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Exploiting a basic chemosensitizing pharmacophore hypothesis. Part 1: Synthesis and biological evaluation of novel arylbromide and bicyclic chemosensitizers against drug-resistant malaria parasites

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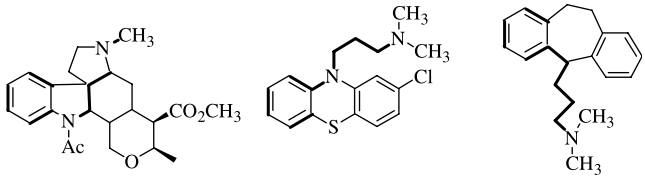
This paper is dedicated to Professor Iwao Ojima on the occassion of his 60th birthday

Abstract—An exploratory series of novel arylbromide and bicyclic chemosensitizers (modulators) against chloroquine-resistant *Plasmodium falciparum* were designed and synthesized on the basis of a basic chemosensitizing pharmacophore hypothesis in malaria. *ortho*-Substituted bromo and biphenyl ether compounds displayed the best activity from the series.

Multi-drug resistant strains of the causative agent of malaria *Plasmodium falciparum* have rendered previously effective drugs such as chloroquine (CQ), once the mainstay of malaria prophylaxis and chemotherapy, essentially useless and malaria impossible to treat in certain areas.[1] Drug combination therapy is an attractive option aimed at prolonging the usefulness of CQ especially in poor countries.[2] Within this context, the use of reversing agents or chemosensitizers is a potential new and effective treatment strategy.[3] However, this strategy has received little attention especially following the failure of initial in vivo experiments with desipramine.[4] In spite of this, it is noteworthy that chlorpheniramine was reported to show clinical effectiveness in reversing CQ resistance in West Africa.[5] Moreover, recent in vitro and in vivo data have provided additional new impetus for research in this area as we march toward the realistic possibility of potent clinically useful CQ resistance reversal agents.[6,7]

In dissecting the structures of malagashanine,[8] a naturally occurring CQ chemosensitizer, and some tricyclic antidepressants[3] exemplified by chloropromazine, we identified the *N*-phenyl-1,3-diamino-propane as a common unit. Likewise, the 1-phenyl-4-amino unit was also identified in verapamil and some synthetic chemosensitizers exemplified by amitriptyline[3] (Fig. 1).

Reduction of the benzene ring of the indoline group of malagashanine did not affect the activity of the parent



Malagashanine **Chloropromazine** **Amitriptyline**

Figure 1. The *N*-phenyl-1,3-diamino-propane and 1-phenyl-4-amino unit as a common unit in the three structures.

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compound,[9] indicating that this functional group was not directly involved in the chemosensitizing activity of the concerned indole alkaloid. This led us to additionally propose a 1,4-diamino unit as another bioactive unit, which is also found in chlorpheniramine (Fig. 2).

These observations led us to postulate a unifying chemosensitizing pharmacophore in malaria as outlined in Figure 3.[10] Previously, we and others have unwittingly exploited this basic chemoreversal pharmacophore hypothesis in the recent design and synthesis of phenothiazine- and xanthene-based chloroquine potentiating agents.[11,12]

In an ongoing and continuing programme aimed at validating the aforementioned hypothesis, we report the synthesis and biological evaluation of exploratory novel arylbromides (**1**), biphenyl (**2**, **3**), and biaryl ether (**4**) chloroquine potentiating agents (Fig. 4). The biphenyl and biaryl ether aromatic templates present in these compounds were selected for exploratory studies due to their ease of synthesis and amenability of the chemistry (Suzuki palladium-catalyzed and copper-mediated, respectively) to chemical library generation. This is expected to facilitate structure–activity relationship studies. Results described in this paper give the first confirmation of the proposed hypothesis, thus providing a preliminary clue for further design and synthesis.

Schemes 1 and 2 depict the straightforward synthesis of target compounds **1**–**4** from commercially available dibromo compounds. The synthesis starts with a Suzuki[13] cross-coupling of a large excess of the respective *o*-, *m*-, and *p*-dibromobenzenes **5** with phenylboronic acid to deliver a small amount (7–12%) of the triphenyl byproducts **6** along with the target intermediate biphenyl bromides **7** in moderate to good yield. Finally, palladium-catalyzed arylamination of dibromobenzenes **5** and biphenyl bromide **7** afforded the respective target compounds in moderate yield. The alternative route of

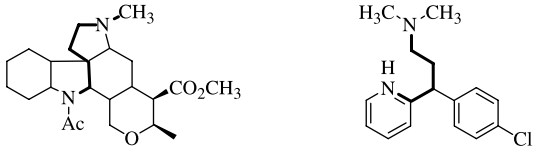


Figure 2. The 1,4-diamino unit in hexahydro-malagashanine and chlorpheniramine.

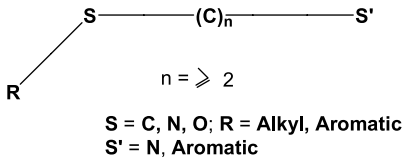
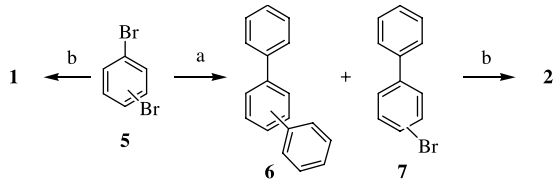


Figure 3. Proposed unifying chemosensitizing pharmacophore in malaria.



Scheme 1. Synthesis of compounds **1** and **2**. Reagents and conditions: (a) (i) 1.2 equiv of $PhB(OH)_2$, *n*-propanol, rt, 15 min; (ii) 0.003 equiv of $Pd(OAc)_2$, 0.01 equiv of Ph_3P, 2.0 equiv of Na_2CO_3, H_2O, reflux, **6** (7–12%) and **7** (58–67%); (b) 1.0 equiv of $H_2N(CH_2)_3NEt_2$, 2.0 equiv of NaO^tBu, 2 mol % of $Pd_2(dba)_3$, 6 mol % of BINAP, dioxane, reflux, 18 h, 50–58%.

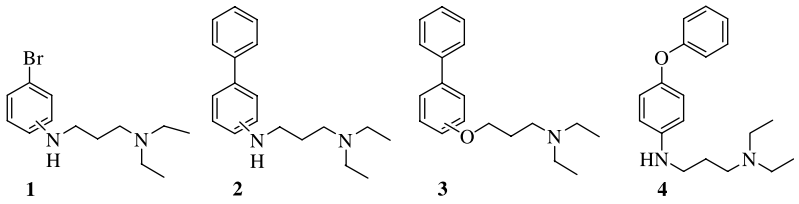
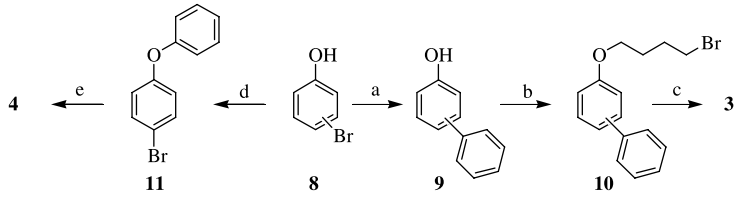


Figure 4. Structures of new bicyclic chloroquine potentiating agents.



Scheme 2. Synthesis of compounds **3** and **4**. Reagents and conditions: (a) (i) 1.2 equiv of $PhB(OH)_2$, *n*-propanol, rt, 15 min; (ii) 0.003 equiv of $Pd(OAc)_2$, 0.01 equiv of Ph_3P, 2.0 equiv of Na_2CO_3, H_2O, reflux, 90–98%; (b) (i) 1.2 equiv of NaH, 0 °C to rt, 30 min; (ii) 2.0 equiv of 1,4-dibromobutane, DMF, 0 °C, 18 h; (c) 2.0 equiv of pyrrolidine, CH_3CN, 18 h, 82–90%; (d) (i) 2.2 equiv of $PhB(OH)_2$, 1.5 equiv of $Cu(OAc)_2$, 2.5 equiv of pyridine, CH_2Cl_2, rt, 18 h 89%; (e) 1.0 equiv of $H_2N(CH_2)_3NEt_2$, 2.0 equiv of NaO^tBu, 2 mol % of $Pd_2(dba)_3$, 6 mol % of BINAP, dioxane, reflux, 18 h, 50–54%.

amination followed by Suzuki coupling resulted in much lower yields of the final target compounds.

Compounds **2** were synthesized from the *o*-, *m*-, and *p*-bromophenols **8** as shown in Scheme 2. The Suzuki cross-coupling reaction of phenyl boronic acid with **8** proceeded in excellent yields (90–98%) of biphenyl **9**, which upon alkylation with 1,4-dibromobutane in the presence of sodium hydride in DMF at 50 °C, followed by reaction of intermediate bromides **10** with pyrrolidine gave the final compounds in generally high yields of 82–90%. On the other hand, the synthesis of biarylether **4** was achieved via $Cu(OAc)_2$-mediated[14,15] arylation of *p*-bromophenol with phenyl boronic acid in the presence of pyridine to afford **10** in 89% yield. Arylamination with 3-(diethylamino)-propylamine led to **4** in yield of 52%.

It is noteworthy that although synthesized, compounds **7**, **9**, and **11** are commercially available.

All new compounds gave ^{1}H NMR, FAB-MS, and purity consistent with their structures.

Both the precursor bromo compounds (**1**-*para*, **1**-*meta*, and **1**-*ortho*) and the target bicyclic compounds **2**–**4** were screened for intrinsic antiplasmodial activity and CQ-potentiating effect using the CQ-resistant *P. falciparum* FCM29, and the results are shown in Table 1. The in vitro antiplasmodial and CQ-enhancing tests based on the inhibition of [^{3}H]-hypoxanthine uptake by *P. falciparum* cultured in human blood were conducted as previously described.[16]

Based on the IC_{50} values presented in Table 1, most compounds generally displayed moderate ($1 < IC_{50} < 5$ μg/ml) to weak ($IC_{50} > 5$ μg/ml) antiplasmodial activity, which may be attributed to the side chain basic terminal nitrogen. Compounds **2**-*para*, **2**-*meta*, **3**-*para*, and **3**-*ortho* having both low IC_{50} and IC_{90} values were comparatively the most active. The nitrogen presumably allows accumulation of the compounds within the acidic compartments of the parasite via the weak base effect. Concerning the chloroquine resistance modulation effects (Table 1), Figure 5A–C shows isobolograms of in vitro interaction between test compounds and chloroquine against the chloroquine-resistant strain FCM29. Regarding the shape of the curves, if the isobologramme graph is a line following roughly the diagonal, it represents an additive effect of the two drugs. If the graph forms a concave curve below the diagonal, it indicates synergy while a curve above the diagonal indicates antagonism between the two drugs. Thus compounds **1** (*ortho*), **1** (*meta*), **1** (*para*), **2** (*para*), **3** (*ortho*), and **4** displayed synergistic effects since they exhibited concave curves. Since the more concave the curve exhibited, the more effective the chemosensitizer, compounds **1** (*meta*), **1** (*para*), and **3** (*ortho*) displayed the best chemosensitizing effects. The brominated compounds showed chemosensitizing activities, but the substitution of the bromine did not significantly improve the activity. Noteworthy was compound **3**-*para* having the most antiplasmodial activity, which exhibited simple additive effects in drug combination. This observation is in agreement with our previous work.[17] Remarkably, isobologramme curves (Fig. 5C) depict the shift of the activity from synergistic, additive to slight antagonistic effect according

Table 1. In vitro antiplasmodial activity and chloroquine-enhancing action of test compounds against the chloroquine-resistant *Plasmodium falciparum* strain FCM29

Concentrations of test compounds (μg/ml)	IC_{50}/IC_{90}	Chloroquine activity (nM)	
		IC_{50}	IC_{90}
1-*para*	6.1 ± 2.2/ 66.7 ± 9.8		
00[a]		160.9 ± 10.1	842.8 ± 174.7
0.5		145.5 ± 22.1	745.3 ± 91.4
1		95.9 ± 8.7	362.5 ± 84.7
2		69.3 ± 8.4	271.5 ± 61.6
4		28.7 ± 6.1	273.1 ± 50.3
2-*para*	2.9 ± 1.8/ 18.5 ± 2.5		
0.375		98.2 ± 21.0	367.6 ± 42.1
0.75		54.0 ± 6.2	198.1 ± 17.5
1.5		25.9 ± 8.1	113.7 ± 20.5
1-*ortho*	3.7 ± 0.4/ 57.4 ± 10.5		
1		58.2 ± 12.4	290.3 ± 42.6
1.5		40.3 ± 7.9	194.9 ± 31.7
2		28.8 ± 4.3	134.0 ± 15.6
3		6.1 ± 0.7	153.4 ± 14.7
2-*ortho*	3.1 ± 0.9/ 94.6 ± 19.2		
0.62		138.4 ± 11.5	820.0 ± 60.7
1.25		72.2 ± 10.2	400.2 ± 71.2
2.50		11.5 ± 2.0	372.7 ± 20.3
2-*meta*	2.3 ± 0.7/ 20.8 ± 7.9		
0.68		159.8 ± 15.7	780.8 ± 104.1
1.36		108.8 ± 12.5	630.4 ± 97.8
1-*meta*	7.6 ± 0.9/ 80.3 ± 11.0		
0.62		109.9 ± 13.4	808.0 ± 101.0
1.25		68.5 ± 5.6	391.7 ± 57.2
2.5		38.5 ± 4.8	442.7 ± 50.8
5		7.2 ± 0.9	210.0 ± 40.7
3-*para*	1.7 ± 0.9/ 24.4 ± 6.3		
0.3		169.3 ± 21.0	655.3 ± 87.4
0.6		101.4 ± 18.7	310.8 ± 56.4
3-*ortho*	3.3 ± 1.3/ 22.3 ± 2.7		
1		53.9 ± 8.7	534.4 ± 71.3
1.5		42.9 ± 3.5	301.4 ± 49.2
2		7.6 ± 2.3	190.6 ± 23.7
3		3.0 ± 0.6	157.3 ± 21.8
3-*meta*	3.1 ± 1.3/ 49.5 ± 8.7		
1		118.6 ± 27.1	744.7 ± 97.6
2		77.7 ± 13.0	556.1 ± 101.0
4	3.1 ± 0.7/ 73.2 ± 3.2		
0.5		151.9 ± 19.1	736.4 ± 97.4
1		100.9 ± 13.4	254.9 ± 81.3
2		70.9 ± 8.7	205.4 ± 40.3

[a] For each compound, control plate with chloroquine alone was made and the mean was calculated as IC_{50} = 160.9 ± 10.1 nM and IC_{90} 842.8 ± 174.7 nM.

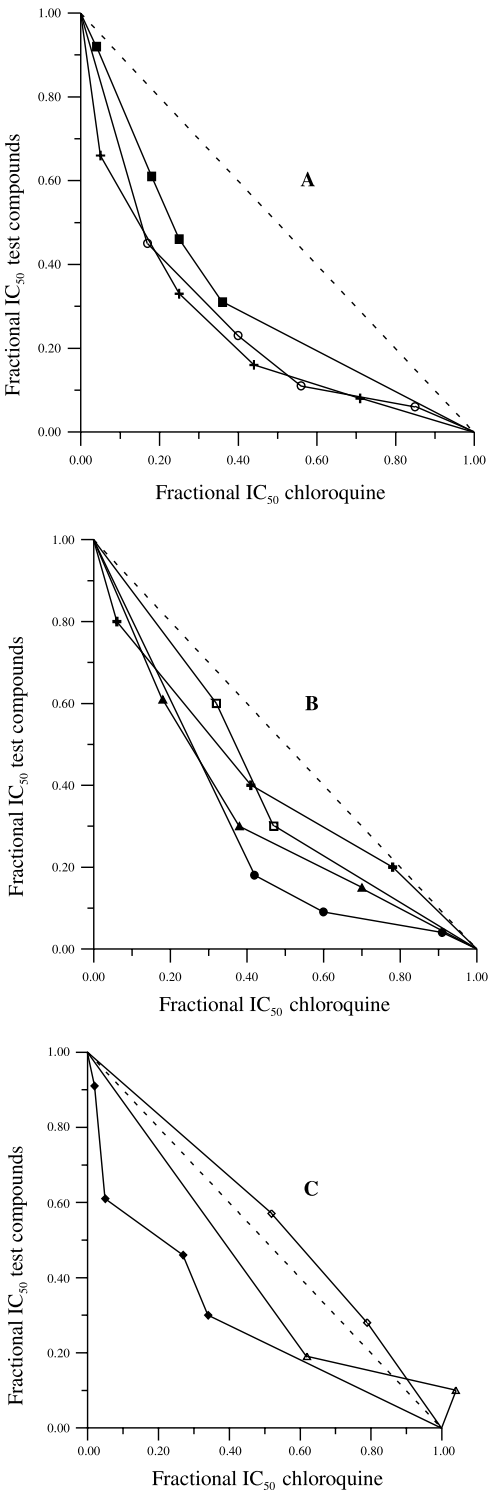


Figure 5. Isobolograms of in vitro interaction between test compounds and chloroquine against the chloroquine-resistant strain FCM29. (A): **1**-*para* (○), **1**-*ortho* (■), **1**-*meta* (+); (B): **2**-*para* (▲), **2**-*ortho* (✚), **2**-*meta* (□), **4** (●); (C): **3**-*para* (△), **3**-*ortho* (♦), **3**-*meta* (◊), Each point in the isobolograms was obtained by dividing IC_{50} for chloroquine plus test compound by IC_{50} for chloroquine alone (abscissa), and the fixed test compound concentration by IC_{50} of its intrinsic antiplasmodial activity (ordinate). Concave curve indicates synergism, curve following the diagonal a simple additive effect and convex curve antagonism. Results are means of assays in triplicate in 2–4 independent experiments until reproducible results were obtained.

to the relative position, *ortho*, *meta*, *para*, of the benzene ring of the biphenyl ether compounds **3**.

In conclusion, we have provided the first confirmation of the proposed chemoreversal hypothesis for drug resistant malaria parasites, thus providing a preliminary clue for further design and synthesis. The Suzuki and copper-catalyzed chemistry utilized to construct the biphenyl biaryl ether templates, respectively, is amenable to chemical library generation due to the commercial availability of structurally diverse boronic acid monomers, which can also be readily synthesized. This is expected to facilitate structure–activity relationship studies to further validate our working hypothesis. Work in this context is currently underway in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.04.033.

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