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New benzimidazole derivatives as antiplasmodial agents and plasmepsin inhibitors: Synthesis and analysis of structure–activity relationships

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ABSTRACT

The newly synthesized benzimidazole compounds were suggested to be inhibitors of *Plasmodium falciparum* plasmepsin II and human cathepsin D by virtual screening of an internal library of synthetic compounds. This was confirmed by enzyme inhibition studies that gave IC_{50} values in the low micromolar range (2–48 μM). Ligand docking studies with plasmepsin II predicted binding of benzimidazole compounds at the center of the extended substrate-binding cleft. According to the plausible mode of binding, the pyridine ring of benzimidazole compounds interacted with S1′ subsite residues whereas the acetophenone moiety was in contact with S1–S3 subsites of plasmepsin II active center. The benzimidazole derivatives were evaluated for capacity to inhibit the growth of intraerythrocytic *P. falciparum* in culture. Four benzimidazole compounds inhibited parasite growth at ⩽3 μM. The most active compound **10**, 1-(4-phenylphenyl)-2[2-(pyridinyl-2-yl)-1,3-benzdiazol-1-yl]ethanone showed an IC_{50} of 160 nM. The substitution of a phenyl group and a chlorine atom at the para position of the acetophenone moiety were shown to be crucial for antiplasmodial activity.

As the most serious tropical parasitic disease, malaria accounts for an estimated 300–500 million cases and up to 2.7 million deaths annually, particularly among young children.[1] Severe malaria is a complex multisystem disorder involving adherence of parasites to blood vessel endothelial cells and severe anemia.[2] Four *Plasmodium* species are responsible for malaria in humans, that is, *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*.[3] Infection caused by *P. falciparum* is the only one normally lethal. *P. falciparum* has developed resistance to nearly all available antimalarial drugs.[4] Therefore, there is an urgent need for new antimalarials based on novel mechanisms of action.[5]

The members of plasmepsin (Plm) family of *Plasmodium* aspartic proteases are involved in hemoglobin degradation during the intra-erythrocytic phase of infection; a process essential for propagation carried out in food vacuole of malarial parasite.[6] Ten Plasmepsins were identified in the genome of *P. falciparum*, and four of them, that is, Plm-I, -II, -III and –IV/(HAP) participate in hemoglobin degradation.[7] Previously, treatment of mouse malaria using Pepstatin A suggested the potential of plasmepsin inhibitors as antimalarial drugs.[8] Recent knockout studies revealed a requisite inhibition of all the four plasmepsins to prevent proliferation of parasite.[9] Plasmepsins (in particular plasmepsin I and II) have been considered as promising target for new antimalarial drugs but Liu et al.[10] have reported that inhibition of food vacuole plasmepsins does not appear to be a promising strategy, unless combined with the inhibitors of malarial parasite cysteine proteases falcipains that are also involved in hemoglobin catabolism. Nevertheless, use of plasmepsin II as a target has led to the identification of a number of potent antimalarial compounds.[11] Therefore, inhibition of this enzyme can be seen as a valuable proxy for inhibition of other targets, most likely nonfood vacuole plasmepsins that have been hard to approach biochemically.[10] Therefore, search for inhibitors broadly active against all hemoglobin-degrading plasmepsins (Plm-I, -II, -III and -IV) while remaining inactive against the closely related human aspartic proteases (cathepsins D and E) continues. Plasmepsin inhibitors with fair selectivity versus human cathepsins have been reported.[12]

A number of chemical functionalities and structural units have been utilized as noncleavable transition-state isosteres in plasmepsin II inhibitors.[11] These inhibitors include statine-derived and reversed-statine based cores,[13,14] tertiary amines based on 4-aminopiperidine-tert-butyl-carbamate,[15] hydroxyethylamines, dihydroxyethylenes and hydroxymethylcarbonyl,[16,17] diphenylurea,[18] acridinyl hydrazides,[19,20] achiral oligoamines[21] and tetrahydro-azepine scaffold compounds.[22]

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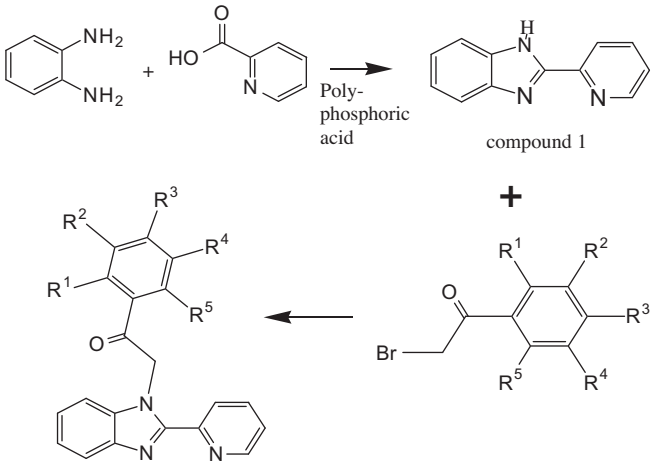
Benzimidazole is a heterocyclic aromatic compound consists of benzene and imidazole rings. The most prominent benzimidazole compound in nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12.[23] Benzimidazole, in an extension of the well-elaborated imidazole system, has been used as a carbon skeleton for *N*-heterocyclic carbenes (NHC). The NHCs are usually used as ligands for transition metal complexes. They are often prepared by deprotonating an N, N′-disubstituted benzimidazolium salt at the two-position with a base.[24,25] Compounds bearing the benzimidazole moiety are reported to have proton pump inhibiting,[26] anticancer,[27] antiallergic,[28] antioxidant,[29] antihistaminic[30] and antimicrobial[31] activities. We synthesized a new class of benzimidazole derivatives (2-pyridine-2-yl-1*H*-benzimidazole) and evaluated their activity against *P. falciparum* (Fig. 1).[32] These compounds were also suggested as potential plasmepsin II and human cathepsin D inhibitors by virtual screening of an in-house compounds database. The ligand docking simulation was confirmed by enzyme inhibition studies.

Virtual screening was carried out by FlexX ligand docking software (version 2.0)[36] using an in-house library of >1000 synthetic compounds and crystal structural coordinate sets of *P. falciparum* plasmepsin II (PDB id; 1M43)[37] and human cathepsin D (PBD id; 1LYB).[38] The in-house library compounds corresponds to thirty different chemical scaffolds that have been synthesized in our laboratory. 3D models of compounds in SYBYL mol2 format were utilized for binding to the active sites of both aspartic proteases. FlexX ligand docking was carried out allowing full flexibility for the ligands, while keeping the proteins fixed. Among the two catalytic aspartates, Asp34 was considered protonated and Asp214 negatively charged during docking (plasmepsin II numberings). After each ligand docking run, 10 top ranking docking solutions were saved and considered for detailed analysis. The enzyme activities of plasmepsin II and cathepsin D were measured as described earlier.[39,40]



(2)	R^1=H	R^2= NO_2	R^3=H	R^4=H	R^5=H
(3)	R^1=H	R^2=OH	R^3=OH	R^4=H	R^5=H
(4)	R^1=H	R^2=H	R^3=Cl	R^4=H	R^5=H
(5)	R^1=NO_2	R^2=H	R^3=H	R^4=H	R^5=H
(6)	R^1=H	R^2=H	R^3= NO_2	R^4=H	R^5=H
(7)	R^1=H	R^2=H	R^3=F	R^4=H	R^5=H
(8)	R^1=F	R^2=H	R^3=F	R^4=H	R^5=H
(9)	R^1=H	R^2=H	R^3= H	R^4=H	R^5=H
(10)	R^1=H	R^2=H	R^3= Ph	R^4=H	R^5=H
(11)	R^1=H	R^2=H	R^3= OMe	R^4=H	R^5=H
(12)	R^1=OMe	R^2=H	R^3=H	R^4=OMe	R^5=H

Figure 1. Synthesis of 2-pyridine-2-yl-1*H*-benzimidazole derivatives (**2**–**12**).

Table 1
Antiplasmodial and aspartic protease inhibitory activities of benzimidazole derivatives

Compd. IDs	Antiplasmodial activity	Plasmepsin-II inhibition	Cathepsin D inhibition
		IC_{50} in μM	
2	⩾3.0	20.4	21.2
3	⩾3.0	19.6	28.5
4	0.7	21.2	31.9
5	⩾3.0	30.6	39.2
6	2.1	30.5	17.8
7	3.0	10	31.9
8	⩾3.0	48	44.5
9	⩾3.0	10	39.2
10	0.16 ± 0.4	14.7	2.0
11	⩽3.0	37.7	14.2
12	⩽3.0	34.0	21.2

Molecular docking of the compounds present in the in-house database predicted benzimidazole derivatives as potential inhibitors of plasmepsin II and cathepsin D. Enzyme inhibition data (Table 1) pointed out that (a) the IC_{50} values of benzimidazole compounds against plasmepsin II and cathepsin D are in the ranges of 10–48 μM and 11–44 μM, respectively, [except cathepsin D inhibition by compound 10] (Fig. 2A and B; Table 1); (b) compounds **7** and **9** are three times more selective towards plasmepsin II compared to cathepsin D with IC_{50} = 10 μM (Table 1) and (c) compound **10** is a comparably potent and selective inhibitor of cathepsin D with IC_{50} = 2.0 μM (compared to IC_{50} = 14.7 for plasmepsin II) (Fig. 2C; Table 1).

3D structural analysis of the predicted binding modes of benzimidazole compounds provided the basis of inhibition from a structural standpoint. The five top binders of benzimidazole compounds were modeled into the active site of plasmepsin II to examine interactions with protein residues. Analysis of FlexX docking solutions predicted binding of benzimidazole compounds at the center of substrate binding cleft (Fig. 3). The benzimidazole moiety of these compounds was docked in the middle with pyridine and acetophenone side chains protruding in different directions. The carbonyl group of the acetophenone side chain is predicted to be pointing towards the side chain of catalytic aspartate Asp34 (Fig. 3). Analysis of top five docking solutions of 11 benzimidazole compounds indicated two binding modes (Fig. 4A and B). In the 'preferable' mode of binding, 9 out of 11 compounds (i.e., except compounds **3** and **11**) were predicted to bind at the active center such that the pyridine moiety interacted with S1′ subsite binding residues whereas acetophenone side chain interacted with S1–S3 subsite forming residues (Fig. 4A). In contrast, compounds **3** and **11** were bound in opposite fashion, that is, the pyridine moiety interacted with S1–S3 subsite residues and the acetophenone side chain interacted with the S1′ subsite (Fig. 4B). Because the majority of benzimidazole compounds (9 out of 11 compounds) were docked in similar fashion, therefore the first mode of ligand binding was considered more plausible.

All antiplasmodial assays were carried out in *P. falciparum* clone HB-3. EC_{50} values of the tested compounds were determined essentially as described earlier.[41] The benzimidazole derivatives with substitutions at the acetophenone ring showed potent

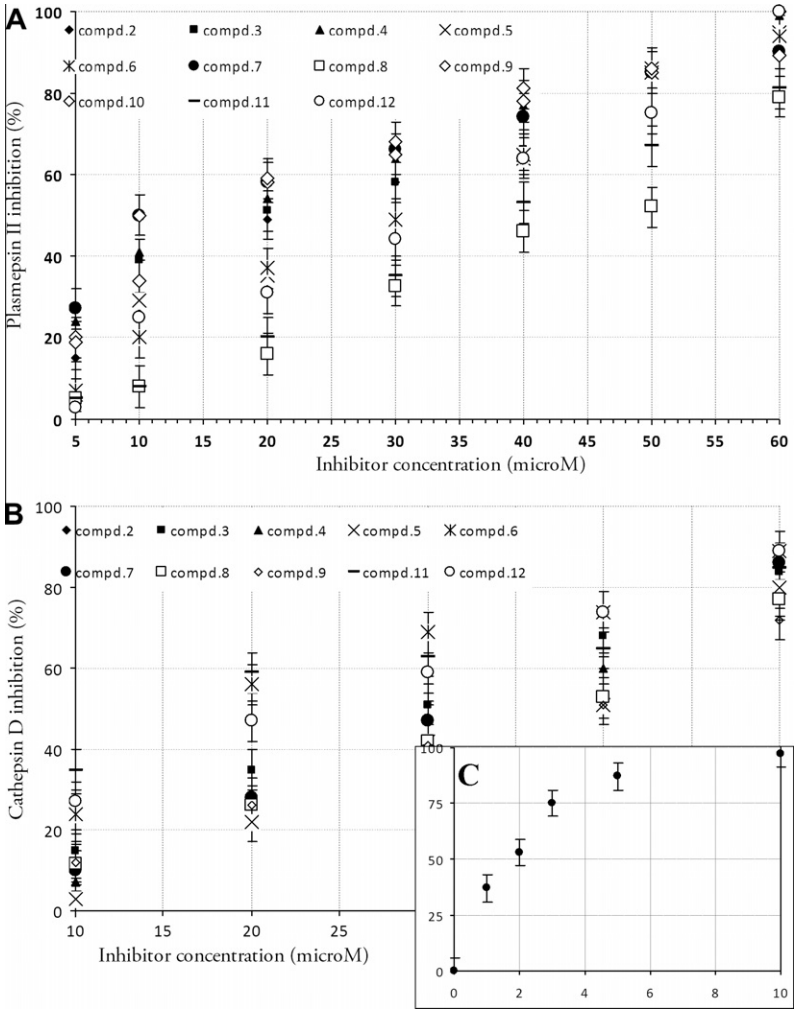


Figure 2. (A) Plasmepsin-II inhibition plots as a function of benzimidazole compounds **2**–**12** concentration; (B) Cathepsin D inhibition plots as a function of benzimidazole compounds **2**–**12** concentration (except compound **10**); (C) Cathepsin D inhibition plot as a function of compound **10** concentration.

antiplasmodial activity. Among the compounds tested, compound **10** had the highest antiplasmodial activity with IC_{50} = 0.16 ± 0.4 μM (Fig. 5A and B) whereas compounds **4** and **6** showed IC_{50} values of 0.7 μM and 2.1 μM, respectively, (Fig. 5C and D). Assessment of killing with two different well-established methods, flow cytometry (Fig. 5A) and hypoxanthine (Fig. 5B) gave similar results. The remaining compounds were considered to be inactive with $IC_{50} \geqslant 3.0$ μM (Table 1). Structure activity relationship (SAR) analysis indicated that the higher antiplasmodial activity of compound **10** was due to the extra phenyl group present at the para position of acetophenone ring (Fig. 1). Furthermore, the presence of a chlorine atom in compound 4 and a nitro group in compound 6 pointed out a role of these substitutions at the para position acetophenone in antiplasmodial activity (Fig. 1).

Our data show that these benzimidazole compounds are clearly aspartic protease inhibitors. However, antiplasmepsin activity did not correlate well with parasite killing. There are 10 aspartic proteases encoded by the *P. falciparum* genome and not all are well characterized. It is likely that the antiparasitic action of these compounds is against one of the less characterized aspartic proteases. The potency of these compounds, especially compound 10 suggests that this is a new class of inhibitors with promise for further antimalarial drug development.

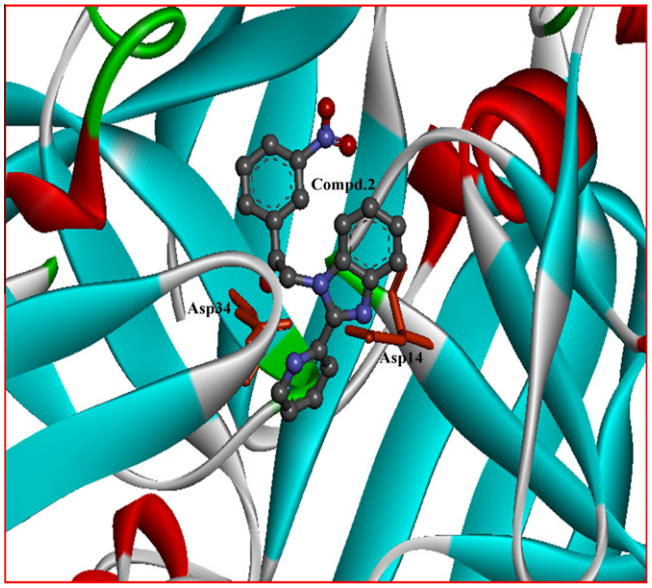


Figure 3. Docking of a benzimidazole derivative (compound **2**) in the substrate binding cleft of plasmepsin II. The compound **2** is shown in ball-stick, plasmepsin-II in solid ribbon and Catalytic aspartates (Asp 34 and Asp214) in stick representation.

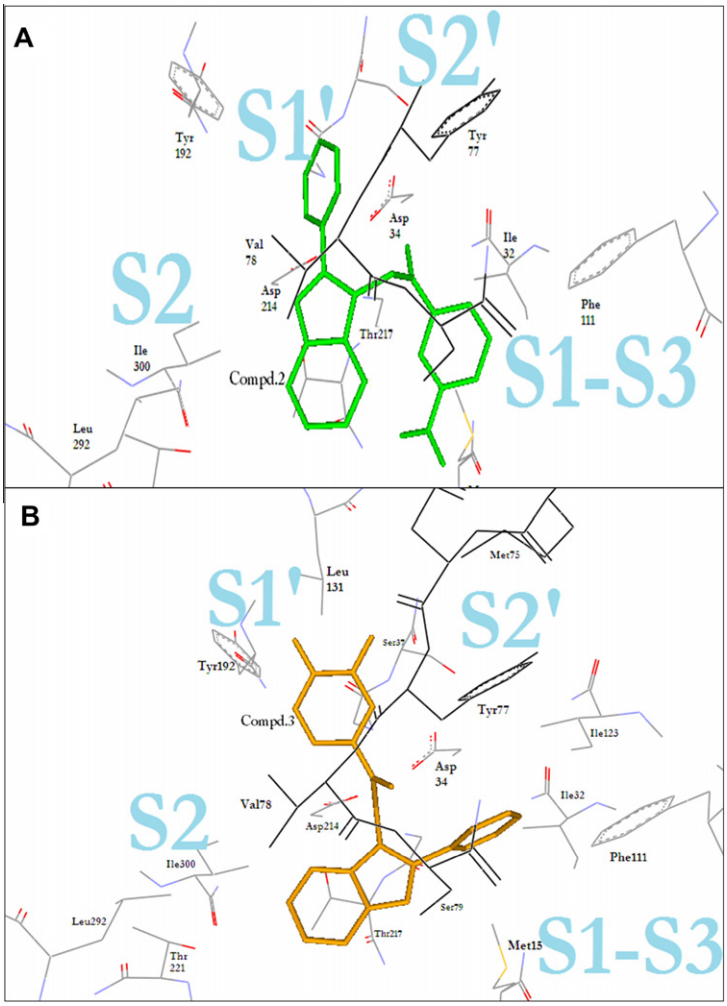


Figure 4. (A) Docking of compound **2** (thick stick) in the active site cleft of plasmepsin-II representing the benzimidazole derivatives binding with prefered mode of binding; (B) Docking of compound **3** (thick stick) in the active site of plasmepsin-II with alternative mode of binding. Enzyme residues involved in binding of inhibitors are shown. Locations of different subsites (S1, S2, S3, S1′ and S2′) are indicated.

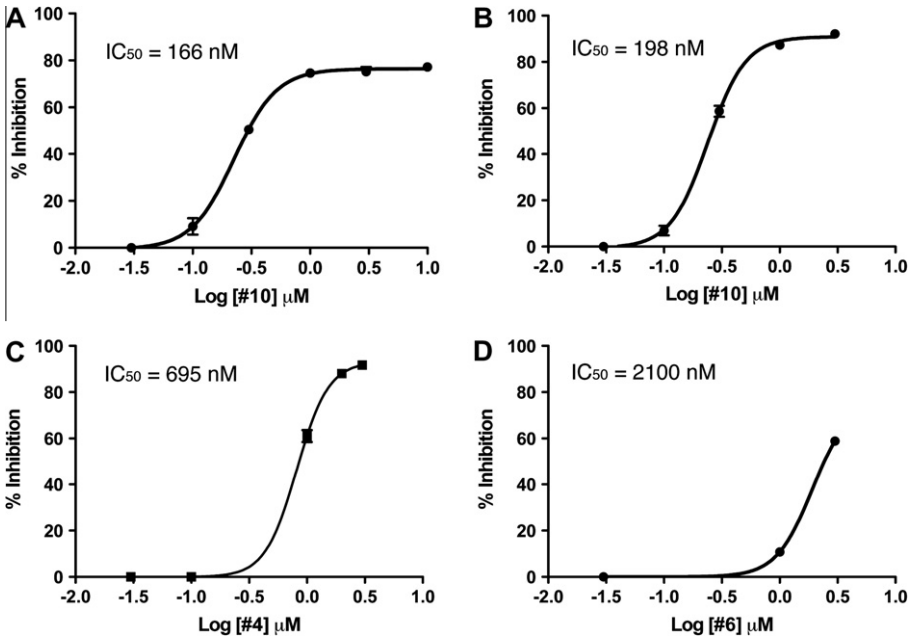


Figure 5. In vitro antiplasmodial activities of compounds **4**, **6** and **10** (designated as #4, #6 and #10). Graphs are log compound concentration versus% inhibition of parasite growth compared to no inhibitor control. (A) Parasite growth measured by flow cytometry; (B–D) parasite growth measured by 3*H*-hypoxanthine incorporation.

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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.2011.10.018.

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