

## **Reinvestigation of Diphenylmethylpiperazine Analogues of Pyrazine as New Class of *Plasmodial* Cysteine Protease Inhibitors for the Treatment of Malaria**

Hari Madhav<sup>1,†</sup>, G. Srinivas Reddy<sup>2,3,†</sup>, Zeba Rizvi<sup>2,3,†</sup>, Ehtesham Jameel<sup>1,†</sup>, Tarosh S. Patel<sup>4</sup>, Abdur Rahman<sup>5</sup>, Vikas Yadav<sup>6</sup>, Sadaf Fatima<sup>1</sup>, Fatima Heyat<sup>1</sup>, Kavita Pal<sup>1</sup>, Amisha Minju-OP<sup>2</sup>, Naidu Subbarao<sup>6</sup>, Souvik Bhattacharjee<sup>5</sup>, Bharat C. Dixit<sup>2</sup>, Puran Singh Sijwali<sup>2,3,\*</sup>, and Nasimul Hoda<sup>1,\*</sup>

<sup>1</sup>Drug Design and Synthesis Laboratory, Department of Chemistry, Jamia Millia Islamia, New Delhi-110025, India.

<sup>2</sup>CSIR-Centre for Cellular and Molecular Biology, Hyderabad-500007, TS, India.

<sup>3</sup>Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, UP, India

<sup>4</sup>Chemistry Department, V. P. & R. P. T. P Science College, Affiliated to Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India.

<sup>5</sup>Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi-110067, India.

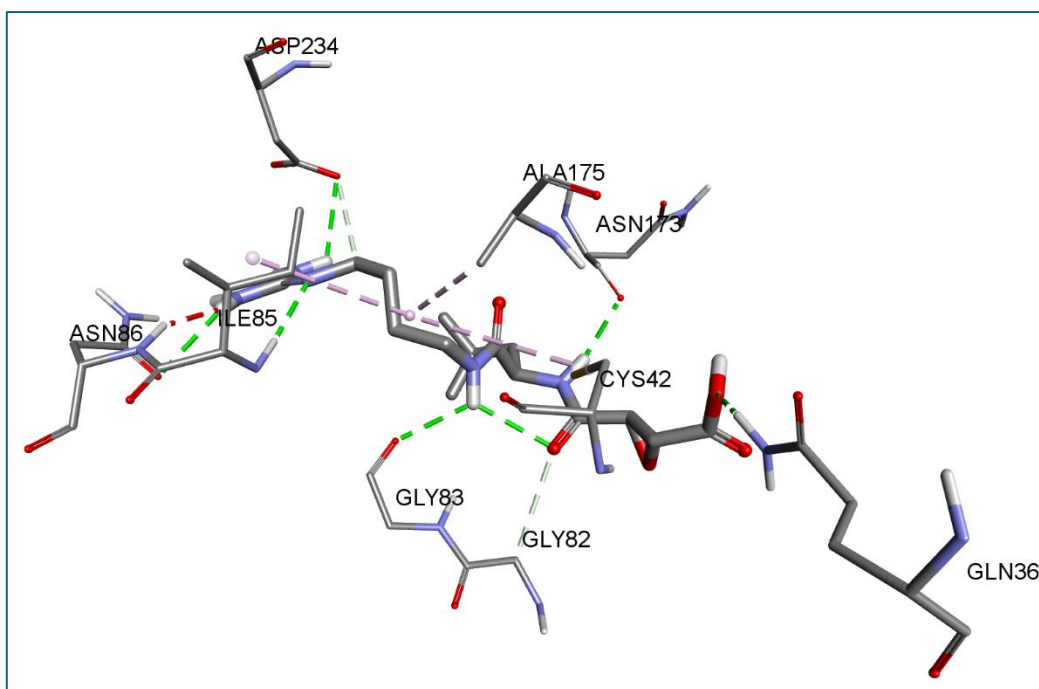
<sup>6</sup>School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi-110067.

<sup>†</sup>These authors contributed equally to the article (Joint first authors).

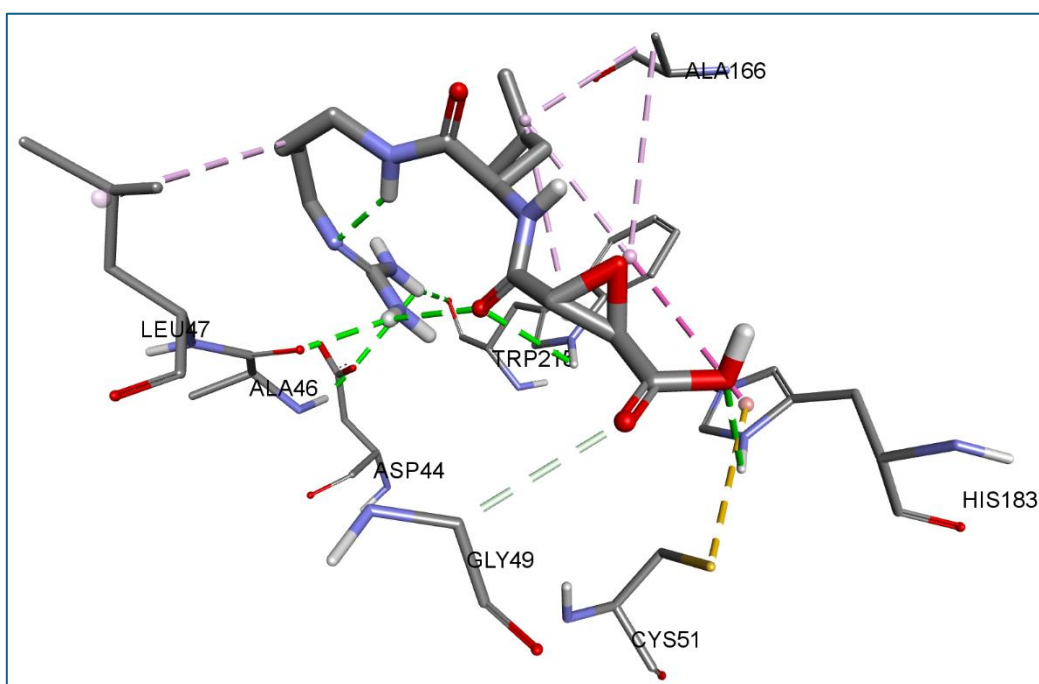
\*Corresponding Authors:

**Prof. Nasimul Hoda**  
Drug Design and Synthesis Laboratory,  
Department of Chemistry,  
Jamia Millia Islamia (A Central University),  
New Delhi-110025, India.  
Email: nhoda@jmi.ac.in

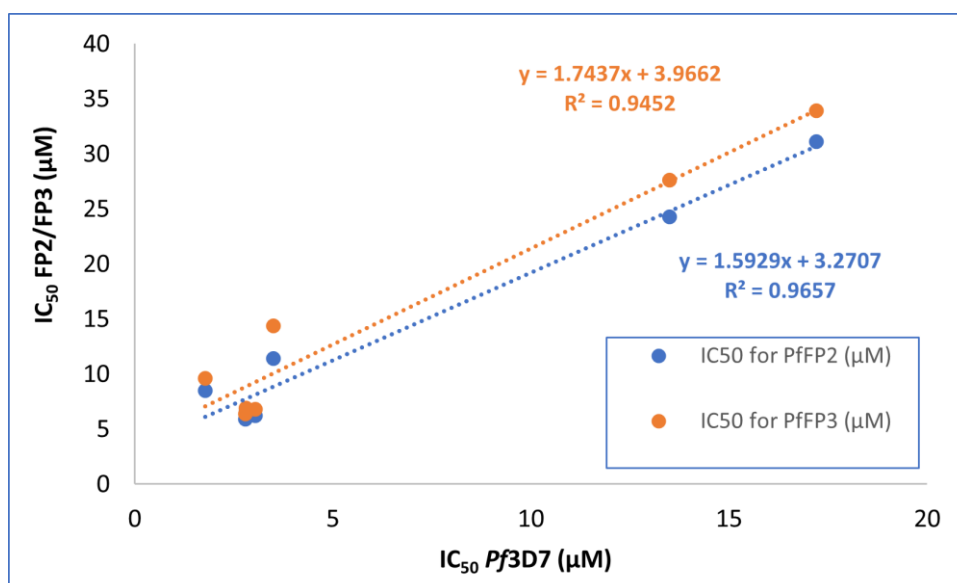
**Dr. Puran Singh Sijwali**  
Senior Principal Scientist  
CSIR-Centre for Cellular and  
Molecular Biology,  
Hyderabad-500007, TS, India.  
Email: psijwali@ccmb.res.in



**Figure S1:** Protein-ligand interactions of *PjFP2* and control **E64**.



**Figure S2:** Protein-ligand interactions of *PjFP3* and control **E64**.



**Figure S3:** Correlation curve for antimalarial potency and enzyme inhibition efficacy of the compounds.

### ADME prediction

One of the key and fundamental steps in the drug discovery process is the evaluation of pharmacokinetics features of an active compound, such as absorption, distribution, metabolism, excretion (ADME), drug-likeness, etc. Most of the molecules were simply refused because of their substandard ADME profiles. To predict various ADME parameters of the tested compounds, Schrödinger's QikProp module was utilized. The findings were compared to the commercial antimalarial CQ and discussed in Table S1.

**Table S1:** Prediction of different pharmacokinetic properties of the compounds.

Compound	#metab	FISA	FOSA	QPlogK <sub>h<sub>sa</sub></sub>	QPlogS	SASA	%HOA	Violations	
								Rule of five	Rule of three
HR1	9	57.17	298.69	0.58	-4.46	693.43	100	0	1
HR2	9	64.12	303.46	0.42	-4.31	710.87	100	0	1
HR3	8	71.52	212.56	0.57	-5.09	702.28	100	0	1
HR4	8	71.58	212.51	0.50	-4.75	688.22	100	0	1
HR5	8	72.53	212.57	0.68	-5.51	723.81	100	0	1
HR6	8	64.77	211.66	0.46	-4.42	679.91	100	0	1
HR7	9	63.85	212.92	0.69	-5.53	733.22	100	0	1
HR8	8	71.54	212.56	0.64	-5.41	715.35	100	0	1
HR9	8	68.87	206.62	0.57	-4.40	664.90	100	0	1
HR10	8	84.67	214.42	0.41	-4.09	672.33	93.59	0	1
HR11	7	74.53	118.14	0.56	-4.83	662.08	100	0	1

<b>HR12</b>	7	74.99	119.86	0.49	-4.52	649.97	100	0	1
<b>HR13</b>	7	76.29	120.81	0.67	-5.35	689.88	100	0	1
<b>HR14</b>	7	56.00	123.45	0.47	-4.09	636.02	100	0	1
<b>HR15</b>	8	76.55	121.51	0.67	-5.37	698.92	100	0	1
<b>HR16</b>	7	75.04	117.78	0.63	-5.14	674.63	100	0	1
<b>CQ</b>	4	24.08	373.47	0.59	-4.55	656.11	100	0	0
<b>E64</b>	3	285.92	333.90	-1.41	-1.40	619.81	17.57	1	1

The calculated total solvent accessible surface area (SASA) for our compounds was in the range of 620 – 733 which represents to surface of the ligand where a solvent molecule can be in contact <sup>[1]</sup>. Schrödinger suggested an identical range for SASA 300.0 – 1000.0 square Å. Similarly, the hydrophobic component of the SASA (FOSA), and a hydrophilic component of the SASA (FISA) exposed due to other atoms were calculated <sup>[2]</sup> which were found in the range of 117.78 – 303.46 and 56.0 – 84.67, respectively. The ideal range for FOSA and FISA is suggested by Schrödinger as 0.0 – 750.0 and 7.0 – 330.0, respectively. Further, prediction of aqueous solubility QPlogS was observed in the range of -5.5 to -4.09 which is preferable in the range of -6.5 to 0.5 mol/dm<sup>3</sup>. The QPlogK<sub>hsa</sub> denotes the binding of the ligands to the human serum albumin and is preferably in the range of -1.5 – 1.5. The tested ligands displayed QPlogK<sub>hsa</sub> within the range of 0.41 – 0.69 which was underlined as acceptable. Likely, the prediction of percent human oral absorption (%HOA) >80% is considered high, and <25% is considered poor and most of our ligands predicted 100% oral absorption.

The prediction of the drug-likeness of the compounds is one of the most important parameters for the development of new therapeutic agents. It was predicted using the rule of five and the rule of three. The rule of five is defined as molecular weight < 500, QPlogPo/w < 5, donorHB ≤ 5, acptHB ≤ 10. Those compounds who follow rule of five are considered to be more drug-like <sup>[3]</sup>. The results of Table S1 demonstrated that the tested ligands do not violate the rule of five. Similarly, rule of three defined as QPlogS > -5.7 mol/dm<sup>-3</sup>, QPPCaco > 22 nm/s (predicted apparent Caco-2 cell permeability), and #primary metabolites (#metab) < 7. Those compounds that follow the rule of three are considered to be more orally available <sup>[4]</sup>. The predicted ADME characteristics of the test compounds fall within the range initially proposed by Schrödinger relying on 95% of approved drugs.

## References

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