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Synthesis and Anti-*Plasmodium* Activity of Benzimidazole Analogues Structurally Related to Astemizole

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A series of compounds structurally related to astemizole were designed and synthesized with the goal of determining their anti-*Plasmodium* activity. Several modifications of the astemizole structure, namely the removal of the 4-fluorobenzyl and/or 4-methoxyphenethyl moieties, substitution of the benzene ring of the benzimidazole scaffold, replacement of the fluorine atom in the 4-fluorobenzyl group, and variation of the 4-aminopiperidine moiety, were explored. In vitro evaluation of the anti-*Plasmodium* activity of these compounds using the ItG strain showed that astemizole and some of its structurally similar derivatives have IC_{50} values in the nanomolar range and exhibit toxicity towards the parasite over Chinese ovarian hamster (CHO) cells with a selectivity as high as 200. The presence of a secondary cyclic amine at position 2 and substitution with chlorine at positions 4 and 5 in the benzimidazole moiety are two modifications that resulted in potent and selective antimalarials based on astemizole.

Introduction

Faced with an average estimated cost of US $800 million required to bring a single drug to market after approximately 15 years of development,[1] the biopharmaceutical industry has recently started to consider other approaches besides de novo drug discovery. Although the use of structure-based drug design, combinatorial chemistry, high-throughput screening and genomics was supposed to result in a significant improvement in productivity, the outcome from these novel discovery technologies has been negligible thus far. On the other hand, the re-evaluation of either drugs-in-use or late-stage clinical failures for a different disease has paid off in several cases. Amongst the most famous examples are duloxetine, a compound that was developed as an antidepressant and has since been repurposed for the treatment of stress urinary incontinence; the sedative thalidomide, which is being considered for the treatment of a severe form of leprosy and multiple myeloma; and sildenafil, a relaxant for coronary arteries that has found a new use in the treatment of erectile dysfunction.

Commonly defined as the process of finding new applications outside the original indication for existing drugs, drug repositioning offers the advantage of faster development times while reducing the development risks, as the safety and pharmacokinetic profiles for the repositioning candidates are already known. Moreover, because chemical optimization, bulk manufacturing, formulation development and, in some cases, even early clinical development and the process of approval by the regulatory agencies for human use, have been finalized, drug repositioning candidates are usually on a fast track to market. Despite the fact that drug repositioning is economically attractive and provides a shortcut between the lab and the clinic, the unique intellectual property issues associated with it can be extremely challenging, unless the drug is off-patent and therefore generic. Some of these candidates are the result of serendipitous observations, but directed efforts could lead also to new indications for old drugs.

Recently, the screening of a library of existing drugs for inhibitors of human malaria parasite *Plasmodium falciparum* identified astemizole (**1**) as a drug that inhibits the proliferation of three parasite strains that differ in chloroquine sensitivity.[2] Astemizole is an off-patent, over-the-counter, nonsedating, selective H_1-histamine receptor antagonist that was voluntarily withdrawn by Janssen Pharmaceutica in most countries, owing to safety concerns related to cardiovascular side effects[3] and the emergence of novel, less dangerous antihistamines.[4] In humans, astemizole appears in serum only briefly (elimination time of approximately 1.1 days) because it undergoes rapid biotransformations by the hepatic cytochrome P450 enzyme system.[5] The major metabolites of astemizole are desmethylastemizole (**2**), 6-hydroxydesmethylastemizole (**3**), and norastemizole (**4**; also known as tecastemizole),[6] which are generated by oxidative O-demethylation, oxidative O-demethylation followed by hydroxylation, and oxidative N-dealkylation of the parent drug, respectively (Figure 1).[7] Desmethylastemizole retains antihistamine properties, has a long elimination time of 9 to 13 days, and its steady-state serum concentration exceeds that of astemizole by more than 30-fold. In contrast, norastemizole appears in the serum in low concentrations following

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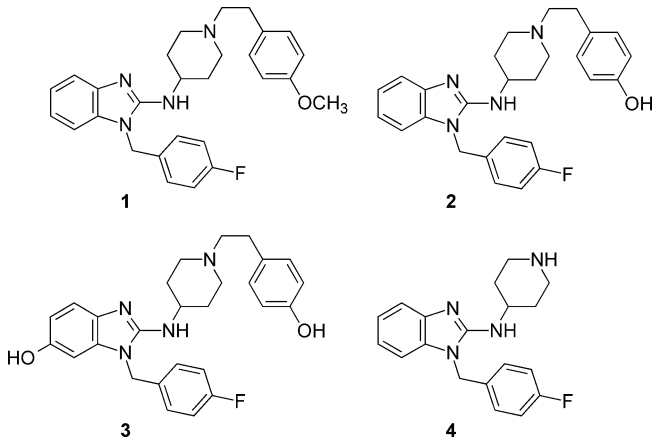


Figure 1. Astemizole (**1**) and its metabolites (**2**–**4**).

astemizole ingestion and has more potent H_1-receptor antagonist properties than desmethylastemizole and astemizole and lesser side effects;[8] therefore, it has undergone development as a new antihistamine drug[9] but was finally dropped by Sepracor Inc. owing to a "nonapprovable" decision by the US Food and Drug Administration (FDA).

In continuation of our interest in developing novel therapeutic candidates for the treatment of malaria,[10–15] a preliminary study aimed at exploring the relevance of astemizole as a starting point for further development was undertaken. The synthesis, characterization, and in vitro anti-*Plasmodium* activity of several compounds structurally related to astemizole are reported herein.

Results and Discussion

Synthesis

The initial aim of this study was to establish the contribution of the different moieties in the structure of astemizole to its anti-*Plasmodium* activity. Therefore, the first three target compounds were norastemizole (**4**), which lacks the 4-methoxyphenetyl moiety of astemizole, compound **5**, which is derived from astemizole by the removal of the 4-fluorobenzyl residue, and finally (1*H*-benzimidazol-2-yl)-(4-piperidinyl)amine (**6**), a molecule that retains the cyclic structures of astemizole but is devoid of both aforementioned appendages (Figure 2).

Several straightforward strategies for the synthesis of norastemizole (**4**) have been reported, starting from either 2-chlorobenzimidazole or from 2-chloro-1-(4-fluorobenzyl)benzimidazole. One of the most recent articles describes the use of microwave irradiation to react the starting material with 4-amino-

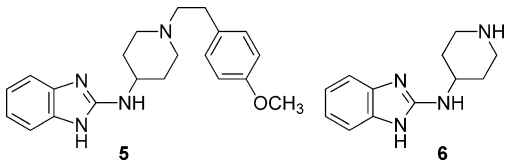
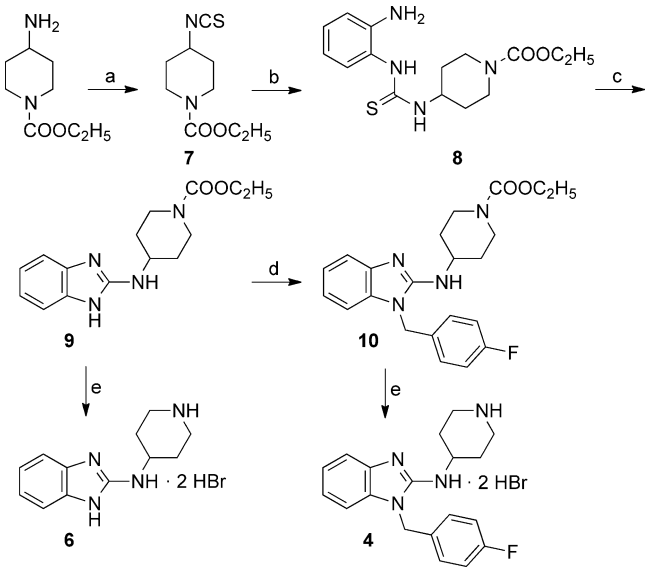


Figure 2. Structures of initial target compounds **5** and **6**.

piperidine,[16] whereas other reports describe a similar coupling reaction at elevated temperatures[17,18] or even under hyperbaric conditions.[19] Lately, the palladium-catalyzed amination of 2-chlorobenzimidazoles, based on Buchwald chemistry, has also been described.[20,21] Despite recent advances towards a more direct synthesis of 2-aminobenzimidazoles, the synthetic approach in Janssen's seminal article[22] was preferred for our work because it does not use expensive catalysts, microwave irradiation, or high temperatures leading to undesired by-products.

Starting from the commercially available ethyl 4-amino-1-piperidinecarboxylate, ethyl 4-((1*H*-benzimidazol-2-yl)amino)-1-piperidinecarboxylate (**9**) was prepared in 34% yield through a three-step sequence comprising the synthesis of isothiocyanate **7**, addition of 1,2-phenylenediamine to the crude product (**7**), and cyclodesulfurization of the resulting thiourea (**8**) using mercury(II) oxide in ethanol, as reported.[22] Because the syrup-like residue obtained after evaporation of solvent in the step leading to thiourea **8** could not be crystallized, column chromatography (silica gel, ethyl acetate/hexanes, 4:1) was used to separate the desired compound from the slight excess of 1,2-phenylenediamine. As presented in Scheme 1, further reaction

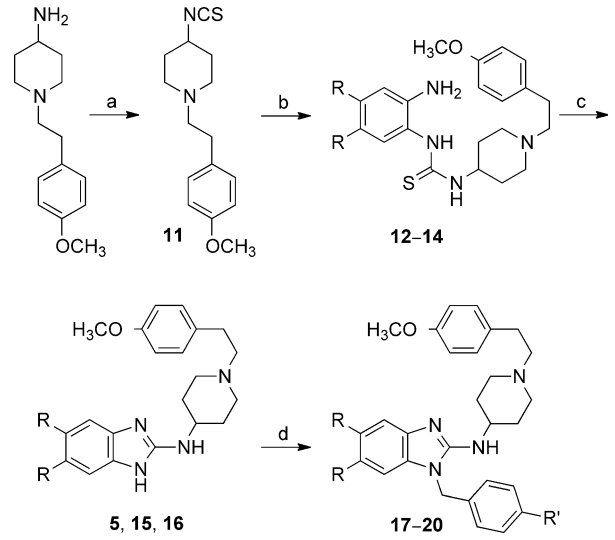


Scheme 1. Synthesis of target compounds **4** and **6**. *Reagents and conditions*: a) NaOH, CS_2, H_2O, 5 °C, 1 h, then ethyl chloroformate, 60–70 °C, 2 h; b) 1,2-phenylenediamine, EtOH, RT, overnight; c) yellow HgO, S, EtOH, reflux, 2 h; d) 4-fluorobenzyl chloride, K_2CO_3, KI, DMF, 70–80 °C, 20 h; e) 48% HBr, reflux, 2 h.

of compound **9** with 4-fluorobenzyl chloride afforded the N1-alkylated benzimidazole **10**, which underwent removal of the protecting group at the piperidine nitrogen atom to give norastemizole (**4**) as a dihydrobromide with a 20% overall yield. Similar deprotection of ethyl 4-((1*H*-benzimidazol-2-yl)amino)-1-piperidinecarboxylate (**9**) yielded target compound **6** as the dihydrobromide.

The same sequence of reactions that was used for the preparation of intermediate **9** was also employed for the synthesis of target compound **5**, starting from commercially available

4-amino-1-(2-(4-methoxyphenyl)ethyl)piperidine. Isothiocyanate **11**, which was obtained in pure form through a combination of extraction and column chromatography, was reacted with 1,2-phenylenediamine to give thiourea **12**, which closed the imidazole ring to form the desired 1*H*-benzimidazol-2-yl-(1-(2-(4-methoxyphenyl)ethyl)piperidin-4-yl)amine (**5**) (R = H) upon desulfurization with mercury(II) oxide (Scheme 2). It is noteworthy that under the conditions described in the literature,[23] the conversion of thiourea **12** was still incomplete even after the reaction mixture had been heated at reflux for 10 h, but when fresh mercury(II) oxide was added after 5 h, only traces of the starting material were noticeable by TLC at the end of the reaction time.

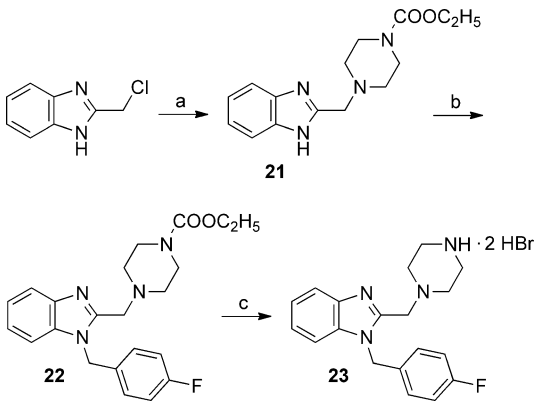


Scheme 2. Synthesis of target compound **5** (R = H), astemizole analogues substituted on the benzimidazole moiety **17** (R = CH_3, R′ = F) and **18** (R = Cl, R′ = F), and astemizole analogues with a substituent other than fluorine on the N1-benzyl moiety **19** (R = R′ = H) and **20** (R = H, R′ = CF_3). *Reagents and conditions*: a) NaOH, CS_2, H_2O, 5 °C, 1 h, then ethyl chloroformate, 70–80 °C, 2 h; b) 1,2-phenylenediamine, EtOH, RT, overnight; c) yellow HgO, S, EtOH, reflux, 10 h; d) 4-fluorobenzyl chloride, K_2CO_3, KI, DMF, 70–90 °C, 18–20 h.

Evaluation of the effect of substituents on the benzimidazole moiety on the anti-*Plasmodium* activity of astemizole analogues was another aim of this study. The synthesis of monosubstituted analogues of astemizole having an unambiguous assignment of the substituent in the benzimidazole moiety could be accomplished following a multistep strategy that starts from the appropriately substituted 2-halo-nitrobenzenes and comprises amination with 4-fluorobenzyl amine, reduction of the nitro group, reaction with isothiocyanate **11**, and ring closure.[24,25] On the other hand, the use of 4-substituted-1,2-phenylenediamine in the outlined synthetic scheme would lead to a mixture of 3- and 4-substituted thioureas,[26] and finally to a mixture of 5- and 6-monosubstituted regiosiomers of astemizole analogues.[24] With the view to avoid the complication arising from the separation of these regioisomers, two symmetrical 4,5-disubstituted-1,2-phenylenediamines, namely 4,5-dimethyl-1,2-phenylenediamine and 4,5-dichloro-1,2-phenylenediamine, were employed in the preparation of astemizole-like compounds with substituents on the benzimidazole moiety. In a manner similar to the one employed for the synthesis of compound **5**, isothiocyanate **11** was reacted with these two symmetrical 4,5-disubstituted-1,2-phenylenediamines to give thioureas **13** (R = CH_3) and **14** (R = Cl), which were subsequently converted into corresponding benzimidazoles **15** and **16**, respectively, using the cyclodesulfurization method. Finally, compounds **15** and **16** were alkylated with 4-fluorobenzyl chloride at N1 of the benzimidazole moiety to yield the novel astemizole analogues **17** (R = CH_3, R′ = F) and **18** (R = Cl, R′ = F).

A brief exploration of the effect of the replacement of the fluorine in the benzyl group appended to N1 of the benzimidazole moiety on the anti-*Plasmodium* activity was also a goal of this study. For this reason, intermediate **5** was alkylated with benzyl bromide and 4-(trifluoromethyl)benzyl bromide to afford compounds **19** (R = R′ = H) and **20** (R = H, R′ = CF_3), respectively, which have a hydrogen and a trifluoromethyl group instead of the typical fluorine of astemizole (Scheme 2).

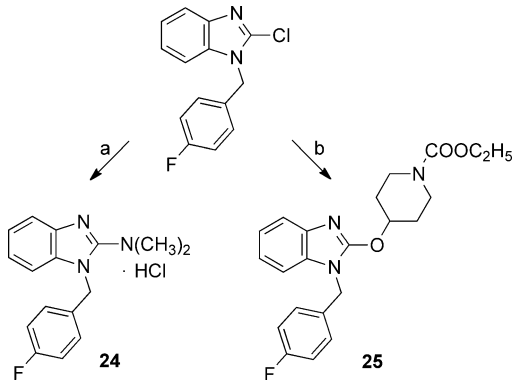
Several modifications of the astemizole aminopiperidine moiety were also considered. Consequently, 2-chloromethylbenzimidazole was condensed with ethyl 1-piperazinecarboxylate to give intermediate **21**, subsequent alkylation of which with 4-fluorobenzyl bromide led to compound **22** (Scheme 3). Deprotection of the piperazine in the former afforded compound **23**, which is structurally very similar to norastemizole (**4**).



Scheme 3. Synthesis of a piperazine-containing analogue of norastemizole. *Reagents and conditions*: a) ethyl 1-piperazinecarboxylate, Na_2CO_3, EtOH, reflux, overnight; b) 4-fluorobenzyl chloride, K_2CO_3, KI, DMF, 75–85 °C, 18 h; c) 48% HBr, reflux, 2 h.

With the aim of replacing the exocyclic nitrogen atom in astemizole with oxygen, commercially available 2-chloro-1-(4-fluorobenzyl)benzimidazole was reacted with ethyl 4-hydroxy-1-piperidinecarboxylate in DMF in the presence of sodium hydride at room temperature, as reported in the literature,[27] but only the starting material was recovered at the end of the reaction time. The replacement of sodium hydride with lithium hydride and an increase in the reaction temperature to approximately 90 °C led to the unexpected 2-dimethylamino-1-(4-fluorobenzyl)benzimidazole (**24**), which presumably arose from

substitution of the chlorine atom in the substrate with the dimethylamine, produced in situ from the solvent in the presence of the base, rather than the normal product. Finally, desired compound **25** was obtained, albeit in a modest yield, by reaction of 2-chloro-1-(4-fluorobenzyl)benzimidazole with ethyl 4-hydroxy-1-piperidinecarboxylate in DMSO in the presence of sodium hydride at 75–85 °C (Scheme 4). Separation from the



Scheme 4. Products arising from the reaction of 2-chloro-1-(4-fluorobenzyl)-benzimidazole with ethyl 4-hydroxy-1-piperidinecarboxylate in different solvents. *Reagents and conditions*: a) ethyl 4-hydroxy-1-piperidinecarboxylate, LiH, DMF, 90 °C, overnight; b) ethyl 4-hydroxy-1-piperidinecarboxylate, NaH, DMSO, 75–85 °C, overnight.

complex reaction mixture proved to be difficult, as **25** co-eluted under different experimental conditions with a minor by-product whose structure was not investigated closely but was revealed by NMR spectroscopy to retain the 1-(4-fluorobenzyl)benzimidazole core, while lacking any aliphatic protons. In the end, use of recurring column chromatography combined with the collection of very small fractions of eluent provided a small amount of compound **25** that was sufficiently pure for biological evaluation.

Structure–anti-*Plasmodium* activity relationship

The effects of astemizole (**1**), norastemizole (**4**), and compounds **5**, **6**, **9**, **10**, **12**, and **14**–**25** on the viability of *P. falciparum* cultures were evaluated (Table 1), and the potency was compared to the toxicity observed in a mammalian line, namely Chinese hamster ovary (CHO) cells. All activities were determined in quadruplicate. The IC_{50} values of the compounds listed in Table 1 were calculated, and a selectivity index was obtained by dividing the IC_{50} values for the CHO cell cultures by the IC_{50} values for the *P. falciparum* cultures. A ratio greater than unity indicates that a compound is preferentially toxic to *P. falciparum* parasites.

With one exception (compound **21**), all of the compounds in this study were found to be selectively toxic to *Plasmodium*; however, only a few displayed reasonably high selectivity. Norastemizole (**4**) was the most selective compound in this collection, with a selectivity index of approximately 200, followed by the structurally related piperazine-containing compound **23** (selectivity index of 130), and astemizole (**1**) and analogues **19** and **20**. These results suggest that a cyclic secondary amine moiety is a structural feature required in a selective antimalarial based on the astemizole scaffold. In terms of potency, several compounds in the collection were found to inhibit parasite proliferation at sub-micromolar concentrations. Astemizole (**1**) is the second most potent compound, its anti-*Plasmodium* activity being surpassed only by the activity of analogue **20**. In contrast to results from the earlier study,[2] norastemizole (**4**) was found to be virtually equipotent to astemizole; moreover, the IC_{50} values determined by us for the in vitro anti-*Plasmodium* activity of both compounds were notably higher (10 times for astemizole and 27 times for norastemizole) than the reported values for the same line. In general, astemizole and all of its close analogues synthesized for this study were highly active towards *P. falciparum*.

Substitution of the benzimidazole moiety (compounds **17** and **18**) slightly decreased the toxicity of the astemizole analogues towards the parasite, as opposed to the activity of the parent compound, and this modification could potentially be used in the future development of an astemizole-based antimalarial drug to separate the antihistaminic activity from the antimalarial action of the candidate. Furthermore, the difference in the anti-*Plasmodium* activities for intermediate thioureas **12** (R = H) and **14** (R = Cl) may be interpreted as the most striking evidence for the importance of the chlorine substituents in the structure of these candidates; indeed, all chlorine-containing compounds have shown a strong inhibition of parasite development that is accompanied by moderate selectivity.

The replacement of the fluorine in astemizole with the bioisosteric hydrogen (compound **19**) led to a compound that is fourfold less potent, whereas the presence of a trifluoromethyl group (compound **20**) slightly improved the potency. Removal of the substituted benzyl group at N1 resulted in divergent trends in terms of anti-*Plasmodium* activity: compounds **5** and **15** were less active than the structurally related astemizole (**1**) and compound **17**, respectively, whereas compound **16** was more potent than the corresponding astemizole-like compound **18**. Nonetheless, these derivatives of astemizole and its analogues still have sub-micromolar IC_{50} values, which seem to indicate that substitution at N1 of the benzimidazole moiety is not essential for anti-*Plasmodium* activity of these compounds. Furthermore, whereas the removal of the 4-methoxyphenethyl group from astemizole is highly beneficial for selectivity towards the parasite without affecting the potency (compound **4**), its replacement with an ethoxycarbonyl group (compound **10**) produced a candidate that is nonselective and has a low toxicity to *P. falciparum*. Removing both the 4-methoxyphenethyl and 4-fluorobenzyl groups from astemizole (as in compound **6**) further increased the anti-*Plasmodium* IC_{50} value into the micromolar range. Although a more comprehensive study is required in order to establish the optimal substitution at N1 and the nitrogen atom of the piperidine moiety, current data suggest that either the absence of a substituent or the presence of a (substituted) phenylalkyl group at these two regions of the astemizole scaffold is highly beneficial for anti-*Plasmodium* activity.

Table 1. In vitro activities of compounds **1**, **4**–**6**, **9**, **10**, **12**, and **14**–**25** in *P. falciparum* and CHO cultures.

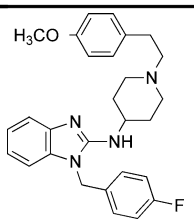
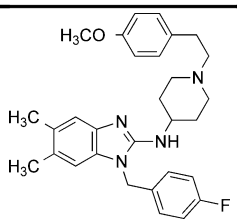
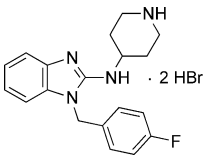
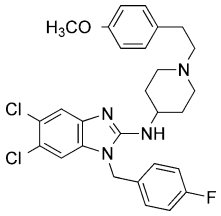
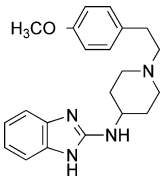
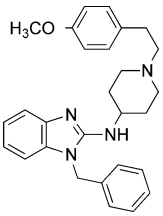
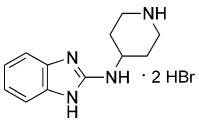
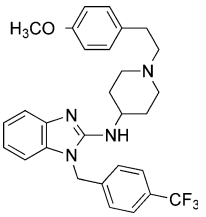
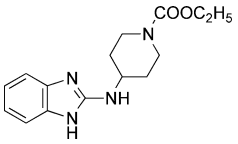
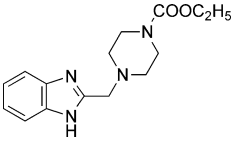
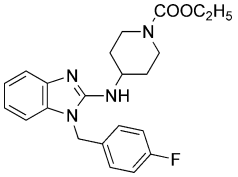
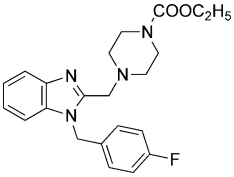
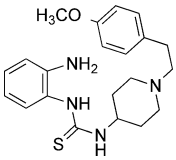
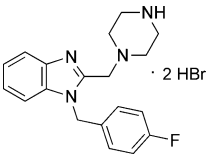
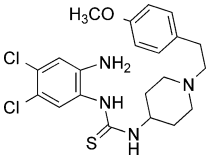
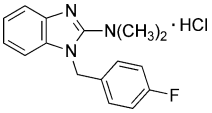
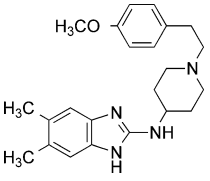
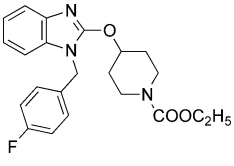
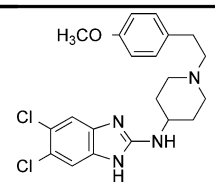
Compd	Structure	IC_{50} [μM][a] *P. falciparum*	IC_{50} [μM][a] CHO cells	SI[b]	Compd	Structure	IC_{50} [μM][a] *P. falciparum*	IC_{50} [μM][a] CHO cells	SI[b]
1		0.072 ± 0.006	2.5 ± 0.1	35	**17**		0.119 ± 0.009	4.9 ± 0.9	41
4		0.08 ± 0.01	17 ± 6	212	**18**		0.35 ± 0.04	3.3 ± 0.7	9
5		0.197 ± 0.006	4.2 ± 0.4	21	**19**		0.36 ± 0.04	6 ± 1	17
6		64 ± 5	271 ± 21	4	**20**		0.07 ± 0.02	3.5 ± 0.2	50
9		68 ± 12	197 ± 116	3	**21**		383 ± 57	276 ± 20	0.7
10		44 ± 4	58 ± 8	1	**22**		28 ± 2	33 ± 2	1
12		18 ± 2	26 ± 1	1	**23**		0.5 ± 0.2	62 ± 2	124
14		0.27 ± 0.02	8 ± 1	30	**24**		11.2 ± 0.9	152 ± 5	14
15		0.182 ± 0.008	0.8 ± 0.1	4	**25**		8 ± 2	12 ± 1	1

Table 1. (Continued)

Compd	Structure	IC_{50} [μM][a] *P. falciparum*	CHO cells	SI[b]	Compd	Structure	IC_{50} [μM][a] *P. falciparum*	CHO cells	SI[b]
16		0.083 ± 0.007	2.3 ± 0.4	28					

[a] Each IC_{50} value represents the mean of four determinations with standard error indicated. The strain line ItG was used. [b] Selectivity index (SI) represents the ratio of activities: IC_{50} (CHO)/IC_{50} (*P. falciparum*). Values greater than unity indicate that the compound has greater potency in *P. falciparum* cultures.

Modifications of the aminopiperidine moiety were, in general, less favorable. Two of the piperazine-containing compounds (**21** and **23**) were found to be less active towards the parasite than their corresponding aminopiperidine-containing counterparts (**9** and **4**, respectively), whereas compound **22** was slightly more active than the analogous **10**. The selectivity in the piperazine-containing series followed a trend that mirrors the selectivity in the aminopiperidine series, with compounds having the nitrogen atom of the cyclic secondary amine protected with a carbethoxy group being virtually nonselective. At the same time, the results for candidate **23** substantiate the observation made for norastemizole (**4**), namely that a cyclic secondary amine moiety is important for both significant anti-*Plasmodium* activity and good selectivity towards the parasite. In addition, intermediate **25** with an oxygen atom was more active than compound **10** featuring the original exocyclic secondary nitrogen, and even more active than its piperazine-containing counterpart **22**; however, the troublesome preparation and separation of **25** would probably deter any further development of antimalarials based on its structure unless a more convenient route for its synthesis is devised. As for compound **24**, which lacks the piperidine moiety, although it exhibits only a moderate anti-*Plasmodium* activity and has a rather poor selectivity towards the parasite, biological evaluation showed that fairly good anti-*Plasmodium* activity could be achieved even with a minimally substituted benzimidazole scaffold, therefore opening new possibilities for rational design based on the aforementioned scaffold.

Conclusions

The series of compounds structurally related to astemizole that were designed with a view to garner some insight concerning the relationship between their anti-*Plasmodium* activity and their structure provided a few valuable indications. While all of the compounds reported in this study showed a good anti-*Plasmodium* activity ($IC_{50} < 0.4$ mM), the most active ones possess IC_{50} values in the nanomolar range. All of the compounds in the series contain the benzimidazole core as the central scaffold, which is probably an essential structural requirement for the manifestation of anti-*Plasmodium* activity; however, the presence of the appropriate appendages at positions 1 and 2 (or the absence thereof) proved to be critical for the fine tuning of their anti-*Plasmodium* toxicity. Notably, compounds having a secondary amine (either 4-aminopiperidine or 4-methylenepiperazine) functionality at position 2 exhibited enhanced toxicity towards the parasite in a selective manner. Both the replacement of fluorine and the substitution of the benzene ring of the benzimidazole core in the astemizole structure could be used to disconnect the antihistaminic and the antimalarial activity of these candidates. Although the present study lays the foundation for the repositioning of astemizole and its analogues as antimalarials, an extension of this investigation that exploits the present findings is needed in order to more accurately define the structural features required for a more active candidate while selectively improving toxicity towards the parasite.

Experimental Section

Chemistry

General: Ethyl 4-amino-1-piperidinecarboxylate was supplied by Alfa Aesar (Ward Hill, MA, USA). 4-Amino-1-(2-(4-methoxyphenyl)-ethyl)piperidine, 4,5-dimethyl-1,2-phenylenediamine, 4,5-dichloro-1,2-phenylenediamine, and 4-hydroxy-1-piperidinecarboxylate were purchased from TCI America Laboratory Chemicals (Portland, OR, USA). All other chemical reagents were obtained from Sigma–Aldrich (Oakville, ON, Canada). Column chromatography was performed on silica gel (230–400 mesh, 60 Å) (SiliCycle, Quebec City, QC, Canada). Analytical TLC was performed on SiliCycle glass-backed precoated silica gel 60 F254 plates, and the compounds were visualized by UV illumination (254 nm). Melting points were recorded on a Mel-Temp II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. The signals from residual protons in deuterated solvents were used as internal standards for the ^{1}H NMR spectra. Chemical shifts for the carbon atoms are given relative to $CDCl_3$ ($\delta = 77.16$ ppm), CD_3OD ($\delta = 49.00$ ppm), or [D_6]DMSO ($\delta = 39.52$ ppm). High-resolution mass spectra were obtained either on a Waters/Micromass GCT mass spectrometer in EI mode or on an Applied Biosystems/MDS Sciex QSTAR XL spectrometer equipped with an Agilent HP1100 Cap-LC system in ESI mode.

Ethyl 4-((1*H*-benzimidazol-2-yl)amino)-1-piperidinecarboxylate (9): Compound **9** was obtained starting from ethyl 4-amino-1-piperidinecarboxylate following the three-step procedure (isothiocya-

nate formation, addition to 1,2-phenylenediamine, cyclodesulfurization) reported by Janssens et al.[22] to yield a colorless solid (975 mg, 34%): m.p.: 237–239 °C (2-butanone) (lit. [22] m.p.: 240.6 °C); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.26$ (t, $J = 7.2$ Hz, 3H), 1.37–1.51 (m, 2H), 2.01–2.10 (m, 2H), 3.02 (br s, 2H), 3.75–3.85 (m, 1H), 4.05–4.17 (m, 4H), 6.92–7.00 (m, 2H), 7.14–7.23 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 15.0$, 33.3, 43.9, 50.8, 62.7, 112.7, 121.3, 155.9, 157.2 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{21}N_4O_2$: 289.1659, found 289.1652.

Ethyl 4-((1-(4-fluorobenzyl)-1*H*-benzimidazol-2-yl)amino)-1-piperidinecarboxylate (10): A mixture of compound **9** (432 mg, 1.5 mmol), 4-fluorobenzyl chloride (325 mg, 2.25 mmol), anhydrous K_2CO_3 (418 mg, 3 mmol), and a few crystals of KI in DMF (4 mL) were heated at 70–80 °C for 20 h. The cooled reaction mixture was partitioned between water (50 mL) and EtOAc (30 mL). The aqueous phase was further extracted with EtOAc (30 mL), then the combined organic phases were washed with water (50 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to give a tan solid. Flash column chromatography (silica gel, EtOAc/hexanes, 4:1, *v/v*) of the crude product afforded **10** as a colorless solid (523 mg, 88%): $R_f = 0.45$ (EtOAc/hexanes, 4:1, *v/v*); m.p.: 175–176 °C (lit. [18] m.p.: 179–180 °C, lit. [22] m.p.: 180.8 °C); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.25$ (t, $J = 7.2$ Hz, 3H), 1.38–1.53 (m, 2H), 2.01–2.10 (m, 2H), 2.99 (br s, 2H), 3.90–4.01 (m, 1H), 4.06–4.17 (m, 4H), 5.22 (s, 2H), 6.91–6.97 (m, 1H), 6.98–7.07 (m, 4H), 7.09–7.16 (m, 2H), 7.31 ppm (d, $J =$ 8.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 15.0$, 33.1, 44.1, 45.3, 51.4, 62.7, 109.1, 116.1, 116.4 (d, $J^2_{C,F} = 21.8$ Hz), 120.9, 122.5, 129.6 (d, $J^3_{C,F} = 8.2$ Hz), 133.8 (d, $J^4_{C,F} = 2.7$ Hz), 135.3, 142.9, 155.2, 157.2, 163.6 ppm (d, $J^1_{C,F} = 243$ Hz); ^{19}F NMR (376 MHz, CD_3OD): $\delta = -118.1$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}FN_4O_2$: 397.2034, found 397.2038.

(1-(4-Fluorobenzyl)-1*H*-benzimidazol-2-yl)-(4-piperidinyl)amine dihydrobromide (4): Compound **10** (397 mg, 1 mmol) was heated at reflux with 48% HBr (25 mL) for 2 h, then the solvent was removed under reduced pressure to give a tan solid that was recrystallized from EtOH to yield **4** as off-white crystals (323 mg, 66%): m.p.: 272–273 °C (dec.) (lit. [18] m.p.: 280 °C, lit. [22] m.p.: 260 °C); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.99$–2.14 (m, 2H), 2.31–2.42 (m, 2H), 3.21–3.31 (m, 2H), 3.53–3.61 (m, 2H), 4.06–4.18 (m, 1H), 5.52 (s, 2H), 7.07–7.16 (m, 2H), 7.27–7.39 (m, 5H), 7.52–7.57 ppm (m, 1H);); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 29.7$, 44.3, 46.6, 50.7, 111.8, 113.0, 116.9 (d, $J^2_{C,F} = 21.8$ Hz), 125.3, 125.6, 130.0 (d, $J^3_{C,F} = 8.1$ Hz), 130.3, 131.3 (d, $J^4_{C,F} = 3.3$ Hz), 131.9, 150.3, 164.0 ppm (d, $J^1_{C,F} =$ 244 Hz); ^{19}F NMR (376 MHz, CD_3OD): $\delta = -116.9$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{22}FN_4$: 325.1823, found 325.1834.

(1*H*-Benzimidazol-2-yl)-(4-piperidinyl)amine dihydrobromide (6): A procedure similar to that employed for the synthesis of compound **4**, but starting from compound **9** (288 mg, 1 mmol), gave compound **6** after recrystallization from EtOH as colorless crystals (355 mg, 94%): m.p.: 310–311 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 1.88–2.04 (m, 2H), 2.28–2.41 (m, 2H), 3.17–3.29 (m, 2H), 3.51–3.61 (m, 2H), 3.92–4.02 (m, 1H), 7.28–7.34 (m, 2H), 7.40–7.46 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 29.6$, 44.0, 49.7, 112.5, 125.0, 130.9, 150.5 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{12}H_{17}N_4$: 217.1448, found 217.1443.

4-Isothiocyanato-1-(2-(4-methoxyphenyl)ethyl)piperidine (11): A well-stirred mixture of 4-amino-1-(2-(4-methoxyphenyl)ethyl)piperidine (4.914 g, 21 mmol) and NaOH (840 mg, 21 mmol) in water (30 mL) was treated dropwise with CS_2 (3.276 g, 42 mmol) at 0 °C. After the mixture was stirred at this temperature for 1 h, ethyl chloroformate (2.29 g, 21 mmol) was added, and the mixture was maintained at 70–80 °C for 2 h. The excess of CS_2 was removed under a stream of nitrogen, then the solvent was decanted, and the brown semisolid residue was extracted in EtOAc (2×40 mL). The combined organic phases were filtered, the solid on the filter paper was washed thoroughly with EtOAc, the filtrate was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to give a residue that was purified by chromatography (silica gel, EtOAc/MeOH, 9:1, *v/v*) to yield the isothiocyanate as a light brown solid (3.60 g, 62%): $R_f = 0.60$ (EtOAc/MeOH, 9:1, *v/v*): m.p.: 64–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.81$–1.92 (m, 2H), 1.93–2.04 (m, 2H). 2.41 (br s, 2H), 2.52–2.61 (m, 2H), 2.63–2.77 (m, 4H), 3.72–3.81 (m, 4H), 6.83 (d, $J = 8.4$ Hz, 2H), 7.11 ppm (d, $J =$ 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 32.5$, 32.9, 50.4, 53.3, 55.4, 60.7, 114.0, 129.7, 131.3, 132.2, 158.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{21}N_2OS$: 277.1369, found 277.1365.

1-(2-Aminophenyl)-3-(1-(2-(4-methoxyphenyl)ethyl)piperidin-4-yl)thiourea (12): Compound **11** (929 mg, 3.36 mmol) was dissolved in EtOAc (5 mL) and treated with a solution of 1,2-phenylenediamine (363 mg, 3.36 mmol) in EtOH (5 mL) at 50 °C. The mixture was stirred at RT for 24 h, then the precipitate was filtered and recrystallized from EtOH to give thiourea **12** (711 mg, 55%): m.p.: 162–163 °C (lit. [23] m.p.: 160–161 °C); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.34$–1.51 (m, 2H), 2.06 (d, $J = 9.6$ Hz, 2H), 2.19 (t, $J = 10.8$ Hz, 2H), 2.48–2.58 (m, 2H), 2.65–2.75 (m, 2H), 2.83 (d, $J = 10.8$ Hz, 2H), 3.77 (s, 3H), 3.91 (s, 2H, exchangeable with D), 4.23–4.37 (m, 1H), 5.69 (d, $J = 7.6$ Hz, 1H, exchangeable with D), 6.73–6.85 (m, 4H), 7.01–7.11 (m, 3H), 7.12–7.20 (m, 1H), 7.46 ppm (s, 1H, exchangeable with D); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 31.7$, 32.9, 52.1, 52.2, 55.4, 60.7, 113.9, 116.7, 119.3, 120.6, 128.5, 129.7, 130.0, 132.3, 143.5, 158.1, 180.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{29}N_4OS$: 385.2057, found 385.2061.

(1*H*-Benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)ethyl)piperidin-4-yl)amine (5): A mixture of thiourea **12** (577 mg, 1.5 mmol), sulfur (10 mg), and yellow mercury(II) oxide (650 mg, 3 mol) was heated at reflux in EtOH (40 mL) for 5 h, then new portions of sulfur and HgO were added, and the mixture was heated at reflux for an additional 5 h. The solids were filtered over a Celite bed, then the solvent was removed under reduced pressure to give a reddish residue that was purified by chromatography (silica gel, EtOAc/MeOH, 2:1, *v/v*). The isolated compound was dissolved in EtOAc (10 mL) and heated at reflux with decolorizing charcoal for 5 min. After the charcoal was removed by filtration, the solution was cooled in an ice bath and slowly diluted with hexanes (50 mL) to afford **5** as a colorless solid (294 mg, 56%): $R_f = 0.13$ (EtOAc/MeOH, 4:1, *v/v*): m.p.: 182–183 °C (lit. [23] m.p.: 104–106 °C; lit. [28] m.p.: 184–185 °C); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.42$–1.57 (m, 2H), 1.98–2.13 (m, 4H), 2.42–2.52 (m, 2H), 2.62–2.71 (m, 2H), 2.82 (d, $J = 11.6$ Hz, 2H), 3.71–3.84 (m, 1H), 3.76 (s, 3H), 4.89 (br s, 1H), 6.81 (d, $J =$ 8.8 Hz, 2H), 7.00–7.10 (m, 4H), 7.28 (dd, $J = 3.2$ and 6.4 Hz, 2H), 8.45 ppm (br s, 1H, exchangeable with D); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 32.9$, 33.0, 50.2, 52.2, 55.4, 60.7, 112.4, 114.0, 120.9, 129.7, 132.3, 154.6, 158.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{27}N_4O$: 351.2179, found 351.2188.

1-(2-Amino-4,5-dimethylphenyl)-3-(1-(2-(4-methoxyphenyl)-ethyl)piperidin-4-yl)thiourea (13): A warm solution of 4,5-dimethyl-1,2-diaminobenzene (476 mg, 3.5 mmol) in EtOH (10 mL) was treated with compound **11** (968 mg, 3.5 mmol) in EtOH (4 mL), and the mixture was stirred at RT for 24 h. After the mixture was refrigerated overnight, the resulting solid was filtered, washed with cold EtOH (10 mL), and recrystallized to yield **13** as an off-white

solid (982 mg, 68%): m.p.: 158–159 °C (EtOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34–1.52 (m, 2H), 2.06 (d, J = 10.0 Hz, 2H), 2.12 (s, 3H), 2.10–2.24 (m, 5H), 2.46–2.58 (m, 2H), 2.63–2.75 (m, 2H), 2.85 (d, J = 10.8 Hz, 2H), 3.69 (s, 2H, exchangeable with D), 3.77 (s, 3H), 4.21–4.35 (m, 1H), 5.73 (d, J = 8.0 Hz, 1H, exchangeable with D), 6.58 (s, 1H), 6.75–6.84 (m, 3H), 7.08 (d, J = 8.8 Hz, 2H), 7.37 ppm (s, 1H, exchangeable with D); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.9, 19.8, 31.8, 33.0, 52.1, 52.3, 55.4, 60.7, 114.0, 118.1, 118.4, 127.7, 128.9, 129.7, 132.3, 138.7, 140.9, 158.1, 180.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{33}N_4OS$: 413.2370, found 413.2370.

1-(2-Amino-4,5-dichlorophenyl)-3-(1-(2-(4-methoxyphenyl)ethyl)-piperidin-4-yl)thiourea (14): A solution of compound **11** (1106 mg, 4 mmol) in EtOH (2 mL) was added to a warm solution of 4,5-dichloro-1,2-diaminobenzene (708 mg, 4 mmol) in EtOH (8 mL), and the mixture was stirred at RT for 19 h. The solid that precipitated was filtered, washed with cold EtOH (5 mL), and recrystallized to afford **14** as a tan solid (871 mg, 48%): m.p.: 171–172 °C (EtOAc/MeOH, 1:1, v/v); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.37–1.58 (m, 2H), 1.87 (d, J = 10.8 Hz, 2H), 1.94–2.15 (m, 2H), 2.45 (t, J = 7.6 Hz, 2H), 2.64 (t, J = 7.6 Hz, 2H), 2.85 (d, J = 9.6 Hz, 2H), 3.71 (s, 3H), 4.10 (br s, 1H), 5.22 (s, 2H, exchangeable with D), 6.82 (d, J = 8.0 Hz, 2H), 6.91 (s, 1H), 7.12 (d, J = 8.0 Hz, 2H), 7.31 (s, 1H), 7.64 (br s, 1H, exchangeable with D), 8.67 ppm (s, 1H, exchangeable with D); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 31.1, 32.1, 51.4, 52.0, 55.0, 60.0, 113.6, 115.8, 116.0, 124.1, 128.2, 129.0, 129.5, 132.4, 144.2, 157.4, 180.2 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{27}Cl_2N_4OS$: 453.1277, found 453.1287.

(5,6-Dimethyl-1*H*-benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)-ethyl)piperidin-4-yl)amine (15): The procedure reported for compound **5** was used, starting from thiourea **13** (825 mg, 2 mmol) to yield a crude residue that was subjected to column chromatography (silica gel, EtOAc/MeOH, 3:1, v/v). The light green solid that was isolated was dissolved in EtOAc (4 mL), treated with charcoal, filtered, and slowly diluted with hexanes (40 mL) to give **15** as a colorless solid (553 mg, 73%): R_f = 0.14 (EtOAc/MeOH, 3:1, v/v); m.p.: 218–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43–1.58 (m, 2H), 2.03–2.18 (m, 4H), 2.28 (s, 6H), 2.47–2.56 (m, 2H), 2.64–2.74 (m, 2H), 2.86 (d, J = 11.6 Hz, 2H), 3.69–3.77 (m, 1H), 3.78 (s, 3H), 4.89 (br s, 1H), 6.78–6.85 (m, 2H), 7.03–7.11 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.2, 32.9, 33.0, 50.2, 52.3, 55.4, 60.8, 113.2, 114.0, 129.0, 129.7, 132.5, 154.1, 158.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{31}N_4O$: 379.2492, found 379.2492.

(5,6-Dichloro-1*H*-benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)-ethyl)piperidin-4-yl)amine (16): The procedure reported for compound **5** was used, starting from thiourea **14** (680 mg, 1.5 mmol) to yield a crude residue that was subjected to column chromatography (silica gel, EtOAc/MeOH, 4:1, v/v) to give **16** as an off-white solid (515 mg, 82%): R_f 0.30 (EtOAc/MeOH, 4:1, v/v); m.p.: 223–225 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 1.55–1.73 (m, 2H), 2.07 (d, J = 11.2 Hz, 2H), 2.26 (t, J = 11.2 Hz, 2H), 2.53–2.65 (m, 2H), 2.70–2.82 (m, 2H), 3.03 (d, J = 11.6 Hz, 2H), 3.59–3.71 (m, 1H), 3.75 (s, 3H), 6.83 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 7.25 ppm (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 32.9, 33.3, 50.8, 53.4, 55.6, 61.8, 114.9, 130.6, 133.1, 157.6, 159.6 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{25}Cl_2N_4O$: 419.1400, found 419.1410.

(1-(4-Fluorobenzyl)-5,6-dimethyl-1*H*-benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)ethyl)piperidin-4-yl)amine (17): Benzimidazole **15** (265 mg, 0.7 mmol) was alkylated with 4-fluorobenzyl chloride (152 mg, 1.05 mmol) in DMF (4 mL) in the presence of anhydrous K_2CO_3 (193 mg, 1.4 mmol) and a few crystals of KI at 80–85 °C for 18 h. Subsequent processing of the reaction mixture as reported for compound **10** afforded a crude residue that, after column chromatography (silica gel, EtOAc/MeOH, 6:1, v/v), yielded **17** as a colorless solid (139 mg, 41%): R_f = 0.20 (EtOAc/MeOH, 6:1, v/v); melting range: 60–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42–1.59 (m, 2H), 2.12 (d, J = 10.8 Hz, 2H), 2.22–2.38 (m, 8H), 2.53–2.63 (m, 2H), 2.70–2.80 (m, 2H), 2.87 (d, J = 9.6 Hz, 2H), 3.78 (s, 3H), 3.86 (br s, 1H), 3.88–4.03 (m, 1H), 5.03 (s, 2H), 6.79–6.87 (m, 3H), 6.97–7.05 (m, 2H), 7.06–7.15 (m, 4H), 7.30 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 20.4, 32.5, 32.7, 45.2, 49.6, 52.3, 55.4, 60.7, 108.1, 114.0, 116.2, 116.3 (d, $J^2_{C,F}$ = 21.5 Hz), 117.5, 128.3 (d, $J^3_{C,F}$ = 8.1 Hz), 128.4, 129.7, 129.9, 131.5 (d, $J^4_{C,F}$ = 2.7 Hz), 132.0, 133.0, 140.6, 152.9, 158.2, 162.6 ppm (d, $J^1_{C,F}$ = 245.6 Hz); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −114.7 ppm (septet); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{36}FN_4O$: 487.2868, found 487.2869.

(5,6-Dichloro-1-(4-fluorobenzyl)-1*H*-benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)ethyl)piperidin-4-yl)amine (18): Benzimidazole **16** (336 mg, 0.8 mmol) was alkylated with 4-fluorobenzyl chloride (174 mg, 1.2 mmol) in DMF (5 mL) in the presence of anhydrous K_2CO_3 (221 mg, 1.6 mmol) and a few crystals of KI at 80–85 °C for 18 h. Subsequent processing of the reaction mixture as reported for compound **10** afforded a crude residue that was subjected to column chromatography (silica gel, EtOAc/MeOH, 9:1, v/v). Treatment of the isolated material with charcoal yielded **18** as a colorless solid (270 mg, 64%): R_f = 0.26 (EtOAc/MeOH, 9:1, v/v); m.p.: 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35–1.49 (m, 2H), 2.08 (d, J = 11.6 Hz, 2H), 2.22 (t, J = 10.8 Hz, 2H), 2.48–2.58 (m, 2H), 2.66–2.75 (m, 2H), 2.80 (d, J = 9.6 Hz, 2H), 3.78 (s, 3H), 3.84–3.97 (m, 2H), 4.98 (s, 2H), 6.82 (d, J = 8.4 Hz, 2H), 7.01–7.15 (m, 7H), 7.53 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.7, 33.0, 45.5, 50.0, 52.1, 55.4, 60.8, 108.5, 114.0, 116.6 (d, $J^2_{C,F}$ = 21.8 Hz), 117.8, 123.1, 125.2, 128.3 (d, $J^3_{C,F}$ = 8.2 Hz), 129.7, 130.3 (d, $J^4_{C,F}$ = 3.2 Hz), 132.4, 134.2, 142.3, 154.6, 158.1, 162.8 ppm (d, $J^1_{C,F}$ = 246.5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −113.7 ppm (septet); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{30}C_{l2}FN_4O$: 527.1775, found 527.1768.

(1-Benzyl-1*H*-benzimidazol-2-yl)-(1-(2-(4-methoxyphenyl)ethyl)-piperidin-4-yl)amine (19): A mixture of compound **5** (280 mg, 0.8 mmol), benzyl bromide (205 mg, 1.2 mmol), anhydrous K_2CO_3 (222 mg, 1.6 mmol), and a few crystals of KI in dry DMF (4 mL) was stirred at 85–100 °C for 20 h. The cooled mixture was diluted with water (50 mL) and extracted with EtOAc (2×50 mL), then the combined organic phases were washed with water (50 mL) and brine (20 mL) and dried over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the residue was purified by chromatography (silica gel, EtOAc/MeOH, 9:1, v/v) to give an oil that was dissolved in EtOAc (1 mL) and gradually diluted with hexanes (50 mL). The solid was filtered and washed with hexanes to afford **19** as a colorless solid (151 mg, 43%): R_f = 0.45 (EtOAc/MeOH, 9:1, v/v); m.p.: 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42–1.58 (m, 2H), 2.11 (d, J = 11.2 Hz, 2H), 2.29 (t, J = 10.4 Hz, 2H), 2.51–2.62 (m, 2H), 2.69–2.94 (m, 4H), 3.78 (s, 3H), 3.88–4.08 (2H), 6.83 (d, J = 8.4 Hz, 2H), 7.01–7.20 (m, 7H), 7.27–7.38 (m, 3H), 7.52 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.3, 32.7, 45.9, 49.5, 52.1, 55.4, 60.7, 107.3, 114.0, 116.6, 119.8, 121.5, 126.7, 128.4, 129.4, 129.7, 132.0, 134.8, 135.5, 142.4, 153.5, 158.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{33}N_4O$ 441.2649, found 441.2645.

(1-(4-Methoxyphenethyl)piperidin-4-yl)-(1-(4-(trifluoromethyl)-benzyl)-1*H*-benzimidazol-2-yl)amine (20): Compound **5** (525 mg, 1.5 mmol), 4-(trifluoromethyl)benzyl bromide (538 mg, 2.25 mmol), anhydrous K_2CO_3 (417 mg, 3 mmol), and a few crystals of KI in dry DMF (5 mL) were combined following a synthetic procedure similar to that described for compound **19** to give a crude material that was purified by chromatography (silica gel, EtOAc/MeOH, 9:1, v/v).

The solvent was removed from the combined fractions to yield a residue containing the desired compound with $R_f = 0.26$ (EtOAc/MeOH, 4:1, *v/v*), which was recrystallized from a small amount of EtOAc to afford **20** as off-white crystals (280 mg, 37%): m.p.: 182–183 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.55$–1.70 (m, 2H), 2.09 (d, $J = 11.6$ Hz, 2H), 2.20–2.32 (m, 2H), 2.53–2.62 (m, 2H), 2.71–2.79 (m, 2H), 3.02 (d, $J = 11.6$ Hz, 2H), 3.75 (s, 3H), 3.78–3.89 (m, 1H), 5.38 (s, 2H), 6.83 (d, $J = 8.8$ Hz, 2H), 6.92–7.08 (m, 3H), 7.12 (d, $J = 8.8$ Hz, 2H), 7.27 (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 7.6$ Hz, 1H), 7.60 ppm (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 32.8$, 33.3, 45.5, 51.4, 53.6, 55.6, 61.8, 109.0, 114.9, 116.2, 120.9, 122.6, 126.6 (m), 128.2, 130.6, 133.2, 135.4, 142.5, 143.1, 155.4, 159.6 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{31}F_3N_4O$ 509.2523, found 509.2510.

Ethyl 4-(1*H*-benzimidazol-2-ylmethyl)-1-piperazinecarboxylate (21): A mixture of 2-chloromethyl-1*H*-benzimidazole (1 g, 6 mmol), ethyl 1-piperazinecarboxylate (948 mg, 6 mmol), and Na_2CO_3 (636 mg, 6 mmol) in EtOH (18 mL) was heated at reflux for 19 h. The solvent was partially removed under reduced pressure, the residue was diluted with water (100 mL), and the resulting solid was filtered, washed with water, and recrystallized from EtOH to give **21** as a white solid (793 mg, 46%): m.p.: 200–201 °C; ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 1.24$ (t, $J = 7.2$ Hz, 3H), 2.51 (t, $J = 4.8$ Hz, 4H), 3.52 (t, $J = 4.8$ Hz, 4H), 3.81 (s, 2H), 4.11 (q, $J = 7.2$ Hz, 2H), 7.18–7.25 (m, 2H), 7.49–7.57 ppm (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 14.9$, 44.7, 54.0, 56.7, 62.7, 123.6, 153.1, 157.2 ppm; HRMS (EI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{21}N_4O_2$: 288.1659, found: 289.1663.

Ethyl 4-((1-(4-fluorobenzyl)-1*H*-benzimidazol-2-yl)methyl)-1-piperazinecarboxylate (22): A mixture of compound **21** (432 mg, 1.5 mmol), 4-fluorobenzyl chloride (434 mg, 3 mmol), K_2CO_3 (414 mg, 3 mmol), and a few crystals of KI in DMF (5 mL) was heated at 75–85 °C for 18 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3×20 mL). The combined organic phases were extracted with 1 M NaOH (2×20 mL), washed with water (2×20 mL) and brine, and then the solvent was removed under reduced pressure to give a residue that was purified by chromatography (silica gel, EtOAc, 100%) to afford a light yellow oil with $R_f = 0.32$ (EtOAc). The oil was redissolved in EtOAc (2 mL), then the solution was cooled in an ice bath and slowly diluted with hexanes (50 mL) under efficient stirring. The solid was filtered and washed with hexanes to yield **21** as colorless crystals (302 mg, 51%): m.p.: 148–149 °C; ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 1.23$ (t, $J = 7.2$ Hz, 3H), 2.43 (br s, 4H), 3.30 (br s, 4H), 3.80 (s, 2H), 4.09 (q, $J = 7.2$ Hz, 2H), 5.62 (s, 2H), 7.01–7.09 (m, 2H), 7.12–7.19 (m, 2H), 7.22–7.29 (m, 2H), 7.33–7.40 (m, 1H), 7.62–7.69 ppm (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 14.9$, 44.6, 47.8, 53.9, 56.0, 62.7, 111.6, 116.5 (d, $J^2_{C,F} = 22.1$ Hz), 119.8, 123.7, 124.5, 129.6 (d, $J^3_{C,F} = 7.3$ Hz), 134.4 (d, $J^4_{C,F} = 2.6$ Hz), 137.0, 142.6, 152.5, 157.1, 163.6 ppm (d, $J^1_{C,F} = 243$ Hz); ^{19}F NMR (CD_3OD, 376 MHz): $\delta = -118.0$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}FN_4O_2$: 397.2034, found: 397.2034.

1-(4-Fluorobenzyl)-2-(piperazin-1-ylmethyl)-1*H*-benzimidazole dihydrobromide (23): A solution of compound **22** (396 mg, 1 mmol) in aq 48% HBr (25 mL) was heated at reflux for 2 h, then the solvent was removed under reduced pressure. After all traces of solvent had been removed under high vacuum overnight, the residue was heated at reflux with EtOH (10 mL) for 10 min, then the mixture was allowed to cool to RT and was filtered to yield a **23** as a yellowish solid (233 mg, 48%): m.p.: 264–265 °C; ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 2.94$ (t, $J = 5.0$ Hz, 4H), 3.24 (t, $J = 5.0$ Hz, 4H), 4.33 (s, 2H), 5.83 (s, 2H), 7.11–7.19 (m, 2H), 7.32–7.40 (m, 2H), 7.58–7.70 (m, 2H), 7.80 (dd, $J = 0.8$ and 7.6 Hz, 1H), 7.91 ppm (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 44.6$, 49.3, 50.9, 53.0, 114.2, 115.6, 117.1 (d, $J^2_{C,F} = 21.9$ Hz), 127.9, 128.1, 130.4 (d, $J^3_{C,F} = 8.4$ Hz), 131.3 (d, $J^4_{C,F} = 3.1$ Hz), 131.7, 134.3, 152.0, 164.1 ppm (d, $J^1_{C,F} = 245$ Hz); ^{19}F NMR (CD_3OD, 376 MHz): $\delta = -116.2$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{22}FN_4$: found: 325.1814.

2-Dimethylamino-1-(4-fluorobenzyl)-1*H*-benzimidazole hydrochloride (24): A mixture of ethyl 4-hydroxy-1-piperidinecarboxylate (693 mg, 4 mmol) and LiH (100 mg, 12.5 mmol) in DMF (5 mL) was stirred at 70 °C for 30 min, then 2-chloro-1-(4-fluorobenzyl)-1*H*-benzimidazole (1043 mg, 4 mmol) was added, and the mixture was stirred at 80–90 °C overnight. Dilution of the reaction mixture with water (80 mL) was followed by extraction with EtOAc (3×25 mL), thorough washing of the organic phase with water and brine, drying over anhydrous Na_2SO_4, and removal of the solvent under reduced pressure to give a residue that was purified by chromatography (silica gel, EtOAc/hexanes, 3:1, *v/v*). Fractions with $R_f = 0.42$ (EtOAc/hexanes, 3:1, *v/v*) were combined, the solvent was removed under reduced pressure, and the resulting oil was redissolved in EtOAc (1 mL), cooled in an ice bath, and slowly diluted with hexanes until precipitation began. The mixture was kept in a freezer overnight, then the resultant precipitate was filtered and air-dried to afford a sticky solid (574 mg) that proved to be mainly the title compound as a free base (purity >90% by NMR). In order to obtain an analytical sample, the free base (269 mg, 1 mmol) was transformed into the hydrochloride by treating its solution in a mixture of acetone/Et_2O (10 mL, 1:1, *v/v*) with 37% HCl (200 mg) in acetone (1 mL). The solvent was removed under a stream of nitrogen, then the residue was dried under high vacuum overnight. Recrystallization from 2-propanol afforded **24** as colorless needles (150 mg, 49%): m.p.: 209–210 °C; ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 3.29$ (s, 6H), 5.56 (s, 2H), 7.11–7.19 (m, 2H), 7.29–7.43 (m, 5H), 7.52 ppm (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 41.6$, 49.6, 112.2, 112.9, 117.1 (d, $J^2_{C,F} = 22$ Hz), 125.5, 126.1, 129.2 (d, $J^3_{C,F} = 8.3$ Hz), 130.5, 132.2 (d, $J^4_{C,F} = 2.8$ Hz), 133.9, 154.3, 164.0 ppm d, $J^1_{C,F} = 244$ Hz); ^{19}F NMR (CD_3OD, 376 MHz): $\delta = -117.0$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{17}FN_3$: 270.1401, found: 270.1412.

Ethyl 4-(1-(4-fluorobenzyl)-1*H*-benzimidazol-2-yloxy)piperidine-1-carboxylate (25): A mixture of ethyl 4-hydroxy-1-piperidinecarboxylate (693 mg, 4 mmol) and NaH (150 mg, 6 mmol) in DMSO (5 mL) was stirred at 70 °C for 30 min, then 2-chloro-1-(4-fluorobenzyl)-1*H*-benzimidazole (1043 mg, 4 mmol) was added. The mixture was stirred at 70–80 °C overnight and then was partitioned between water (80 mL) and EtOAc (30 mL). The aqueous phase was further extracted with EtOAc (2×20 mL), the combined organic phase was washed with water (30 mL) and brine and dried over anhydrous Na_2SO_4 before the solvent was removed under reduced pressure. Flash column chromatography (silica gel, EtOAc/hexanes, 1:1, *v/v*) of the residue afforded **25** as a dense colorless oil (494 mg, 31%): $R_f = 0.32$ (EtOAc/hexanes, 1:1, *v/v*); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.27$ (t, $J = 7.2$ Hz, 3H), 1.76–1.90 (m, 2H), 2.03–2.15 (m, 2H), 3.34–3.45 (m, 2H), 3.70 (br s, 2H), 4.15 (q, $J = 7.2$ Hz, 2H), 5.11 (s, 2H), 5.32–5.40 (m, 1H), 6.95–7.03 (m, 2H), 7.08–7.21 (m, 5H), 7.55 ppm (d, $J = 7.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.8$, 30.8, 40.9, 45.2, 61.6, 75.6, 108.5, 115.9 (d, $J^2_{C,F} = 21.5$ Hz), 117.9, 121.3, 122.0, 128.9 (d, $J^3_{C,F} = 8.2$ Hz), 132.1 (d, $J^4_{C,F} = 2.9$ Hz), 133.5, 140.2, 155.6, 156.3, 162.4 ppm (d, $J^1_{C,F} = 245$ Hz); ^{19}F NMR ($CDCl_3$, 376 MHz): $\delta = -115.2$ ppm (septet); HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}FN_3O_3$: 398.1874, found: 398.1875.

Biology

General: RPMI 1640, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), DMSO, hypoxanthine, and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma–Aldrich (Oakville, ON, Canada). Fetal calf serum and gentimicin were purchased from Wisent (St Bruno, QC, Canada). Human serum was kindly provided by the Chemo Day Care department of the Princess Margaret Hospital, Toronto, Canada. RPMI-A and RPMI-10 were obtained by mixing RPMI 1640 with human and fetal calf serum, respectively. The absorbance of the biological samples was determined using a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Nonlinear regression analysis of the data was performed using SigmaPlot (Jandel Scientific, Corte Madera, CA, USA).

Determination of anti-Plasmodium *activity*: *P. falciparum* cultures were grown in O+ blood obtained by venipuncture of volunteers. Cultures of the laboratory line ItG were maintained by the method of Trager and Jensen[29] using RPMI 1640 supplemented with 10% human serum and 50 μM hypoxanthine (RPMI-A). The effects of the test compounds on the viability of *P. falciparum* cultures were determined using a lactate dehydrogenase (LDH) enzyme assay specific for the enzyme found in *P. falciparum* (pLDH).[30,31] Briefly, test compounds were dissolved in DMSO to afford a solution having a concentration of 10 mg mL^{-1}. Twofold serial dilutions were then produced in 50 μL of RPMI-A in a 96-well plate, then 50 μL of parasite culture (2% hematocrit, 2% parasitemia) was added to each well, and the plates were then incubated at 37 °C in an atmosphere of 95% nitrogen, 3% carbon dioxide, and 2% oxygen for 72 h. The contents of the wells were then resuspended using a multichannel pipette, and a 15 μL sample was removed from each well and added to 100 μL of pLDH enzyme assay mixture.[30] After 1 h, the absorbance of the wells at 595 nm was measured. The IC_{50} values of individual compounds were determined by nonlinear regression analysis of the data using SigmaPlot.[32] The IC_{50} values represent means ± standard error calculated from four independent determinations.

In vitro CHO cell activity assay: CHO cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 25 mM HEPES, and gentimicin (RPMI-10). Cells were seeded in 96-well plates and grown to 50% confluency in 100 μL of RPMI-10 per well prior to the addition of either DMSO alone or a 10 mg mL^{-1} solution of a test compound in DMSO. Compound gradients were prepared by adding 90 μL of RPMI-10 mixed with 10 μL of the test compound solution to the first well in the series, mixing, transferring 100 μL to the next well, and repeating this until the next-to-last well was reached. After 48 h, the viability of the cells was determined by discarding the media in the wells and adding 100 μL of 10 mg mL^{-1} of MTT in RPMI-10, incubating the plates for a further 1 h, and then removing the media, adding 100 μL of DMSO and reading the absorbance at 650 nm.[33] The IC_{50} values of individual compounds were determined by nonlinear regression analysis of the dose–response curve using SigmaPlot.

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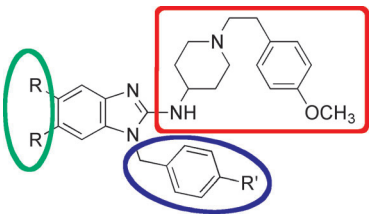
Derivatization iteration: Several modifications of astemizole were considered in order to better define the optimal structure for anti-*Plasmodium* activity in a series of structurally related astemizole derivatives. The presence of a secondary cyclic amine at position 2 and substitution with chlorine at positions 4 and 5 of the benzimidazole moiety resulted in potent activity.



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Synthesis and Anti-*Plasmodium* Activity of Benzimidazole Analogues Structurally Related to Astemizole