Lerisetron Analogues with Antimalarial Properties: Synthesis, Structure−Activity Relationship Studies, and Biological Assessment

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ABSTRACT: A phenotypic whole cell high-throughput screen against the asexual blood and liver stages of the malaria parasite identified a benzimidazole chemical series. Among the hits were the antiemetic benzimidazole drug Lerisetron 1 (IC50 NF54 = 0.81 μM) and its methyl-substituted analogue 2 (IC50 NF54 = 0.098 μM). A medicinal chemistry hit to lead effort led to the identification of chloro-substituted analogue 3 with high potency against the drug-sensitive NF54 (IC50 NF54 = 0.062 μM) and multidrug-resistant K1 (IC50 K1 = 0.054 μM) strains of the human malaria parasite Plasmodium falciparum. Compounds 2 and 3 gratifyingly showed in vivo efficacy in both Plasmodium berghei and P. falciparum mouse models of malaria. Cardiotoxicity risk as expressed in strong inhibition of the human ether-a-go-go-related gene (hERG) potassium channel was identified as a major liability to address. This led to the synthesis and biological assessment of around 60 analogues from which several compounds with improved antiplasmodial potency, relative to the lead compound 3, were identified.

INTRODUCTION

Malaria, caused by the Plasmodium parasite genus and transmitted to humans by the bite of Plasmodium-infected female Anopheles mosquitoes, remains a life-threatening disease. Among the five species of Plasmodium parasites (Plasmodium falciparum (Pf), Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi) that cause malaria in humans, P. falciparum is the most serious and often leads to death. A milder form of malaria is initiated in humans by P. vivax, P. ovale, and P. malariae and less frequently by P. knowlesi. According to the 2018 World Malaria Report, globally, approximately 219 million malaria cases and about 435,000 deaths were reported in 2017. On the basis of the figures published by the Institute of Health Metrics and Evaluation (IHME) and World Health Organization (WHO), 90% of reported deaths caused by malaria occur in sub-Saharan Africa. Worldwide, children under 5 years of age are the most vulnerable age group and accounted for 61% of the total malaria deaths in 2017. Malaria can be prevented by the use of indoor insecticide sprays and mosquito nets and avoiding the accumulation of stagnant water in the house or treatment using improved antimalarial medications.

Among the various antimalarial medications used for the treatment of malaria, artemisinin-based combination therapies (ACTs) have been found to be the most effective, especially against P. falciparum and have allowed millions of patients to be cured over the last few decades. Unfortunately, the emerging resistance to all existing antimalarials has become a recurring challenge for the goal of malaria eradication. In 2008, delayed parasite clearance by ACTs, which hinted at the emergence of resistance, were reported in patients from the eastern Thai-Cambodian border. Hence, there is a critical and urgent need to develop novel and affordable antimalarial therapeutic agents to tackle this rising problem.

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Compounds possessing a benzimidazole core possess a broad spectrum of biological activities, including antimalarial activity\(^{10}\) (Figure 1). This scaffold is present in astemizole (brand name Hismanal), a second-generation antihistamine drug and antimalarial lead that was withdrawn from the market due to rare but potentially fatal side effects, such as QTc interval prolongation and related arrhythmias due to human ether-a-go-go related gene (hERG) channel blockade.\(^{11}\) Nor-astemizole is an active metabolite of astemizole with supposedly lower cardiac risks.\(^{12}\) Lerisetron, a related benzimidazole derivative, is an effective antagonist of the 5-HT\(_3\) receptor and was used in the treatment of chemotherapy-induced nausea.\(^{13}\)

![Figure 1. Pharmacologically active molecules containing the benzimidazole structure.](https://dx.doi.org/10.1021/acsomega.0c00327)

**Chemistry.** The 1,2,5-trisubstituted benzimidazoles were synthesized using a literature protocol, which leads to the final target compounds in five consecutive steps as shown in Scheme 1.

The first step involves a nucleophilic aromatic substitution reaction (S\(_{NAr}\)) between suitable amines, o-halo-nitro compounds 6, and an additional base [K\(_2\)CO\(_3\) or Et\(_3\)N] at elevated temperatures to obtain an amino-nitro intermediate 7,\(^{15}\) followed by a reduction step [Pt/C and H\(_2\)] or iron powder and NH\(_4\)Cl\(^{17}\) or hydrazine hydrate\(^{18}\) to afford amines 8. The cyclization of 8 to the 1,3-dihydro-2H-benzo[d]imidazol-2-one 9 was carried out with triphosgene\(^{19}\) or with trimethyl orthoformate/HCOOH or triethyl orthoformate/PTSA to access benzimidazole intermediate 11.\(^{20}\)

Transformation to the 2-halogen derivatives was performed on 9 using POCl\(_3\) and HCl or PCl\(_3\) to yield 10.\(^{21}\) Unfortunately, this reaction did not work well for some substrates. Alternatively, deprotonation of the benzimidazole 11 using lithium diisopropylamide (LDA) followed by subsequent halogenation with Cl\(_2\)C–CCl\(_3\) or CBr\(_4\) yielded 12.\(^{22}\) The desired halogenated derivatives 12 were cleanly isolated and in high yield albeit initially an undesired side product 13 was observed when more than 1 equiv of LDA was used. Interestingly, double deprotonation/halogenation opened the door to cleanly attach additional halogen atoms to the benzene ring in position 4. These derivatives were further used for the attachment of aromatic rings via Pd-mediated coupling with boronic acids.\(^{23}\) The halogenated compounds 10, 12, and 13 were subjected to amination reactions using a broad range of amines in t-BuOH and triethylamine to access the corresponding final products 14–15 and 16–42 in good yields.

Furthermore, compound 43 was synthesized following a sodium triacetoxyborohydride-mediated reductive amination protocol (Scheme 2),\(^{24}\) which was carried out by the treatment of the amine 33 and a variety of aldehydes. The corresponding products (43) were obtained in generally low yields (16–49%).

Carbon-linked benzimidazole derivatives were synthesized as depicted in Scheme 3. The amide coupling between 44 and 8 was accomplished via the treatment with 1-[bis-(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]-pyridinium 3-oxide hexafluorophosphate (HATU) and diisopropyl ethyl amine (DIEPA) to yield amidines 45.\(^{25}\) Ring closure and Boc-deprotection of 45 were accomplished in acetic acid when heated to 100 °C in a sealed tube. The resulting C-linked benzimidazoles 46 were isolated in low to good yields.\(^{26}\)

2-Amino benzimidazole derivatives 49 and 52 were synthesized by a Suzuki coupling reaction\(^{23}\) between the bromo compounds 47 or 50 with the appropriate aryl boronic acids, followed by Boc-deprotection using 4 N HCl in 1,4-dioxane in low to moderate yields (Scheme 4,ii). The incorporation of a nitrogen atom into the phenyl ring was carried out by selecting an appropriate commercially available chloropyridine isomer 53a and 53b (Scheme 5). Using an identical protocol carried out as mentioned earlier in

![Figure 2. Exploration of SAR studies based on lead compound 3.](https://dx.doi.org/10.1021/acsomega.0c00327)
the Scheme 1, the reaction between 53 with benzylamine yielded the desired amino-nitro products 54. These compounds (54) were subsequently reduced via catalytic hydrogenation with platinum on carbon and hydrogen to furnish diamino derivatives followed by the cyclization with trimethyl orthoformate and formic acid at elevated temperatures to access imidazopyridines 55 in low to good yields in a one-pot reaction. Deprotonation of Imidazopyridines 55 was realized with in situ generated LDA followed by treatment with hexachloroethane to afford chloro-imidazopyridines 56 in 91 and 37% yields, respectively. The chloro compounds 56, piperazine, and Et₃Ni n-t-BuOH were heated to 120 °C in a pressure tube to yield the desired products 57.

**RESULTS AND DISCUSSION**

**In Vitro Antiplasmodium Activity, Solubility, and Cytotoxicity.** All of the synthesized compounds were evaluated for in vitro antiplasmodium activity against the drug-sensitive strain (NF54) of *P. falciparum*. Compounds with *Pf* NF54 <0.400 μM were also evaluated against a multidrug-resistant strain (K1) using chloroquine and artesunate as the reference drugs. Selected compounds were also assessed for cytotoxicity in a Chinese Hamster Ovary (CHO) cell line using emetine as a reference drug. Aqueous solubility was determined at pH 2.0 and 6.5. Additionally, selected compounds were further tested for their activity against the drug-resistant strain (K1) using chloroquine and artesunate as the reference drugs.
hERG channel and in vitro microsomal metabolic stability was assessed as a percentage of the test compound remaining after 30 min.

**SAR of the Eastern Substituent.** On the basis of the literature precedence, hERG inhibition liabilities can be drastically reduced by the removal of polar/basic moieties (e.g., reduction of the basicity of amines), formation of zwitterions (e.g., introduction of −COOH groups), disrupting the π−π interaction (e.g., replacing electron-donating groups with electron-withdrawing groups and introducing steric hindrance), and by discrete structural modifications (e.g., imparting rigidity). On the basis of the aforementioned strategies, we began exploring the Eastern Substituent by replacing the basic piperazine moiety with nonbasic ones, or replacing it with a variety of basic cyclic/bicyclic amines or introducing open-chain amines (2-, 3- or 4-carbon linkers) or adding acidic groups. For the investigation of the Eastern Substituent, we left the Western Substituent (phenyl with 5-chloro substituent) and the Southern Substituent (benzyl) unchanged, to have matched pairs for comparison.

A broad range of 2-amino benzimidazoles were synthesized and evaluated for in vitro Pf activity, solubility, CHO cytotoxicity for comparison with 3 (Table 1), and for activity on the hERG channel. The morpholine derivative 16a displayed a noteworthy drop-in potency with an IC50 value of >1 μM (NF54) compared with 3. The piperazine derivatives 17 and 18 containing a nonbasic nitrogen also showed a substantial loss of antiplasmodium activity, demonstrating the importance of a basic nitrogen in the Eastern side chain. Compound 20, with a zwitterionic character, was inactive, whereas the related nitrile derivative 19 retained reduced activity, presumably due to the reduced basicity of the

**Scheme 3. General Synthetic Route for the C-Linked Benzimidazoles 46**

**Scheme 4. General Synthetic Route for the Synthesis of 5-Substituted (49) and 4,5-Disubstituted 2-Amino Benzimidazoles (52)**

**Scheme 5. Synthetic Route for the Synthesis of Imidazopyridines (57)**
piperazine nitrogen. In a publication from the University of Helsinki and GlaxoSmithKline (GSK) Tres Cantos, a compound similar to 21 but without the Southern Substituent benzyl group was reported as being active; however, our derivative 21 proved to be completely inactive. Examples 22 and 24 without a benzyl group were resynthesized since they had been reported as active (185 and 66 nM); however, in our assays, these compounds were completely inactive. The follow-on derivatives containing a Bn-group (23 and 25) were also completely inactive in our assays. We also resynthesized the literature compound 26, which was at least weakly active in our assays despite having no basic nitrogen in the Eastern substituent. We did not pursue this approach any further, since the compounds without a basic nitrogen did not show improved hERG activity profiles (see the discussion later on and the data in Table 7). It is clear from Table 1 that incorporating a nonbasic Eastern substituent led to a considerable drop in the in vitro Pf activity for the above-mentioned compounds, demonstrating the importance of a basic nitrogen for antiplasmodium activity.

When compounds with a basic nitrogen in the Eastern substituent are compared against each other (Table 2), additional methyl groups on the ring were detrimental to activity (27) but connecting the Eastern substituent via a carbon linker results in the retention or improvement of potency (46a). A potency improvement was seen with the piperidine-4-amine side chain (28) where the basic nitrogen was moved slightly away from the benzimidazole core, leading to a 3-fold boost in potency. Consistent with this observation was the 2-fold shift in activity for the bicyclic Eastern substituent in 29 both for NF54 and K1.

We next investigated the effect of open-chain analogues on antiplasmodium activity. Opening the piperazine ring to the ethylenediamine derivative 33 led to a 4-fold drop-in potency. However, we did see an improvement relating to the hERG activity of the compound, which will be discussed later. Potency dropped even further in the case of the related butanediamine (30) and propanediamine (32) derivatives. Methylation of the nitrogen (34) or dimethylation (35) resulted in nearly equipotent derivatives. Incorporation of two benzyl groups (43a) on the terminal nitrogen proved to be

| Compound | X | R² | R³ | IC₅₀ (µM) | Solubility (µM) | IC₅₀ (CHO) (µM) | Si² |
|----------|---|----|----|----------|----------------|-----------------|----|
| Chloroquine |   |    |    | 0.016    | 0.194          |                 |    |
| Artesunate |   |    |    | 0.004    | 0.003          |                 |    |
| Emetine   |   |    |    |          | 0.095          |                 |    |

Table 1. Eastern Substituents: In Vitro Antiplasmodium Activity, Solubility, and Cytotoxicity Data of the Compounds with Reduced Basicity

Mean from n values of ≥2 independent experiments with sensitive (NF54) and multidrug-resistant (K1) strains of P. falciparum. Aqueous solubility using high-performance liquid chromatography (HPLC)-diode array detector (DAD)-mass spectrometry (MS). CHO = Chinese hamster ovarian cell line. Selectivity index (SI) = IC₅₀ (CHO)/IC₅₀ (Pf/NF54). Data from Singh et al. ND = not determined.
Table 2. Eastern Substituents—In Vitro Antiplasmodium Activity, Solubility, and Cytotoxicity Data of the Compounds Retaining a Basic Nitrogen

| Compound | R<sup>1</sup> | IC<sub>50</sub>(μM)<sup>a</sup> | Solubility<sup>b</sup> (μM) | IC<sub>50</sub>(CHO)<sup>c</sub> (μM) | SI<sup>d</sup> |
|----------|-------------|------------------|-----------------|-----------------|--------|
|          | P/NF54     | P/K1            | pH 2.0          | pH 6.5          |        |
| 3        | 0.062      | 0.054           | 150             | 140             | 45     | 726   |
| 27       | 0.216      | ND              | 150             | 140             | ND     | ND    |
| 46a      | 0.038      | 0.076           | 190             | 125             | 21     | 553   |
| 19       | 0.306      | 0.454           | >150            | <5              | 48     | 157   |
| 28       | 0.022      | 0.014           | >150            | >150            | 45     | 2045  |
| 29       | 0.037      | 0.028           | >150            | 140             | 44     | 1189  |
| 30       | 1.341      | ND              | ND              | 200             | ND     | ND    |
| 31       | 0.751      | ND              | ND              | 200             | ND     | ND    |
| 32       | 0.348      | 0.717           | 145             | 200             | 40     | 115   |
| 33       | 0.245      | 0.339           | 150             | 200             | 4      | 16    |
| 34       | 0.233      | 0.507           | >150            | 150             | 4      | 17    |
| 35       | 0.357      | ND              | 150             | 200             | 46     | 129   |
| 43a      | 0.882      | ND              | ND              | 5               | ND     | ND    |
| 43b      | 0.086      | 0.212           | 135             | 25              | 3      | 35    |
| 43c      | 2.281      | ND              | ND              | ND              | ND     | ND    |
| 43d      | 2.775      | ND              | ND              | ND              | ND     | ND    |
| 36       | 7.060      | ND              | ND              | 200             | ND     | ND    |
| 37       | 8.519      | ND              | ND              | 53              | ND     | ND    |
| 38       | 0.276      | 1.124           | >150            | 125             | 41     | 149   |
| 39       | 5.824      | ND              | ND              | ND              | ND     | ND    |
| 40       | >10        | ND              | ND              | 15              | ND     | ND    |

<sup>a</sup>Mean from n values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of <i>P. falciparum</i>.<sup>b</sup>Aqueous solubility using HPLC-DAD-MS. <sup>c</sup>CHO = Chinese hamster ovarian cell line. <sup>d</sup>Selectivity index (SI) = [IC<sub>50</sub> (CHO)/IC<sub>50</sub> (Pf/NF54)]. ND = not determined.

detrimental to activity. However, the compound with a single benzyl group on the terminal nitrogen (43b) displayed reasonably good activity, which was comparable to that of 3. Replacement of the phenyl group of 43b with a pyridyl or CF<sub>3</sub>-phenyl (43c and 43d) was not successful. When the terminal nitrogen was incorporated into a ring system (36–38), compounds were found to be less active. Overall, acyclic derivatives on the eastern end were less potent than their cyclic counterparts. Selected, potent compounds (IC<sub>50</sub> NF54 < 0.400 μM) were screened against a multidrug-resistant strain (K1) to determine if these compounds are still effective against a resistant strain. All derivatives tested were also active against K1 within a twofold window, except for 38 (4-fold difference). In general, most of the analogues showed good solubility across both acidic (pH = 2) and neutral (pH = 6.5) conditions, with few exceptions. This active set of compounds (NF54 < 0.400 μM) was also screened in a cytotoxicity assay using Chinese hamster ovarian (CHO) cells. The most potent compounds had a good safety window (SI = CHO CC<sub>50</sub>/NF54 IC<sub>50</sub> > 500), except for compound 43b (SI < 35-fold).
Overall, in vitro cytotoxicity was in general not a concern for the series.

**SAR of the Western Substituent.** For the investigation of the Western substituent, Eastern substituents that gave active compounds based on the known derivatives (piperazine, piperidine, and diaminopropane) were chosen. The Southern substituent (benzyl) was left unchanged to have matched pairs for comparison (Table 3).

In a series of compounds where the Eastern substituent is piperazinyl, the switch from a 5-methyl substituent (2) to 5-Cl (3) or 5-Br (16b) led to a ~2-fold improvement in potency, whereas a 5-CF$_3$ substituent (14c) led to a ~3-fold loss of potency and a reduced safety index. As mentioned earlier, introducing steric hindrance on the core is a hERG mitigation strategy. Aromatic/heteroaromatic substituents were introduced in the 5-position (49a−d and 49ea), via coupling of the bromo derivative with the respective boronic acid derivatives. The consequence was a 25-fold or greater loss in potency. In a series of compounds wherein the Eastern substituent is piperidinyl, the 5-Cl (46a) and 5-Br (46b) derivatives picked up some potency in comparison to the piperazine derivatives in the NF54 assay but were equally potent in the K1 assay. There was a ~4-fold drop-in activity for the corresponding 5-CF$_3$ (46c) derivative, whereas an ester (46d) or sulfone (46e) substituent in the 5-position led to inactive compounds. In a series of compounds wherein the Eastern substituent is diaminopropyl, this trend was confirmed where the chloro and bromo derivatives are equipotent but a 5−6-fold drop-in potency was observed for the corresponding 5-CF$_3$ substituent while there was more than a 10-fold drop-in potency for the unsubstituted derivative.

As mentioned earlier, introducing steric hindrance is a known hERG mitigation strategy. We accordingly synthesized and evaluated double halogenation products of the Western substituent. Several substituents were introduced in the 4-position of the benzimidazole scaffold while keeping the piperazinyl as the Eastern substituent (Table 4). Compound 42a with an additional chlorine atom at the 4-position showed equal or slightly improved $P_f$ activity against both NF54 and K1 strains in comparison to the 4-unsubstituted derivative 3, whereas the 4-Br derivative 42k was equipotent or slightly less potent. Unfortunately, both derivatives were ~4-fold more cytotoxic than the 4-unsubstituted compound 3 and the SI index dropped below 500 as a consequence. Introducing larger aromatic substituents in compounds 52a and 52b was not tolerated.

**Table 3. In Vitro Antiplasmodium Activity, Solubility, and Cytotoxicity Data of the Western Substituent with Different Substituents in 5-Position**

| Compd | $R^1$ | X | $IC_{50}$ (μM)$^a$ | Solubility$^b$ (μM) | $IC_{50}$ (CHO)$^c$ (μM) | Si$^d$ |
|-------|-------|---|-----------------|---------------------|--------------------------|------|
|       |       |    | PF54 | PK1 | pH 2.0 | pH 6.5 | ND | ND | ND | ND | ND | ND |
| 2*    | Me    | 0.098 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 3     | Cl    | 0.062 | 0.054 | 150 | 140 | 45 | 726 | ND | ND | ND | ND | ND |
| 16b   | Br    | 0.042 | 0.064 | 185 | 105 | 41 | 976 | ND | ND | ND | ND | ND |
| 14c   | CF$_3$| 0.310 | 0.415 | 150 | 200 | 7 | 3 | ND | ND | ND | ND | ND |
| 49a   | >10.00| ND | ND | ND | 195 | ND | ND | ND | ND | ND | ND | ND |
| 49b   |       | 6.962 | ND | ND | ND | 25 | ND | ND | ND | ND | ND | ND |
| 49c   |       | 5.077 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 49d   |       | 4.282 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 49ea  |       | 1.655 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 46a   | Cl    | 0.038 | 0.076 | 190 | 125 | 21 | 553 | ND | ND | ND | ND | ND |
| 46b   | Br    | 0.028 | 0.061 | 190 | 110 | 20 | 714 | ND | ND | ND | ND | ND |
| 46c   | CF$_3$| 0.138 | ND | ND | ND | 135 | 185 | 30 | 217 | ND | ND | ND |
| 46d   | CO$_2$Me | 3.827 | ND | ND | ND | 200 | ND | ND | ND | ND | ND | ND |
| 46e   | SO$_2$Me | >10 | ND | ND | ND | 200 | ND | ND | ND | ND | ND | ND |
| 49e   | Cl    | 0.348 | 0.717 | 145 | 200 | 40 | 115 | ND | ND | ND | ND | ND |
| 41    | Br    | 0.382 | 1.010 | 195 | 100 | 32 | 84 | ND | ND | ND | ND | ND |
| 15    | CF$_3$| 2.074 | ND | ND | ND | 190 | ND | ND | ND | ND | ND | ND |
| 58    | H     | 4.050 | ND | ND | ND | 200 | ND | ND | ND | ND | ND | ND |

$^a$Mean from $n$ values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of *P. falciparum*. $^b$Aqueous solubility using HPLC-DAD-MS. $^c$CHO = Chinese hamster ovarian cell line. $^d$Selectivity index (SI) = [IC$_{50}$ (CHO)/IC$_{50}$ (Pf/NF54)]. $^e$Obtained from NOVARTIS screening library and Pf activity determined at NITD. ND = not determined.
On the basis of literature precedence,34 we reasoned that incorporation of a nitrogen atom into the Western substituent might result in improved Pf potency and reduce hERG activity. Hence, a couple of changes to the core were investigated by incorporating an additional nitrogen (Table 5). Unfortunately, these modifications (57a and 57b) led to inactive compounds.

SAR of the Southern Substituent. For the investigation of the Southern substituent, the piperazinyl moiety was fixed as the Eastern substituent on the basis that compounds with this moiety showed activity. The Western substituent was left unchanged, or we used the corresponding unsubstituted derivative, to have matched pairs for comparison (Table 6). Removal of the 1-carbon spacer in the N-benzyl group (16f) or introducing an additional spacer (16g) led to inactive compounds. Replacing the phenyl group with a hydrogen (60), cyclohexyl (14h), or 2-/4-pyridyl (59 and 14i) groups also resulted in inactive compounds. The combination of a one-carbon spacer and a phenyl ring (3) was found to be optimal for maintaining the Pf activity as the other N-replacements were not tolerated.

In Vitro, hERG Channel Activity, and Metabolic Stability. Selected compounds with good antiplasmodium activity were further evaluated for hERG channel inhibition (Table 7). Unfortunately, only one compound (33) showed some marginal improvement. The nonbasic compounds 18 and 19 showed only marginal improvement in the hERG stability.

Table 4. In Vitro Antiplasmodium Activity, Solubility, and Cytotoxicity Data of the Disubstituted Western Substituent

| Compd | R² | IC₅₀ (µM)³ | Solubility (µM) | IC₅₀ (CHO) (µM) | SI² |
|-------|----|------------|-----------------|----------------|-----|
|       |    |            | P/FNF4 | P/K1 | pH 2.0 | pH 6.5 | P/FNF4 | P/K1 |
| 3     | H  | 0.062      | 0.054 | 150 | 140 | 45 | 726 |
| 42a   | Cl | 0.055      | 0.032 | 140 | 70 | 12 | 218 |
| 42k   | Br | 0.071      | ND | 145 | 50 | 13 | 183 |
| 52a   |   | 3.652      | ND | ND | 195 | ND | ND |
| 52b   |   | 2.166      | ND | ND | 200 | ND | ND |

³Mean from n values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of P. falciparum. ⁴Aqueous solubility using HPLC-DAD-MS. ⁵CHO = Chinese hamster ovarian cell line. ⁶Selectivity index (SI) = [IC₅₀ (CHO)/IC₅₀ (Pf/NF54)]. ND = not determined.

Table 5. In Vitro Antiplasmodium Activity of the Modified Cores Containing Additional Nitrogen Atoms

| Compd | Structure | IC₅₀ (µM)³ | Solubility (µM) | IC₅₀ (CHO) (µM) | SI² |
|-------|-----------|------------|-----------------|----------------|-----|
|       |           |            | P/FNF4 | P/K1 | pH 2.0 | pH 6.5 | P/FNF4 | P/K1 |
| 3     |           | 0.81       | ND | ND | ND | ND |
| 16f   |           | >1.0       | ND | ND | ND | ND |
| 16g   |           | >1.0       | ND | ND | ND | ND |
| 14h   |           | 7.884      | ND | ND | ND | ND |
| 1³    |           | 0.81       | ND | ND | ND | ND |
| 59    |           | 9.77       | ND | ND | ND | ND |
| 14i   |           | >10        | ND | ND | ND | ND |
| 60    |           | >10        | ND | ND | ND | ND |

³Mean from n values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of P. falciparum. ⁴Obtained from NOVARTIS screening library and Pf activity determined at NITD. ND = not determined.

Table 6. SAR of the Southern Substituent

| Compound | R² | X | IC₅₀ (µM)³ | Solubility (µM) | IC₅₀ (CHO) (µM) | SI² |
|----------|----|---|------------|-----------------|----------------|-----|
|          |    |   | P/FNF4 | P/K1 | pH 2.0 | pH 6.5 | P/FNF4 | P/K1 |
| 3        | H  |   | 0.062 | 0.054 | 150 | 140 | 45 | 726 |
| 16f      | Cl |   | 2.083 | ND | 140 | 70 | 12 | 218 |
| 16g      |   |   | >1.0 | ND | 145 | 50 | 13 | 183 |
| 14h      |   |   | 7.884 | ND | ND | ND | ND |
| 1³      | H |   | 0.81 | ND | ND | ND | ND |
| 59      |   |   | 9.77 | ND | ND | ND | ND |
| 14i      | Me |   | >10 | ND | ND | ND | ND |

³Mean from n values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of P. falciparum. ⁴Obtained from NOVARTIS screening library and Pf activity determined at NITD. ND = not determined.
liability but substantial loss in antiplasmodium activity. The only compound with a better hERG profile was the one containing an ethylenediamine side chain (33), leading to a still unsatisfactory 10-fold safety window.

The microsomal metabolic stability of the active analogues was evaluated in human, mouse, and rat liver microsomes and was found to be generally high for most compounds (except 19 and 27) across the three species.

**Pharmacokinetic Studies in Rats.** On the basis of the good in vitro antimalarial activity and solubility data, in vivo pharmacokinetics (PK) studies in rats were initiated for compound 3, with the goal to assess the exposure and oral bioavailability in rodent species. Following intravenous administration at 1 mg/kg dose, compound 3 displayed high total systemic clearance (>100% hepatic blood flow) and high volume of distribution (>5 L/kg), resulting in a moderate half-life (1.98 h) (Table 8). Following oral administration at 5 mg/kg dose, compound 3 showed rapid absorption, reasonable exposure, and moderate oral bioavailability (42%).

Table 7. hERG Inhibition and Microsomal Stability Data of the Active Compounds

| Compd | R<sup>1</sup> | R<sup>2</sup> | X | IC<sub>50</sub> (μM)<sup>a</sup> | hERG IC<sub>50</sub> (μM) | Metabolic Stability<sup>b</sup> (% remaining after 30 minutes) | HLMs | MLMs | RLMs |
|-------|---------------|--------------|-------|-----------------|-----------------|-----------------------------|-------|-------|-------|
| 2<sup>α</sup> | H Me | 0.098 | ND | - | - | - |
| 1<sup>α</sup> | H H | 0.81 | 0.32 | - | - | - |
| 3 | H Cl | 0.062 | 0.009 | 74.4 | 63.0 | 42.1 |
| 17 | H Cl | >1.0 | 2.3 | - | - | - |
| 18 | H Cl | >1.0 | 0.39 | - | - | - |
| 19 | H Cl | 0.306 | 0.18 | 8.6 | 2.0 | 1.2 |
| 27 | H Cl | 0.216 | 0.017 | 23.2 | 31.3 | 36.5 |
| 29 | H Cl | 0.037 | 0.02 | 92.2 | 85.9 | 85.7 |
| 28 | H Cl | 0.022 | 0.07 | 80.9 | 76.0 | 39.9 |
| 46a | H Cl | 0.0379 | 0.46 | 91.8 | 97.6 | 92.9 |
| 38 | H Cl | 0.276 | ND | 98.9 | 98.9 | 96.4 |
| 33 | H Cl | 0.245 | 2.2 | 98.9 | 71.2 | 88.0 |
| 43b | H Cl | 0.086 | ND | - | - | - |
| 32 | H Br | 0.348 | 1.63 | 97.0 | 89.8 | 93.3 |
| 41 | H Br | 0.382 | ND | 99.4 | 98.6 | 97.0 |
| 16b | H Br | 0.0424 | 0.03 | 88.2 | 94.0 | 89.8 |
| 14c | H CF<sub>3</sub> | 0.310 | 0.15 | 92.2 | 82.0 | 95.6 |
| 46b | H Br | 0.028 | ND | 99.3 | 97.4 | 97.7 |
| 42a | Cl Cl | 0.055 | 0.59 | 76.3 | 72.5 | 66.1 |
| 42k | Br Cl | 0.071 | ND | 97.4 | 91.6 | 93.8 |

<sup>a</sup>Mean from n values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of P. falciparum. <sup>b</sup>hERG determined at Novartis Institute for Tropical Diseases (NITD). Human, mouse, and rat liver microsomes. Obtained from NOVARTIS screening library and Pf activity determined at NITD. ND = not determined.

Table 8. Pharmacokinetic Parameters for Compound 3 Following Intravenous Dosing at 1 mg/kg and Oral Dosing at 5 mg/kg in Rats<sup>c</sup>

| PK parameters | intravenous PK parameters | oral PK parameters |
|---------------|---------------------------|--------------------|
| V<sub>x</sub> (L/kg) | CL (mL/(min kg)) | T<sub>1/2</sub> (h) | C<sub>max</sub> (μM) | T<sub>max</sub> (h) | AUC (μM h) | F (%) |
| 17.69 | 144 | 1.98 | 0.80 | 0.08 | 1.5 | 42 |

<sup>c</sup>V<sub>x</sub> = volume of distribution at steady state; CL = total systemic clearance; T<sub>1/2</sub> = half-life; C<sub>max</sub> = maximum concentration of drug in plasma; T<sub>max</sub> = time to maximum concentration of drug in plasma; AUC = area under the curve; F = oral bioavailability; compounds were formulated in 30% N-methyl pyrrolidone/70% PEG200 for intravenous administration and 0.5% methyl cellulose/0.5% Tween 80 in water for oral administration.

**In Vivo Efficacy Studies.** On the basis of the potent in vitro activity and promising in vitro physicochemical properties (Table 2 and 9), the frontrunner compounds were further profiled in vivo for efficacy in a Plasmodium berghei and P. falciparum infection model.
Table 9. In Vitro Physicochemical Properties

| parameters               | 1             | 2             | 3             |
|--------------------------|---------------|---------------|---------------|
| structure                | Leriostroton  | (R = H)       | (R = Me)      |
| (R = H)                  |               | (R = Cl)      |               |
| molecular weight†        | 292.4         | 306.4         | 326.8         |
| PSA†                     | 35.56         | 35.56         | 35.56         |
| cLogP‡                   | 3.3           | 3.8           | 4.1           |
| solubility (mM), (pH 6.8)| >1            | >0.1‡         | 0.77          |
| PAMPA (%FA)              | 99            | 100           | 99            |
| mouse†/rat*/human‡       | 29/−/26.6     | 36.7/172/43.5 | 45.3/79/≤25  |
| mPPB (%)                 | 93.7          | 97.8          |               |

†In silico predictions; PSA = polar surface area; PAMPA = parallel artificial membrane permeability assay (fraction absorbed). ‡In vitro metabolic stability in liver microsomes (μM/min mg). §In vitro metabolic stability in hepatocytes (μL/min per 10⁶ cells). mPPB = mouse plasma protein binding.

Table 10. In Vivo Efficacy of Selected Analogues on Plasmodium Mouse Malaria Models

| parameters               | 2             | 3             |
|--------------------------|---------------|---------------|
| malaria model            | P. berghei    | P. berghei    | P. falciparum |
| dose (mg/kg)             | 1 × 100       | 1 × 100       | 4 × 25        |
| activity (%)             | 94            | 99            | >99.9         |
| survival (days)          | 6             | 7             |               |
| Cmax (μM)                | 13.1          | 20.6          | 5.2           |
| AUC (μM h)               | 165.4         | 377.1         | 86.0          |

*Activity = average parasitemia reduction compared with untreated controls; survival = average lifespan after infection (6−7 days for untreated control mice); Cmax = maximum concentration of drug in plasma; AUC = area under the curve; compounds were formulated in 0.5% methyl cellulose/0.5% Tween 80 in water for oral administration.

Figure 3. P. falciparum SCID mouse efficacy study for compound 3 compared with chloroquine (CQ).

A novel series of 1,2,5-trisubstituted benzimidazoles was identified from phenotypic whole cell high-throughput screening of the Novartis chemical library. A broad range of structurally diverse benzimidazoles were synthesized and assessed for in vitro antiparasitic activity. SAR analysis indicated that several of the synthesized compounds displayed promising activity (IC50 NF54 <0.1 μM). As expected, addition of a functional group (H → Me → Cl) increased lipophilicity and plasma protein binding (Table 9). Overall, these compounds displayed good solubility, permeability, and metabolic stability in mouse and human species. However, in vitro clearance was moderate to high in rat and that translated to high in vivo total systemic clearance (Table 8). Additionally, the frontrunner compound displayed good parasitemia reduction in two malaria mouse models (Table 10 and Figure 3); however, survival rates as determined in the P. berghei model were poor and equated to the vehicle-treated animals. Further lead optimization needs to be carried out to address the unresolved hERG liability of the series, as well as the high plasma clearance and short half-life of the compounds (Scheme 6).

**EXPERIMENTAL SECTION**

All commercially available chemicals were purchased from either Sigma-Aldrich or Combi-Blocks. All solvents were dried by appropriate techniques. Unless otherwise stated, all solvents used were anhydrous. 1H NMR spectra were recorded on a Bruker Spectrometer at 300 or 400 MHz. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed silica-gel 60 F254 (70−230 mesh) plates. Flash column chromatography was performed with Merck silica-gel 60 (70−230 mesh). Chemical shifts (δ) are given in ppm downfield from trimethylsilane (TMS) as the internal standard. Coupling constants, J, are recorded in hertz (Hz). Purity was determined by HPLC, and all compounds were confirmed to have >95% purity. The data that is not shown below is supplied in the Supporting Information.

**Aqueous Solubility.** Water solubility was analyzed using a miniaturized shake flask method. Ten millimolar stock solutions of each of the compounds were used to prepare calibration standards (10−220 μM) in DMSO. The same 10 mM stock solutions were accurately dispensed in duplicate into 96-well plates, and the DMSO, dried down (MiVac GeneVac, 90 min, 37 °C). Thereafter, the samples were reconstituted (200 μM) in an aqueous solution and shaken (20 h, 25 °C). The solutions were analyzed by means of HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector). Best fit calibration curves were constructed using the calibration standards, which were used to determine the aqueous samples’ solubility.

**Metabolic Stability Assay.** The metabolic stability assay was performed in duplicate in a 96-well microtiter plate. The
test compounds (0.1 μM) were incubated (37 °C) in mouse, rat, and pooled human liver microsomes (final protein concentration of 0.4 mg/mL; XenoTech, Lenexa, KS) suspended in 0.1 M phosphate buffer (pH 7.4) for predetermined time points, in the presence and absence of the cofactor-reduced nicotinamide adenine dinucleotide phosphate (NADPH) (1 mM). The reactions were quenchned by the addition of ice cold acetonitrile containing an internal standard (carbamazepine, 0.0236 μg/mL). The samples were centrifuged, and the supernatant was analyzed by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Agilent Rapid Resolution HPLC, AB SCIEX 4500 MS). The relative loss of the parent compound over time was determined by the addition of ice cold acetonitrile containing an internal standard (carbamazepine, 0.0236 μg/mL). The samples were centrifuged, and the supernatant was analyzed by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Agilent Rapid Resolution HPLC, AB SCIEX 4500 MS). The relative loss of the parent compound over time was monitored and plots were prepared for each compound of Ln% remaining versus time to determine the first-order rate constant for compound depletion. This was used to calculate the degradation half-life and in vitro intrinsic clearance value and subsequently to predict the in vivo intrinsic clearance and in vivo hepatic extraction ratio (Em). Metabolite searches were not conducted during the metabolic stability assay.35

In Vitro Antiplasmodium Assay. All parasite strains were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, VA). Compounds were tested using parasite lactate dehydrogenase assay as a marker for parasite survival.37 Briefly, the respective stock solutions of CQ diphasate and test compounds were prepared to 2 mg/mL in distilled water (for CQ) and 100% DMSO for test compounds and then stored at −20 °C, and further dilutions were prepared on the day of the experiment. The cultures were synchronized in the ring stage as described previously using 15 mL of 5% (w/v) D-sorbitol in water.38 Synchronous cultures of Pf/NF54 (CQS) and Pf/K1 (CQR) in the late trophozoite stage were prepared to 2% parasitemia and 2% hematocrit. Compounds were tested at starting concentrations of 10 000 ng/mL (1000 ng/mL for CQ), which were then serially diluted twofold in complete medium to give 10 concentrations with a final volume of 200 μL in each well. Parasites were incubated in the presence of the compounds at 37 °C in a specialized atmosphere of 4% CO2 and 3% O2 in nitrogen for 48 h. Following incubation, 100 μL of MalStat reagent and 15 μL of resuspended culture were combined, followed by addition of 25 μL of nitro blue tetrazolium chloride (NBT). The plates were kept in the dark for about 10 min to fully develop, and absorbance was measured at 620 nm on a microplate reader. Raw data were exported to Microsoft Excel for dose-response analysis.

Cytotoxicity Assay. Compounds were screened for in vitro cytotoxicity against Chinese hamster ovarian (CHO) mammalian cell lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The reference standard, emetine, was prepared to 2 mg/mL in distilled water while stock solutions of test compounds were prepared to 20 mg/mL in 100% DMSO with the highest concentration of solvent to which the cells were exposed having no measurable effect on the cell viability. The initial concentration of the compounds and control was 10 μg/mL, which was serially diluted in complete medium with 10-fold dilutions to give six concentrations, the lowest being 0.001 μg/mL. Plates were incubated for 48 h with 100 μL of drug and 100 μL of cell suspension in each well and developed afterward by adding 25 μL of sterile MTT (Thermo Fisher Scientific) to each well, followed by 4 h of incubation in the dark. The plates were then centrifuged; the medium was aspirated, and 100 μL of DMSO was added to dissolve crystals before reading the absorbance at 540 nm. Data were analyzed, and the sigmoidal dose-response was derived using GraphPad Prism v 4.0 software (La Jolla). All experiments were performed for at least three independent biological repeats, each with technical triplicates.

In Vivo Efficacy Studies. P. berghei Mouse Model. In vivo antimalarial activity was assessed for groups of five female NMRI mice (20–22 g) intravenously infected on day 0 with P. berghei GFP ANKA malaria strain (2 × 107 parasitized erythrocytes, donation from AP Waters and CJ Janse, Leiden University). Untreated control mice die typically between day 6 and day 7 post infection. Experimental compounds were formulated (in 0.5% methyl cellulose/0.5% Tween 80) and administered orally in a volume of 10 mL/kg as a single dose (24 h postinfection) of 100 mg/kg. Parasitaemia was determined 72 h post infection using standard flow cytometry techniques. Activity was calculated as the difference between the mean percent parasitaemia for the control and treated groups expressed as a percent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites by slide reading.

P. falciparum SCID Mouse Model. Compound efficacy was assessed in the murine P. falciparum SCID model essentially as described.39 Briefly, compound 3, formulated in 7% Tween 80, 3% ethanol, was administered to a cohort of age-matched female immunodeficient NOD-scid IL-2Rγnull mice (The Jackson Laboratory, Bar Harbor, ME) previously engrafted with human erythrocytes (generously provided by the Blood Bank in Zürich, Switzerland). The mice were intravenously infected with 2 × 107 P. falciparum Pf3D7/09-infected erythrocytes (day 0).40 On day 3 after infection, mice were randomly allocated to treatments that were administered once a day for 4 days (n = 2 mice) by oral gavage at 25 mL/kg.
Parasitemia was measured by microscopy. Chimerism was monitored by flow cytometry using anti-murine erythrocyte TER119 monoclonal antibody (Pharmingen, San Diego, CA) and SYTO-16 and then analyzed by flow cytometry in serial 2 µL blood samples.

In vivo studies conducted at the Swiss TPH, Basel were approved by the veterinary authorities of the Canton Basel-Stadt (permit no. 1731 and 2303) based on Swiss cantonal (Verordnung Veterinärmab Basel-Stadt) and national regulations (the Swiss animal protection law, Tierschutzgesetz).

General Procedure 1 (GP1) for the Synthesis of 3, 14c, 14h, 14i, 15, 16a, 16b, 16f, 16g, 17–41, 42a, and 42k (Scheme 1); (Synthesis of Starting Material; See the Supporting Information). Step 7: General Procedure for Synthesis of 16a, 16b, 16g, and 41. A mixture of appropriate 2-CI-benzimidazole (1 equiv), corresponding amine (10–12 equiv), and Et,N (10 equiv) in tert-butanol (0.15 M) was heated in a pressure tube at a temperature of 120 °C for 6 h to 18 days. Then, the reaction mixture was allowed to cool to room temperature and saturated aqueous NaHCO₃ solution (40 mL) and EtOAc (50 mL) were added. The organic phase was separated and the aqueous phase was added with an excess (40 mL) and EtOAc (50 mL) were added. The organic phase was combined and washed with an aqueous solution of NaHCO₃ and concentrated in vacuo at reduced pressure. The residue was puriﬁed by flash column chromatography using ISCO Teledyne RediSep Rf column eluting DCM/MeOH (0.5 M NH₃ solution) or hexane/EtOAc to get pure products.

N1-(1-Benzyl-5-chloro-1H-benzo[d]imidazol-2-yl)-propane-1,2-diamine (41). Flash column chromatography using DCM/MeOH (0.5 M NH₃ solution) (0–25% gradient). (52 mg, 31% yield), off white solid. 1H NMR (300 MHz, CDCl₃) δ (ppm) 7.15 (dd, J = 8.3, 1.8 Hz, 1H), 7.13–7.15 (m, 2H), 7.10 (dd, J = 9.0, 0.3 Hz, 1H), 6.99 (dd, J = 8.3, 0.4 Hz, 1H), 5.2 (s, 2H), 3.56 (t, J = 6.7 Hz, 2H), 2.85 (t, J = 7.0 Hz, 2H), 1.91 (quin, J = 6.8 Hz, 2H); HPLC purity 99%, retention time (Rt) = 3.513 min; method used (general positive); LC–MS (APCI) m/z = found 359.1 (M + H)+ and 361.1 (M + 2)+ on a positive mode M 280 nm [calcld for C₁₇H₂₀BrN₄ m/z = 359.0871 (M + H)+].

General Procedure 2 (GP2) for the Reductive Amination Reaction (Scheme 2) (43a–d). To a mixture of N1-(1-benzyl-5-chloro-1H-benzo[d]imidazol-2-yl)ethane-1,2-diamine (33) (1 equiv), benzaldehyde (1.05 equiv), and acetic acid (2.0 equiv) in dichloroethane (DCE) (0.027 M) was added sodium triacetoxyborohydride (2.25 equiv) and acetic acid (2.0 equiv) in dichloroethane (DCE) (0.027 M) and stirred for 2–24 h at RT. TLC and LC–MS indicated the complete consumption of the starting material and showed a mass peak for monobenzylated (major) and in some cases, a dibenzylated (minor) product. Then, the reaction was quenched by the addition of NaOH (1 N solution (30 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography using ISCO Teledyne elution DCM/MeOH (0.5 M NH₃ solution) (0–10% gradient) to afford product. The purified oily/gummy compounds were dissolved in MeOH (5 mL), and a 2.0 M solution of HCl/ether (4 mL) was added and concentrate in vacuo to dryness. Et₂O (6 mL) was added and stirred at room temperature of 25 °C for 1 h; the precipitate (ppt) was filtered and dried to afford a pure salt.

N1-Benzyl-N₁-(1-benzyl-5-chloro-1H-benzo[d]imidazol-2-yl)ethane-1,2-diamine Hydrochloride (43b). Flash column chromatography using DCM/MeOH (0.5 M NH₃ solution) (0–10% gradient) (44 mg, 44% yield), yellow solid. 1H NMR (300 MHz, CDCl₃) δ (ppm) 7.62–7.59 (m, 2H), 7.56–7.55 (m, 1H), 7.50–7.46 (m, 3H), 7.42–7.30 (m, 7H), 5.53 (s, 2H), 4.34 (s, 2H), 3.98 (t, J = 6.1 Hz, 2H), 3.51 (t, J = 6.2 Hz, 2H); HPLC purity 99%, retention time (Rt) = 0.911 min; method used (general positive); LC–MS (APCI) m/z = found 391.2 (M + H)+ and 393.2 (M + 2)+ on a positive mode M 280 nm [calcld for C₁₇H₂₀BrN₄ m/z = 427.1456 (M + H)+].

Note: Dibenzylated (4 mg, 3.57%) product was also obtained.

N¹-(1-Benzyl-5-chloro-1H-benzo[d]imidazol-2-yl)-N²-(pyridin-4-ylmethyl)ethane-1,2-diamine Hydrochloride (43c). Flash column chromatography using DCM/MeOH (0.5 M NH₃ solution) (0–5% gradient) (40 mg, 49% yield), yellow solid. 1H NMR (300 MHz, CDCl₃) δ (ppm) 8.95–8.92 (m, 2H), 8.27 (d, J = 6.5 Hz, 2H), 7.57–7.56 (m, 1H), 7.42–7.33 (m, 7H), 5.57 (s, 2H), 4.66 (s, 2H), 4.06 (t, J = 5.9 Hz, 2H), 3.64 (t, J = 5.9 Hz, 2H); HPLC purity 99%, retention time (Rt) = 1.054 min; method used (general positive); LC–MS (APCI) m/z = found 392.2 (M + H)+ and 394.2 (M + 2)+ on a positive mode M 280 nm [calcld for C₁₉H₂₃Cl₂N₄ m/z = 428.1049 (M + H)+].

Note: Dibenzylated product was not observed.
General Procedure 3 (GP3) for the Synthesis of C-Linked Compounds (46a–e) (Scheme 3). Step 1: General Procedure for Amide Coupling (45a). Synthesis of tert-Butyl 4-((2-(benzylamino)-5-chlorophenyl)carbamoyl)piperidine-1-carboxylate (45a). To a solution of the N1-benzyl-4-chlorobenzene-1,2-diamine (8a) (400 mg, 1.719 mmol) in DMS (8.0 mL) was added 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid (394 mg, 1.719 mmol), HATU (784 mg, 2.063 mmol), and DIPEA (0.751 mL, 4.30 mmol). The reaction mixture was stirred at 35 °C. After 16 h, TLC indicated that the reaction was completed. H2O (30 mL) and EtOAc (60 mL) were added, the organic phase separated, and the aqueous phase extracted with a further portion of EtOAc (2 × 20 mL). The organic phases were combined and washed sequentially with saturated aqueous NaHCO3 (30 mL) and brine (30 mL), dried over anhydrous Na2SO4, and concentrated in vacuo to a crude residue of 45a (450 mg, 39% yield) as a brown oil, which was used directly to the next step without any further purification. TLC system Rf = 0.25 [hexane/EtOAc (4/6)]. HPLC purity 67%, retention time (Rt) = 4.756 min; method used (general negative); LC–MS (APCI) m/z = found 442.2 (M – H)+ on a negative mode M 280 nm [calcd for C26H31N6O2 m/z = 442.1897 (M – H–)].

Step 2: General Procedure for Cyclization and Deprotection of Boc Group (46a). Synthesis of 1-Benzyl-5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (46a). A suspension of the crude tert-butyl 4-((2-(benzylamino)-5-chlorophenyl)carbamoyl)piperidine-1-carboxylate 45a (1.0 equiv) in acetic acid (60 equiv) was stirred and heated in a sealed tube at a temperature at 100 °C. After 6 h, TLC indicated that the reaction is completed. LC–MS indicated a corresponding mass ion peak. The reaction mixture was allowed to cool to 25 °C and concentrated in vacuo to afford a crude residue. EtOAc (50 mL) was added, and aqueous NaHCO3 solution (50 mL) was saturated. The organic phase was separated, and the aqueous phase, extracted, with a further portion of EtOAc (2 × 30 mL). The organic phases were combined, dried over anhydrous Na2SO4, and concentrated in vacuo to afford a residue. Purification by flash chromatography was carried out using ISCO Teledyne on a 12 g RediSep Rf column, and elution was carried out using DCM/MeOH (0.5 M NH4 solution) (0–25% gradient) to afford 46a (64 mg, 28% yield) as an off white solid. 1H NMR (300 MHz, CD3OD) δ (ppm) 7.67 (dd, J = 1.9, 0.5 Hz, 1H), 7.45 (dd, J = 8.7, 0.6 Hz, 1H), 7.40–7.27 (m, 4H), 7.15–7.09 (m, 2H), 5.61 (s, 2H), 3.54–3.47 (m, 2H), 3.45–3.40 (m, 1H), 3.18–3.05 (m, 2H), 2.15–1.98 (m, 4H); HPLC purity 98%, retention time (Rt) = 0.434 min; method used (general positive); LC–MS (APCI) m/z = found 326.2 (M+) and 328.2 (M + 2)+ on a positive mode M 280 nm [calcd for C19H21ClN3 m/z = 326.1424 (M + H)+].

General Procedure 4 (GP4) for the Synthesis of 49a–e, 52a, and 52b (Scheme 4). Step 1: General Procedure for Suzuki Cross-Coupling. Bromo compounds 47/50 (1.0 equiv) and appropriate aryl boronic acids (1.3 equiv) were taken in a pressure vial in 1,4-dioxiane (0.1 M) and water (0.318 M) (5:1) followed by the addition of cesium carbonate (3.0 equiv) and [1,1′-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (0.1 equiv) and heated to 95 °C until TLC of the reaction indicated complete consumption of the starting material (approximately 4–21 h). The reaction mixture was cooled to room temperature and a saturated solution of NaHCO3 (30 mL) was added and extracted with EtOAc (2 × 40 mL). The combined organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude residue was purified by flash chromatography using eluting hexane/EtOAc or DCM/MeOH gradient to afford the desired Suzuki cross-coupled product in moderate yields, which were used directly in the next step.

**t**-**Butyl 4-((1-Benzyl-5-(1H-pyrazol-3-yl)-1H-benzo[d]imidazo[2-3,1-d]imidazol-2-yl)piperazine-1-carboxylate (48a).** Flash column chromatography using hexane/EtOAc (0–100% gradient), (66 mg, 45% yield), off white solid. HPLC purity 99%, retention time (Rt) = 1.226 min; method used (general positive); LC–MS (APCI) m/z = found 459.2 (M + H)+ on a positive mode M 280 nm [calcd for C29H23ClN3O2 m/z = 459.25 (M + H)+].

**Step 2: General Procedure for Boc-Deprotection.** A Boc-protected compound (1 equiv) in 4 N HCl in dioxane (4 mL) was stirred for 2–20 h. TLC and LC–MS of the reaction indicated the complete consumption of the starting material. The reaction solvent was evaporated under reduced pressure and diethyl ether (5 mL) was added. Then, the ppt was filtered off and washed with an excess of diethyl ether (5 mL) to afford the desired hydrochloride salt in low to moderate yields.

1-Benzyl-2-((piperazin-1-yl)-5-(1H-pyrazol-3-yl)-1H-benzo[d]imidazole Hydrochloride (49a). (35 mg, 67% yield), yellow solid. 1H NMR (300 MHz, CD3OD) δ (ppm) 8.08 (dd, J = 1.6, 0.6 Hz, 1H), 7.90–7.86 (m, 2H), 7.51–7.35 (m, 6H), 6.88 (d, J = 2.5 Hz, 1H), 5.63 (s, 2H), 3.90–3.87 (m, 4H), 3.50–3.46 (m, 4H); HPLC purity 99%, retention time (Rt) = 2.688 min; method used (general positive); LC–MS (APCI) m/z = found 359.1 (M + H)+ on a positive mode M 280 nm [calcd for C19H21ClN3 m/z = 359.1751 (M + H)+].
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Notes
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ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; hERG, human ether-a-go-go related gene; ACN, acetonitrile; DCM, dichloromethane; DCE, dichloroethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; LDA, lithium disopyropylamide; PTSA, para-toluenesulfonic acid; HATU, 1-[[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; DIPEA, disopropyl ethyl amine; CHO cells, chinese hamster ovarian cells; ER, extraction ratio; SAR, structure–activity relationships; CQ, chloroquine; PK, pharmacokinetics; NMR, nuclear magnetic resonance; TMS, trimethylsilane; RT, room temperature; NITD, Novartis Institute for Tropical Diseases; GSK, GlaxoSmithKline; WHO, World Health Organization

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