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Structure-Based Approach to Falcipain-2 Inhibitors: Synthesis and Biological Evaluation of 1,6,7-Trisubstituted Dihydroisoquinolines and Isoquinolines

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Abstract—1,4,7-Trisubstituted isoquinolines were designed, synthesized and evaluated for their inhibition against *Plasmodium falciparum* cysteine protease falcipain-2. The 1-benzyloxyphenyl-dihydroisoquinoline and -isoquinoline derivatives were found to exhibit better activity against falcipain-2 than their corresponding 1-hydroxyphenyl or 1-methoxyphenyl analogues. The docking scores correlate with the IC_{50} values of compounds and give a high coefficient correlation of 0.94.

Introduction

Malaria remains to be one of the most deadly parasitic diseases affecting 300 million people worldwide and leading to more than 2 million deaths per year.[1] The management of continuing upsurge of malaria is further complicated due to widespread resistance of the parasite to common antimalarials and cross resistance to structurally unrelated drugs.[2–4] To prevent resistance against the new drugs, artemisinin and its analogues are restricted to the institutional use in the malaria-prone areas. In addition to overcome resistance, usage of combination therapy[5] and development of resistance modulators[6,7] have also been attempted. Recently, the feasibility of generating populations of transgenic mosquitoes that have diminished potential to carry and transmit parasite[8] has been explored. Therefore, chemotherapy of malaria remains a challenge to medicinal chemists. Extensive research to identify newer targets for chemotherapy has led to the identification of protease inhibitors as an attractive alternative.[9–15] The acidic food vacuole within the parasite contains two major peptide hydrolases (proteases), namely aspartic and cysteine proteases along with other enzymes. These proteases are considered to be responsible for an ordered hemoglobin degradation that produce essential amino acids required for parasite survival.[16–19] Therefore, the disruption of the hemoglobin degradation pathway in the malaria parasite seems to be a logical approach to antimalarial chemotherapy.[20] Though the various steps in the degradation pathway can be targeted individually, there are several reports indicating that inhibition of the plasmepsins (aspartic protease) and falcipains (cysteine vacuolar proteases) could prove lethal to the parasite.[21–27] In our endeavors toward development of antimalarials following a structure-based design approach against the cysteine proteases we have recently reported the homology modeling of falcipain-2.[28] This model was built with great accuracy and validated by docking studies of the known peptidyl vinyl sulfone inhibitors. Based on this three-dimensional model, a new non-peptide inhibitor having isoquinoline motif (Fig. 1) was designed, synthesized and assayed for inhibition of falcipain-2. In our studies interaction of 1-(4-hydroxy-phenyl) group with the Asp 234 in the S-2

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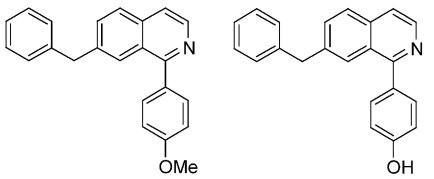


IC_{50}= 10μM IC_{50}= 8μM

Figure 1. Structures of reported isoquinolines active at micromolar concentration.[28]

pocket[29] was considered essential, therefore it was decided to synthesize and evaluate simpler 1,6,7-trisubstituted isoquinoline analogue against falcipain-2 as prelude to discover new antimalarial agents. The details of this study are presented herein.

Chemistry

The synthetic strategy adopted to obtain various isoquinoline derivatives is described in Scheme 1. The substituted benzaldehydes (**1a–e**) used as starting substrates were converted to corresponding nitroalkenes (**2a–e**) via Henry reaction under reported conditions.[30] These nitroalkenes (**2a–e**) were then reduced to benzeneethanamines (**3a–e**) in the presence of $LiAlH_4$. These amines (**3b–c**, **e**) were converted to amides (**4b–c**, **e**) using *p*-anisoyl chloride followed by cyclization in the presence of phosphorous oxychloride to afford 3, 4-dihydro-isoquinolines (**6b–c**). Interestingly the cyclizations to dihydroisoquinolines depend on the substitution on phenyl ring (Table 1) as 4-bromo derivative failed to cyclize (entry 4). The 3,4-dihydoisoquinolines were then subjected to dehydrogenation in the presence of palladium–carbon to furnish the isoquinoline analogues

Table 1. Effect of substituent on the cyclization of amides to dihydroiso-quinolines

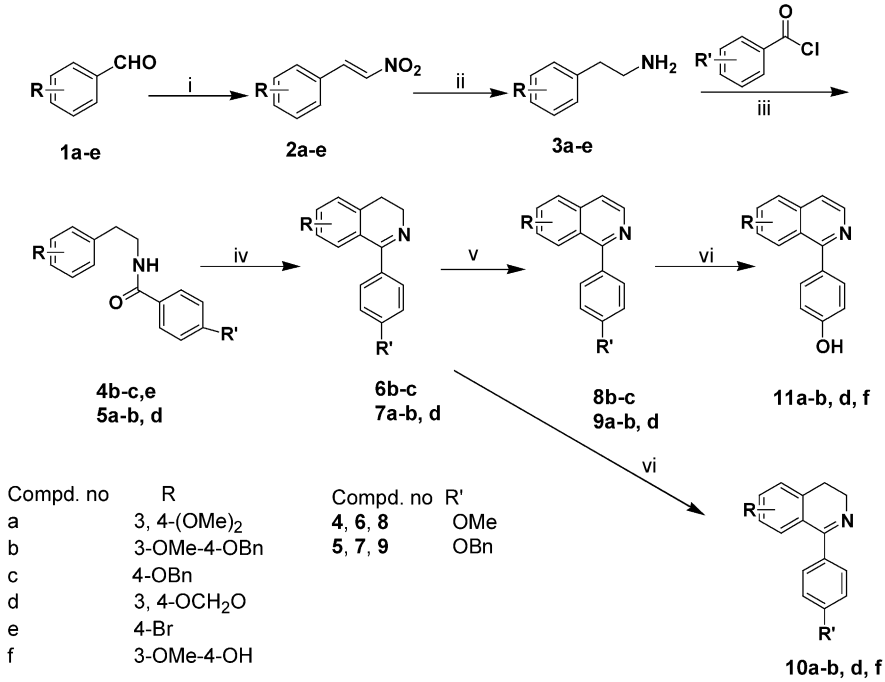
Entry	R	R′	Time	Yield (%)
1	3,4-$(OMe)_2$	OMe	1 h	85
2	3-OMe-4-OBn	OMe	3 h	68
3	4-OBn	OMe	27 h	30
4[a]	4-Br	OMe	No reaction	—
5[28]	4-Bn	OMe	3 days	25
6	3,4-$(OMe)_2$	OBn	1.5 h	72
7	3-OMe-4-OBn	OBn	16 h	26
8h	3,4-OCH_2O	OBn	8	43

[a]No data since it did not gave the corresponding dihydroisoquinoline.

(**8b–c**). These isoquinolines could not be regioselectively demethylated at position 4 of the phenyl ring present at position 1 of isoquinolines as any such attempt led to polymeric material. Therefore, in an improved synthetic methodology instead of *p*-anisoyl chloride, *p*-benzyloxy benzoyl chloride was used to obtain the ethanamides **5a–b**, **d** followed by cyclization to furnish the dihydroisoquinolines (**7a–b**, **d**). These were then dehydrogenated to afford the quinolines (**9a–b**, **d**) (Table 2). The deprotection of the benzyl group in the dihydroisoquinolines **7a** led to **10a** while similar deprotection in isoquinolines **9a**, **d** yielded the corresponding hydroxy derivatives **11a**, **d** in good yields. However deprotection in **9b** resulted in mixture of **11b** and **11f**. Thus, the hydroxy quinolines obtained herein were in better yields than that obtained through deprotection of methoxy group.[28]

Results and Discussion

The dihydroisoquinoline and isoquinoline analogues synthesized were subjected to in vitro evaluation for



Scheme 1. Reagents and conditions; (i) CH_3NO_2, NH_4OAc, AcOH, ultrasound, 3 h; (ii) $LiAlH_4$, dry THF, 65 °C, 1.5 h; (iii) dry DCM, 6 h, rt; (iv) $POCl_3$, dry toluene; (v) 10% Pd/C, dry decalin; (vi) 10% Pd/C, H_2, 30 psi, 8–12 h.

Table 2. Effect of substituents on the dehydrogenation of dihydroisoquinolines to quinolines

Entry	R	R′	Time (h)	Yield (%)
1	3,4-$(OMe)_2$	OMe	3	57
2	3-OMe-4-OBn	OMe	4.5	48
3	4-OBn	OMe	15	39
4[28]	4-Bn	OMe	16	35
5	3,4-$(OMe)_2$	OBn	30	78
6	3-OMe-4-OBn	OBn	38	76
7	3,4-OCH_2O	OBn	36	72

inhibition of protease activity. The results of biological activity are summarized in Table 3. The activities of these new analogues were comparable to that of the previous compounds (Fig. 1) with a few analogues showing better potency.

The docking scores for these compounds were calculated as described in the experimental section and are summarized in Table 3. From the graph between the log IC_{50} values and docking scores, it is evident that docking scores indeed correlate to the IC_{50} values of the compounds, giving a high coefficient correlation of 0.94 (Fig. 3). This trend was analogous with that of vinyl sulfone inhibitors, which were originally used to validate our homology model.[28] From results of the activity it could be speculated that substitutions on 6, 7 positions of the isoquinoline does not play a significant role toward the activity. All compounds with *p*-methoxy phenyl substitution at position 1 of the isoquinoline were almost equipotent as the earlier reported ligands.[28] On the other hand compounds with *p*-benzyloxy phenyl group were found to be more potent as compared to

Table 3. In vitro activity against Falcipain-2 along with the docking scores in the homology model

Compd	Compd		IC_{50} (μM)	Docking scores (kcal/mol)
	R	R'		
6b	3-OMe-4-OBn	OMe	10	−200
6c	4-OBn	OMe	10	−233
7a	3,4-$(OMe)_2$	OBn	10	−216
7b	3-OMe-4-OBn	OBn	4	−328
7d	3,4-OCH_2O	OBn	3	−406
8b	3-OMe-4-OBn	OMe	10	−178
9a	3, 4-$(OMe)_2$	OBn	3	−401
9b	3-OMe-4-OBn	OBn	3	−399
9d	3,4-OCH_2O	OBn	3	−408
10b	3-OMe-4-OBn	OH	NS	
11a	3,4-$(OMe)_2$	OH	10	−176
11a	3,4-$(OMe)_2$	OH	10	−176
11b	3-OMe-4-OBn	OH	8	−298
11f	3-OMe-4-OH	OH	10	−244
11d	3,4-OCH_2O	OH	NS	

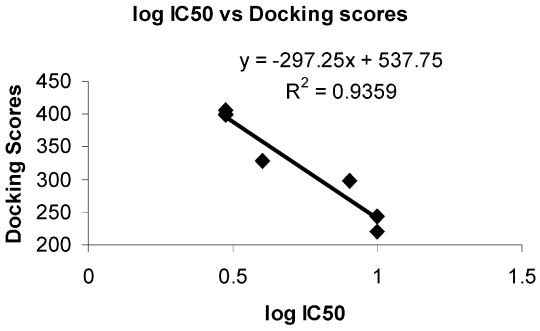


Figure 3. Correlation between log IC_{50} versus docking scores.

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 FALCI ( ---> )                                                                                                  ( ---> )
P_1AIM ( ---> )                                                                                                  ( ---> )
P_1BY8 (    A5) EILDTHWELWKKTHRKQYNNKVDEISRRLIWEKNLKYISIHNLEASLGVHTYELAMNHLGDMTS (A68   )
P_1EWP ( ---> )                                                                                                  ( ---> )
P_1MEG ( ---> )                                                                                                  ( ---> )
P_1PPO ( ---> )                                                                                                  ( ---> )

 FALCI (     1)                                          AYDWRLHSGVTPVKDQKNCGSCWAFSSIGS (30    )
P_1AIM (     1)                                    APAAVDWRARGAVTAVKDQGQCGSCWAFSAIGN (33    )
P_1BY8 (   A69) EEVVQKMTGLKVPLSHSRSNDTLYIPEWEGRAPDSVDYRKKGYVTPVKNQGQCGSCWAFSSVGA (A132  )
P_1EWP (    A1)                                    APAAVDWRARGAVTAVKDQGQCGSCWAFSAIGN (A33   )
P_1MEG (     1)                                    LPENVDWRKKGAVTPVRHQGSCGSCWAFSAVAT (33    )
P_1PPO (     1)                                    LPENVDWRKKGAVTPVRHQGSCGSCWAFSAVAT (33    )

 FALCI (    31) VESQYAIRKNKLITLSEQELVDCSFKNYGCNGGLINNAFEDMIELGGICPDGDYPYVSDIAPNL (93    )
P_1AIM (    34) VECQWFLAGHPLTNLSEQMLVSCDKTDSGCSGGLMNNAFEWIVQENNGAVYTEDSYPYASGEGI (91    )
P_1BY8 (  A133) LEGQLKKKTGKLLNLSPQNLVDCVSENDGCGGGYMTNAFQYVQKN--RGIDSEDAYPYVGQ--E (A192  )
P_1EWP (   A34) VECQWFLAGHPLTNLSEQMLVSCDKTDSGCSGGLMNNAFEWIVQENNGAVYTEDSYPYASGEGI (A91   )
P_1MEG (    34) VEGINKIRTGKLVELSEQELVDCERRSHGCKGGYPPYALEYVAKN---GIHLRSKYPYKAKQGT (94    )
P_1PPO (    34) VEGINKIRTGKLVELSEQELVDCERRSHGCKGGYPPYALEYVAKN---GIHLRSKYPYKAKQGT (94    )

 FALCI (    94) CNIDRCTEKYGIKNYLSVPDNKLKEALRFLGPISISVA|VSDDFAFYKEGIFDGECGDQLNHAV (156   )
P_1AIM (    92) SPPCTTSGHTVGATITGHVELPQDEAQIAAWLAVNGPVAVAVD----ASSWMTYTGGVMTSCVS (155   )
P_1BY8 (  A193) ESCMYNPTGKAAKCRGYREIPEGNEKALKRAVARVGPVSVAIDASLTSFQFYSKGVYYDESCNS (A256  )
P_1EWP (   A92) SPPCTTSGHTVGATITGHVELPQDEAQIAAWLAVNGPVAVAVD----ASSWMTYTGGVMTSCVS (A155  )
P_1MEG (    95) CRAKQVGGPIVKTSGVGRVQ-PNNEGNL-LNAIAKQPVSVVVESKGRPFQL--YKGGIFEGPCG (154   )
P_1PPO (    95) CRAKQVGGPIVKTSGVGRVQ-PNNEGNL-LNAIAKQPVSVVVESKGRPFQL--YKGGIFEGPCG (154   )

 FALCI (   157) MLVGFGMKEIVNPLTKKGEKHYYYIIKNSWGQQWGERGFINIETDESGLMRKCGLGTDAFIPLI (220   )
P_1AIM (   156) EALDHGVLLVGYND----------SAAVPYWIIKNSWTTQWGEEGYIRIAKGSNQCLVKEEASS (208   )
P_1BY8 (  A257) DNLNHAVLAVGYGI----------QKGNKHWIIKNSWGENWGNKGYILMARNK---NNACGIAN (A307  )
P_1EWP (  A156) EQLDHGVLLVGYND----------SAAVPYWIIKNSWTTQWGEEGYIRIAKGSNQCLVKEEASS (A208  )
P_1MEG (   155) TKVEHAVTAVGYGK----------SGGKGYILIKNSWGTAWGEKGYIRIKRAPGNSPGVCGLYK (208   )
P_1PPO (   155) TKVDHAVTAVGYGK----------SGGKGYILIKNSWGTAWGEKGYIRIKRAPGNSPGVCGLYK (208   )

 FALCI (   221) E                                                                                                (221   )
P_1AIM (   209) AVVG                                                                                             (212   )
P_1BY8 (  A308) LASFPKM                                                                                          (A314  )
P_1EWP (  A209) AVVGL                                                                                            (B281  )
P_1MEG (   209) SSYYPTKN                                                                                         (216   )
P_1PPO (   209) SSYYPTKN                                                                                         (216   )
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Figure 2. Sequences having significant scores.

their corresponding hydroxyl analogues suggesting that S-2 pocket prefer to accommodate hydrophobic groups. Also the planarity of the isoquinolines is not important since both the dihydroisoquinoline and isoquinoline were found to have almost similar IC_{50} values. The narrow range of IC_{50} of all compounds described herein may be from the fact that all these inhibitors share common skeleton with little variation on their side chains. On the basis of these results the new ligand having benzyloxy phenyl group at position 1 and 6, 7 disubstitution in the isoquinoline moiety was docked into the active pocket of the falcipain homology model (Figs 4 and 5). The results from the assay of these compounds could be used to further gain insight of the nature of binding interactions they have with the enzyme, which may promote the development of better inhibitors. Future efforts will focus on the synthesis and evaluation of other heterocyclic derivatives with similar structural framework with an objective to have better inhibition against falcipain-2 correlating to the antimalarial activity.

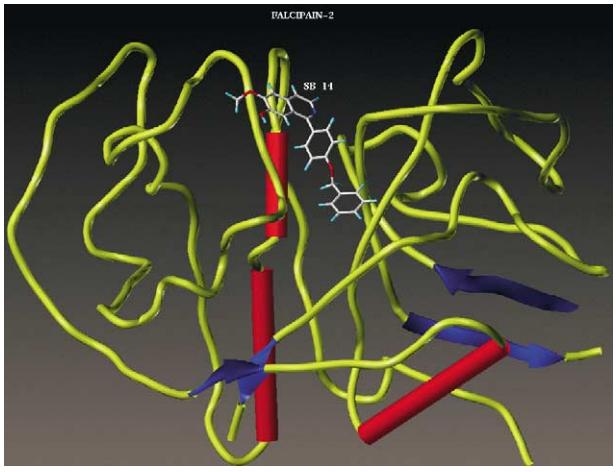

Figure 4. 1,4,7-Substituted isoquinoline docked in the active pocket.

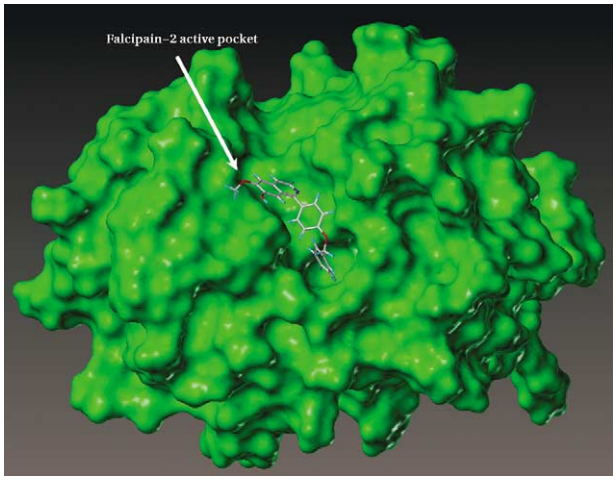

Figure 5. The falcipain-2 active pocket with OBn group in the S-2 subsite.

Experimental

Melting points were determined in capillary tubes on a hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 400 FT spectrometer, using TMS as an internal reference; chemical shifts values are in δ ppm and *J* values are in Hz. Mass spectra were recorded on a Waters-Micromass LCMS system using direct flow injections. Reactions were run in oven-dried glassware and dried solvents that were prepared by distillation over calcium hydride.

Nitroalkenes (2a–e). A mixture of appropriately substituted benzaldehyde (10 mmol) ammonium acetate (1.76 g, 22 mmol), nitromethane (7.0 mL, 100 mmol), acetic acid (1.7 mL) was sonicated for 3 h at room temperature. The separated solid was filtered off and washed with ethanol–water (50:50) mixture and air dried to obtain the product in 76–88% yields.

Benzeneethanamines (3a–e). To the stirred suspension of $LiAlH_4$ (470 mg, 12.2 mmol) in THF (3 mL) was added dropwise, a solution of appropriately substituted nitroalkene (3.5 mmol) in THF (25 mL) and the reaction mixture was stirred at 65 °C for 6 h. It was then cooled down to 0 °C and decomposed with 30% aq sodium hydroxide solution. To this solution ethyl acetate was added and the mixture was stirred for another 30 min. The organic layer was separated, dried over sodium sulfate and evaporated to obtain pure amines as oils in 70–82% yields. These amines were used for further reaction without any purification.

Benzeneethanamides (4a–c, e and 5a–b, d). To the stirred solution of amine (6.8mmol) in dry dichloromethane (15 mL) was added freshly baked potassium carbonate (2.25 g, 13.6 mmol) and the reaction mixture was cooled to 0 °C. A solution of acid chloride (6.8 mmol) in dichloromethane (15 mL) was added dropwise and the reaction was continued to stir at room temperature for 8 h. Thereafter the reaction mixture was extracted with water and dichloromethane. The organic layer was separated, washed with brine dried over sodium sulfate and evaporated to obtain a residue. This upon column chromatography over silica gel using dichloromethane–methanol (99:1) furnished the pure product.

Dihydroisoquinolines (6b–c, 7a–b, d). To an appropriate solution of substituted ethanamide (1.3 mmol) in toluene (10 mL) was added phosphorous oxychloride (3.37 mL, 3.62 mmol) and the reaction mixture was refluxed under stirring at 110 °C as per the time indicated in Table 1. The reaction was cooled down and was poured onto ice and decomposed using 20% aq sodium hydroxide solution. It was then extracted with ethyl acetate (2×50 mL) and water. The organic layers were combined, dried over sodium sulfate and evaporated to obtain a residue. This residue upon column chromatography over silica gel employing dichloromethane–methanol (98:2) furnishes the pure product.

1-(4-Methoxyphenyl)-6-methoxy-7-benzyloxy-3, 4-dihydroisoquinoline (6b). Mp 188–189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 3.06 (t, 2H, J = 6.0 Hz, –CH_2), 3.91 (s, 3H, OMe), 3.95 (m, 2H, J = 6.0 Hz, –NCH_2), 4.06 (s, 3H, OMe), 5.09 (s, 2H, –OCH_2), 6.95 (s, 3H, Ar–H), 7.30 (s, 5H, Ar–H), 7.51, 7.53(d, 2H, J = 8.0 Hz, Ar–H); mass (ESMS+) 374.17 (calcd), 374.25 (found).

1-(4-Methoxyphenyl)-7-benzyloxy-3, 4-dihydroisoquinoline (6c). Mp 98–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 2.71(t, 2H, J = 6.0 Hz, –CH_2), 3.79 (m, 2H, J = 6.0 Hz, –NCH_2), 3.86 (s, 3H, OMe), 4.99 (s, 2H,–OCH_2), 6.90, 6.92(d, 2H, J = 8.0 Hz, Ar–H), 7.01, 7.03 (d, 1H, J = 8.0 Hz, Ar–H), 7.17, 7.19 (d, 1H, J = 8.0 Hz, Ar–H), 7.34 (s, 5H, Ar–H), 7.51, 7.53 (d, 2H, J = 8.0 Hz, Ar–H); mass (ESMS+) 356.16 (calcd), 356.00 (found).

1-(4-Methoxyphenyl)-6-methoxy-7-hydroxy-3, 4-dihydroisoquinoline (6f). Mp 206–207 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 2.71 (t, 2H, J = 6.0 Hz, –CH_2), 3.78 (t, 3H, J = 6.0 Hz, –NCH_2), 3.85 (s, 3H, OMe), 4.02 (s, 3H, OMe), 6.76 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 6.92, 6.94 (d, 2H, J = 8.0 Hz, Ar–H), 7.54, 7.56 (d, 2H, J = 8.0 Hz, Ar–H); mass (ESMS+) 284.12 (calcd), 284.13 (found).

1-(4-Benzyloxyphenyl)-6, 7-dimethoxy-3, 4-dihydroisoquinoline (7a). Mp 147–149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 2.71 (t, 2H, J = 7.2 Hz,–CH_2), 3.76 (m, 5H, –NCH_2 and OMe), 3.95 (s, 3H, OMe), 5.12 (s, 2H, –OCH_2), 6.78 (s, 1H, Ar–H), 6.84 (s, 1H, Ar–H), 7.02, 7.04 (d, 2H, J = 8.0 Hz, Ar–H), 7.39 (m, 5H, Ar–H), 7.56, 7.58 (d, 2H, J = 8.0 Hz, Ar–H), 8.39, 8.40 (d, 1H, J = 5.6 Hz, Ar–H); mass (ESMS+) 374.17 (calcd), 374.38 (found).

1-(4-Benzyloxyphenyl)-6-methoxy-7-benzyloxy-3, 4-dihydroisoquinoline (7b). Mp oil; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 2.68 (t, 2H, J = 7.2 Hz,–CH_2), 3.75 (t, 2H, J = 7.2 Hz, –NCH_2), 3.96 (s, 3H, OMe), 5.03 (s, 2H, –OCH_2), 5.13 (s, 2H, –OCH_2), 6.78 (s, 1H, Ar–H), 6.83 (s, 1H, Ar–H), 6.91, 6.93 (d, 2H, J = 8.0 Hz, Ar–H), 7.36 (m, 10H, Ar–H), 7.46, 7.48 (d, 2H, J = 8.0 Hz, Ar–H), 8.39, 8.40 (d, 1H, J = 5.6 Hz, Ar–H); mass (ESMS+) 450.20 (calcd), 450.45 (found).

1-(4-Benzyloxyphenyl)-6, 7-methylenedioxy-isoquinoline (7d). Mp 175–176 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 2.91 (t, 2H, J = 6.0 Hz, –CH_2), 3.83 (t, 2H, J = 6.0 Hz, –NCH_2), 5.18 (s, 2H, –OCH_2), 5.88 (s, 2H, OCH_2O), 6.58 (s, 1H, Ar–H), 6.93 (s, 1H, Ar–H), 7.01, 7.03 (d, 2H, J = 8.0 Hz, Ar–H), 7.34 (s, 5H, Ar–H), 7.74, 7.76 (d, 2H, J = 8.0 Hz, Ar–H); Mass (ESMS+) 358.40 (calcd), 358.29 (found).

Isoquinolines (8b–c and 9a–b, d). A mixture of appropriate dihydroisoquinoline (0.3 g) and 10% palladium on carbon in 2 mL of decalin was heated at 230 °C under stirring for a period of time as indicated in Table 2. To the reaction mixture dichloromethane was added and the catalyst was filtered off over Celite. The filtrate was evaporated and subjected to column chromatography over silica gel. Elution with dichloromethane–methanol (98:2) furnished the pure product.

1-(4-Methoxyphenyl)-6-methoxy-7-benzyloxy-isoquinoline (8b). Mp 167–168 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 3.90, 4.05 (2s, 6H, 2×OMe), 5.06 (s, 2H, –OCH_2), 6.96, 6.98 (d, 2H, J = 8.0 Hz, Ar–H), 7.37 (m, 7H, Ar–H), 7.43, 7.45 (d, 1H, J = 8.0 Hz, Ar–H), 8.41, 8.42 (d, 1H, J = 6.0 Hz, Ar–H); mass (ESMS+) 372.11 (calcd), 372.27 (found).

1-(4-Methoxyphenyl)-6-methoxy-7-hydroxy-isoquinoline (8e). Mp 100–102 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 3.86, 4.06 (2s, 6H, 2×OMe), 7.01, 7.03 (d, 2H, J = 8.0 Hz, Ar–H), 7.12 (s, 1H, Ar–H), 7.45, 7.47 (d, 1H, J = 6.0 Hz, Ar–H), 7.51 (s, 1H, Ar–H), 7.58, 7.60 (d, 2H, J = 8 Hz, Ar–H), 8.40, 8.42 (d, 1H, J = 8.0 Hz, Ar–H); mass (ESMS+) 282.11 (calcd), 282.19 (found).

1-(4-Benzyloxyphenyl)-6, 7-dimethoxy-isoquinoline (9a). Mp oil; ^{1}H NMR ($CDCl_3$, 400 MHz): 3.88 (s, 3H, OMe), 4.05 (s, 3H, OMe), 5.17 (s, 2H, –OCH_2), 7.14 (m, 4H, Ar–H), 7.43 (m, 5H, Ar–H), 7.65, 7.67 (d, 2H, J = 8.0 Hz, Ar–H), 8.44, 8.46 (d, 1H, J = 8.0 Hz, Ar–H); mass (ESMS+) 372.15 (calcd), 372.23 (found).

1-(4-Benzyloxyphenyl)-6-methoxy-7-benzyloxy-isoquinoline (9b). Mp oil; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 4.05 (s, 3H, OMe), 5.18 (s, 4H, OCH_2O), 7.03, 7.05 (d, 2H, J = 8.0 Hz, Ar–H), 7.12 (s, 1H, Ar–H), 7.34 (m, 12H, Ar–H), 7.49, 7.51 (d, 2H, J = 8.0 Hz, Ar–H), 8.41, 8.42 (d, 1H, J = 6.0 Hz, Ar–H); mass (ESMS+) 448.19 (calcd), 448.27 (found).

1-(4-Benzyloxyphenyl)-6, 7-methylenedioxy-isoquinoline (9d). Mp 96–97 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 5.18 (s, 2H, –OCH_2), 5.83 (s, 2H, OCH_2O), 6.87, 6.89 (d, 2H, J = 8.0 Hz, Ar–H), 7.02 (m, 3H, Ar–H), 7.21 (s, 5H, Ar–H), 7.68, 7.70 (d, 2H, J = 8.0 Hz, Ar–H), 8.31, 8.32 (d, 1H, J = 6.0 Hz, Ar–H); mass (ESMS+) 356.38 (calcd), 356.57 (found).

Debenzylation (10a–b, d, f and 11a–b, d, f). A mixture of appropriate compound (0.1 g) in methanol (5 mL) and 10% palladium (20 mg) on carbon was hydrogenated at 40 psi for 8–12 h. The catalyst was filtered off through a bed of Celite and the filtrate was evaporated to obtain a residue. This residue upon column chromatography over silica gel using dichloromethane: methanol (95:5) furnished the pure product in 90–95% yields.

1-(4-Hydroxyphenyl)-6, 7-dimethoxy-3, 4-dihydroisoquinoline (10a). Mp 120–121 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 2.79 (t, 2H, J = 7.2 Hz,–CH_2–), 3.64 (s, 3H, OMe), 3.76 (m, 3H, –NCH_2), 3.96 (s, 3H, OMe), 6.64, 6.66 (d, 2H, J = 8.0 Hz, Ar–H), 6.78 (s, 1H, Ar–H), 6.86 (s, 1H, Ar–H), 7.35, 7.37 (d, 2H, J = 8.0 Hz, Ar–H); Mass (ESMS+) 284.12 (calcd), 284.35 (found).

1-(4-Hydroxyphenyl)-6, 7-dimethoxy-isoquinoline (11–a). Mp 212–214 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 3.87 (s, 3H, OMe), 4.05 (s, 3H, OMe), 6.78, 6.80 (d, 2H, J = 8.0 Hz, Ar–H), 7.13 (s, 1H, Ar–H), 7.36 (s, 1H, Ar–H), 7.37, 7.39 (d, 2H, J = 8.0 Hz, Ar–H),

7.51, 7.52 (d, 2H, $J=5.2$ Hz, Ar–H), 8.41, 8.42 (d, 2H, $J=5.2$ Hz, Ar–H); Mass (ESMS+) 282.11 (calcd), 282.11 (found).

1-(4-Hydroxyphenyl)-6-methoxy-7-benzyloxy-isoquinoline (11b). Mp 212–214 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 4.06 (s, 3H, OMe), 5.16 (s, 2H, $-OCH_2$), 6.65, 6.67 (d, 2H, $J=8.0$ Hz, Ar–H), 7.13 (s, 1H, Ar–H), 7.32 (m, 5H, Ar–H), 7.49, 7.51 (d, 2H, $J=5.6$ Hz, Ar–H), 8.39, 8.40 (d, 1H, $J=5.6$ Hz, Ar–H); mass (ESMS+) 358.14 (calcd), 358.31 (found).

1-(4-Hydroxyphenyl)-6, 7-methylenedioxy-isoquinoline (11d). Mp 66–67 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 5.88 (s, 2H, OCH_2O), 6.69, 6.71 (d, 2H, $J=8.0$ Hz, Ar–H), 7.03 (m, 3H, Ar–H), 7.66, 7.68 (d, 2H, $J=8.0$ Hz, Ar–H), 8.40, 8.41 (d, 1H, $J=5.6$ Hz, Ar–H); mass (ESMS+) 266.07 (calcd), 266.09 (found).

1-(4-hydroxyphenyl)-6-methoxy-7-hydroxy-isoquinoline (11f). Mp 175–176 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): 3.93 (s, 3H, OMe), 6.82, 6.84 (d, 2H, $J=8$ Hz, Ar–H), 6.99 (s, 1H, Ar–H), 7.31, 7.33 (d, 2H, $J=5.6$ Hz, Ar–H), 7.40 (m, 3H, Ar–H), 8.24, 8.25 (d, 2H, $J=5.6$ Hz, Ar–H); mass (ESMS+) 268.09 (calcd), 268.08 (found).

Computational method

Construction of homology model, docking, molecular dynamics and energy minimization studies were performed on a Silicon Graphics Octane 2 workstation with dual R12000 processors. Homology modeling was performed within the COMPOSER module of SYBYL 6.7 (Tripos Asso. Inc. St. Louis, MO, USA). The model was theoretically validated by the PROTABLE module of BIOPLOYMER. DOCK 4.0[31] was employed for docking the designed inhibitors into the active pocket of falcipain-2.

Homology modeling

The falcipain-2 sequence was obtained from SWISS-PROT and TrEMBL databases of ExPASy Molecular Biology Server.[32] Only the mature sequence (Q9N6S8) was considered for deriving the homology model. A WU-BLAST 2.0 search was performed on the mature falcipain-2 sequence with default parameters of BLASTP gapped alignment. All the homologous proteins identified by this search (a total of 13 sequences), belonged to the cysteine protease family. The sources for these homologs included *Homo sapiens*, *Carica papaya*, *Trypanosoma cruzi* and *Leishmania major*.

The model sequence was aligned against each sequence in the database using the reported method of Needleman and Wunsch.[33] Gaps were inserted into either sequence to find an optimal alignment. A significance score in the range of 19.8–27.6 was obtained for the query sequence. Only those sequences having a significance score of 25 or more were considered for structure alignment (Fig. 2). The resulting alignment was manually refined to optimize the matching of several characteristics including (a) conserved protease hydrophobic side chains and buried positions in the template structures, (b) functionally important sites such as the active site cysteine, and (c) insertions/deletions.

Five SCRs were conceived based on this alignment and are shown in Fig. 2. Cruzain, having an overall percent identity of 55% and more than 90% in the SCRs with falcipain-2 (Fig. 1), was chosen to derive coordinates for building the SCRs of falcipain-2.

The last step in the generation of the 3-D model was building the structurally variable regions (SVRs) or the loops onto the SCRs. The Tweak Loop approach[34,35] was used to build loops onto the SCRs. The loop residue torsion angles were then manually adjusted to achieve an exact fit to the model anchor coordinates.

Further refinement of the model was performed using Kollman charges[36] by first minimizing the backbone atoms to a gradient of 0.01 Å followed by complete side-chain minimization. All molecular dynamic (MD) simulations assisted by simulated annealing (SA) were performed at a constant temperature of 300 K. A time step of 1 fs (10^{-15}) for a total length of 1000 fs, with a snapshot at every 5 fs, a long non-bonding cutoff distance of 12 Å, and a distance dependent dielectric of $\varepsilon=4$ were used. The non-bonding pair list was updated every 25 fs. In SA, the protein was heated at 700 K for 10,000 fs and annealed to 200 K for 10,000 fs for a total of 10 cycles. The different conformations generated by MD-SA were further minimized and the structure with lowest energy was validated using the PROTABLE and was then used for docking studies.

Docking studies/protocol

In order to further validate the falcipain-2 model, the designed inhibitors (Table 3) were docked into the active site of falcipain-2. MD-SA was performed on these inhibitors using a protocol similar to the one described for protein refinement above, except that the snapshots were taken at every 25 fs and length of simulation was 5000 fs. This resulted in 20 conformations for each of the inhibitor, which were then subjected to rigid docking. For docking, a Connolly surface of the protein's active site (all residues within a radius 5 Å around Cys 42) was created using a 1.4 Å probe radius and further used to generate a cluster of 46 overlapping spheres. To compute interaction energies, a 3-D grid of 0.3 Å resolution was centered on the active site. Gasteiger–Hückel charges for the ligands and Kollman charges for the protein were employed. A 6-12 Lennard–Jones potential was used for the vander Waals component of the scoring function. A distance-dependent dielectric function ($e=r$), energy cutoff distance of 10.0 Å and contact cutoff distance of 4.5 Å were used. A 0.75 Å of van der Waals overlap was allowed.[15] All other parameters were set to their default values. Different orientations of the ligands were searched and ranked based on their energy scores.

Assay for protease activity

Assays of the hydrolysis of the fluorogenic substrate Z-Phe-Arg-AMC (Enzyme Systems Products, Livermore, CA, USA), by soluble parasite extracts containing falcipain were performed as previously described. For all assays, 4 nM falcipain was incubated with 10 nM dithiothreitol and compounds added from 100× stocks (in DMSO) in 0.1 M sodium acetate, pH 5.5, for 30 min at room temperature before the *Z*-Phe-Arg-AMC substrate (final concentration 50 μM) was added. Fluorescence caused by the cleavage of the substrate (excitation 380 nm, absorbance 460 nm) was then monitored continuously over a period of 30 min. The rate of substrate (increase in fluorescence over time) in the presence of isoquinolines inhibitors was compared with the rate of hydrolysis in controls incubated with an equivalent volume of DMSO. In each experiment, multiple concentrations of dihydroisoquinolines and isoquinolines were evaluated in duplicate or triplicate, and IC_{50} values were extrapolated from curves of percent control activity over concentration.

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