of a variety of primary and secondary amines with alkyl chloride **4**. Finally, R^2 could be varied while holding R^3 as an ethyl substituent, by reductive alkylation of secondary amine **5** with a series of aldehydes. As these synthetic sequences were efficient and generated sufficient material for biological testing, they were not further optimized. All compounds were purified by standard silica gel chromatography, and converted to the HCl salts for biological testing.

Diversity at the R^1 position was investigated through derivatization of **3** (Scheme 1). *N*-Benzyl-4-piperidone was reductively alkylated with 3-chloroaminopropane, and the resultant amine was Boc protected. Primary alkyl chloride **6** was then reacted with ethylamine, and the benzyl group was subsequently removed under transfer hydrogenation conditions to afford **3**. The free piperidine **3** was then reductively alkylated with a series of four aldehydes, followed by acidic deprotection, to afford compounds **7a**–**d**.



Scheme 1. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, Et_3N, 3-chloroaminopropane; (b) Boc_2O, THF; (c) Et_2NH, NaI, DMF, 70 °C; (d) NH_4HCO_2, Pd/C, MeOH, EtOAc; (e) $NaBH(OAc)_3$; (f) HCl, dioxane.

Diversity at the R^2 and R^3 positions was investigated through reaction of a variety of primary and secondary amines with compound **4** (Scheme 2). 4-Piperidone monohydrate hydrochloride was reductively alkylated with 1,1-diphenylacetaldehyde to afford amino piperidone **8**. This was then subjected to reductive amination with 3-chloroaminopropane, and the resultant secondary amine was Boc protected to afford **4**. Primary alkyl chloride **4** was reacted with a series of five amines, followed by cleavage of the Boc group under acidic conditions to afford compounds **9a**–**e**.

Finally, R^3 was varied while holding R^2 constant (Scheme 3). Primary alkyl chloride **4** was reacted with

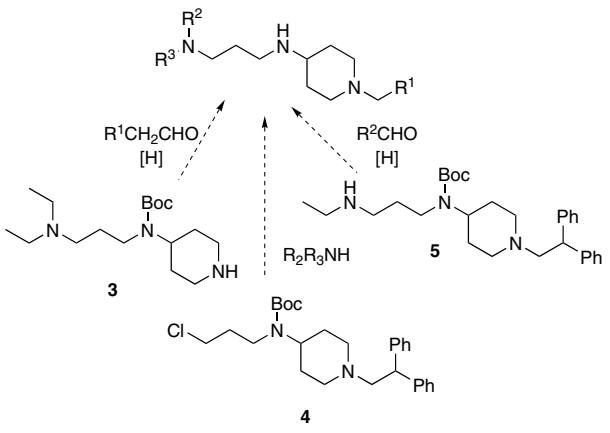
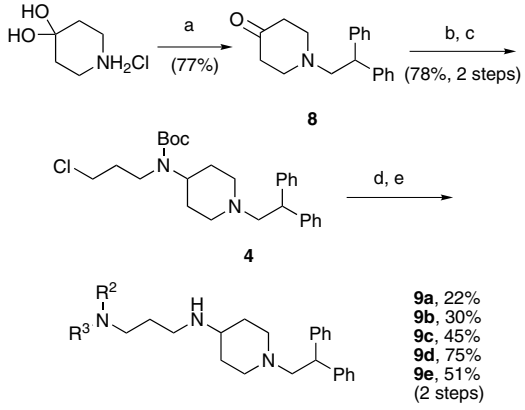
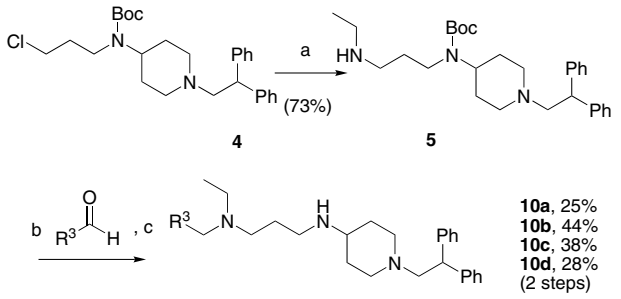


Figure 2. Modification of **2** to assess SAR.



Scheme 2. Reagents and conditions: (a) 1,1-diphenylacetaldehyde, $NaBH(OAc)_3$, Et_3N, AcOH, MeOH; (b) $NaBH(OAc)_3$, Et_3N, AcOH, CH_3CN, 3-chloroaminopropane; (c) Boc_2O, THF; (d) NaI, DMF, 70 °C; (e) HCl, dioxane.



Scheme 3. Reagents and conditions: (a) ethylamine, NaI, DMF, 70 °C; (b) $NaBH(OAc)_3$, $(ClCH_2)_2$; (c) HCl, dioxane.

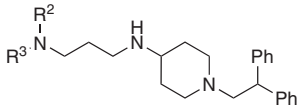
ethylamine to afford secondary amine **5**, which was reductively alkylated with a series of four aldehydes. The Boc group was removed under acidic conditions to afford compounds **10a**–**d**.

The antimalarial activity of **7a**–**7d**, **9a**–**9e**, and **10a**–**d** were measured in red-blood-cell-based assays. Efficacy was monitored by parasite ^{3}H-hypoxanthine incorporation using parasite-infected human erythrocytes in late ring stage.[6] All compounds were assayed in duplicate against the multi-drug-resistant parasite, Dd2. The cytotoxicities of these new compounds were measured in quadruplicate against a human foreskin fibroblast assay. Average values for both of these assays are reported.

As shown in Table 1, compounds with aromatic functionality at R^1 showed improved antimalarial activity. While compounds **7c** and **7d** had better activity than parent compound **2**, they had worse activity to toxicity ratios. Consequently, further optimization of activity was carried out through varying substituents at positions R^2 and R^3.

The data in Table 2 demonstrates the striking change in activity and toxicity observed through small permutations in the R^2 and R^3 substituents of compound **2**. Smaller substituents at R^2 and R^3 resulted in increased activity, but with even greater increases in toxicity (compound **9a**). Secondary amine **9b** has decreased activity and a worse activity to toxicity ratio relative to tertiary amine **2**, underlying the importance of having a tertiary amine at this position. Cyclic amines **9c** and **9d** showed increases in activity and maintained an approximate activity to toxicity ratio of 10:1. Compound **9e**[7] showed a 15-fold increase in activity when compared to parent compound **2**, while improving the activity to toxicity ratio from 11:1 to over 78:1.

Table 2. SAR with symmetrically substituted amines



Compd	R^2	R^3	IC_{50} (nM)[a]	Tox. (nM)[b]
2	Ethyl	Ethyl	900	9900
9a	Methyl	Methyl	300	1300
9b	Ethyl	H	2500	2600
9c	$-(CH_2)_4-$	—	200	2500
9d	$-(CH_2)_5-$	—	200	1200
9e	*n*-Propyl	*n*-Propyl	>60	4700

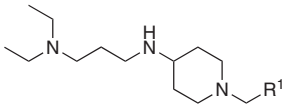
[a] Dd2 *P. Falciparum*.
[b] Human foreskin fibroblast.

The final set of data is shown in Table 3. All changes from the diethylamine to unsymmetrically substituted tertiary amines resulted in increases in activity, and most compounds maintained similar levels of activity to toxicity ratios. However, compound **10d**[8] showed over a 10-fold increase in activity when compared to **2**, while also improving its activity to toxicity ratio from only 11:1 to over 100:1.

We next attempted to better understand the activity of these compounds through correlation of their activity with their lipophilicity profile, or Log *D*. A subset of the compounds was tested and their Log *D* plotted versus their activity (Table 4). As the lipophilicity increases, the activity of the compound increases. However, there is no obvious trend when examining lipophilicity versus toxicity.

Two of the most promising compounds, **9e** and **10d**, were also tested to determine their metabolic vulnerability. This assay uses LC/MS/MS to determine the degree to which the compounds are metabolized upon exposure

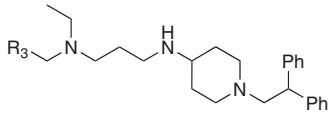
Table 1. SAR in the R^1 region



Compd	R^1	IC_{50} (nM)[a]	Tox. (nM)[b]
2	$CH(C_6H_5)_2$	900	9900
7a	C_6H_{11}	4300	6000
7b	$CH_2C_6H_{11}$	920	2900
7c	$CH_2C_6H_4$ (4-Cl)	850	2700
7d	CH_2–C_6H_4 (4-C_6H_5)	400	1200

[a] Dd2 *P. Falciparum*.
[b] Human foreskin fibroblast.

Table 3. SAR with unsymmetrically substituted amines



Compd	R^1	IC_{50} (nM)[a]	Tox. (nM)[b]
2	$CH(C_6H_5)_2$	900	9900
10a	C_6H_{11}	200	2200
10b	$CH_2C_6H_{11}$	250	2100
10c	$CH_2C_6H_4$(4-Cl)	200	2000
10d	CH_2–C_6H_4(4-C_6H_5)	75	7600

[a] Dd2 *P. Falciparum*.
[b] Human foreskin fibroblast.

Table 4. Comparison of IC_{50} and toxicity with Log *D*

Compd	IC_{50} (μM)[a]	Tox. (μM)[b]	Log *D*[c]
7a	4.3	6.0	−0.25
9b	2.5	2.6	−0.44
7b	0.92	2.9	−0.59
7c	0.85	2.7	−0.70
7d	0.40	1.2	0.7
9a	0.30	1.3	0
9c	0.20	2.5	0.11
9d	0.20	1.2	0.77
10a	0.20	2.2	2.11
10d	0.075	7.6	3.12
9e	>0.060	4.7	1.41

[a] Dd2.
[b] Human Foreskin Fibroblast.
[c] Determined by Robertson Microlit Laboratories, Inc. (Madison, NJ).

Table 5. Metabolism by liver microsomes

Compd	Human S9% remaining[a]	Mouse S9% remaining[a]
9e	56	118
10d	45	113

Experiment performed by PPD™ Discovery (Morrisville, NC).
[a] Single time point at 1 h.

to murine and human liver S9-fractions for one hour. As seen in Table 5, both compounds are only 50% degraded by human S9 fractions, and are not degraded at all by murine S9 fractions.

We have further improved the activity of our new class of non-aminoquinoline antimalarial compounds. These compounds are easily and rapidly synthesized from simple, commercially available starting materials. In addition to improving the activity of initial hit **2** with an IC_{50} of 900 nM to lead compound **9e** with an IC_{50} of less than 60 nM against a multi-drug-resistant strain of *P. falciparum*, we improved the activity to cytotoxicity ratio from 11:1 to 78:1. The most promising compounds had acceptable log *D* values and were reasonably metabolically stable in both human and murine liver S-9 fractions.

Acknowledgements

We gratefully acknowledge the support of the Medicines for Malaria Venture. The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as a Sponsoring Member.

References and notes

1. Woster, P. M. *Annu. Rep. Med. Chem.* **2001**, *36*, 99.
2. Krogstad, D. J. In *Mechanisms of Microbial Disease*, 2nd Edn.; Schaechter, M., Medoff, G., Eisenstein, B. I., Eds.; Williams and Wilkins: Baltimore, MD, 1993; pp 597–615.
3. Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. *Science* **2000**, *404*, 307.
4. Choi, C. Y. H.; Schneider, E. L.; Kim, J. M.; Gluzman, I. Y.; Goldberg, D. E.; Ellman, J. A.; Marletta, M. A. *Chem. Biol.* **2002**, *9*, 881; For recent advances in new heme-polymerization inhibitors, see: O'Neill, P. M.; Mukhtar, A.; Stocks, P. A.; Randle, L. E.; Hindley, S.; Ward, S. A.; Storr, R. C.; Bickley, J. F.; O'Neil, I. A.; Maggs, J. L.; Highes, R. H.; Winstanley, P. A.; Bray, P. G.; Park, B. K. *J. Med. Chem.* **2003**, *46*, 4933, and references cited therein.
5. Brinner, K. M.; Kim, J. M.; Habashita, H.; Gluzman, I. Y.; Goldberg, D. E.; Ellman, J. A. *Bioorg. Med. Chem.* **2002**, *10*, 3649.
6. Gluzman, I. Y.; Francis, S. E.; Oksman, A.; Smith, C. E.; Duffin, K. L.; Goldberg, D. E. *J. Clin. Invest.* **1994**, *93*, 1602.
7. ^{1}H NMR (400 MHz, MeOD-d_3) δ 0.91 (t, J = 7.2 Hz, 6H), 1.51–1.79 (m, 6H), 1.80 (q, J = 6.8 Hz, 2H), 1.90 (m, 2H), 2.11 (m, 2H), 2.60 (m, 4H), 2.76 (t, J = 6.8 Hz, 2H), 2.80 (m, 1H), 2.95 (d, J = 6.8 Hz, 2H), 3.02 (m, 4H), 4.21 (t, J = 7.6 Hz, 1H), 7.12 (m, 2H), 7.23 (m, 8H). ^{13}C NMR (125 MHz, MeOD-d_3) δ 11.9, 20.0, 24.2, 30.7, 45.8, 49.5, 50.3, 53.1, 54.0, 56.3, 64.1, 127.3, 129.1, 129.4, 145.2. ESI-MS (LR) $[M+H]^+$ calcd for $C_{28}H_{43}N_3$, 422.7; found, 422.5.
8. ^{1}H NMR (500 MHz, MeOD-d_3) δ 1.07 (t, J = 7.5 Hz, 3H), 1.24 (m, 2H), 1.66 (m, 4H), 1.91 (t, J = 12 Hz, 2H), 2.39 (m, 1H), 2.62 (m, 6H), 2.77 (m, 4H), 2.83 (m, 2H), 2.93 (d, J = 7.5 Hz, 2H), 4.13 (t, J = 7.5 Hz, 1H), 7.12 (m, 2H), 7.23 (m, 8H), 7.32 (m, 3H), 7.42 (m, 2H), 7.56 (m, 2H), 7.61 (m, 2H). ^{13}C NMR (125 MHz, MeOD-d_3) δ 11.5, 25.9, 31.4, 33.2, 46.0, 48.1, 50.3, 52.7, 53.5, 55.5, 56.2, 64.3, 127.2, 127.8, 128.0, 128.3, 129.1, 129.4, 129.9, 130.03, 140.2, 140.7, 142.1, 145.3. ESI-MS (LR) $[M+H]^+$ calcd for $C_{38}H_{47}N_3$, 546.8; found, 546.5.