Original Article

Insignificant *in vitro* falcipain-2 inhibitory activity of novel 2-(4-(substituted benzoyl) piperazine-1-yl)-*N*-phenylacetamide derivatives

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Abstract

Background: The cysteine protease, falcipain-2 (FP-2) is an important drug target for the management of infection by the human malaria parasite *Plasmodium falciparum*. The rapid emergence of resistance is the main problem with all antimalarial agents. Hence, the discovery of novel, effective drugs to counter the spread of malaria parasites that are resistant toward existing agents, especially drugs that can act on new targets, is urgently necessary. **Materials and Methods:** A novel series of 2-(4-(substituted benzoyl) piperazine-1-yl)-*N*-phenylacetamide derivatives was designed using the ligand-based approach, employing a three-point pharmacophore model. It consists of an aromatic group (monocyclic/bicyclic), which is attached to the hydrophobic moiety; commonly an aromatic residue through hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) atom(s) acting as the linker. The new chemical entities were synthesized from the key intermediate *N*-phenyl-2-(piperazine-1-yl) acetamide, by coupling it with various substituted acids in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) and 1 hydroxybenzotriazole (HOBt). The obtained compounds' structures were confirmed by ^{1}H NMR and by mass spectral data. **Results:** All the synthesized compounds were evaluated for their *in vitro* FP-2 inhibitory activity. Two compounds 5l and 5q showed very weak enzyme inhibition activities (3-5%) and the remaining 15 compounds showed no inhibition at 10 μm concentrations. However, unlike other reported FP-2 inhibitors, none of these molecules showed potent activity. **Conclusion:** This series of compounds did not have or had very less antimalarial activity.

Key words: Antimalarial, falcipain-2 (FP-2), ligand-based drug design

INTRODUCTION

The morbidity and mortality due to malaria in humans have made it a major public health problem in tropical and subtropical countries. Despite more than a century of efforts to eradicate or control malaria, the disease remains a major threat to public health. The National Malaria Control and Eradication Programmes have been hampered by technical, operational, and socioeconomic difficulties, and the spread of drug resistance in parasite and insecticide resistance in mosquito vectors is making malaria a formidable challenge.[1,2] The World Health Organization (WHO) Malaria Report 2012 indicates that in 2010, there were 219 million malaria cases leading to approximately 660,000 malaria deaths, mostly among African children.[3]

A number of drugs are available to treat malaria;[4] however, drug resistance, toxicity, and high costs lead to complications in malaria treatment. Recently, drug resistance against artemisinin, the key compound in artemisinin combination therapies (ACTs), is also emerging,[5,6] and we need a safe

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and cost-effective novel chemical class of antimalarial agents for treatment.[7]

Malaria proteases are attractive antimalarial targets owing to their crucial roles in parasite growth, development, and infection. On the basis of *in silico* approaches, 123 putative proteases are identified in the *Plasmodium falciparum* genome, and these are grouped into five major classes[8,9] (aspartic, cysteine, metallo, serine, and threonine). Among these proteases, the cysteine and aspartic proteases of *P. falciparum*, known as falcipains and plasmepsins, respectively, play a major role in parasite food assimilation by their ability to degrade hemoglobin and are promising targets for antimalarial drug design.[10-12]

Numerous studies showed that malaria cysteine proteases are important for the hydrolysis of hemoglobin, erythrocyte rupture, and erythrocyte invasion.[13] In *P. falciparum*, four falcipains (falcipain-1, -2, -2', and -3) play an important role in the intraerythrocytic stage of the parasite life cycle.[14-16] Among these, falcipain-2 (FP-2) is the most intensely studied enzyme, and its structural and functional data suggest that it is an attractive target for therapeutic intervention.[17,18] FP-2 possesses an unusually long prosequence that is 2-3 times longer than any in other papain family of proteases,[19] and it does not require a prodomain for acquiring a catalytically competent conformation.[19] However, the pro region plays an important role in the folding and regulation of activity of the protease region; the recombinant pro region of FP-2 has been shown to inhibit FP-2 activity effectively.[20] Leupeptin (N-acetyl-L-leucyl-L-leucyl-L-argininal) and E-64 (N-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide) are potent inhibitors of falcipain-2, but do not possess drug-like characteristics [Figure 1].[21-23] Along with this, many reports indicate that most of the FP-2 inhibitors are generated from peptides analogs, have nanomolar IC_{50}S (due to the formation of a covalent bond with thiolate at the catalytic site), and produce toxicity.[24,25]

The aforementioned discussion motivated us to develop a novel series of nonpeptidic FP-2 inhibitors as antimalarial agents. A new pharmacophore model was generated, and based on this pharmacophore, a series of 17 structurally novel piperazine-containing molecules were synthesized toward the inhibition of FP-2 protease.

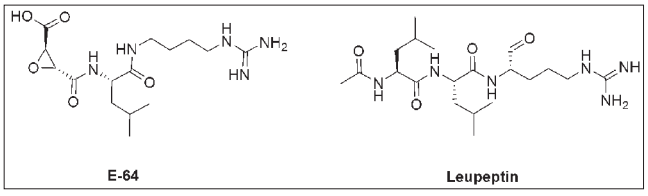


Figure 1: Existing standard ligands for FP-2 inhibition

MATERIALS AND METHODS

Chemistry

Melting points (m.p.) were determined in open capillary tubes and on a Buchi 530 melting point apparatus (Texas City, TX), and were uncorrected. Thin-layer chromatography (TLC) was carried out to monitor progress of the reaction. Spots were identified by their absorption under ultraviolet (UV) light. ^{1}H NMR spectra were recorded with Bruker DPX (Germany)operating at 400 MHz in $CDCl_3$ or DMSO-d_6 solvent, with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown as δ values (ppm); the J values are expressed in hertz (Hz). Signals are represented as s (singlet), d (doublet), t (triplet), q (quintet), or m (multiplet). The mass spectra (ESI) of most of the compounds exhibited molecular ions as $(M + 1)^+$/ $(M + Na)^+$. The purity of the final compounds (5a-q) was evaluated on a Waters™ liquid chromatography/mass spectrometry (LC/MS) system (USA) equipped with a photodiode array detector using an XBridge C18 5µm 4.6 mm × 150 mm column. The methods used were of the following three types: Method A, 10% MeOH in H_2O (1 mL/min, isocratic); method B: 25% MeOH in H_2O (1 mL/min, isocratic); and method C: 15% CH_3CN (0.05% TFA) in H_2O (1 mL/min, isocratic). High-performance liquid chromatography (HPLC) purity and physical constants of the final compounds are presented in Tables 1 and 2. The chemicals were purchased commercially from Aldrich (Bangalore), Fluka (Switzerland), Spectrochem (Mumbai).

Experimental

Synthesis of 2-Chloro-N-phenylacetamide (2)

To a solution of compound 1 (1 g, 10.7 mmol), and K2CO3 (7.4 g, 53.6 mmol) in 200 mL dichloromethane

Table 1: Purity data for final synthesized compounds (5a-q)

Compounds	Method	Purity (in %)
5a	A	95.1
5b	B	96.0
5c	B	95.6
5d	A	94.2
5e	C	96.8
5f	A	98.4
5g	A	94.9
5h	A	91.0
5i	B	90.1
5j	A	98.2
5k	A	98.5
5l	A	99.1
5m	B	95.1
5n	A	95.2
5o	A	94.4
5p	A	95.1
5q	B	95.1

Table 2: Physical constants of final derivatives (5a-q)

Compound	R	Molecular weight	Molecular formula	% Yield[a]	m.p. in °C	Log *P*[b]
5a	*m*-F	341.1	$C_{19}H_{20}FN_3O_2$	70	144-146	1.84
5b	*p*-CH_3	337.2	$C_{20}H_{23}N_3O_2$	56	142-144	2.17
5c	*m*-Cl	357.1	$C_{19}H_{20}ClN_3O_2$	65	134-136	2.24
5d	*o*-CH_3	337.2	$C_{20}H_{23}N_3O_2$	76	143-145	2.17
5e	*p*-Cl	357.1	$C_{19}H_{20}ClN_3O_2$	65	136-138	2.24
5f	*o*-OCH_3	353.2	$C_{20}H_{23}N_3O_3$	71	141-143	1.56
5g	*p*-OCH_3	353.2	$C_{20}H_{23}N_3O_3$	66	134-136	1.56
5h	*m*-Br	401.1	$C_{19}H_{20}BrN_3O_2$	68	140-142	2.51
5i	*p*-Br	401.2	$C_{19}H_{20}BrN_3O_2$	64	138-140	2.51
5j	*m*-CF_3	391.1	$C_{20}H_{20}F_3N_3O_2$	64	132-134	2.60
5k	*o*-CF_3	391.1	$C_{20}H_{20}F_3N_3O_2$	64	124-126	2.60
5l	*p*-CF_3	391.1	$C_{20}H_{20}F_3N_3O_2$	64	154-160	2.60
5m	*m*-OCH_2CH_3	367.2	$C_{21}H_{25}N_3O_3$	62	124-126	1.89
5n	*p*-OCH_2CH_3	367.2	$C_{21}H_{25}N_3O_3$	62	120-122	1.89
5o	*p*-F	341.1	$C_{19}H_{20}FN_3O_2$	69	146-148	1.84
5p	*o*-Cl	357.1	$C_{19}H_{20}ClN_3O_2$	65	134-136	2.24
5q	*p*-CH_2CH_3	351.2	$C_{21}H_{25}N_3O_2$	75	130-132	2.59

[a]Yields refer to isolated pure compounds, [b]Log *P* values are calculated by using ChemDraw ultra 10.0 (cambridgesoft, USA)

was taken in a round bottom flask (500 mL) and cooled to 0 °C. To this solution, chloroacetyl chloride (1.7 mL, 21.4 mmol) was added dropwise over 10 min and allowed to stir for 20 min at room temperature (RT), quenched the reaction mixture with saturated sodium hydrogen carbonate (15 mL), and washed with water. The organic layer was separated, dried over sodium sulfate and evaporated under reduced pressure. The crude reaction mixture was purified by column chromatography using dichloromethane and methanol as a mobile phase to obtain a pure compound 2 as a white solid. Yield 66%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.10 (t, J = 8.0 Hz, 1H), 4.12 (s, 2H).

Synthesis of tert-butyl 4-(2-oxo-2-(phenylamino) ethyl) piperazine-1-carboxylate (3)

In a round-bottom flask (250 mL) compound 2 (1.0 g, 5.8 mmol), and K2CO3 (4.0 g, 29.4 mmol) were suspended in anhydrous acetonitrile and allowed to stir at 90 ºC for 10 min. To this solution N-Boc-piperazine (1.3 g, 7.0 mmol) was added and refluxed for 4 h. On completion of the reaction monitored by TLC, acetonitrile was removed under reduced pressure and the reaction crude was diluted with ethyl acetate. The organic layer was then extracted with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to achieve a crude mixture. This mixture was purified by column chromatography using ethyl acetate and hexane as a mobile phase to obtain a pure compound 3 as a light brown solid. Yield 78%, ^{1}H NMR ($CDCl_3$) δ: 8.96 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.36 (t, J = 8.0 Hz, 2H), 7.21 (t, J = 8.0 Hz, 1H), 3.52 (m, 4H), 3.23 (S, 2H), 2.60 (m, 4H), 1.50 (s, 9H).

Synthesis of N-phenyl-2-(piperazine-1-yl) acetamide (4)

To a solution of compound 3 (1.0 g, 3.0 mmol) in DCM (20 mL), in a round-bottom flask at 0 °C, trifluoroacetic acid (1.1 mL, 15.0 mmol) was added dropwise and allowed to stir at RT for 16 h. Upon completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure. A saturated solution of sodium bicarbonate was added to the residue and extracted with ethyl acetate. The ethyl acetate layer was evaporated to obtain the free amine as off-white solid. Yield 75%, ^{1}H NMR (DMSO-d_6) δ: 9.49 (s, 1H), 7.55 (d, J = 7.8 Hz, 2H), 7.23 (t, J = 7.8 Hz, 2H), 7.01 (t, J = 7.4 Hz, 1H), 3.13 (s, 2H), 3.10 – 3.01 (m, 4H), 2.72-2.61 (m, 4H).

General procedure for the synthesis of 2-(4-(substituted benzoyl) piperazine-1-yl)-N-phenylacetamide derivatives (5a-q)

In a round-bottom flask (250 mL), an appropriate carboxylic acid (1 g), triethyl amine (2.4 equiv), EDC. HCl (1.5 equiv), and HOBt (0.8 equiv) were suspended in DCM and allowed to stir for 10 min at RT, followed by the addition of compound 4 (1.1 equiv). The reaction mixture was stirred for 6 h at RT, after completion of the reaction solvents were removed under reduced pressure. The crude mixture was diluted in ethyl acetate and the organic layer was washed with saturated sodium bicarbonate (twice) and brine (once). The organic layer was dried over sodium sulfate, evaporated under vacuum and crude compound was purified by column chromatography.

2-(4-(3-fluorobenzoyl) piperazine-1-yl)-N-phenylacetamide (5a)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 8.88 (s, 1H), 7.59 (d, J = 8.0 Hz, 3H), 7.35 (t, J = 7.2 Hz, 1H), 7.26 (t, J = 8.0 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 7.09 (q, 8.0 Hz, 2H), 3.82 (m, 2H), 3.37 (m, 2H), 3.14 (s, 2H), 2.66 (m, 2H), 2.51 (m, 2H). ESI MS: m/z = 342.1 $[M + 1 H]^+$.

2-(4-(4-methylbenzoyl) piperazine-1-yl)-N-phenylacetamide (5b)

Brown solid, ^{1}H NMR ($CDCl_3$) δ: 9.75 (s, 1H), 7.62 (d, J = 7.6 Hz, 2H), 7.27 (m, 6H), 7.05 (t, J = 7.2 Hz, 1H), 3.64 (m, 4H), 3.17 (s, 2H), 2.54 (m, 4H), 2.32 (s, 3H). ESI MS: m/z = 338.2 $[M + 1 H]^+$.

2-(4-(3-chlorobenzoyl) piperazine-1-yl)-N-phenylacetamide (5c)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.86 (s, 1H), 7.49 (d, J = 7.6 Hz, 2H), 7.34 (m, 2H), 7.28 (m, 3H), 7.22 (m, 1H), 7.06 (t, J = 7.6 Hz, 1H), 3.62 (m, 4H), 3.14 (s, 2H), 2.59 (m, 4H). MS (ESI): m/z 357.0 $[M]^+$ and 358.2 $[M + 1H]^+$.

2-(4-(2-methylbenzoyl) piperazine-1-yl)-N-phenylacetamide (5d)

Brown solid, ^{1}H NMR ($CDCl_3$) δ: 8.94 (s, 1H), 7.56 (m, 2H), 7.31 (m, 3H), 7.22 (m, 2H), 7.14 (m, 2H), 3.92 (m, 2H), 3.35 (m, 2H), 3.19 (s, 2H), 2.73 (m, 2H), 2.53 (m, 2H), 2.33 (s, 3H). ESI MS: m/z = 338.2 $[M + 1 H]^+$.

2-(4-(4-chlorobenzoyl) piperazine-1-yl)-N-phenylacetamide (5e)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.92 (s, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.38 (m, 6H), 7.13 (t, J = 7.4 Hz, 1H), 3.69 (m, 4H), 3.20 (s, 2H), 2.66 (m, 4H). MS (ESI): m/z 357.1 $[M]^+$ and 358.1 $[M + 1H]^+$.

2-(4-(2-methoxybenzoyl) piperazine-1-yl)-N-phenylacetamide (5f)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 8.92 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.26 (m, 2H), 7.18 (d, J = 8.0 Hz, 2H), 7.05 (t, J = 7.2 Hz, 1H), 6.93 (t, J = 8.0 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 3.83 (m, 2H), 3.78 (s, 3H), 3.27 (m, 2H), 3.11 (s, 2H), 2.64 (m, 2H), 2.47 (m, 2H). ESI MS: m/z = 354.2 $[M + 1 H]^+$.

2-(4-(4-methoxybenzoyl) piperazine-1-yl)-N-phenylacetamide (5g)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.98 (s, 1H), 7.56 (dd, J = 8.6, 1.2 Hz, 2H), 7.37 (m, 4H), 7.13 (m, 1H), 6.92 (m, 2H), 3.84 (s, 3H), 3.69 (m, 4H), 3.20 (s, 2H), 2.65 (m, 4H). ESI MS: m/z = 354.1 $[M + 1 H]^+$.

2-(4-(3-bromobenzoyl) piperazine-1-yl)-N-phenylacetamide (5h)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 8.91 (s, 1H), 7.59 (t, J = 7.2 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.21 (t, J = 8.0 Hz, 2H), 7.12 (m, 1H), 7.03 (m, 2H), 3.82 (m, 2H), 3.31 (m, 2H), 3.10 (s, 2H), 2.64 (m, 2H), 2.51 (m, 2H). ESI MS: m/z = 402.1 $[M + 1 H]^+$.

2-(4-(4-bromobenzoyl) piperazine-1-yl)-N-phenylacetamide (5i)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.94 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.37 (m, 2H), 7.27 (m, 2H), 7.15 (t, J = 8.0 Hz, 1H), 7.09 (m, 2H), 3.89 (m, 2H), 3.36 (m, 2H), 3.13 (s, 2H), 2.64 (m, 2H), 2.50 (m, 2H). ESI MS: m/z = 402.2 $[M + 1 H]^+$.

2-(4-(3-trifluoromethyl) piperazine-1-yl)-N-phenylacetamide (5j)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.84 (s, 1H), 7.63 (m, 2H), 7.51 (m, 4H), 7.28 (t, J = 8.0 Hz, 2H), 7.07 (t, J = 7.4 Hz, 1H), 3.64 (m, 4H), 3.14 (s, 2H), 2.61 (m, 4H). ESI MS: m/z = 392.1 $[M + 1H]^+$.

2-(4-(2-trifluoromethyl) piperazine-1-yl)-N-phenylacetamide (5k)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.55 (t, J = 7.2 Hz, 1H), 7.48 (m, 3H), 7.27 (m, 3H), 7.06 (m, 1H), 3.84 (m, 2H), 3.20 (m, 2H), 3.12 (s, 2H), 2.65 (m, 2H), 2.46 (m, 2H). ESI MS: m/z = 392.1 $[M + 1 H]^+$.

2-(4-(4-trifluoromethyl) piperazine-1-yl)-N-phenylacetamide (5l)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.84 (s, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 8.5 Hz, 4H), 7.26 (t, J = 8.0 Hz, 2H), 7.05 (t, J = 7.4 Hz, 1H), 3.80 (m, 4H), 3.41 (m, 2H), 3.12 (s, 2H), 2.58 (m, 2H). ESI MS: m/z = 392.2 $[M + 1 H]^+$.

2-(4-(3-ethoxybenzoyl) piperazine-1-yl)-N-phenylacetamide (5m)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 8.98 (s, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.15 (m, 2H), 6.92 (m, 1H), 6.90 (m, 1H) 6.87-691 (m, 1H), 6.67 (m, 1H), 6.56 (d, J = 8.0 Hz, 1H), 3.98 (q, J = 7.0 Hz, 2H), 3.62 (m, 2H), 3.41 (m, 4H), 3.20 (s, 2H), 2.46 (m, 2H),1.36 (t, J = 7.0 Hz, 3H). ESI MS: m/z = 368.1 $[M + 1 H]^+$.

2-(4-(4-ethoxybenzoyl) piperazine-1-yl)-N-phenylacetamide (5n)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.98 (s, 1H), 7.59 (m, 2H), 7.30 (m, 3H), 7.12 (m, 4H), 4.18 (q, J = 7.0 Hz, 2H), 3.93 (m, 2H), 3.40 (m, 2H), 3.21 (s, 2H), 2.74 (m, 2H), 2.54 (m, 2H), 1.40 (t, J = 7.0 Hz, 3H). ESI MS: m/z = 368.2 $[M + 1 H]^+$.

2-(4-(4-fluorobenzoyl) piperazine-1-yl)-N-phenylacetamide (5o)

White solid, ^{1}H NMR ($CDCl_3$) δ: 8.89 (s, 1H), 7.60 (d, J = 7.9 Hz, 2H), 7.34 (t, J = 7.2 Hz, 2H), 7.27 (t, J = 7.9 Hz,

2H), 7.15 (m, 1H), 7.05 (m, 2H), 3.84 (m, 4H), 3.11 (s, 2H), 2.66 (m, 2H), 2.53 (m, 2H). ESI MS: m/z = 342.2 [M + 1 H]$^+$.

2-(4-(2-chlorobenzoyl) piperazine-1-yl)-N-phenylacetamide (5p)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 9.75 (s, 1H), 7.62 (d, J = 7.4 Hz, 2H), 7.27 (m, 5H), 7.05 (t, J = 7.2 Hz, 2H), 3.64 (m, 4H), 3.18 (s, 2H), 2.54 (m, 4H). (ESI): m/z 357.0 [M]$^+$ and 358.1 [M + 1H]$^+$.

2-(4-(4-ethylbenzoyl) piperazine-1-yl)-N-phenylacetamide (5q)

Yellow solid, ^{1}H NMR ($CDCl_3$) δ: 8.98 (s, 1H), 7.56 (dd, J = 8.6, 1.2 Hz, 2H), 7.37 (m, 4H), 7.13 (m, 1H), 6.92 (m, 2H), 3.93 (m, 2H), 3.40 (m, 4H), 3.21 (s, 2H), 2.60 (m, 2H), 2.54 (q, J = 7.0 Hz, 2H), 1.42 (t, J = 7.0 Hz, 3H). ESI MS: m/z = 352.1 [M + 1 H]$^+$.

Enzyme assay

The inhibitory potency of each of the 17 novel molecules was analyzed by its ability to block the activity of FP-2. Active recombinant FP-2 was produced by following a protocol described by Shenai *et al.*[19] and Korde *et al.*[26] Fluorimetric assay for FP-2 activity was carried out as described previously by Kumar *et al.*[27] The release of fluorescence over time leads to the comparison of activities. Percentage inhibition was calculated based on dimethyl sulfoxide (DMSO) as control and is represented in Table 3.

RESULTS AND DISCUSSION

The reported FP-2 inhibitors [Figure 2][28-30] share three common elements as a pharmacophore. It consists of an aromatic group (monocyclic/bicyclic), which is attached to the hydrophobic moiety; commonly an aromatic residue through hydrogen bond donor (HBD) and HBA atom(s) acting as the linker. The distance between the aromatic residue and the hydrophobic group ranged 9-14 Å. The HBD and HBA atom(s) are present as in either heterocyclic/alicyclic or open-chain form.

The numbers of HBD and HBA atoms ranged 0-2 and 2-5, respectively. By considering these common features as pharmacophoric for FP-2 inhibitors, a pharmacophore model was constructed [Figure 3]. Based on this model, structurally novel FP-2 inhibitors were designed. The designed 2-(4-(substituted benzoyl) piperazine-1-yl)-*N*-phenylacetamide (5a-q) analogs were synthesized from the key intermediate N-phenyl-2-(piperazine-1-yl) acetamide (4) according to the reported method[5,6] The intermediate compound 4 was synthesized on a large scale from the starting material aniline in a sequence of reactions, depicted in Scheme 1.

First, chloroacetyl chloride was subjected to nucleophilic substitution reaction with aniline to mask the chloro functionality, which yielded compound 2. This intermediate was reacted with *N*-boc-protected piperazine to obtain compound 3, Subsequently, deprotection of intermediate 3 with trifluoroacetic acid yielded the intermediate *N*-phenyl-2-(piperazine-1-yl) acetamide (4). The resultant compound was coupled with the appropriate carboxylic acid in the presence of coupling agents 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBt) under nitrogen atmosphere to yield the final derivatives (5a-q). The synthesized compounds were isolated as pure and characterized by ^{1}H NMR, mass, and HPLC analysis data. The analytical and spectral data of the compounds are found to be in compliance with the structure of the synthesized compounds. All new chemical entities were evaluated for their FP-2 inhibition.

Overall, using the traditional pathway of a drug design tool by connecting two hydrophobic groups with a simple linker, we synthesized a series of molecules and screened these against the cysteine protease FP-2. The screening results are summarized in Table 3.

Table 3: Percentage inhibition values of final synthesized compounds (5a-q)[C']

Compound	R	Inhibition rate at 10 μm %	Compound	R	Inhibition rate at 10 μm %
5a	*m*-F	No inhibition	5j	*m*-CF_3	No inhibition
5b	*p*-CH_3	No inhibition	5k	*o*-CF_3	No inhibition
5c	*m*-Cl	No inhibition	5l	*p*-CF_3	3.0
5d	*o*-CH_3	No inhibition	5m	*m*-OCH_2CH_3	No inhibition
5e	*p*-Cl	No inhibition	5n	*p*-OCH_2CH_3	No inhibition
5f	*o*-OCH_3	No inhibition	5o	*p*-F	No inhibition
5g	*p*-OCH_3	No inhibition	5p	*o*-Cl	No inhibition
5h	*m*-Br	No inhibition	5q	*p*-CH_2CH_3	5.0
5i	*p*-Br	No inhibition	E-64		97.4

C' Data are mean values of three independent experiments and the deviations are <10% of the average value

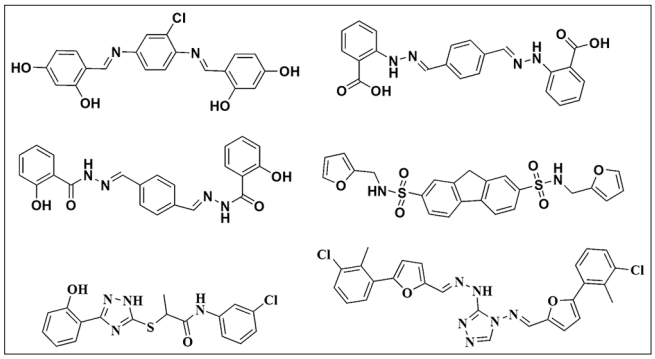


Figure 2: (A-G) Structures of some reported FP-2 inhibitors

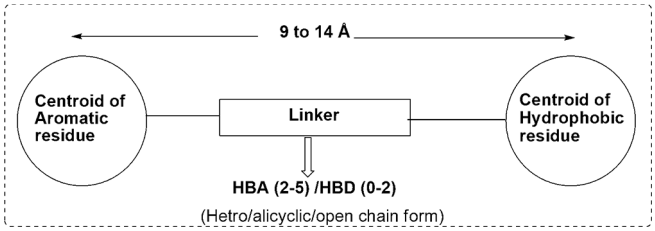
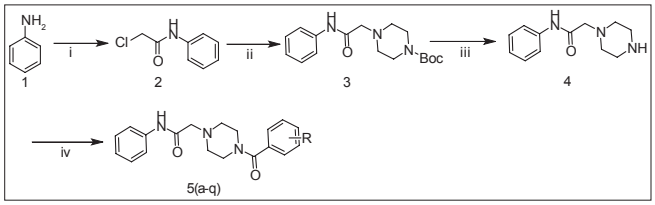


Figure 3: Pharmacophoric features necessary for FP-2 inhibitor



Scheme 1: Synthetic route of 2-(4-(substituted benzoyl)piperazine-1-yl)-*N*-phenylacetamide derivatives (5a-q). Reagents and conditions: i) Chloroacetylchloride, K_2CO_3, DCM, 0°C-RT, 20 min; ii) *N*-Boc-piperazine, K_2CO_3, CH_3CN, 90°C, 4 h, iii) TFA/DCM, 0°C-RT, 16 h; iv) EDC.HCL, HOBT/TEA-DCM, RT, 6 h

CONCLUSION

In summation, 2-(4-(substituted benzoyl) piperazine-1-yl)-*N*-phenylacetamide derivatives have been designed, synthesized, and screened for FP-2 inhibition. The structures of the compounds were assigned on the basis of ^{1}H NMR, and mass spectral data. All the analytical data correspond to their respective compounds. From the results, as presented in Table 3, on close perlustration and analysis, it was found that none of the synthesized compounds based on the piperazine linker showed any significant antimalarial activity owing to insignificant binding. Hence, further detailed investigation will be necessary, using the modern drug design tools to find out the exact reason for the insignificant antimalarial activity of the synthesized compounds, as well as to generate new analogs having potent FP-2 inhibitory activity.

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