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Synthesis, characterization and in vitro evaluation of novel enantiomerically-pure sulphonamide antimalarials

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Abstract

Malaria parasites currently gain resistance rapidly, across countries and continents. Hence, the discovery and development of novel chemical compounds, as well as scaffolds, with superior antimalarial activity remains an important priority, not only for the developing world. Our report describes the development, characterization and evaluation of novel bepotastine-based sulphonamide antimalarials inhibiting asexual stage development of *Plasmodium (P.) falciparum* parasites *in vitro*. Screening results showed potent inhibitory activity of a number of novel sulphonamides against *P. falciparum* at low micromolar concentrations, in particular in late-stage parasite development, as well as selectivity over mammalian cells. Based on computational studies we hypothesize N-myristoyltransferase as the target of the compounds developed here. Our results demonstrate the value of novel bepotastine-based sulphonamide compounds for targeting th asexual developmental stages of *P. falciparum*.

Keywords: *Plasmodium falciparum*, Anti-malarials, *in vitro* screening, Drug Discovery, Giemsa-staining, Microscopy, Flow cytometry,

Background

Malaria caused by parasitic protozoans of the family *Plasmodium* continues to be a major threat to millions of people across mainly Africa, India, and South East Asia. Based on recent reports, at least 700,000 people, the majority of which are children under the age of 8, die every year due to malaria-associated pathology **[1].** There are 4 major classes of plasmodia that cause human malaria: *Plasmodium (P.) falciparum, P. vivax, P. ovale* and *P. malariae.* Among these, *P. falciparum* and *P. vivax* are the most commonly found parasitic variants in Africa and Asia, respectively **[2].** Current treatment for malaria relies on the use of a series of chemotypes that operate through distinct mechanisms impairing parasitic survival within human hosts **[3-4],** primarily by targeting pathways directly related to hemoglobin degradation. However, drug resistant strains for all of the common antimalarials including artemisinin are currently emerging **[5-6].** Of late, given the emergence of multidrug-resistant malaria, even combination therapy shows to be ineffective in many situations as well **[2]**. Thus, the identification of new chemical entities targeting specific stages of parasite development is of extreme importance to global human health.

Classical anti-malarial drugs include chloroquine, artemisinin and pyrimethamine. In combination with pyrimethamine, folic acid antagonists such as sulfadoxine were and are widely used for malaria treatment; however resistance against this combination has been reported extensively **[7].** Histamine-1 receptor antagonists like bepotastine were shown to be effective against the proliferation of *Plasmodia* both *in vitro* and *in vivo* **[8].** Thus, we in this work designed and evaluated novel chemical scaffolds, which combine structural features of both sulphonamides and bepotastine, for their efficacy against the asexual development stages of *P. falciparum.* Our results revealed efficient inhibition of parasite development and replication by these compounds at low micromolar concentrations, interfering at the final stages of intra-erythrocytic life cycle progression. These molecules showed low toxicity to mammalian cells (>10 fold selectivity), and therefore represent potential lead scaffolds for developing novel anti-malarials.

Compound Synthesis and Characterization

We previously developed many new molecules of diverse structure that targets cancer pathways [**9-14**]. Here, to synthesize the reported compounds, we used commercially available 2-bromopyridine and 4-chlorobenzaldehyde to yield racemic (4-chlorophenyl)methyl)(pyridine-2-yl)methanol (**1)** via Grignard

reaction **[15],** which upon reacting with 1-benzyl-4-chloropiperidine (**2)** in DMF using potassium carbonate as base under reflux condition resulted in the racemic compound 2-((1-benzylpiperidin-4-yloxy)(4-chlorophenyl)methyl)pyridine **(3).** Compound **3** was then subjected to debenzylation followed by resolution using R(-)/S(+) mandelic acid in methanol to yield 2-((4-chlorophenyl)(piperidin-4-yloxy) methyl)pyridine (**4a-b)**. Other reported procedures for the synthesis of 2-((4-chlorophenyl)(piperidin-4-yloxy) methyl)pyridine in literature mostly involve the use of organolithium reagents and other extreme conditions, such as longer reaction times and low temperature **[16]**. The resolution using mandelic acid was less laborious compared to other resolution methods *eg.* where repeated crystallizations were involved in achieving the required chiral purity **[17].** The reaction of chirally pure (**4a-b)** with different commercially available benzene sulfonyl chlorides (**5a-i)** in the presence of triethylamine and dichloromethane at room temperature gave sulphonamides (**6a-r**) in 82-90% yield (**Scheme 1**). The compounds (**6a-r**) were characterized by melting point, LC-MS, HRMS, ^{1}H NMR, ^{13}C NMR, and chiral HPLC analysis (**Supplementary Information, S1**).

***In vitro* screening against *Plasmodium falciparum* parasites**

To identify active molecules among the newly synthesized sulphonamides, we initially chose to conduct a pre-screening against laboratory strains (3D7) of *P. falciparum*. Three different concentrations of the compounds dissolved in DMSO were added to actively metabolizing parasites (at trophozoite stage, ~30 hours post invasion (hpi)). DMSO-treated parasites were included as negative controls. 48h post-treatment, parasitemia was scored (after staining with Hoechst 33342) using a standard flow cytometric method **[18]**. Giemsa-stained smears prepared in parallel were examined microscopically to validate the flow cytometry results. From this, we were able to identify ten molecules (**6b, 6c, 6e, 6f, 6g, 6k, 6l, 6n, 6p** & **6r**) with significant inhibitory activity at 20 μM against *P. falciparum* (**Supplementary Figure S2**), which were chosen for subsequent experiments.

In order to accurately determine inhibitory potential of the compounds, trophozoite stage parasites (3D7) were treated at concentrations ranging from 1.6 μM to 200 μM. DMSO-treated and chloroquine-treated parasites served as negative and positive controls respectively. After 48h of incubation with the inhibitors, parasites were fixed with gluteraldehyde and stained with Hoechst for flow cytometry. The sensitivity profiles obtained against 3D7 and chloroquine-resistant (K1) strains are provided in **Figures 1A and 1B,**

and corresponding IC_{50} values are summarized in **Table 1**. Based on these results, three of the tested compounds (6e, 6k, 6n) showed inhibitory activity in the low micromolar range (<4μM), while all the other compounds (6b, 6c, 6f, 6g, 6l, 6p, 6r) were effective only below 15μM. Apart from 6k, all other compounds showed comparable inhibitory effect on both parasites (**Table 1**). Cytotoxicity assays (MTT) **[19]** were performed for all ten selected compounds using a Madin-Darby Canine Kidney (MDCK) cell line. For all compounds except **6c,** a selectivity (IC_{50} MDCK/IC_{50} 3D7) of larger than 10-fold was observed (see **Table 1** and **Supplementary Figure S3**). Compound **6n** turned out to be most selective, with a selectivity index of 92.

To further examine putative biological mechanisms that these molecules are interfering with, we performed experiments probing their stage-specific effects. In order to do this, parasites were continuously treated with each compound **(6b, 6c, 6e, 6f, 6g, 6k, 6l, 6n, 6p, 6r**) at various life-stages (ring, trophozoite and schizonts). In brief, parasites were treated at 4*IC_{50}, 2*IC_{50}, IC_{50} and 0.25*IC_{50} (as determined from Table 1) and monitored using Giemsa-stained images and flow cytometry counting. The results are displayed in Figures **2A, 2B and** 2C for the different development stages. It can be seen that most of the compounds showed an effect on schizont to ring transition at concentrations at IC_{50} and at 2* IC_{50}. This means that these molecules were primarily interfering with the late stages of merozoite development and merozoite rupture.

Giemsa-stained smears from each experiment were examined for specific phenotypes in order to understand the lead compound (**6n**) action further. A representative panel is shown in **Supplementary Figure S4**. Uninfected RBCs looked morphologically indistinct even after addition of the compound. Control-treated (DMSO) parasites developed as anticipated over 46 hours post invasion to form multinucleated schizonts. However, parasites treated with the compounds presented here appear to become trapped in a unique phenotype whereby they fail to segment out into multiple schizonts. At 52 hours post invasion, all DMSO-treated parasites had egressed and formed ring-stage parasites, whereas inhibitor-treated parasites appear to remain locked-in, thereby resulting in parasite death.

Based on the screening results, most of the R and S forms of 2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine (CPMP) attached to the substituted-phenyl sulphonyls showed significant inhibition of malaria growth. Molecules with 2,5-dichloro, 4-nitro, 2,4,6-trymethyl, and 2-nitro-substituted phenyl

sulphonyl groups attached to CPMP inhibited parasite growth with IC_{50} values of 2.1, 3.2, 7.4, and 13.33 μM, respectively. The IC_{50} value for the positive control sulfadoxine is 1 μM, and it is hence comparable in activity to the newer scaffolds presented here. On the other hand, the R forms of 4-methoxy- as well as the S forms of 4-methyl-substituted phenylsulphonyl groups were found to have slightly higher IC_{50} values, of 6.8 and 8.8μM respectively. Additionally, 3-nitro, and 3-trifluoromethyl-substituted phenyl sulphonyls attached to CPMP significantly reduced antimalarial properties. To evaluate potential *in vivo* efficacy of the most effective compound **6n**, BALB/c mice infected with *P. yoelii*, were given a daily dosage of 10 mg/kg body weight or 50mg/kg body weight via i.p. injection. The compound only had mild inhibitory effect on parasite replication (see **Supplementary Figure S5**).

***In silico* mode-of-action analysis**

Aiming to rationalize antimalarial activity of the bepotastine-based sulfonamides, we searched the Protein Data Bank [**20**] for co-crystal structures of aromatic sulfonamide ligands bound to proteins of plasmodial origin and retrieved two hits. Based on ligand similarity beyond the sulfonamide functionality we decided to investigate N-myristoyltransferase. Further, given that it has recently been validated as a novel antimalarian drug target in *Plasmodium vivax* by Wright et al. [**21**]. In this work it was shown on a structural basis that aromatic sulfonamides are capable of inhibiting N-myristoyltransferase, and hence we chose the published co-crystal structure of enzyme and sulfonamide inhibitor as the basis for our *in silico* analysis (PDB 2YND). Induced fit docking of our series of sulfonamides to *P. vivax* N-myristoyltransferase yielded consistent binding modes, in contrast to static docking runs that often led to severely distorted ligand conformations. Additionally, flexible docking enabled successful redocking of the cognate ligand with an RMSD <0.6Å.

In silico docking predicted our compounds to occupy a very similar region of the binding pocket (see **Figure 3**). The inverted sulfonamide functionality allows accommodation of the substituted benzene in the region occupied by the pyrazole ring of the cocrystallized ligand **(Figure 3A).** As this region is particularly involved in π-π interactions with Phe-103 and Phe-105, the steric and electronic structure of the substituted benzenes is deemed crucial for optimal molecular interactions [**22**]. The Y-shaped tail group of the ligands is predicted to overlap with the long hydrophobic stretch of the co-crystallized ligand, thereby filling major

parts of the enzyme's binding cavity and forming multiple additional hydrophobic contacts **(Figures 3B and 3C)**. Moreover, we find polar contacts to Ser-319 as well as contacts to the C-terminal Leu-410 for some ligands. Several structural water molecules are involved in anchoring the polar interaction sites in the binding site. Docking scores of our ligands were found in similar range as the cognate ligand, thus pointing towards similar interaction energies.

Summary

In this work, we report the synthesis, characterization and *in vitro* testing of a novel library of sulphonamides for their antimalarial activity. To this end, number of bepotastine-based sulphonamides were synthesized, as both of these compound classes are known to efficiently inhibit parasitic development **[7, 8].** Subsequently, these compounds were tested to establish their inhibitory potential on asexual stages of human malaria parasite. To prioritize potential antimalarial scaffolds within the synthesized library, we conducted preliminary screening experiments, based on which the ten most active molecules were selected for detailed analyses. It was found in dose-response experiments that the majority of the selected compounds killed malaria parasites at lower micromolar concentrations, were in most (but not all) cases comparably effective on regular and drug-resistant parasitic variants, and demonstrated selectivity over mammalian cells (>10-fold, and up to 92-fold). The compounds reported here in general appeared to primarily manipulate later stages of asexual development, based on preliminary phenotypic screening results reported. These phenotypes were, however, not entirely comparable to typical egress-impaired phenotypes induced by serine/cysteine protease inhibitors **[23]**. Based on computational work we hypothesize the potential target for the compounds reported here to be N-myristoyltransferase. Further work is needed to accurately document the precise molecular mechanism, and to ensure that these molecules are indeed interfering with this molecular target.

Materials and Methods

All solvents used were of analytical grade and reagents used were purchased from Sigma-Aldrich.^{1}H and ^{13}C NMR spectra were recorded on a Bruker WH-200 (400 mHz or 300 mHz) spectrometer in $CDCl_3$ or DMSO-d_6 as solvent, using TMS as an internal standard and chemical shifts are expressed as ppm. High resolution mass spectra were determined on a Bruker Daltonics instrument. SOR was recorded using

Rudolph autopol IV polarimeter. The progress of the reaction was monitored by TLC pre-coated silica gel G plates.

Synthesis & Characterization

Typical procedure for the synthesis of (4-chlorophenyl)methyl)(pyridine-2-yl)methanol (**1**): In a 300mL round bottom-3-neck flask fitted with a mechanical stirrer, a reflux condenser, and an immersion thermometer were charged32 mL of tisopropyl magnesium bromide (1M in THF, 0.03 mol) under nitrogen atmosphere. The reaction mass was cooled to 10 ^{0}C and 2-bromopyridine 5g (0.03 mol) was added slowly maintaining the temperature below 20 ^{0}C. Then the reaction mass was brought to room temperature and stirred for 1hr. 4-chlorobenzaldehyde 4.4g (0.03 mol) was added drop wise and the reaction mixture was stirred for 4h until completion of the reaction was observed by TLC. The reaction was quenched with ammonium chloride and the organic layer was separated, dried over sodium sulphate and concentrated under reduced pressure. The obtained crude product was purified by flash column chromatography in hexane/ethyl acetate.Yield (6g, 86%); ^{1}H NMR (300 MHz, $CDCl_3$) 8.58 (d, 1H), 8.57-7.20(m, 7H), 5.75 (s,1H), 1.60(s, 1H); LCMS (MM:ES+APCI) 220.3$(M+H)^+$; $C_{12}H_{10}ClNO$: C, 65.61; H, 4.59; N, 6.38; Found: C, 65.78; H, 4.69; N, 6.32.

Typical procedure for the synthesis of 2-((1-benzylpiperidin-4-yloxy)(4-chlorophenyl)methyl)pyridine (**3**): 5g (22.7mmol) of **1** and 5.7g (27.3mmol) of 1-benzyl-4-chloropiperidine (**2)** was dissolved in 30mL of DMF. Dried Potassium carbonate 12.56g (91.04mmol) was charged to the above reaction mass in a round bottom flask equipped with a magnetic stir bar. The reaction mass was heated to reflux for 20h. Completion of the reaction was monitored by TLC. The reaction mass was quenched to water extracted with ethyl acetate 80mL three times. The combined organic phase was subjected to brine wash to remove DMF, dried over anhydrous sodium sulphate and concentrated under vacuum. The crude product (**3**) was flash chromatographed on a short silica gel column in hexane/ethyl acetate. Yield (8.1g, 90.8%).%) ^{1}H NMR (300 MHz, $CDCl_3$) 8.48-7.25 (m, 13H), 5.69(s, 1H), 3.97 (s,2H), 3.64(m, 1H), 3.14(m, 2H), 2.92(m, 2H), 1.96(m, 2H), 1.74(m, 2H); LCMS (MM:ES+APCI) 394.4$(M+H)^+$; Anal.Calcd for $C_{24}H_{25}ClN_2O$: C 73.36; H 6.41; N 7.13; Found: C, 73.26; H, 6.45; N, 7.16.

Typical procedure for the synthesis of 2-((4-chlorophenyl)(piperidin-4-yloxy) methyl)pyridine (4): The compound 3 (7g) obtained in the above process was diluted in 200 ml methanol and transferred under nitrogen atmosphere into an autoclave, charged with 5% Pd/C catalyst (0.8g). The mixture was pressurized with hydrogen gas at 40psi maintaining the reaction temperature below 30°C. Hydrogen uptake initially was ~40 psi per 30 min for about 4h. The uptake reduced to ~40 psi per 2 to 3 h. The reaction was continued for 16 h until the hydrogen uptake stopped. TLC analyses were performed to check for completion of the reaction. The reaction mixture was cooled, and the catalyst was filtered off. The methanolic solution was dried over sodium sulphate and concentrated to dryness.

2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine (4): Yield (5g, 94%) ^{1}H NMR (300 MHz, $CDCl_3$) 8.50-7.12 (m, 8H), 5.64(s, 1H), 3.53 (m,1H), 3.09(m, 2H), 2.59-2.46 (m,3H,-CH_2,-NH) 1.9(m, 2H), 1.6(m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 162.04, 148.82, 140.37, 136.94, 133.20, 128.48, 128.21, 122.46, 120.64, 80.59, 73.76, 44.17, 32.94, 32.91, 29.66; DEPT attached; LCMS (MM: ES+APCI) 303 $(M+H)^+$; Chiral HPLC (% ee) 49.91: 50.09.

Typical procedure for the separation of the R and S forms of 4: 5 g of the syrupy racemic 2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine (4) was taken in 15ml methanol and heated to 60 ^{0}C, a hot solution of S(+) mandelic acid (2eq) in 10ml methanol was added and entire reaction mass was refluxed for 2h. The reaction mixture was concentrated to half the volume and the S(-)-2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine (4b) was precipitated out by adding 2-propanol. The maximum chiral purity of the S isomer was achieved by recrystallization from methanol/isopropyl alcohol mixture. The mother liquor collected after the filtration of the S isomer was concentrated to dryness and the crude mass was treated in the same mannr as above with R(-)mandelic acid to separate the R(+)-2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine (**4a**).

Characterization of **(4a)**: Yield (2g, 40%). Anal.Calcd for $C_{17}H_{19}ClN_2O$: C 67.43; H 6.32; N 9.25; Found: C, 67.49; H, 6.28; N, 9.29; Chiral HPLC (%ee) 98.4; $[\alpha]_D$= +11(C 0.05, MeOH);

Characterization of (**4b**): Yield 2.1g, 42%); Anal.Calcd for $C_{17}H_{19}ClN_2O$: C 67.43; H 6.32; N 9.25; Found: C, 67.38; H, 6.37; N, 9.24; Chiral HPLC (%ee) 100; $[\alpha]_D$= -11(C 0.05, MeOH).

General procedure for the synthesis of 6(a-r): The salt forms of the compounds **(4a-b)** were made free bases as follows: The salt was taken in dichloromethane (DCM) in a beaker, cooled in an ice bath and basified using aqueous sodium hydroxide (NaOH) solution (6M) until the pH reached ~12. The free amine was extracted two times using DCM, the combined organic phase was washed with water, followed by brine solution. The entire organic phase was dried over anhydrous sodium sulphate and concentrated under vacuum .200mg (0.6mmol) of free amine was dissolved in 5 mL DCM. Triethylamine (0.5 mL) was added to the above reaction mass in a round bottom flask equipped with a magnetic stir bar. Sulphonyl chloride (**5a-i**) (0.65mmol) were added to the reaction mass and stirred at 30 ^{0}C for 14 h. Completion of the reaction was monitored by TLC analysis. The reaction mass was quenched with water and then extracted with 10 ml DCM three times. The combined organic phase was subjected to saturated sodium bicarbonate wash, brine wash, dried over anhydrous sodium sulphate, and concentrated under vacuum. The crude product (**6a-r)** was flash chromatographed on a short silica gel column in hexane/ethyl acetate.

Characterization of (R)-2-((4-chlorophenyl)((1-((3-nitrophenyl)sulfonyl)piperidinyl)oxy)methyl)pyridine (**6a**): Yield (270mg, 84.3%); Tan colored solid; mp: 78 ^{0}C; 1H-NMR (400 MHz, DMSO-d_6) 8.55- 8.53(d,J=8.0Hz,1H), 8.43- 8.42(d, J=4.0Hz,1H), 8.35 (s,1H), 8.17- 8.15 (d,J=8.0Hz,1H), 7.95- 7.91(t,J=8.0Hz,1H),7.75-7.71(m,1H), 7.42-7.40(d,J=8.0Hz,1H),7.31-7.21(m, 5H), 5.59(s, 1H), 3.45-3.41(m, 1H), 3.25-3.23(m, 2H), 2.83- 2.78(m, 2H) 1.89- 1.88 (m, 2H) 1.67- 1.60 (m, 2H); 13C-NMR (DMSO-d_6);161.53, 149.29, 148.53, 140.98, 137.58, 137.51, 133.83, 132.45, 131.97, 129.11, 128.64, 128.18, 123.10, 122.43, 120.72, 80.18, 71.11, 43.74, 30.53, 30.38; HRMS Calcd 510.086; Found: 510.086(M+Na^+); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.63; H, 4.57; N, 8.65; Chiral HPLC (%ee) 99.9.

Characterization of (R)-2-((4-chlorophenyl)((1-((4-nitrophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (6b): Yield (287mg, 89.6%); Tan coloured solid; mp: 110 ^{0}C; 1H-NMR (400 MHz, DMSO-d_6) 8.45- 8.44 (d,J=4Hz,1H), 7.81- 7.73(m,3H), 7.45- 7.35 (m,7H), 7.25- 7.22(d,J=8.0Hz,1H), 5.59(s, 1H), 3.47- 3.39(m, 1H), 3.04- 3.02(m, 2H), 2.78- 2.76(m, 2H), 1.86- 1.84 (m, 2H), 1.66- 1.62 (m, 2H); 13C-NMR (DMSO-d_6);161.67, 149.11, 148.63, 140.38, 137.58, 137.45, 133.13, 132.45, 132.09, 129.11, 128.64, 128.13, 123.39, 122.43, 120.12, 80.09, 71.01, 43.74,

30.50, 30.22; HRMS Calcd 510.086; Found: 510.086 ($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.64; H, 4.53; N, 8.64; $[\alpha]_D$= -37.6(C 0.01, MeOH);

Characterization of (R)-2-((4-chlorophenyl)((1-((2-nitrophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (6c): Yield (267mg, 83.4%); Tan coloured solid; mp: 85 ^{0}C; 1H-NMR (400 MHz, DMSO-d_6) 8.43- 8.41 (d,J=8Hz,1H), 7.73- 7.70(m,1H), 7.60- 7.58 (d,J=8.0Hz, 2H), 7.43- 7.32(m, 3H),7.30- 7.21 (m, 5H), 5.57(s, 1H), 3.35(m, 1H), 3.11- 3.07(m, 2H), 2.73- 2.69(m, 2H), 1.84- 1.83 (m, 2H), 1.64- 1.61 (m, 2H); ^{13}C NMR (DMSO-d_6);161.55, 149.29, 148.53, 140.98, 137.18, 137.11, 133.83, 132.48, 131.97, 129.11, 128.64, 128.18, 123.10, 122.43, 120.72, 79.99, 71.74, 43.84, 30.54, 30.28; HRMS Calcd 510.086; Found: 510.086($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.64; H, 4.55; N, 8.69; Chiral HPLC (%ee) 96.0

Characterization of (R)-2-((chlorophenyl)((1-((3- (trifluoromethyl)phenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (6d): Yield (290mg, 86.5%); Off-white solid; mp: 108 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.43- 8.42(d, J=4Hz,1H), 8.14- 8.12(d, J=8.0Hz, 1H), 8.07- 8.05(d, J=8.0Hz, 1H), 7.94- 7.90(m, 2H),7.72- 7.70 (m, 1H),7.40- 7.38(d, J=8Hz,1H),7.30- 7.21(m,5H), 5.59(s, 1H), 3.47(m, 1H), 3.19- 3.16(m, 2H), 2.84- 2.82(m, 2H), 1.88- 1.86 (m, 2H), 1.64- 1.62 (m, 2H); 13C-NMR (DMSO-d_6);161.56, 149.29, 141.02, 137.46, 137.23, 132.43, 132.00, 131.56, 130.64, 130.38, 130.35, 130.31, 129.07, 128.61, 125.18, 124.15, 124.11, 123.08, 122.47, 120.63, 80.12, 70.96, 43.60, 30.46, 30.31; HRMS Calcd 511.107; Found: 511.1067(M+H) Anal.Calcd for $C_{24}H_{22}ClFN_3O_3S$: C 56.42; H 4.34; N 5.48; Found: C, 56.39; H, 4.37; N, 5.47; $[\alpha]_D$= -39.7(C 0.01, MeOH);

Characterization of (R)-2-((4-chlorophenyl)((1-((2,5- dichlorophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (6e): Yield (296 mg, 88.3%); Brown low melting solid; 1H-NMR (400 MHz, DMSO-d_6) 8.44- 8.42(d, J=8.0Hz,1H), 7.73- 7.70(m,1H), 7.60- 7.58 (d, J=8.0Hz, 1H), 7.43- 7.32(m, 3H),7.30- 7.21 (m, 5H), 5.57(s, 1H), 3.36(m, 1H), 3.11- 3.07(m, 2H), 2.74- 2.69(m, 2H), 1.84- 1.83 (m, 2H), 1.63- 1.62 (m, 2H); 13C-NMR (DMSO-d_6);160.59, 151.29, 150.53, 139.96, 137.48, 137.11, 133.83, 132.48, 131.43, 129.18, 128.83, 128.19, 123.04, 122.38, 80.18, 73.64, 43.81, 30.78, 30.57; HRMS Calcd 511.0417; Found: 511.0411(M+H); Anal.Calcd for $C_{23}H_{21}Cl_3N_2O_3S$: C 53.97; H 4.14; N 5.47; Found: C,53.94 ; H, 4.19; N, 5.50; Chiral HPLC (%ee) 98.7.

Characterization of (R)-2-((4-chlorophenyl)((1-((4-methoxyphenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6f**): Yield (260 mg, 83.8%); Off-white solid; mp: 114 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.44-8.43 (d, J=4Hz, 1H), 7.74-7.70 (m,1H), 7.65- 7.63 (d, J=8.0Hz, 2H), 7.39-7.37 (d, J=8.0Hz, 1H), 7.32-7.22 (m, 5H), 7.14-7.12 (d, J= 8.0 Hz, 1H), 5.56 (s, 1H), 3.84 (s, 3H), 3.44 (m, 1H), 3.07 (m, 2H), 2.73- 2.69(m, 2H), 1.84- 1.83 (m, 2H), 1.64- 1.62 (m, 2H); 13C-NMR (DMSO-d_6); 162.96, 161.06, 148.99, 141.00, 137.40, 132.41, 130.05, 129.09, 128.61, 126.90, 123.17, 120.72, 115.00, 79.88, 71.16, 55.99, 43.52, 30.42, 30.21; HRMS Calcd 495.112; Found: 495.112 ($M+Na^+$); Anal.Calcd for $C_{24}H_{25}ClN_2O_4S$: C 60.94; H 5.33; N 5.92; Found: C, 60.98; H, 5.31; N, 5.96; $[\alpha]_D$= -40.5(C 0.01, MeOH).

Characterization of (R)-2-((4-chlorophenyl)((1-(mesitylsulfonyl)piperidin-4- yl)oxy)methyl)pyridine (6g): Yield (280mg, 88%); White solid; mp: 106 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.46- 8.45 (d, J=4Hz, 1H), 7.81- 7.77(m, 1H), 7.53- 7.51 (d, J= 8.0Hz, 1H), 7.39- 7.34 (m, 4H),7.27- 7.24 (m, 1H),7.05 (s, 2H), 5.64(s, 1H), 3.56 (m, 1H), 3.32 (s, 6H),3.24(m, 2H), 2.93- 2.88 (m, 2H), 2.26 (s, 3H), 1.83-1.80 (m, 2H), 1.60- 1.57 (m, 2H); ^{13}C-NMR (DMSO-d_6) 161.71, 149.32, 142.85, 141.17, 140.03, 137.60, 132.45, 132.31, 132.14, 129.16, 128.70, 120.80, 80.34, 71.79, 41.63, 30.68, 30.59, 22.76, 20.90; HRMS Calcd 507.148; Found: 507.148 ($M+Na^+$); Anal.Calcd for $C_{26}H_{29}ClN_2O_3S$: C 64.38; H 6.03; N 5.78; Found: C, 64.41; H, 6.01; N, 5.79;Chiral HPLC (%ee) 96.2.

Characterization of (R)-2-((4-chlorophenyl)((1-((4- fluorophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (6h): Yield (268mg, 88.7%);Tan coloured solid; mp: 106 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.44- 8.43 (d, J= 4Hz, 1H), 7.81- 7.73 (m,3H), 7.49- 7.22 (m, 8H), 5.59 (s, 1H), 3.46 (m, 1H), 3.14- 3.10 (m, 2H), 2.78- 2.76 (m, 2H), 1.85- 1.84 (m, 2H), 1.66-1.63 (m, 2H); ^{13}C-NMR (DMSO-d_6): 166.29, 163.80, 161.56, 149.30, 141.02, 137.48, 132.43, 132.16, 132.13, 131.03, 130.94, 129.09, 128.63, 123.10, 120.68, 117.10, 116.88, 80.11, 70.99, 43.57, 30.41, 30.29; HRMS Calcd 483.092; Found: 483.092 ($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClFN_2O_3S$: C 59.93; H 4.81; N 6.08; Found: C, 59.89; H, 4.77; N, 6.12; Chiral HPLC (%ee) 97.2.

Characterization of (R)-2-((4-chlorophenyl)((1-tosylpiperidin-4- yl)oxy)methyl)pyridine (**6i**): Yield (254mg, 84.3%); Tan coloured solid; mp: 98 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.39- 8.37 (d, J=8Hz, 1H), 7.72-7.70 (m, 1H), 7.59-7.57 (d, J=8.0 Hz, 2H), 7.43-7.38 (m, 3H), 7.32-7.21 (m, 5H), 5.56 (s, 1H), 3.34 (m, 1H), 3.11-3.07 (m, 2H), 2.73-2.69 (m, 2H), 2.40 (s, 3H), 1.85-1.83 (m, 2H), 1.64-1.62 (m, 2H);

^{13}C-NMR (DMSO-d_6): 160.96, 149.30, 143.43, 141.94, 137.49, 132.42, 132.21, 130.13, 128.96, 128.63, 127.96, 122.94, 120.71, 79.99, 71.11, 43.29, 30.51, 30.32, 21.57; HRMS Calcd 479.117; Found: 479.117 ($M+Na^+$); Anal.Calcd for $C_{24}H_{25}ClN_2O_3S$: C 63.08; H 5.51; N 6.13; Found: C, 63.06; H, 5.48; N, 6.14; Chiral HPLC (%ee) 99.

Characterization of (S)-2-((4-chlorophenyl)((1-((3-nitrophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6j**): Yield (265mg, 82%); Tan colored solid; mp: 78 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.55- 8.53 (d, J=8.0 Hz, 1H), 8.42- 8.41(d, J=4.0 Hz, 1H), 8.35 (s, 1H), 8.19-8.17 (d, J=8.0Hz, 1H), 7.94-7.90 (t, J=8.0Hz, 1H), 7.75-7.71 (m, 1H), 7.42-7.40 (d, J=8.0Hz, 1H), 7.32-7.21 (m, 5H), 5.63(s, 1H), 3.45-3.41(m, 1H), 3.25-3.23(m, 2H), 2.83- 2.77(m, 2H) 1.89- 1.87 (m, 2H) 1.67- 1.61 (m, 2H); ^{13}C-NMR (DMSO-d_6); 162.53, 149.59, 148.03, 140.88, 137.88, 137.11, 133.93, 132.54, 132.07, 130.07, 128.64, 128.10, 123.13, 122.43, 120.72, 80.96, 71.99, 43.70, 30.55, 30.36; HRMS Calcd 510.086; Found: 510.086($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.58; H, 4.57; N, 8.67; Chiral HPLC (%ee) 97.2.

Characterization of (S)-2-((4-chlorophenyl)((1-((4-nitrophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6k**): Yield (283mg, 88%); Tan coloured solid; mp: 110 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.45-8.44 (d,J=4Hz, 1H), 7.81-7.73 (m, 3H), 7.45-7.35 (m,7H), 7.25- 7.22(d,J=8.0Hz,1H), 5.61(s, 1H), 3.47- 3.39(m, 1H), 3.04- 3.03(m, 2H), 2.78- 2.76(m, 2H), 1.86- 1.84 (m, 2H), 1.66-1.62 (m, 2H); 13C-NMR (DMSO-d_6);161.99, 148.99, 148.63, 140.93, 137.57, 137.47, 133.11, 132.45, 132.09, 129.11, 128.64, 128.13, 123.33, 122.44, 120.11, 80.99, 71.70, 43.75, 30.50, 30.22; HRMS Calcd 510.086; Found: 510.086($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.68; H, 4.53; N, 8.69; $[\alpha]_D$= +37.6(C 0.01, MeOH).

Characterization of (S)-2-((4-chlorophenyl)((1-((2-nitrophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6l**): Yield (273mg, 85%); Tan coloured solid; mp: 85 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.43-8.41 (d,J=8Hz,1H), 7.73-7.70 (m,1H), 7.60-7.58 (d, J=8.0Hz, 2H), 7.43-7.32 (m, 3H),7.30-7.21 (m, 5H), 5.61 (s, 1H), 3.35 (m, 1H), 3.11- 3.07 (m, 2H), 2.73-2.69 (m, 2H), 1.84-1.83 (m, 2H), 1.64-1.61 (m, 2H); ^{13}C-NMR (DMSO-d_6): 162.05, 149.29, 148.53, 140.98, 137.12, 137.11, 133.83, 132.42, 130.07, 129.11, 128.64, 128.18, 123.10, 122.43, 120.79, 80.89,

71.98, 43.84, 30.54, 30.29; HRMS Calcd 510.086; Found: 510.086($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClN_3O_5S$: C 56.61; H 4.54; N 8.67; Found: C, 56.56; H, 4.59; N, 8.71; Chiral HPLC (%ee) 97.6.

Characterization of (S)-2-((4-chlorophenyl)((1-((3-(trifluoromethyl)phenyl)sulfonyl piperidin-4-yl)oxy)methyl)pyridine (**6m**): Yield (288 mg, 86%); Off-white solid; mp: 108 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.44- 8.43(d, J=4Hz,1H), 8.14- 8.12(d, J=8.0Hz, 1H), 8.07- 8.05(d, J=8.0Hz, 1H), 7.94- 7.90(m, 2H),7.72- 7.70 (m, 1H),7.40- 7.38(d, J=8.0Hz,1H),7.30- 7.21(m,5H), 5.60(s, 1H), 3.47(m, 1H), 3.19-3.16(m, 2H), 2.84- 2.82(m, 2H), 1.88- 1.86 (m, 2H), 1.66- 1.63 (m, 2H); ^{13}C-NMR (DMSO-d_6);162.00, 149.26, 141.04, 137.46, 137.23, 132.43, 132.08, 131.55, 130.64, 130.38, 130.35, 130.31, 129.03, 128.62, 125.18, 124.15, 124.11, 123.00, 122.44, 120.63, 80.99, 71.00, 43.60, 30.48, 30.32; ; HRMS Calcd 511.107; Found: 511.1067(M+H) ; Anal.Calcd for $C_{24}H_{22}ClFN_3O_3S$: C 56.42; H 4.34; N 5.48; Found: C, 56.47; H, 4.33; N, 5.52; $[\alpha]_D$= +39.7(C 0.01, MeOH).

Characterization of (S)-2-((4-chlorophenyl)((1-((2,5- dichlorophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6n**): Yield (291mg, 87%); Brown low melting solid; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.43- 8.42(d, J=8.0Hz,1H), 7.73- 7.70(m,1H), 7.60- 7.58 (d, J=8.0Hz, 1H), 7.43- 7.32(m, 3H),7.30- 7.21 (m, 5H), 5.59(s, 1H), 3.35(m, 1H), 3.11- 3.07(m, 2H), 2.73- 2.69(m, 2H), 1.84- 1.83 (m, 2H), 1.64- 1.61 (m, 2H); 13C-NMR (DMSO-d_6);160.99, 151.99, 150.93, 139.98, 137.48, 137.11, 133.83, 132.48, 131.43, 129.18, 128.83, 128.11, 123.01, 122.33, 80.99, 73.98, 43.81, 30.78, 30.91; HRMS Calcd 511.0417; Found: 511.0411(M+H); Anal.Calcd for $C_{23}H_{21}Cl_3N_2O_3S$: C 53.97; H 4.14; N 5.47; Found: C,53.93 ; H, 4.16; N, 5.56; Chiral HPLC (%ee) 98.

Characterization of (S)-2-((4-chlorophenyl)((1-((4- methoxyphenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6o**): Yield (266mg, 86%); Off-white solid; mp: 114 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6): 8.43- 8.42 (d, J=4Hz,1H), 7.74-7.70 (m,1H), 7.65- 7.63 (d, J=8.0Hz, 2H), 7.39-7.37 (d, J=8.0Hz, 1H), 7.32- 7.22 (m, 5H), 7.14- 7.12 (d, J=8.0Hz, 1H), 5.57 (s, 1H), 3.84 (s,3H), 3.44(m, 1H), 3.07(m, 2H), 2.73- 2.69(m, 2H), 1.84- 1.83 (m, 2H), 1.64- 1.62 (m, 2H); 13C-NMR (DMSO-d_6);163.13, 161.56, 149.29, 141.04, 137.49, 132.41, 130.15, 129.09, 128.62, 127.22, 123.11, 120.72, 114.90, 80.08, 71.05, 56.16, 43.56, 30.36, 30.30; HRMS Calcd 495.112; Found: 495.112($M+Na^+$); Anal.Calcd for $C_{24}H_{25}ClN_2O_4S$: C 60.94; H 5.33; N 5.92; Found: C, 60.91; H, 5.37; N, 5.95; $[\alpha]_D$= +40.5(C 0.01, MeOH).

Characterization of (S)-2-((4-chlorophenyl)((1-(mesitylsulfonyl)piperidin-4- yl)oxy)methyl)pyridine (**6p**) : Yield (281mg, 88.5%); White solid; mp: 106 ^{0}C; 1H-NMR (400 MHz, DMSO-d_6) 8.46- 8.45(d,J=4Hz,1H), 7.81- 7.77(m,1H), 7.53- 7.51 (d,J=8.0Hz, 1H), 7.39- 7.34(m, 4H),7.27- 7.24 (m, 1H),7.05 (s, 2H), 5.67(s, 1H), 3.56(m, 1H), 3.32(s, 6H),3.24(m, 2H), 2.93- 2.88(m, 2H), 2.26 (s,3H), 1.83- 1.80 (m, 2H), 1.60- 1.57 (m, 2H); 13C-NMR (DMSO-d_6) 160.03, 149.85, 142.14, 141.17, 140.03, 137.17, 132.45, 132.31, 132.16, 129.16, 128.60, 120.80, 80.99, 71.99, 41.63, 30.62, 30.53, 22.75, 20.96; HRMS Calcd 507.148; Found: 507.148 ($M+Na^+$); Anal.Calcd for $C_{26}H_{29}ClN_2O_3S$: C 64.38; H 6.03; N 5.78; Found: C, 64.42; H, 5.99; N, 5.81; Chiral HPLC (%ee) 97.1.

Characterization of (S)-2-((4-chlorophenyl)((1-((4- fluorophenyl)sulfonyl)piperidin-4-yl)oxy)methyl)pyridine (**6q**): Yield (259mg, 86%); Tan coloured solid; mp: 98 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.44- 8.43(d,J=4Hz,1H), 7.81- 7.73(m,3H), 7.49- 7.22 (m, 8H), 5.61(s, 1H), 3.46(m, 1H), 3.14- 3.10(m, 2H), 2.78- 2.76(m, 2H),1.85- 1.84 (m, 2H)1.66 1.63(m, 2H); ^{13}C-NMR (DMSO-d_6)166.39, 163.83, 161.54, 149.34, 141.01, 137.48, 132.43, 132.16, 132.13, 131.03, 130.94, 129.04, 128.61, 123.10, 120.48, 117.40, 116.18, 80.94, 71.03, 43.57, 30.46, 30.31; HRMS Calcd 483.092; Found: 483.092 ($M+Na^+$); Anal.Calcd for $C_{23}H_{22}ClFN_2O_3S$: C 59.93; H 4.81; N 6.08; Found: C, 59.85; H, 4.87; N, 6.11; Chiral HPLC (%ee) 98.9.

Characterization of (S)-2-((4-chlorophenyl)((1-tosylpiperidin-4- yl)oxy)methyl)pyridine (6r): Yield (263mg, 87.3%); Tan coloured solid; mp: 98 ^{0}C; ^{1}H-NMR (400 MHz, DMSO-d_6) 8.43- 8.41(d,J=8Hz,1H), 7.73- 7.70(m,1H), 7.60- 7.58 (d,J=8.0Hz, 2H), 7.43- 7.38(m, 3H),7.32- 7.21 (m, 5H), 5.57(s, 1H), 3.35(m, 1H), 3.11- 3.07(m, 2H), 2.73- 2.69(m, 2H), 2.40(s,3H), 1.84- 1.83(m, 2H), 1.64- 1.61 (m, 2H); ^{13}C-NMR (DMSO-d_6)161.56, 149.30, 143.93, 141.04, 137.49, 132.82, 132.41, 130.23, 129.10, 128.63, 127.96, 123.10, 120.70, 80.11, 71.09, 43.29, 30.41, 30.32, 21.48; HRMS Calcd 479.117; Found: 479.117($M+Na^+$); Anal.Calcd for $C_{24}H_{25}ClN_2O_3S$: C 63.08; H 5.51; N 6.13; Found: C, 63.11; H, 5.55; N, 6.09; Chiral HPLC (%ee) 97.5.

Blood Collection and malaria experiments

Fresh blood was collected from healthy donors with their informed consent in EDTA tubes (VACUETTE® EDTA Tubes, Greiner Bio-One). The blood collection procedure was verified and approved by the

Institutional Review Board (IRB) of the National University of Singapore (NUS). Blood was washed three times by centrifugation (600g for 10 minutes) using RPMI 1640 (R8758 Sigma-Aldrich) to remove the buffy coat. Washed RBCs were stored at 50% hematocrit in RPMI 1640. *P. falciparum* (3D7 clone of the NF54 strain) was cultured in 2.5% haematocrit, RPMI-HEPES medium at pH 7.4 supplemented with hypoxanthine 50 μg/mL, $NaHCO_3$ 25 mM, gentamicin 2.5 μg/mL, and Albumax II (Gibco, Singapore) 0.5% wt/vol in an atmosphere containing 5% CO_2, as routinely followed **[24].**

Determination of parasitemia by flow cytometry

For the determination of parasitemia, 100 μL aliquots of the culture from the respective wells were collected and fixed overnight with 0.1% glutaraldehyde (Sigma-Aldrich) at 4°C. Fixed cells were washed twice with PBS (400xg, 5 min), permeablised with 0.25% Triton X-100 (Sigma-Aldrich) for 10min at RT and then washed again with PBS. Samples were then incubated with 25μg/ml Hoechst 3342 for 30 minutes and washed twice with PBS and then analyzed by flow cytometry using an LSRII (BD) flow cytometer as reported elsewhere **[25-27].** The samples were excited using a 355nm UV laser for detection and 75,000 RBCs were analyzed for each sample. Data acquisition and analysis was carried out using FlowJo (Tree Star, Inc., Ashland, OR).

Phenotypic Analysis through microscopy

Thin blood smears were prepared on glass slides and fixed with 100% methanol. Smears were stained with filtered 1/10 dilution of Giemsa (Sigma) solution in phosphate buffered saline (PBS), incubated for 10 min at room temperature, washed with distilled water, dried, and observed under an oil immersion lens (100x).

Cytotoxicity Assay

To determine cell viability the colorimetric MTT metabolic activity assay was used. MDCK cells were seeded in a 96-well plate at a density of 10^4 cells/well at 37°C, 5% CO_2 in medium comprising of DMEM (Sigma-Aldrich), 10% FBS (Sigma) and Pen-Strep-Glut and left to adhere overnight. This medium was removed after 24 h and medium containing varying concentrations of compound was added and incubated overnight. Cells treated with medium only served as a negative control group. The supernatant of each well

was then aspirated and the wells were washed with PBS. This was replaced with medium containing 0.5mg/ml MTT dye (Sigma) and the plates were left to incubate at 37°C for 4 h. The supernatant was carefully aspirated and the resultant formazan crystals were dissolved in dimethyl sulfoxide (100 µL) and the absorbance intensity measured using a microplate reader at 495 nm with a reference wavelength of 620 nm. All experiments were performed in duplicates, and the relative cell viability was expressed as a percentage relative to the untreated control cells.

***In vivo* efficacy testing in rodent malaria parasites**

Male BALB/c 6-8weeks old mice were obtained from InVivos Singapore, and subsequently bred under specific pathogen free (SPF) condition at Nanyang Technological University Animal Holding Unit. Genetically modified *Plasmodium yoelii* (*P.yoelii*) lethal strain YM, which is expressing mcherry (YM-mcherry) throughout the life cycle, ws used for infection. When parasitemia reached around 1% mice were subjected to drug treatment by intraperitoneal (i.p.) injection for 4 consecutive days. Positive control groups were treated with Chloroquine (CQ, dissolved in distilled water) of 10mg/kg body weight or 50mg/kg body weight, and negative control mice were treated with DMSO, while experimental groups received compound 6n (dissolved in DMSO) of 10mg/kg body weight or 50mg/kg body weight. Parasitemia was monitored daily 4 hours after drug treatment by flow cytometry. All animal experiments were carried out in accordance with institutional guidelines of Nanyang Technological University.

Molecular Docking analysis

Transferability of *in silico* docking results from *P. vivax* to *P. falciparum* N-myristoyltransferase was ensured by performing a sequence alignment of sequences using BLASTP as provided by UniProt [**28**]. We found 80% overall sequence identity and 93% sequence similarity. As all amino acids in a radius of 4.5Å around the ligand binding site are identical and second sphere amino acid exchanges are highly conservative, we expect validity of our *in silico* analysis for *P. falciparum.* We prepared chain A of the crystal structure for docking using protonate3d in MOE [**29-31**]. Since several rigid docking attempts did not yield consistent binding modes for our compound series, we performed induced fit docking runs for all compounds using MOE's default parameters (Molecular Operating Environment 2014.0901, Chemical

Computing Group, Montreal, Canada). Thereby, we allowed for conformational flexibility of the protein side-chains in addition to the ligand during energy minimization [**32**]. Furthermore, we introduced pharmacophore filters to reduce search space by enforcing a hydrogen bond acceptor function at the position of the sulfonamide and a hydrophobic group at the pyrazole position (the tolerance radius for both features was set to 2.5Å).

Figure & Table Legends

Scheme 1: Schematic representation for the synthesis of the novel sulphonamides reported here.

Figure 1: Dose response curve for the most effective 10 compounds identified from the library screen. Shortlisted compounds were screened against common laboratory strains of malaria parasites, 3D7 **(Fig. 1A)** and chloroquine resistant parasite variant, K1 **(Fig. 1B).** Screening was performed at concentrations ranging from 0μM to 100μM. Both parasite strains showed comparable sensitivity to most inhibitors (all except 6k).

Figure 2: Effect of the selected compounds on distinct stages of parasite development. To document stage-specificity of the compounds tested, experiments were carried out using parasites at early stage during ring-trophozoite transition **(Fig. 2A),** trophozoite-schizont transition **(Fig. 2B)** and schizont-ring transition **(Fig. 2C),** at four different compound concentrations **(**4*IC_{50}, 2*IC_{50}, IC_{50} and 0.25*IC_{50}). It can be seen that the majority of compounds has an effect during the later stages of parasite development.

Figure 3: **Predicted protein-ligand interactions of the sulphonamides with N-myristoyltransferase.** Induced fit docking of the bepotastine-derived sulfonamide series to *Plasmodium vivax* N-myristoyltransferase yielded a binding mode in agreement with an X-ray structure of another sulfonamide (PDB: 2YND). Crystal structure of the enzyme (green cartoon) with the template ligand (atomic coloring with carbon in white). Side-chains of Phe-103 and Phe-105 are shown to indicate π-π stacking in this region **(Fig. 3A).** Predicted binding poses for compounds **6l** and **6i** that showed similar occupation of the binding pocket **(Fig. 3B & 3C).** The substituted benzene rings of the title compounds forming π-π interactions of variable strength, which occupies the similar positions of the pyrazole ring of the co-crystallized ligand.

Table 1: Overall susceptibility to each molecules of *Plasmodium falciparum* strains 3D7 and K1. Sensitivity of parasites (3D7 and K1) within a 0 μM to 100 μM compound concentration range was

evaluated. Further, cytotoxicity of the same compounds was tested on the MDCK cell line, and the resulting selectivity is evaluated as 3D7 IC_{50}/MDCK IC_{50}.

Table 2: Stage-specific effects of the compounds presented here on *Plasmodium falciparum*. '-' represents no effect; '+' indicates effect at 4*IC_{50}; '++' indicates effect at 4*IC_{50} and 2*IC_{50}; '+++' indicates effect at 4*IC_{50}, 2*IC_{50} and IC_{50}. It can be seen that most compound are active on the later stages of parasite development.

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Table 1

Compound	**IC_{50} 3D7 (µM)**	**95% Confidence Interval**	**IC_{50} K1 (µM)**	**95% Confidence Interval**	**Cytotoxicity IC_{50} MDCK (µM)**	**Selectivity Index**
6b	8.85	8.086 to 9.687	7.345	6.444 to 8.371	>100	>11.3
6c	15.61	14.42 to 16.90	9.231	8.589 to 9.921	>100	>6.4
6e	**2.908**	**2.677 to 3.160**	**4.187**	**3.386 to 5.178**	**>200**	**>68.8**
6f	6.812	5.938 to 7.816	6.952	6.060 to 7.976	>100	>14.7
6g	11.41	9.937 to 13.10	9.777	8.703 to 10.98	>200	>17.5
6k	**3.271**	**2.623 to 4.079**	**7.219**	**6.218 to 8.380**	**>200**	**>61.1**
6l	13.33	11.67 to 15.22	13.22	12.27 to 14.24	>200	>15.0
6n	**2.168**	**1.876 to 2.506**	**2.379**	**2.171 to 2.606**	**>200**	**>92.3**
6p	7.414	6.856 to 8.018	6.342	5.936 to 6.776	>200	>27.0
6r	8.84	6.622 to 11.80	9.111	7.820 to 10.62	>200	>22.60

Table 2

Compounds	Ring to Trophozoite	Trophozoites to Schizont	Schizont to Ring
6b	-	-	++
6c	-	-	+++
6e	-	-	++
6f	-	-	+
6g	-	+	+
6k	-	-	+
6l	+	+	++
6n	-	-	+++
6p	+	-	+++
6r	+	-	++
Control	-	-	-