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RESEARCH ARTICLE

Pyrazole Schiff Base Hybrids as Anti-Malarial Agents: Synthesis, *In Vitro* Screening and Computational Study

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Abstract: ***Background*:** Malaria is one of the most vital infectious diseases caused by protozoan parasites of the *Plasmodium* genus. As *P. falciparum*, the cause of most of the severe cases of malaria, is increasingly resistant to available drugs such as amodioquine, chloroquine, artemisinin, and antifolates, there is an urgent need to identify new targets for chemotherapy

***Objective*:** This study screened novel pyrazole derivatives carrying iminium & benzothiazole group for antimalarial potential against *P. falciparum* chloroquine sensitive (3D7) strain.

***Materials & Methods*:** Several pyrazole schiff base hybrids with a wide range of substitution have been synthesized via condensation of substituted aniline with substituted 4-formylpyrazole and evaluated for their *in vitro* antimalarial activity against asexual blood stages of human malaria parasite, *Plasmodium falciparum*. The interaction of these conjugate hybrids was also investigated by molecular docking studies in the binding site of *P. falciparum* cystein protease falcipain-2. The pharmacokinetic properties were also studied using ADME prediction.

***Results*:** Among all compounds, **6bf** and **6bd** were found to be potential molecules with EC_{50} 1.95µg/ml and 1.98µg/ml respectively. Docking study results reveal that the pyrazole schiff base derivatives occupy the *PfFP* binding sites and they show good interactions with significant values of binding energies.

***Conclusion*:** We provide evidence which implicates pyrazole Schiff base hybrids as potential prototypes for the development of antimalarial agents.

Keyword: Pyrazole, *Plasmodium falciparum*, antimalarial, Falcipain-2, docking, ADME.

1. INTRODUCTION

Malaria is one of the prehistoric parasitic infectious diseases which are caused by the protozoan parasite of the *Plasmodium* genus and is transmitted to human by the infected female *Anopheles* mosquito. According to World Health Organization malaria reports, half of the world population is at threat to malarial infection [1]. *P. falciparum* is becoming resistant to the existing drugs in the market; therefore, there is an imperative need for the introduction of new therapeutic agents to act against the disease. Enzyme cysteine protease falcipain-2 (FP-2) that is involved in *P falciparum* development emerges to be a good target. Mounting facts suggest that cysteine proteases are concerned with host cell rupture and release of merozoites. In the presence of such inhibitors, merozoites mature usually but are unable to run off from host erythrocytes [2, 3]. This makes FP-2 an attractive target for antimalarial drug designs. Computational methodologies have emerged as a vital component of many drug discovery programs, from hit identification to lead optimization and beyond [4-6], and approaches such as ligand- [4] or structure based virtual screening [7] techniques are widely used in many discovery efforts. Along this line, there is also great inclination of using distinctive heterocyclic skeletons for the development of dual therapeutic drugs. The rationale of this approach relies on the hybridization of two drugs, both dynamic compounds and pharmacophoric units familiar and derived from known bioactive molecules [8].

Schiff bases are the prospective molecules, which can be affective against the problem of drug resistance [9]. In addition to the synthetic derivatives, ancistrocladidine having iminium group moiety, is a natural product formed by plants belonging to the family *Dioncophyllaceae* and *Ancistrocladiceae* and is known as an anti-malarial agent with activity against *P. falciparum* strains 3D7 and K1 [10]. Schiff bases obtained by the condensation of 2,6-diarylsubstituted piperidin-4-ones with 7-chloro-4-hydrazinoquinoline have also been tested for antimalarial activities and reported to show strong antimalarial activity against the *P. falciparum* strains [11]. Therefore, in the quest for new effective drug molecules against malaria, Schiff bases can be a potential possibility of research. Furthermore,

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numerous reports have shown that pyrazoles have significant antimalarial activities. For illustration, chloroquine substituted 4-formylpyrazole analogues have been accounted for to show a huge *in vitro* anti-malarial activity using an experimental model against *P. falciparum* [12]. In another research, antiparasitic activities of the pyrazole azomethines were also reported [13]. There are likewise different reports that demonstrated the antimalarial activities of pyrazole derivatives [14]. Further, it has been reported that compounds containing benzothiazole moiety displayed intense anti-plasmodial activity against *P. falciparum* [15, 16]. Hence, based on the literature review [17], the present work aims to synthesize novel pyrazole derivatives carrying iminium & benzothiazole groups and evaluated as potential antimalarial agents against *P. falciparum* chloroquine sensitive (3D7) strain. Furthermore, we investigated the interaction of these hybrids in the binding site of *P. falciparum* falcipain-2 (*PfFP*) protein structures using molecular docking studies and ADME prediction was carried out computationally to get an insight of the structural parameters leading to activity.

2. MATERIALS & METHODS

2.1. Chemistry

All the reagents were purchased from commercial sources and were used without purification. Chromatography was carried on silica gel (60-120 and 100-200 mesh). All the reactions were monitored by thin layer chromatography (TLC). Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected.^{1}H NMR and ^{13}C NMR spectra were recorded either in pure DMSO-d_6 or in $CDCl_3$/DMSO-d6 mixture on Bruker NMR spectrometers at 300/400 MHz and 75.5/100 MHz respectively using tetra methylsilane (TMS) as internal standard. Chemical shifts are expressed in δ, ppm. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Abbreviations 's' for singlet and 'm' for multiplet were used for NMR assignments. The mass spectral data were obtained on Q-TOF MICROMASS (LC-MS) spectrometer at Punjab University of Chandigarh India. The antimalarial activity of synthesized compounds was assessed against chloroquine-sensitive *P. falciparum* (3D7) isolate obtained from the National Institute of Malaria Research (NIMR), New Delhi, India. *P. falciparum* were cultivated in human A Rh+ red blood cells using RPMI 1640 medium (Sigma, India) supplemented with AB Rh+ serum (10%), 5% sodium bicarbonate (Sigma, India) and 40 μg/mL of gentamycin sulfate (Sigma, India).

*General procedure for the synthesis of hydrazones (**3a-c**)*

An equimolar amount of compound 2-hydrazino-benzothiazole **2** (1.5mmol), substituted with methyl ketone **1** (1.5mmol) and glacial acetic acid (2-3drop) was taken in absolute ethanol (20ml) and refluxed for 4-5 hrs. On cooling, a solid was separated which was filtered, dried and crystallized from ethanol to afford hydrazones **3**.

*2-[(Z)-2-(1-phenylethylidene)hydrazin-1-yl]-1,3-benzothiazole (**3a**)*

205-207°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 9.78 (s, 1H, NH), 7.32-7.71 (m, 9H, ArH), 2.37 (s, 3H, CH_3).

*2-[(Z)-2-[1-(4-methylphenyl)ethylidene]hydrazin-1-yl]-1,3-benzothiazole (**3b**)*

210-212°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 9.57 (s, 1H, NH), 7.28-7.82 (m, 8H, ArH), 2.53 (s, 3H, CH_3), 2.32 (s, 3H, CH_3).

*2-[(Z)-2-[1-(4-fluorophenyl)ethylidene]hydrazin-1-yl]-1,3-benzothiazole (**3c**)*

230-232°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 9.73 (s, 1H, NH), 7.32-7.73 (m, 8H, ArH), 2.32 (s, 3H, CH_3).

*General procedure for the synthesis of 4-formylpyrazoles (**4a-c**)*

To a cold, stirred solution of dimethylformamide (6 mL) and phosphorous oxychloride (2.5 mmol) was added hydrazone **3** (6, 1 mmol) following the literature procedure [18]. The reaction mixture was stirred at 55-60°C for 5 h, cooled to room temperature, poured into ice chilled water and neutralized with saturated aqueous sodium bicarbonate solution where upon a solid was separated that was filtered, washed with excess of chilly water, dried and crystallized from acetic acid to afford aldehydes.

*1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4a**)*

175-177°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 10.07(s, 1H, CHO), 8.86 (s, 1H, pyrazole C_5-H), 7.32-7.82 (m, 9H, ArH).

*1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde (**4b**)*

166-168°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 10.15 (s, 1H, CHO), 8.83 (s, 1H, pyrazole C_5-H, 7.33-7.78 (m, 8H, ArH), 2.28 (s, 3H, CH_3).

*1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde (**4c**)*

196-198°C, yield 73%. ^{1}H-NMR (DMSO-d_6) δ 10.09 (s, 1H, CHO), 8.82 (s, 1H, pyrazole C_5-H), 7.28-7.85 (m, 8H, ArH).

*General procedure for the conversion of 4-formylyrazole into 4-arylidene pyrazole derivatives (**6**)*

An equimolar mixture of substituted aromatic amines **5** and 4-formylpyrazoles **4** was refluxed in solvent benzene for 10-12 hrs in the presence of a catalytic amount of sulphuric acid. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction and washed with cooled ethanol, dried and recrystallized from ethanol [19].

*(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)benzenamine (**6aa**)* m.p. 172-174°C, yield 81%. ^{1}H-NMR (DMSO-d_6) δ 8.91 (s, 1H, CH=N), 8.86 (s, 1H, CH, pyrazole), 7.39-8.18 (m, 14H, ArH). ^{13}C NMR (DMSO-d_6): δ 160.46, 152.74, 151.63, 150.58, 148.63, 133.40, 131.37, 129.41, 129.10, 128.93, 128.63, 127.68, 127.36, 126.76, 124.94, 124.21, 121.55, 121.06, 119.86. MS

(ESI) m/z = 381.16 (M +H^+). Anal. Calc. for $C_{23}H_{16}N_4S$: C, 72.61; H, 4.24; N, 14.73; S 8.43 Found: C, 72.66; H, 4.27; N, 14.80; S 8.49

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)-4-methylbenzenamine ***(6ab)*** m.p.= 178-180°C, yield 89%. ^{1}H-NMR (DMSO-d_6) δ 8.92 (s, 1H, CH=N), 8.86 (s, 1H, CH, pyrazole), 7.39-8.18 (m, 13H, ArH), 2.49 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.35, 152.37, 151.96, 151.83, 148.07, 133.26, 132.53, 132.46, 130.42, 129.38, 128.83, 128.27, 127.86, 127.55, 126.64, 124.64, 121.06, 121.04, 119.74, 21.28. MS (ESI) m/z = 395.16 (M +H^+). Anal. Calc. for $C_{24}H_{18}N_4S$: C, 73.07; H, 4.60; N, 14.20; S 8.13 Found: C, 73.16; H, 4.57; N, 14.18; S 8.19

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)-4-methoxybenzenamine ***(6ac)*** m.p.=184-185 °C, yield 79%. ^{1}H-NMR (DMSO-d_6) δ 8.84 (s, 1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.24-8.06 (m, 13H, ArH), 3.79 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 160.36, 156.17, 152.13, 151.83, 151.68, 148.26, 133.46, 132.60, 129.42, 128.83, 128.74, 127.86, 127.55, 126.21, 124.96, 122.64, 121.06, 119.04, 114.74, 55.47. MS (ESI) m/z = 411.49 (M +H^+). Anal. Calc. for $C_{24}H_{18}N_4OS$: C, 70.22; H, 4.42; N, 13.65; S 7.81 Found: C, 70.16; H, 4.47; N, 13.68; S 7.89

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)-4-chlorobenzenamine ***(6ad)*** m.p.= 198-199 °C, yield 73% . ^{1}H-NMR (DMSO-d_6) δ 8.86 (s, 1H, CH=N), 8.81 (s, 1H, CH, pyrazole), 7.30-8.09 (m, 13H, ArH). ^{13}C NMR (DMSO-d_6): δ 160.46, 152.86, 151.74, 151.58, 148.20, 133.76, 132.27, 129.60, 129.42, 129.38, 128.83, 128.87, 127.96, 127.56, 126.86, 124.64, 121.06, 121.04. 119.74. MS (ESI) m/z = 415.09 (M +H^+). Anal. Calc. for $C_{23}H_{15}ClN_4S$: C, 66.58; H, 3.64; N, 13.50; S 7.73 Found: C, 66.56; H, 3.67; N, 13.58; S 7.81

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)-2-chlorobenzenamine ***(6ae)*** m.p.= 205-207 °C, yield 71% . ^{1}H-NMR (DMSO-d_6) δ 8.84 (s, 1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.24-8.06(m, 13H, ArH), 3.79 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 160.76, 152.83, 151.74, 151.48, 149.56, 133.20, 132.76, 130.29, 129.60, 129.42, 128.86, 128.64, 127.74, 127.58, 127.22, 126.36, 125.15, 121.38, 119.56, 118.27. MS (ESI) m/z = 415.11 (M +H^+). Anal. Calc. for $C_{23}H_{15}ClN_4S$: C, 66.58; H, 3.64; N, 13.50; S 7.73 Found: C, 66.59; H, 3.69; N, 13.55; S 7.78

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)-3-chlorobenzenamine ***(6af)*** m.p.=202-204 °C, yield 72% . ^{1}H-NMR (DMSO-d_6) δ 8.83 (s, 1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.23-8.03 (m, 13H, ArH). ^{13}C NMR (DMSO-d_6): δ. 160.76, 152.83, 152.74, 151.58, 151.20, 134.76, 133.27, 132.60, 130.42, 129.38, 128.83, 128.27, 127.86, 127.64, 126.56, 124.74, 123.48, 121.22, 121.07, 121.04, 119.29. MS (ESI) m/z = 415.05 (M +H^+). Anal. Calc. for $C_{23}H_{15}ClN_4S$: C, 66.58; H, 3.64; N, 13.50; S 7.73 Found: C, 66.55; H, 3.67; N, 13.57; S 7.76

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)benzenamine ***(6ba)*** m.p.= 158-160 °C, yield 65% . ^{1}H-NMR (DMSO-d_6) δ 8.93 (s, 1H, CH=N), 8.86 (s, 1H, CH, pyrazole), 7.33-8.12 (m, 13H, ArH), 2.46 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.35, 152.37, 151.96, 151.83, 148.07, 133.26, 132.53, 132.46, 130.42, 129.38, 128.83, 128.37, 127.86, 127.55, 126.64, 124.64, 121.06, 121.04, 119.74. MS (ESI) m/z = 395.14 (M +H^+). Anal. Calc. for $C_{24}H_{18}N_4S$: C, 73.07; H, 4.60; N, 14.20; S 8.13 Found: C, 73.14; H, 4.52; N, 14.26; S 8.11

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)-4-methyl-benzenamine ***(6bb)*** m.p.= 173-175 °C, yield 73% . ^{1}H-NMR (DMSO-d_6) δ 8.83 (s, 1H, CH=N), 8.77 (s, 1H, CH, pyrazole), 7.24-8.09 (m, 13H, ArH), 2.29 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 160.74, 152.20, 151.99, 151.78, 148.94, 139.41, 133.53, 132.46, 132.42, 130.38, 129.83, 129.27, 127.86, 126.55, 125.64, 124.64, 121.06, 121.04, 119.77. MS (ESI) m/z = 409.14 (M +H^+). Anal. Calc. for $C_{25}H_{20}N_4S$: C, 73.50; H, 4.93; N, 13.71; S 7.85 Found: C, 73.54; H, 4.93; N, 13.76; S 7.79

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)-4-methoxy-benzenamine ***(6bc)*** m.p.= 180-182 °C, yield 72% . ^{1}H-NMR (DMSO-d_6) δ 8.86 (s, 1H, CH=N), 8.76 (s, 1H, CH, pyrazole), 7.36-8.06 (m, 12H, ArH), 3.79 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.78, 156.53, 152.17, 151.93, 151.83, 148.68, 139.46, 133.60, 132.42, 129.83, 129.74, 127.86, 126.55, 125.21, 124.96, 122.64, 121.06, 119.04, 114.74, 55.26, 21.26. MS (ESI) m/z = 425.16 (M +H^+). Anal. Calc. for $C_{25}H_{20}N_4OS$: C, 70.73; H, 4.75; N, 13.20; S 7.55 Found: C, 70.78; H, 4.73; N, 13.26; S 7.59

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)-4-chloro-benzenamine ***(6bd)*** m.p.= 184-185 °C, yield 79% . ^{1}H-NMR (DMSO-d_6) δ 8.87 (s, 1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.23-8.02 (m, 12H, ArH), 2.13 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.75, 152.37, 151.96, 151.87, 148.77, 139.27, 133.73, 132.46, 129.42, 129.38, 129.13, 127.86, 126.55, 125.67, 125.64, 121.27, 121.04, 119.74, 21.27. MS (ESI) m/z = 429.14 (M +H^+). Anal. Calc. for $C_{24}H_{17}ClN_4S$: C, 67.20; H, 3.99; N, 13.06; S 7.48 Found: C, 67.14; H, 3.93; N, 13.14; S 7.49

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)-2-chloro-benzenamine ***(6be)*** m.p.= 179-181 °C, yield 69% . ^{1}H-NMR (DMSO-d_6) δ 8.85 (s, 1H, CH=N), 8.73 (s, 1H, CH, pyrazole), 7.29-8.04 (m, 12H, ArH), 2.13 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.86, 152.17, 151.83, 151.41, 149.20, 139.46, 133.27, 132.60, 130.07, 129.96, 129.26, 129.13, 127.36, 127.31, 126.86, 125.64, 125.21, 124.04, 121.74, 119.87, 118.47, 21.27. MS (ESI) m/z = 429.12 (M +H^+). Anal. Calc. for $C_{24}H_{17}ClN_4S$: C, 67.20; H, 3.99; N, 13.06; S 7.48 Found: C, 67.16; H, 3.91; N, 13.09; S 7.51

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-1H-pyrazol-4-yl)methylene)-3-chloro-benzenamine ***(6bf)*** m.p.= 185-187 °C, yield 79% . ^{1}H-NMR (DMSO-d_6) δ 8.95 (s, 1H, CH=N), 8.81 (s, 1H, CH, pyrazole), 7.28-8.04 (m, 12H, ArH), 2.15 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.83, 152.86, 152.17, 151.49, 151.20, 139.46, 134.27, 133.60, 132.30, 130.96, 129.41, 129.13, 127.36, 126.21, 125.86, 124.64, 123.21, 121.87, 121.74, 121.27, 119.74. MS (ESI) m/z = 429.13 (M +H^+). Anal. Calc. for $C_{24}H_{17}ClN_4S$: C, 67.20; H, 3.99; N, 13.06; S 7.48 Found: C, 67.24; H, 4.03; N, 13.11; S 7.41

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)benzenamine ***(6ca)*** m.p.= 193-195

°C, yield 73% . ^{1}H-NMR (DMSO-d_6) δ 8.95 (s, 1H, CH=N), 8.86 (s, 1H, CH, pyrazole), 7.27-8.07 (m, 13H, ArH). ^{13}C NMR (DMSO-d_6): δ 163.38, 160.07, 152.44, 151.83, 151.22, 148.74, 133.34, 132.35, 129.86, 129.14, 127.96, 127.41, 126.07, 124.36, 124.15, 121.14, 121.06, 119.04, 115.74. MS (ESI) m/z = 399.07 (M +H$^+$). Anal. Calc. for $C_{23}H_{15}FN_4S$: C, 69.33; H, 3.79; N, 14.06; S 8.05 Found: C, 69.24; H, 3.83; N, 14.11; S 8.11

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)-4-methyl-benzenamine ***(6cb)*** m.p.= 209-211 °C, yield 67% . ^{1}H-NMR (DMSO-d_6) δ 8.89 (s, 1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.24-8.09 (m, 12H, ArH), 2.16 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 163.35, 160.37, 152.53, 151.83, 151.07, 148.26, 133.46, 132.80, 132.40, 130.48, 129.39, 127.96, 127.55, 126.86, 124.64, 121.64, 121.06, 119.04, 115.74, 21.11. MS (ESI) m/z = 413.15 (M +H$^+$). Anal. Calc. for $C_{24}H_{17}FN_4S$: C, 69.88; H, 4.15; N, 13.58; S 7.77 Found: C, 69.84; H, 4.13; N, 13.61; S 7.74

(Z)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)-4-methoxy-benzenamine ***(6cc)*** m.p. 181-183 °C, yield 74% . ^{1}H-NMR (DMSO-d_6) δ 8.84 (s,1H, CH=N), 8.75 (s, 1H, CH, pyrazole), 7.33-8.05 (m, 12H, ArH), 3.73 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 163.36, 160.17, 156.53, 152.83, 151.68, 151.26, 148.46, 133.60, 132.42, 129.38, 127.74, 127.47, 126.55, 124.96, 122.86, 121.64, 119.06, 115.04, 114.74, 55.37. MS (ESI) m/z = 429.15 (M +H$^+$). Anal. Calc. for $C_{24}H_{17}FN_4OS$: C, 67.27; H, 4.00; N, 13.08; S 7.48 Found: C, 67.24; H, 4.08; N, 13.11; S 7.44

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)-4-chloro-benzenamine ***(6cd)*** m.p. 229-231 °C, yield 71% . ^{1}H-NMR (DMSO-d_6) δ 8.86 (s,1H, CH=N), 8.78 (s, 1H, CH, pyrazole), 7.30-8.07 (m, 12H, ArH). ^{13}C NMR (DMSO-d_6): δ 163.20, 160.41, 152.86, 151.64, 151.21, 148.46, 133.47, 132.67, 129.78, 129.39, 129.16, 127.74, 127.41, 126.21, 124.86, 121.64, 121.21, 119.04, 115.74. MS (ESI) m/z = 433.13 (M +H$^+$). Anal. Calc. for $C_{23}H_{14}ClFN_4S$: C, 63.81; H, 3.26; N, 12.94; S 7.41 Found: C, 63.84; H, 3.28; N, 12.88; S 7.47

(E)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-chloro-benzenamine ***(6ce)*** m.p. 245-247 °C, yield 78% . ^{1}H-NMR (DMSO-d_6) δ 8.85 (s, 1H, CH=N), 8.78 (s, 1H, CH, pyrazole), 7.28-8.05 (m, 12H, ArH). ^{13}C NMR (DMSO-d_6): δ 163.86, 160.17, 152.83, 151.86, 151.20, 149.46, 133.27, 132.69, 130.07, 129.39, 129.26, 127.96, 127.55, 127.21, 126.86, 125.64, 124.21, 121.07, 119.74, 118.87, 115.47. MS (ESI) m/z = 433.09 (M +H$^+$). Anal. Calc. for $C_{23}H_{14}ClFN_4S$: C, 63.81; H, 3.26; N, 12.94; S 7.41 Found: C, 63.87; H, 3.29; N, 12.98; S 7.46

*(E)-N-((1-(1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)-3-chloro-benzenamine****(6cf)*** m.p. 223-225 °C, yield 78% . ^{1}H-NMR (DMSO-d_6) δ 8.82 (s,1H, CH=N), 8.78 (s, 1H, CH, pyrazole), 7.28-8.06 (m, 12H, ArH). ^{13}C NMR (DMSO-d_6): δ. 163.46, 152.97, 152.53, 151.58, 151.20, 134.76, 133.27, 132.60, 130.42, 129.38, 127.26, 127.21, 126.55, 124.96, 123.86, 121.64, 121.06, 121.04, 119.74, 115.47. MS (ESI) m/z = 433.17 (M +H$^+$). Anal. Calc. for $C_{23}H_{14}ClFN_4S$: C, 63.81; H, 3.26; N, 12.94; S 7.41 Found: C, 63.86; H, 3.31; N, 12.88; S 7.48

2.2. Assay for *in vitro* Antimalarial Activity

2.2.1. Parasite Cultivation

The antimalarial activity of synthesized compounds was assessed against chloroquine-sensitive *P. falciparum* (3D7) isolated obtained from the National Institute of Malaria Research (NIMR), New Delhi, India. *P. falciparum* are cultivated in human A (+) red blood cells using RPMI 1640 medium supplemented with AB (+) serum (10%), 5% sodium bicarbonate and 40 μg/mL of gentamycin sulfate [20].

2.2.2. In vitro Test for Antimalarial Activity

The *in vitro* activity of *P. falciparum* intra erythrocytic stage on test compounds was evaluated by Schizont Maturation Inhibition method as described previously [21]. Firstly, a stock solution of synthesized compounds was prepared by dissolving in DMSO and serially diluted with RPMI 1640 medium to reach 1 mg/mL prior to use. Serial double dilutions were made in 96-well microliter plates with a concentration range of 1.98-500 μg/mL against a control containing incomplete medium with the same concentration of DMSO. The cultures, before testing, were synchronized by treatment with 5% D-sorbitol with a parasitemia of 0.6-0.8%. Each well received 10 μL of parasite-infected erythrocytes, 5% hematocrit and 90 μL of different drug dilutions. The plates were incubated at 37°C for 24 hours. After confirmation of the presence of 10% mature schizonts in control well (without drug), the blood from each well was harvested, and a thick blood smear was prepared on a glass slide. Growth of the parasites from duplicate wells of each concentration was monitored in Giemsa stained blood smears by counting the number of schizonts per 200 asexual parasites. Growth inhibition was expressed as the percentage of schizonts in each concentration, compared with controls.

2.2.3. Antimalarial Activity Calculation and Analysis

The number of schizonts counted per well was directly entered into the nonlinear regression software, HN NonLin V 1.1 [22], which was particular for the analysis of *in vitro* drug sensitivity assay for malaria. Individual dose response curves were generated and their EC_{50} values were determined.

2.3. Molecular Docking Study

Docking study is used to diminish false positive and recognize suitable orientation for the ligand in a protein active site. For the docking of ligands to protein active sites, an advanced molecular docking program, AutoDock [23] was used in the present study. The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site. The ligands were built in Marvin Sketch. All hydrogens in the structure were added, 2D molecules were cleaned into 3D and conformational energy of the molecules was minimized using MMFF94 force field and the ligands were saved as pdb files. MGL Tools 1.5.4 was used to prepare the ligands' pdbqt files, setting the number of rotatable bonds to

maximum, since most of the ligands used in this study have fewer than eight rotatable bonds (http://mgltools.scripps.edu/). The 3D crystal structure of *PfFP* with good resolution of 3.1 Å was taken from Protein Data Bank (www.rcsb.org) with (PDB Id: 2GHU). The crystal structures of protein were prepared for docking by adding hydrogen atoms, assigning bond orders, removal of all ions, water molecules and all the ligands from the structure. The polypeptide chain was processed in MGL Tools 1.5.4 to obtain the grid.pdbqt files. A grid-box was generated that was large enough to cover the entire receptor binding site. In the present study, the dimensions of the grid were 40×40×40 grid points with spacing of 0.375 A° between grid points and center on the ligands (51.36, 0.384, -31.12 coordinates). Docking simulations were performed using Lamarckian Genetic Algorithm (LGA) [24]. For each ligand, 10 docking poses were generated out of which best docked confirmation was considered for further analysis about hydrogen bonding. Further characterization via MD simulations was conducted using complexes that were selected according to their binding energy values and the interactions made with the surrounding residues. The resulting conformations were visualized in the PyMol Viewer tool (http://www.pymol.org/).

2.4. *In silico* ADME Prediction Method

The pharmacokinetic profile of the synthesized compounds showing good antimalarial activity was predicted by using programs Qikprop v3.6 (Schrodinger, Inc., New York, NY, 2012). All the compounds prepared by LigPrep were used for the calculation of pharmacokinetic properties by QikProp. The program QikProp, utilizes the method of Jorgensen [25] to calculate pharmacokinetic properties and descriptors such as octanol/water partitioning coefficient (logP), aqueous solubility (QPlogS), brain/blood partition coefficient (QPlogBB), human serum albumin binding (QPlogKhsa) and others.

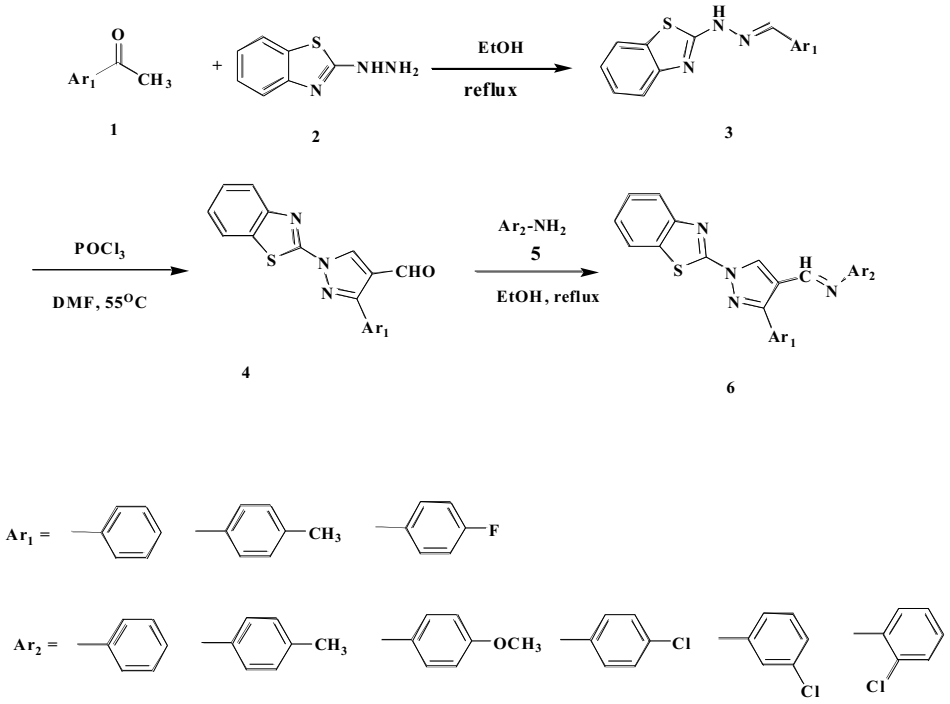
3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic approach adopted to obtain the intermediate and targeted compounds is outlined in Scheme **1**. First, 2-hydrazinobenzothiazole was condensed with various substituted methyl ketones in aqueous ethanol affording corresponding hydrazone. The subsequent reaction of hydrazone under Vilsmeiere-Hack condition afforded 4-formylpyrazole [18]. Further condensation of 4-formylpyrazole with aromatic amine using concentrated H_2SO_4 gave the target product [19]. Spectral data (^{1}H NMR, ^{13}C NMR, mass and elemental analysis) of the newly synthesized compounds were in complete agreement with the proposed structures. The ^{1}HNMR spectra in general displayed a characteristic signal at δ 9.96-10.18 assigned for aldehydic proton and a singlet at δ 8.66-8.95 for C_5-H of pyrazole ring. The formation of schiff base in ^{1}HNMR was ascertained based on a singlet at δ 8.49-8.86.

3.2. *In vitro* Antimalarial Activity

All the eighteen synthesized compounds were evaluated for their *in vitro* antimalarial activity against the asexual blood stage of human malaria parasite, *P. falciparum* as described previously [21]. EC_{50} value of these compounds is depicted in Table **1**. To get structural insights, two variations were made in the pyrazole hybrid. In the structural motif of



Scheme 1. Synthesis of Pyrazole schiff base hybrid.

these hybrids, pyrazole and benzothiazole substitution at position N-1 was kept common and variations were made in phenyl ring substitution at position 3 and 4. Most of the compounds showed good to moderate activity. Among the series, compounds **6ba-6bf** having *p*-CH_3 substitution at the phenyl ring on pyrazole nucleus at position 3 showed maximum antimalarial activity in the range 1.953-3.518 µg/ml while compounds **6ca-6cf** having a *p*-F substitution showed moderate antimalarial activity in the range 2.59-5.9 µg/ml. Unsubstituted compounds **6aa-6af** showed least antimalarial activity in the range 2.7-8.3 µg/ml. Among the synthesized compounds, compound with *m*-Cl substitution at position 4 was found to be the most active compound **6bf** with EC_{50} 1.953µg/ml followed by compounds **6bd**, **6bb** with EC50 1.98µg/ml and 2.24µg/ml respectively. Substitution of phenyl ring at position 4 of pyrazole nucleus with halogen group, showed potential antimalarial activity in the range 1.95-4.03 µg/ml. Compound **6aa** was found to be the least active compound of the series with EC_{50} 8.34 µg/ml.

Table 1. ***In vitro*** **antimalarial activity of Pyrazole Schiff base hybrid against** ***P. falciparum*** **(3D7) strain.**

Compounds	Ar_1	Ar_2	EC_{50}(µg/ml)
6aa	Ph	Ph	8.342
6ab	Ph	*p* -CH_3Ph	2.760
6ac	Ph	*p* -OCH_3Ph	4.126
6ad	Ph	*p* –ClPh	2.769
6ae	Ph	*o*-ClPh	4.038
6af	Ph	*m*-ClPh	2.702
6ba	*p*-CH_3Ph	Ph	3.518
6bb	*p* -CH_3Ph	*p* -CH_3Ph	2.241
6bc	*p* -CH_3Ph	*p* -OCH_3Ph	3.923
6bd	*p* -CH_3Ph	*p* –ClPh	1.983
6be	*p* -CH_3Ph	*o*-ClPh	2.621
6bf	*p* -CH_3Ph	*m*-ClPh	1.953
6ca	*p* -FPh	Ph	5.920
6cb	*p*-FPh	*p*-CH_3Ph	2.726
6cc	*p* -FPh	*p*-OCH_3Ph	5.009
6cd	*p* -FPh	*p*-ClPh	2.594
6ce	*p* -FPh	*o*-ClPh	3.753
6cf	*p* -FPh	*m*-ClPh	2.621
Chloroquinine	-	-	1.3[a]

[a] Activity reported [21]

3.3. Structure Activity Relationship

The SAR study revealed that various substitutions at position 3 and 4 on pyrazole motif were responsible for a range of antimalarial activities. Substitutions of phenyl ring at position 3 of pyrazole nucleus with electron donating group like methyl, showed good antimalarial activity while substitution of electron withdrawing group like fluoro partially decreased antimalarial activity. Substitutions of phenyl ring at position 4 of pyrazole nucleus with electron withdrawing group like halogen, showed potential antimalarial activity. *Para* and *Meta* substitution of chloro group showed excellent activity whereas ortho substitution led to partially decreased antimalarial activity. Compounds containing moderate electron donating group like methyl also showed potential antimalarial activity while methoxy group and no substitution at phenyl ring decreased the biological activity. The antimalarial activity among pyrazole derivatives showed that substituted aromatic ring showed maximum activity than the unsubstituted aromatic ring. The SAR studies developed from the screening data are displayed in Fig. (**1**).

3.4. Docking Studies

To study the probable mode of action for antimalarial activity and to predict orientation of the molecules at the active site, docking simulations were performed using Auto Dock Vina program. A cysteine protease inhibitor obstructing the development of cultured *P. falciparum* parasites was chosen for docking because it is an important target for antimalarial chemotherapy [26, 27]. All the eighteen compounds were docked into the crystal structure of *P. falciparum* falcipain-2. The scoring functions and interactions with the surrounding amino acids were used to predict their binding modes, binding affinities and orientation of these compounds at the active site. The results of docking studies for the interaction between Falcipain-2 and the representative schiff base of pyrazole derivatives are given in Table **2**. The result reveals that the pyrazole Schiff base derivatives occupy the *PfFP* binding sites and they show good interactions with significant values of binding energies. Binding energies for these compounds were in the range of -7.9 to -8.6 kcal/mol. Important hydrophobic bond interactions were observed with the energy minimized structure of ligands and *PfFP* protein.

According to the binding affinity, the most active compound was **6bf** which matched with our results of *in vitro* studies on *P. falciparum.* Fig. (**2**) and (**3**) illustrate the binding pose as well as interactions of compound **6ba** and **6bf** with docking score -8.5 and -8.6, respectively. All compounds showed π-π stacking with the phenyl ring of amino acid TRP206. In compound **6ba**, pyrazole ring showed π-π stacking with phenyl ring of amino acid PHE158 and phenyl ring at position 4 of the pyrazole ring showed π-π interaction with amino acid TRP206. The fair interactions with good binding energies suggest that schiff base of pyrazole derivatives ligands are potential compounds in inhibiting falcipain-2 protein.

3.5. Prediction of Pharmacokinetic Properties

All Pyrazole Schiff base hybrids were screened for their different pharmacokinetic parameters of compounds using ADME predictions by QIKProp 3.6. All the synthesized compounds were primarily screened considering the primary parameters of Lipinski's rule of 5. All the synthesized hybrids had drug-likeness properties per Lipinski's rules specified in Table **3** and ADME properties is depicted in Table **4**. An orally active drug should not have more than 1

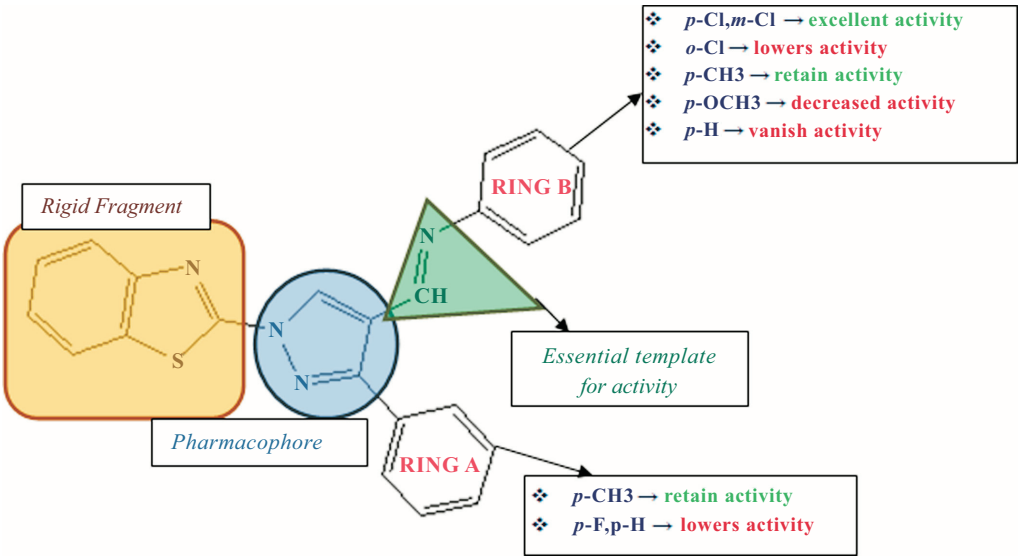


Fig. (1). Structure-Activity Relationship of Pyrazole Schiff base hybrid.

Table 2. Docking score with the π-π interactions of Pyrazole Schiff base hybrid with amino acids.

Compound	Docking Score	Interaction Type	Amino Acid Involved	Interaction with Structural Feature
6aa	-8.2	π-π π-π	TRP206 TRP206	Pyrazole ring Ph at position 3
6ab	-8.1	π-π π-π	TRP206 TRP206	Ph at position 3 *p*-CH_3Ph at position 4
6ac	-7.9	π-π	TRP206	*p*-OCH_3Ph at position 4
6ad	-8.0	π-π	TRP206	*p*-ClPh at position 4
6ae	-8.1	π-π	TRP206	*o*-ClPh at position 4
6af	-8.1	π-π π-π	TRP206 TRP206	Ph at position 3 *m*-ClPh at position 4
6ba	-8.5	π-π π-π	PHE158 TRP206	Pyrazole ring Ph at position 4
6bb	-8.3	π-π	TRP206	*p*-CH_3Ph at position 4
6bc	-7.9	π-π	TRP206	*p*-OCH_3Ph at position 3
6bd	-8.2	π-π	TRP206	*p*-ClPh at position 4
6be	-8.6	π-π	TRP206	*o*-ClPh at position 4
6bf	-8.6	π-π π-π	TRP206 TRP206	Pyrazole ring *p*-CH_3Ph at position 4
6ca	-8.4	π-π π-π	TRP206 TRP206	Pyrazole ring *p*-FPh ring at position 3
6cb	-8.2	π-π	TRP206	*p*-CH_3Ph at position 4
6cc	-7.9	π-π	TRP206	*p*-OCH_3Ph at position 4
6cd	-8.4	π-π π-π	TRP206 TRP206	Pyrazole ring *p*-FPh at position 3
6ce	-8.1	π-π π-π	TRP206 TRP206	Pyrazole ring *p*-FPh at position 3
6cf	-8.1	π-π π-π	TRP206 TRP206	Pyrazole ring *p*-FPh at position 3

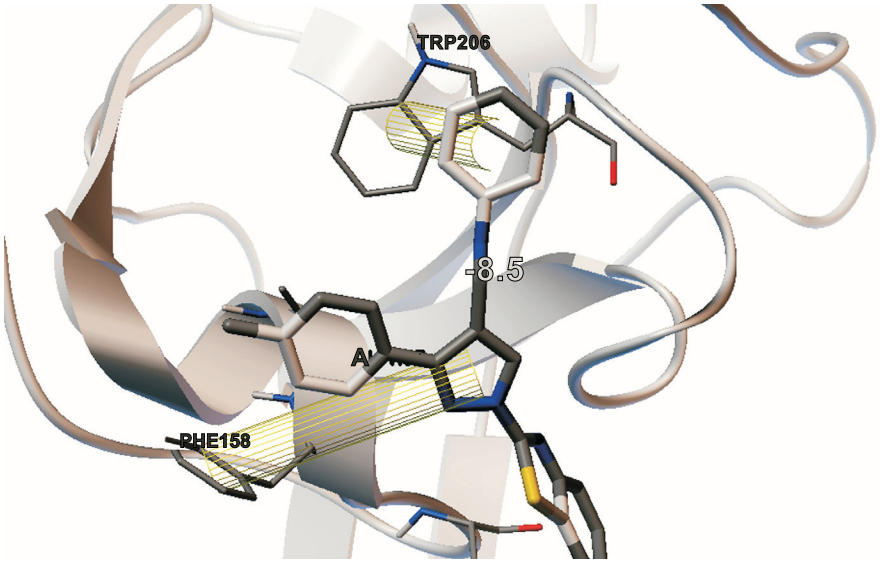


Fig. (2). Binding mode of Compound **6ba** in pocket of*PfFP* (PDB: 2GHU).

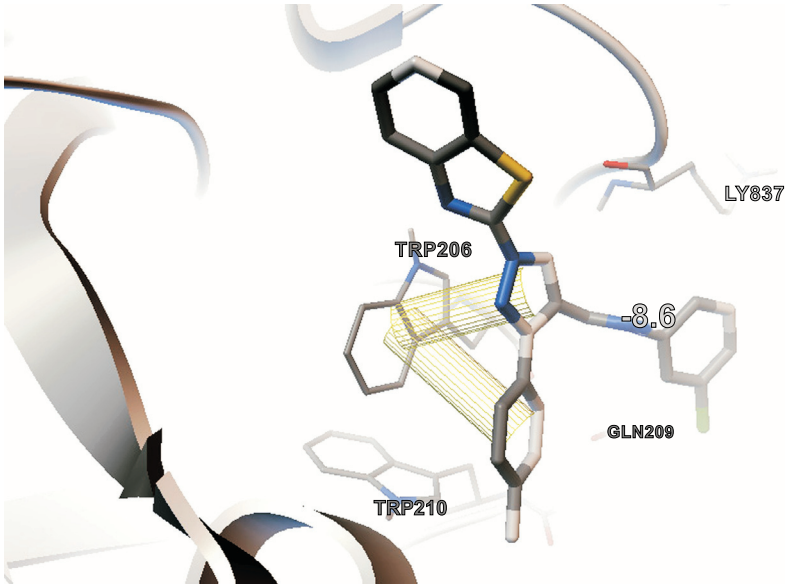


Fig. (3). Binding mode of Compound **6bf** in pocket of*PfFP* (PDB: 2GHU).

violation of these rules [28]. The most favorable values of the descriptors like polar surface area and rotatable bonds have profound influence on the oral bioavailability of the molecules [29]. In the present study, all the synthesized compounds possessed several rotatable bonds less than 15 and polar surface area fell suitably in the acceptable range (7-200 Å). Caco-2 cell model and Madin-Darby canine kidney (MDCK) cell model have been suggested as a reliable *in-vitro* model for the calculation of oral drug absorption, present as a model for the gut-blood barrier [30]. QPPCaco and QPP MDCK predictions for all the test compounds showed significant values. The solubility (QPlogS) of organic molecules in water has a major impact on numerous ADME-related properties like absorption, distribution, transport, and ultimately bioavailability. All the synthesized compounds showed solubility values within the limits (-6.5 to 0.5). Further, the results for the QPlogkhsa descriptor of Qikprop representing predicted values of human serum albumin binding indicated that synthesized molecules were found to fall in the permitted range (1.5 to 1.5). Blood-Brain Barrier (BBB) penetration is crucial in the pharmaceutical sphere because CNS-active compounds must cross it and all the synthesized compounds showed trustworthy prediction for brain/blood partition coefficient.

CONCLUSION

In the present work, we have developed a series of molecules comprised of pyrazole schiff base hybrid where most of the compounds showed significant *in vitro* antimalarial activity against CQS strains of *P. falciparum*. Among all compounds, **6bf** and **6bd** were found to be

Table 3. Prediction of Lipinski's 'Rule of 5' for the Pyrazole Schiff base hybrid.

Compound	M.W.	Polar Surface Area	logP	HBD	HBA	'N' of violation
6aa	380.10	43.08	5.78	0	4	1
6ab	394.12	43.08	6.22	0	4	1
6ac	410.12	52.32	5.83	0	5	1
6ad	414.07	43.08	6.45	0	4	1
6ae	414.07	43.08	6.41	0	4	1
6af	414.07	43.08	6.43	0	4	1
6ba	394.12	43.08	6.22	0	4	1
6bb	408.14	43.08	6.67	0	4	1
6bc	424.13	52.32	6.28	0	5	1
6bd	428.08	43.08	6.90	0	4	1
6be	428.08	43.08	6.85	0	4	1
6bf	428.08	43.08	6.88	0	4	1
6ca	398.10	43.08	5.94	0	4	1
6cb	412.11	43.08	6.30	0	4	1
6cc	428.11	52.32	6.00	0	5	1
6cd	432.06	43.08	6.62	0	4	1
6ce	432.06	43.08	6.57	0	4	1
6cf	432.06	43.08	6.59	0	4	1

Table 4. Calculated ADME properties.

Comps	QlogBB (-3.0 to 1.5)	QP logKHSA	QPP MDCK (<25 poor, >500 great)	QPP caco(<25 poor, >500 great)	QPlogS (-6.5 to 5)	No of rotatable bond <15	Percent Human oral absorption (>80%— high and <25%—poor)
6aa	0.181	1.179	508	5297	-6.273	4	100
6ab	0.172	1.153	508	5297	-6.505	4	100
6ac	0.049	1.353	4057	804	-6.399	5	100
6ad	0.294	1.307	921	529	-6.649	4	100
6ae	0.295	0.843	924	531	-5.360	4	100
6af	0.287	1.518	721	429	-5.475	4	100
6ba	0.159	1.493	100	531	-4.994	4	100
6bb	0.174	1.692	510	527	-4.607	4	100
6bc	0.166	1.648	510	527	-4.945	5	100
6bd	0.039	1.429	404	429	-4.102	4	100
6be	0.287	1.166	923	527	-4.217	4	100
6bf	0.109	1.263	506	531	-4.227	4	100
6ca	0.101	1.237	506	531	-4.512	4	100
6cb	-0.021	1.437	404	430	-5.012	4	100
6cc	0.222	1.391	916	539	-5.045	5	100
6cd	0.553	1.237	906	859	-4.489	4	100
6ce	0.789	1.391	100	859	-4.639	4	100
6cf	0.690	1.177	100	856	-4.724	4	100

potential molecules with EC_{50} 1.95µg/ml and 1.98µg/ml respectively. The observed activity was further correlated via molecular docking study. The molecular modeling studies revealed that the pyrazole Schiff base analogues completely occupy the site of *PfFP*-2 with different modes. The ascertained ADME parameters for the synthesized compounds showed good pharmacokinetic properties. This outcome shows that the pyrazole Schiff base hybrids may be promising leads and provide a significant model for further structural as well as biological optimization.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content of the manuscript.

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