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Synthesis, antimalarial activity, and target binding of dibenzazepine-tethered isoxazolines

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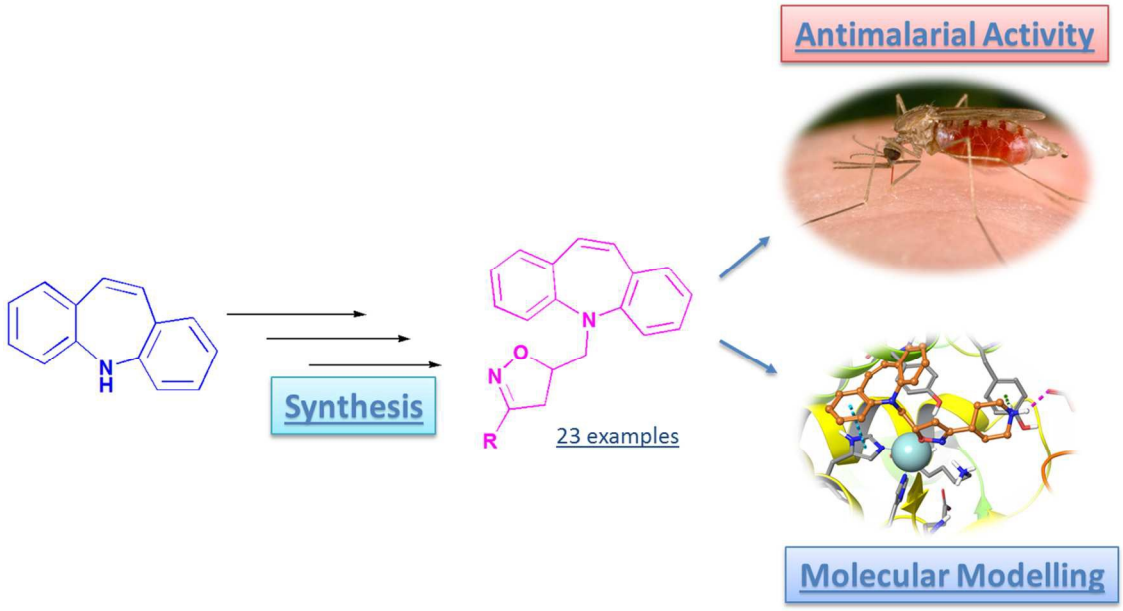
Library of dibenzazepine tethered 3,5-disubstituted isoxazolines were synthesised *via* 1,3-dipolar cycloaddition reaction and further an additional diversified group of dibenzazepine derivatives were accessed by Suzuki coupling of **6d** with various organoboronic acids. All these compounds were evaluated for their antimalarial activity against drug-sensitive *Plasmodium falciparum* 3D7 strain. Among the tested molecules, five compounds (**6j, 6k, 8c, 8k** and **8l**) that highly inhibited the parasite growth were further assessed for antimalarial activity using an additional chloroquine-sensitive (D6) and two chloroquine-resistant (W2 and 7G8) *P. falciparum* strains. Molecular docking and dynamics simulation studies were performed to understand the binding mode and binding strengths of the selected compounds with the enzyme.



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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Malaria, a complex and deadly parasitic infectious disease, is a huge public health problem in many endemic countries around the globe. The prevailing extensive resistance of malaria parasites to traditional drugs and emergence of resistance to the currently used frontline artimisinin-based chemotherapy calls for the development of new drugs. Towards this objective and since compounds containing dibenzazepine moiety are effective in treating both gametocyte and asexual stage malaria parasites, including multi drug resistant parasites, a library of dibenzazepine tethered 3,5-disubstituted isoxazolines were synthesised *via* 1,3-dipolar cycloaddition reaction. An additional diversified group of dibenzazepine derivatives were accessed by Suzuki coupling of one the above dibenzazepine derivatives with various organoboronic acids. All compounds were structurally characterized and were evaluated for their antimalarial activity. They exhibited good to excellent inhibitory activity against the growth of drug-sensitive *Plasmodium falciparum* 3D7 strain with IC_{50} values ranging from 0.2 to 7.7 µM. About 50% of the compounds were either minimally or not toxic to human cell lines. Five of the compounds (**6j, 6k, 8c, 8k** and **8l**) that highly inhibited the parasite growth were further assessed for antimalarial activity using an additional chloroquine-sensitive (D6) and two chloroquine-resistant (W2 and 7G8) *P. falciparum* strains. These compounds were effective against all four strains (3D7, D6, W2 and 7G8), exhibiting IC_{50} values of 0.1 to 1.75 µM. The dibenzazepines were identified to target metalloamino-peptidase of parasites. Molecular docking and dynamics simulation studies were performed to understand the binding mode and binding strengths of the selected compounds with the enzyme. In agreement with their excellent antimalarial activity, the data suggested that the compounds can strongly bind to the active site of the enzyme.

Introduction

Malaria is a deadly infectious disease in many tropical and subtropical countries of the world. Approximately 50% of the global population is at risk of contracting the disease. According to a recent WHO report, in 2013, ~200 million clinical cases of malaria were diagnosed, and ~600,000 deaths have occurred worldwide, most of which were in young children and in pregnant women.[1] In addition to the huge death toll it exerts, malaria vastly curtails socioeconomic progress of people in the endemic areas, which are mostly underdeveloped, by causing frequent debilitating clinical conditions and thus, contributing to the loss of enormous productive manpower. Although five species of *Plasmodium* family of protozoan parasites can infect humans to cause malaria, *P. falciparum* and *P. vivax* are responsible for almost all malarial deaths. While *P. falciparum* is most prevalent in Africa, both *P. vivax* (majorly) and *P. falciparum* (to variable degrees) are prevalent in Southeast Asia including Indian subcontinent, some parts of Africa, and South America. Further, *P. falciparum,* the most virulent among malaria parasites that can infect human, accounts for >90% of all malarial deaths, whereas *P. vivax* is responsible for most of the remainder of deaths.

Traditionally chloroquine and other quinine derivatives have been the most commonly used drugs to treat malaria. However, parasites have developed widespread resistance against these drugs.[2] To overcome this problem, artemisinin-based combination therapy (ACT) has been developed as a frontline treatmnet.[3,4] Although ACT has been effective in treating malaria and is still being widely used, resistance has emerged against this treatment in several Southeast Asian countries.[5,6] Hence, development of new, effective antimalarial drugs is urgently needed. Towards this goal, our research group has recently reported the potential utility of 1,2-disubstituted-4-quinolones and abietane diterpenoids as new antimalarial agents,[7,8] and we have been continuing efforts to identify new classes of antimalarial agents.

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

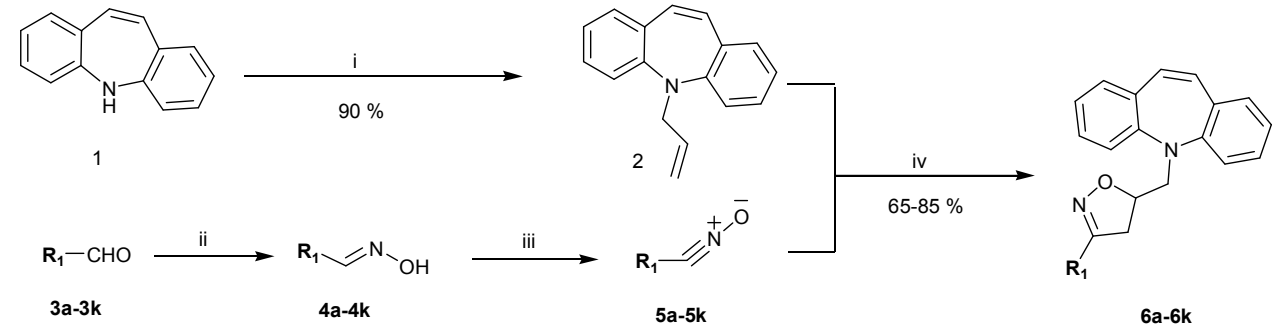
The blood stage infection of *Plasmodium* parasites is responsible for the clinical symptoms and severe illnesses of malaria. The initial infection involves the entry of merozoites released from the matured liver stage parasites into erythrocytes, where they grow into trophozoites, which upon maturation undergo schizogony to form merozoites. At the end of 48 h intraerythrocytic life cycle of parasites, the merozoites released from infected erythrocytes invade new erythrocytes to start the next 48 h life cycle.[9,10] During their growth in erythrocytes, parasites depends on the host products for nutritional need. For example, amino acids formed by the degradation of host cell haemoglobin by aminopeptidases are the main source for parasite protein synthesis.[11,12] *P. falciparum* M1 alanyl aminopeptidase (PfA-M1), a terminal zinc-metalloamino-peptidase of hemoglobin digestion converts the cleaved hemoglobin peptides into an amino acid pool, which is used by parasites for its protein synthesis.[13-15] Since hemoglobin-derived amino acids are critical for parasite growth, PfA-M1 is considered as a potential target for the development of antimalarial agents.[16-19]

Compounds containing dibenzazepine **1** scaffold exhibit a wide range of biological activity and hence, a variety of derivatives have been synthesized and evaluated as antimicrobial and antifungal,[20] antioxidant,[21,22] anticancer,[23] antiepileptic,[24] anticonvulasent,[25-27] and serotonin agents.[28] Some examples of clinically important dibenzazepine-based drugs are carbamazepine,[25,26,29] opipramol,[30] and oxacarbazepine[24] that used for treating epilepsy, anxiety, and mood disorders. A recent report describes dibenzazepine derivatives as efficient antimalarial agents for both the gametocyte stage and the blood stage *P. falciparum*.[31] The dibenzazepine moiety has also been used for the development of agents against multi drug resistant *Plasmodium* parasites.[32] Given the potential usefulness of dibenzazepines as target-specific antimalarial agents and in view of exploring various new classes of compounds for antimalarial activity, we synthesized a library of dibenzazepines having substituted five-membered heterocyclic rings at the *N*-position of the azepine ring and evaluated their antimalarial activity.

Results and Discussion

Chemistry

At first, a series of 3,5-disubstituted isoxazolines (**6a-6k**) bearing aryl, heteroaryl and alicyclic group at the C-3 position of isoxazoline ring and having dibenzazepine moiety at the C-5 position were synthesized[36] through the reactions depicted in Scheme 1. The synthon, dipolarophile 5-allyl-*5H*-dibenzazepine **2** was prepared by *N*-allylation of 5*H*-dibenzo[*b,f*]azepine **1** using allyl bromide in presence of tetra-*n*-butylammonium bromide (TBAB) as a phase transfer catalyst, sodium hydroxide as a base in the biphasic mixture of toluene-water in 1:1 ratio at 55 °C. The dipolar aryl nitrile oxides (**5a-5k**) was generated *in-situ* in two-step processes by the addition of hydroxylamine hydrochloride to aryl, heteroaryl or alicyclic aldehydes (**3a-3k**) in the presence of sodium acetate in methanol followed by the action of *N*-chlorosuccinimide (NCS) on oximes formed (**4a-4k**) in the presence of triethyl amine base (Scheme 1). Coupling of synthon **2** with aryl, heteroaryl or alicyclic nitrile oxides in the presence of triethyl amine afforded dibenzazepine tethered 3,5-disubstituted isoxazolines (**6a-6k**). The compounds were characterised by LC-MS, ^{1}H and ^{13}C NMR, and elemental analyses (see the supplementary file)



Reagents and conditions: i) allyl bromide, TBAB, NaOH, toluene-water (1:1), 55 °C, 4h; ii) $NH_2OH.HCl$, CH_3COONa, CH_3OH, RT, 3h; iii) NCS, Et_3N, $CHCl_3$, RT, 3h; iv) Et_3N, $CHCl_3$, 0 °C-RT, 6h.

Scheme 1 Synthesis of 3,5-disubstituted isoxazolines (**6a-6k**) from 5-allyl-5*H*-dibenzo[*b,f*]azepine **2**.

Dibenzazepine derivatives carrying substituents on the dibenzazepine ring were found to exhibit effective biological and pharmacological properties with minimal side effects.[27,32] Therefore, these class of compounds have been of special interest for exploiting as drug candidates. Considering this, an additional series of compounds (**8a-8l**) were prepared by introducing various substituents on the *para*-position of the C-3 phenyl moiety of isoxazoline ring in **6d**. This was accomplished *via* Suzuki coupling of **6d** with various organoboronic acids (**7a-7l**) in the presence of $Pd(dppf)Cl_2.CH_2Cl_2$ catalyst[37] using Cs_2CO_3 as base in dioxane-water biphasic solvent under nitrogen atmosphere (Scheme 2). The products were purified by column chromatography on silica gel (60-120 mesh) using mixtures of ethyl acetate and hexane in suitable ratios and were characterised by LC-MS, ^{1}H and ^{13}C NMR, and elemental analyses (see the supplementary file). Additionally, compound **6d** was characterized by single crystal XRD studies and the crystallographic data have been deposited at the CCDC No 1049585[38] (Fig. S1, see the supplementary file).

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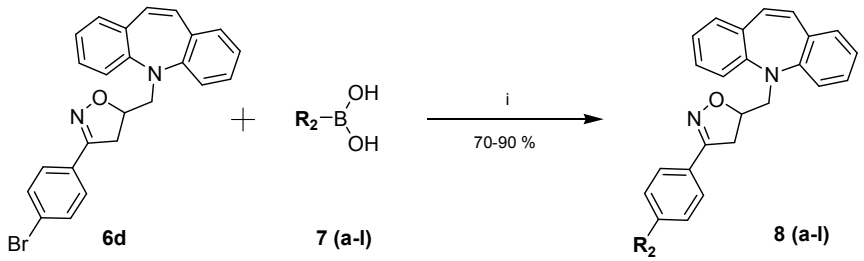


Where, R_2 = **8a**= *p*-(Cl)Ph **8b**= *p*-(OH)Ph **8c**= *p*-(OH)Ph **8d**= *p*-(CH_2OH)Ph **8e**= *p*-(CHO)Ph **8f**= *p*-(OMe)Ph **8g**= *p*-(OCF_3)Ph **8h**= *p*-(CN)Ph **8i**= *p*-(CH_2NHPh)Ph **8j**= 2-thiophene **8k**= 4-pyridyl **8l**= 5-(6-Chloro-1,3-dihydro-indol-2-one)

Reagents and conditions: i) Pd(dppf)Cl_2.CH_2Cl_2, Cs_2CO_3, dioxane/water (6:1), 100 °C, 12 h.

Scheme 2 Suzuki coupling of 5-[3-(4-bromo-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine **6d** with various boronic acids.

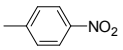
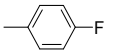
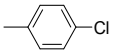
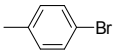
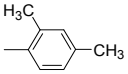
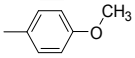
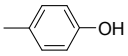
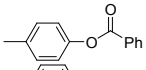
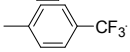
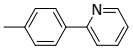
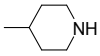
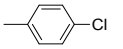
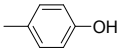
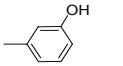
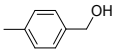
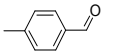
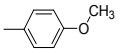
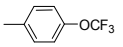
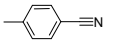
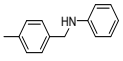
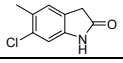
Biology

All the synthesized compounds (**6a-6k** and **8a-8l**) were initially examined for antimalarial activity against chloroquine-sensitive *P. falciparum* 3D7 strain. The parasite growth inhibition was assessed at concentrations ranging from 0.1 to 100 µM by a SYBR Green assay.[39,40] All compounds showed good to excellent antimalarial activity with IC_{50} values of 0.2 to 7.7 µM (Table 1). The cytotoxicity of the compounds was examined using human kidney embryonic (HEK 293) cells and human lung tumor epithelial cell (A549) by the MTS assay. Many of the compounds exhibited either minimal or no cytotoxicity (IC_{50} >40 µM), and others displayed low cytotoxicity (20 to 40 µM) (Tables 1). The compounds, **6j**, **6k**, **8c**, **8k**, and **8l** that showed inhibitory activity less than 2 µM were further assessed for their ability to inhibit an additional chloroquine-sensitive parasite (D6) strain and two chloroquine-resistant (W2 and 7G8) strains at concentrations ranging from 0.10 µM to 1.75 µM. Each of the five compounds inhibited both chloroquine-sensitive and chloroquine-resistant parasites to similar extents (Table 2). Further, we tested **6j**, **6k**, **8c**, **8k** and **8l** for lysis of red blood cells at concentration ranging from 2.5 µM to 40 µM. The compounds were not lytic at or below 20 µM, suggesting that the antimalarial activity of dibenzazepines is not due to the lysis of red blood cells.

Structure-activity relationship

In efforts to understand the structure-activity relationship, first the activity of dibenzazepine **6**-series derivatives having substituents at the *para*-position of the phenyl moiety at the C-3 position of isoxazoline ring was compared. Regardless of whether the substituents are electron-withdrawing groups such as -NO_2 (**6a**), -F (**6b**), -Cl (**6c**), -Br (**6d**) and -CF_3 (**6i**) or electron-releasing groups such as -CH_3 (**6f**) and -OH (**6g**) groups, the compounds showed good to excellent parasite growth inhibitory activity. Interestingly, nitrogen-containing hetero-alicyclic substituent piperidyl (**6k**) or hetero-aromatic substituent pyridyl (**6j**) in place of phenyl moiety exhibited much higher inhibitory activity than the above compounds. However, compounds containing hydrophobic methyl substituents (**6e**) or ester substituent (**6h**) were relatively less active. This could be due to their expected lower solubility compared to other compounds. In **8a-8l** series, in general, compounds having hydroxy- or methoxy-phenyl substituents (**8b**, **8c**, **8d**, and **8f**) showed excellent inhibitory activity. As is the case of **6**-series, compounds **8k** and **8l**, bearing pyridyl or 2-indolone substituents, exhibited more potent activity. The remaining compounds, **8a**, **8e**, **8g**, **8i**, and **8j**, were relatively less active than others and there was no clear distinct structure-activity relationship was evident.

Table 1 Antimalarial activity of 3,5-disubstituted isoxazolines **(6a-6k** and **8a-8l)** against drug-sensitive *P. falciparum* 3D7 strain and cytotoxicity against A549 and HEK 293 cells.

		Parasite growth inhibition IC_{50} (μM ± SD)		
Product	**R_1**	**3D7 Strain** [a]	**A549 cells** [b]	**HEK293 cells** [b]
6a		2.65 ± 0.95	>100	>100
6b		2.11 ± 0.74	18.40 ± 2.61	21.60 ± 1.51
6c		1.69 ± 0.28	18.60 ± 2.30	16.60 ± 3.14
6d		1.42 ± 0.96	26.80 ± 4.51	31.60 ± 4.53
6e		3.35 ± 0.95	>100	>100
6f		2.27 ± 0.88	43.20 ± 3.66	40.16 ± 6.23
6g		2.27 ± 0.88	24.10 ± 3.16	23.60 ± 4.36
6h		4.17 ± 1.20	42.10 ± 5.03	43.40 ± 5.20
6i		2.23 ± 0.85	23.40 ± 4.41	26.60 ± 3.22
6j		0.58 ± 0.18	28.40 ± 3.17	22.10 ± 3.22
6k		0.19 ± 0.06	45.60 ± 5.62	44.80 ± 6.11
8a		7.70 ± 1.46	20.10 ± 3.94	36.40 ± 5.27
8b		2.69 ± 1.49	36.70 ± 4.68	42.30 ± 5.09
8c		1.75 ± 0.84	41.60 ± 3.13	31.90 ± 6.73
8d		2.83 ± 1.06	33.60 ± 4.43	41.20 ± 3.50
8e		5.67 ± 1.63	21.20 ± 3.82	31.20 ± 3.61
8f		2.70 ± 0.94	56.20 ± 6.22	>100
8g		4.79 ± 0.93	18.60 ± 4.20	29.10 ± 5.36
8h		2.63 ± 1.22	39.50 ± 4.63	44.30 ± 5.03
8i		5.20 ± 1.10	46.40 ± 6.63	41.30 ± 5.15
8j		3.14 ± 0.93	42.40 ± 4.93	58.40 ± 4.66
8k		0.995 ± 0.21	>100	>100
8l		1.15 ± 0.67	56.6 ± 6.72	50.10 ± 4.70

[a] The fluorescence intensity was measured in a microliter plate reader.
[b] The absorbance was measured in a microliter plate spectrophotometer.

Target identification

PASS Online structure-activity predictive program was used to identify the target molecule involved in blocking of malaria parasite growth by dibenzazepine derivatives.[33,34] This program predicts many types of biological activity, including pharmacological effects, interaction with metabolic enzymes, transporters, and mechanisms of action by comparing the structures of test compounds with the data base. The prediction is based on statistical probability data that was developed based on the structure-activity relationships of a collection of structurally similar molecules. Searching for the probable targets suggested that PfA-M1 aminopeptidase is the most likely target candidate. Hence, binding mode of the dibenzazepine derivatives with PfA-M1 was analyzed by computational modeling studies.

Table 2 Activity of selective dibenzazepine derivatives against drug-sensitive and drug-resistant *P. falciparum*.

	Parasite growth inhibition (IC_{50}, μM ± SEM)			
Compound	**3D7**	**D6**	**W2**	**7G8**
6j	0.58 ± 0.18	0.40 ± 0.08	0.36 ± 0.09	0.42 ± 0.06
6k	0.19 ± 0.06	0.16 ± 0.04	0.12 ± 0.09	0.10 ± 0.06
8c	1.75 ± 0.84	1.66 ± 0.16	1.22 ± 0.09	1.30 ± 0.12
8k	0.99 ± 0.21	1.66 ± 0.04	1.10 ± 0.12	1.20 ± 0.02
8l	1.15 ± 0.67	1.06 ± 0.13	0.92 ± 0.09	0.93 ± 0.15
Chloroquine	0.13 ± 0.06	0.12 ± 0.08	0.32 ± 0.09	0.28 ± 0.03
Mefloquine	0.04 ± 0.02	0.03 ± 0.06	0.16 ± 0.07	0.11 ± 0.03

Molecular docking analysis

The aim of the molecular docking study was to elucidate how dibenzazepine derivatives bind to the target PfA-M1, which cleaves hemoglobin-derived peptides. The docking results provided appropriate information about the binding affinity, binding energy and orientation of ligand-enzyme interactions to inhibit the function of PfA-M1. The molecular docking was carried out for all substituted dibenzazepine tethered 3,5-disubstituted isoxazoline derivatives with PfA-M1 and the interaction of ligands with protein was analyzed (Table S1, Supplementary file). The docking protocol was validated using the reported structure of PfA-M1 bound to its ligand. The root mean square (RMS) deviation between the actual and the predicted pose was 0.8 Å, which was well within the acceptable limit of 2.0 Å.[39]

The compound **6d** showed docking score of -6.68 kcal/mol. It exhibited combination of both electrostatic and hydrophobic interactions resulting in strong binding affinity. The oxygen and nitrogen atom of isoxazoline ring formed co-ordination bond with the Zn^{+2} ion with the bond length 2.2 Å and 2.5 Å, respectively; the dibenzazepine ring formed π–π parallel staking interactions with His496; the terminal aromatic ring formed π–π staking interactions with Tyr575; the bromine formed week halogen interactions with backbone carbonyl of Glu319; the residues Val493, Val523, Tyr580, Tyr575, Val459, Met462 formed hydrophobic interaction with ligand (Fig. 1, Table 3).

The compound **6j** exhibited docking score of -8.06 kcal/mol; the oxygen and nitrogen atom of isoxazoline ring of **6j** formed co-ordination bond with the Zn^{+2} ion with the bond length 2.3 Å and 2.6 Å, respectively; the nitrogen atom of isoxazoline ring formed

week H-bond with hydroxyl of Tyr580; the dibenzazepine ring formed π–π interactions with His496; the terminal pyridine ring formed edge to face type of π–π interactions with Tyr575; the residues Val493, V523, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320 formed hydrophobic interaction with ligand (Table 3).

The compound **6k** exhibited potent *in vitro* antimalarial activity. The docking pose of **6k** with protein showed strong combination of electrostatic and hydrophobic interactions with active site amino acid residues of PfA-M1, the observed docking score was -7.90 kcal/mol. The oxygen and nitrogen atom of isoxazoline ring formed co-ordination bond with the Zn^{+2} ion with the bond length 2.3 Å and 2.8 Å, respectively; the dibenzazepine ring formed π–π interactions with His496; the terminal piperidine ring formed strong ionic interaction (like salt bridge) with Glu572 and the protonated nitrogen of the piperidine ring formed π-cation interaction with Tyr575 resulting in high gain in binding affinity; the residues Val493, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320 formed hydrophobic interaction with ligand (Fig. 1, Table 3).

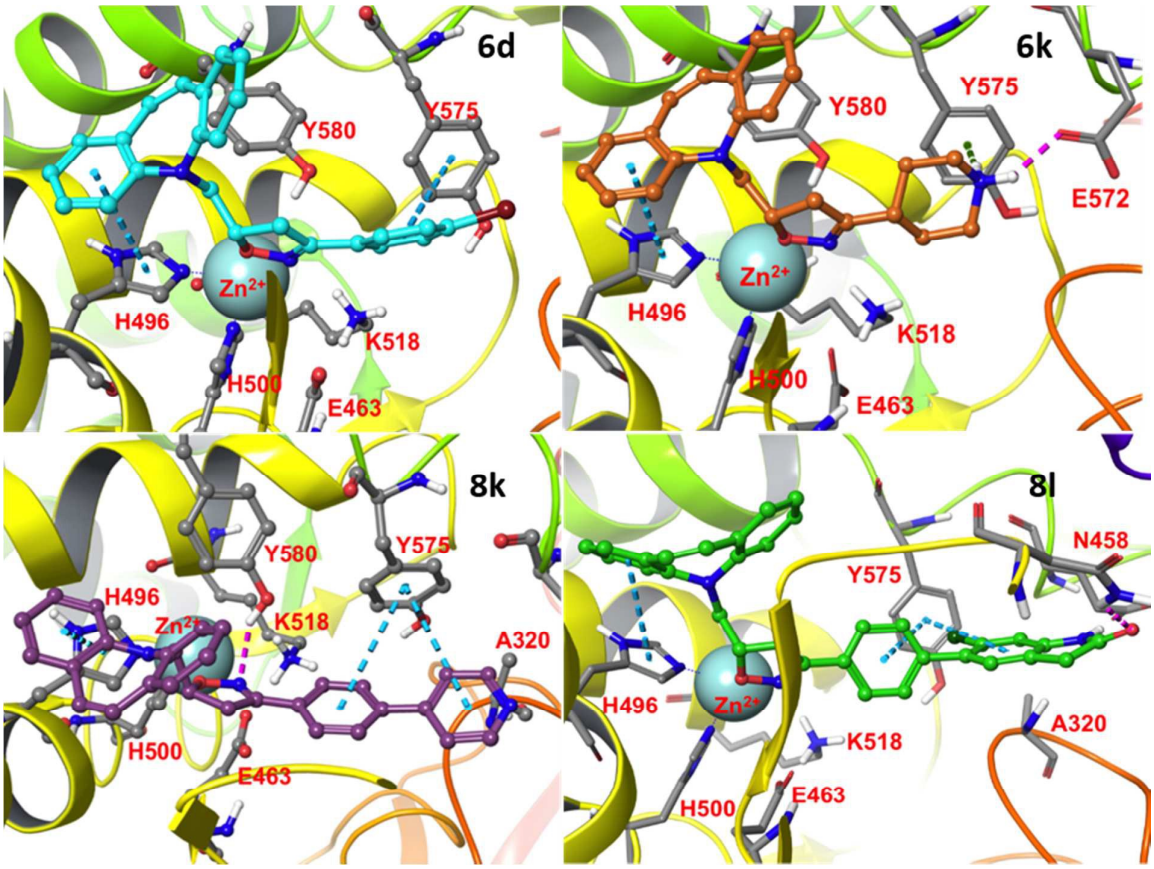


Fig. 1 Docking pose for compounds **6d**, **6k**, **8k** and **8l** with PfA-M1. The H-bonds are shown in pink colour dotted lines, π–π interactions are shown in blue colour dotted lines and π-cation interactions are shown in green colour dotted lines.

To improve the mode of interaction and binding ability with the target, the structural modification on compound **6d** was carried out and obtained several additional compounds (**8** series) and tested for antimalarial activity. Of these, two compounds, **8k** and **8l**, that showed excellent activity were analysed for binding to PfA-M1. The docking score for compound **8k** was -7.80 kcal/mol. The oxygen and nitrogen atom of isoxazoline ring formed co-ordination bond with the Zn^{+2} ion with the bond length 2.1 Å and 2.5 Å, respectively; the nitrogen atom of isoxazoline ring formed weak H-bond with hydroxyl group of Tyr580; the dibenzazepine ring formed π–π interactions with His496; the terminal pyridine ring and the penultimate phenyl ring formed edge to face π-π interaction with Tyr575; the residues V523, Val493, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320 formed hydrophobic interaction with ligand (Fig. 1, Table 3).

The compound **8l** showed a docking score of -7.30 kcal/mol. The oxygen and nitrogen atom of isoxazoline ring formed co-ordination bond with the Zn^{+2} ion with the bond length 2.2 Å and 2.3 Å respectively; the dibenzazepine ring formed π–π interactions with His496; the phenyl ring formed π–π staking interactions with Tyr575, the terminal carbonyl of 2-indolone formed H-bond with Asn458 and formed π–π staking interactions with Tyr575; the residues Val493, Val523, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320 formed hydrophobic interaction with ligand (Fig. 1, Table 3). The ligand displayed combination of electrostatic and hydrophobic interactions, but the binding pose bumped on Van der Waals radii of the protein atoms, which could be the reason for observed decreased activity of **8l** compared to **8k** (Fig. 1, Table 3).

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Table 3 Docking score and interaction residues for the binding of dibenzazepine derivatives with PfA-M1.

Compound	Docking score (kcal/mol)	Metal coordinate bond	π–π stacking or π–cation interaction	H-bond forming residues	Hydrophobic interaction residues
6d	-6.68	With Zn^{+2}	His496, Tyr575	-	Val493, Val523, Tyr580, Tyr575, Val459, Met462
6j	-8.06	With Zn^{+2}	His496, Tyr575	Tyr580	Val493, V523, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320
6k	-7.90	With Zn^{+2}	His496, Tyr575	Glu572	Val493, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320
8k	-7.80	With Zn^{+2}	His496, Tyr575	Tyr580	Val493, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320
8l	-7.30	With Zn^{+2}	His496, Tyr575	Asn458	Val493, Val523, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320

Molecular dynamics simulation

To confirm the stability of binding mode predicted by Glide docking of the compounds to PfA-M1 and to monitor the structural changes in the form of conformations and ligand-protein interactions, molecular dynamics (MD) simulation was performed. The docked complex of the most potent compound **6k** was considered for the MD simulation. The complex was adequately soaked into simulation box consisted of 24,244 water molecules. The system was simulated for 50 ps for equilibration. The final simulation run was for a total of 10 ns, during which 1000 structures enumerated were saved in the trajectory. To understand the stability of the complex during MD simulation, the protein backbone frames were aligned to the backbone of initial frame and then the RMS deviation was calculated with respect to the initial frame. The RMS deviations between the original structure and the structure enumerated during MD simulation were plotted (Fig. 2). The protein backbone RMS deviation recorded during simulation shows large deviation for the initial 500 ps due to the initial protein structural stabilization; after about 500 ps the system showed a steady state dynamics. The backbone structural deviations observed in protein for the latter phase of 2 to 10 ns was in the range of 1.28 Å to 1.69 Å compared to that of original structure. From 2 ns to till the end of simulation, the total RMS deviation of protein was within a range of 0.41 Å. This clearly suggests that 10 ns of simulation are sufficient for stabilizing this complex. To further confirm the structure stability of the protein, the RMS deviations between the side chain conformations of the amino acid residues was measured and found minimal RMS deviations of 0.43 Å after 2 ns (Fig. 2).

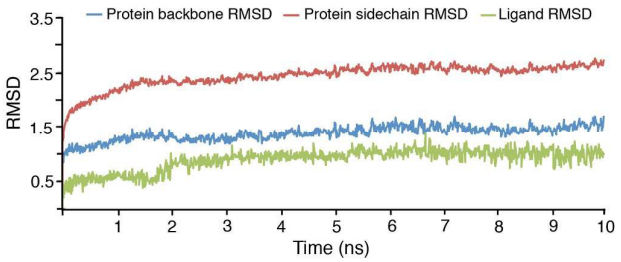

Fig. 2 The RSM deviations between the original structure and the structure enumerated during MD simulation; the backbone fluctuations are shown in blue, ligand fluctuations are shown in green colour and the protein side chain fluctuations are shown in red colour.

Further the RMS deviation of the ligand as shown in the Fig. 2, drastically reduced to 0.52 Å after 2 ns simulation. This strongly demonstrates that the ligand is well stabilized in binding site of the protein. Various inter-molecular interactions, including H-bond, hydrophobic, ionic, and electrostatic interactions were formed between ligand and protein during the MD simulation, making the ligand well stabilized in the binding pocket. The Fig. 3 shows the summary of the total interactions observed during the MD simulation. The stacked bar charts are normalized over the course of the trajectory. Values over 1.0 are possible as some amino acid residues could make multiple contacts of same subtype with the ligand. All the ligand-protein interactions found in the docking study were retained throughout the MD simulation. Fig. 4 shows a schematic diagram of the ligand interacting with the amino acid residues of protein structure evolved during MD simulation. Residues with the ligand interactions that occurred more than 25% of the simulation time in the trajectory are shown. The total number of specific contacts the protein made with the ligand over the course of the trajectory is shown in Fig. 5. From the structure evolved from the MD simulation, it is evident that ionic interaction

with Glu572, co-ordination bond formation with Zn^{+2}, the π-cation interaction with Tyr575 and hydrophobic interaction with Tyr580 of ligand is contributing strongly for the binding affinity; the π-cation interaction with Arg489 and π−π interactions with His496 were also observed. The water mediated bridged interaction between the amino acid residues Glu319 and Glu463 further stabilizing the ligand in the pocket.

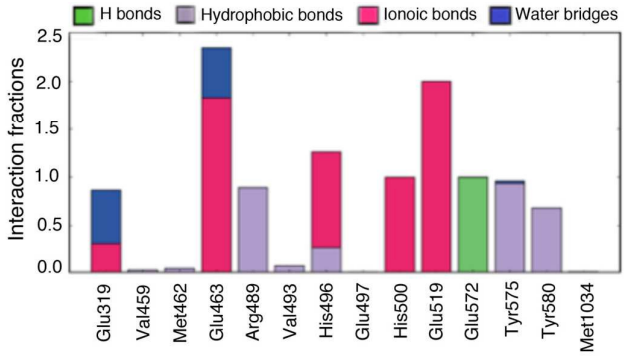

Fig. 3 Interaction between PfA-M1 and **6k** evolved during MD simulation.

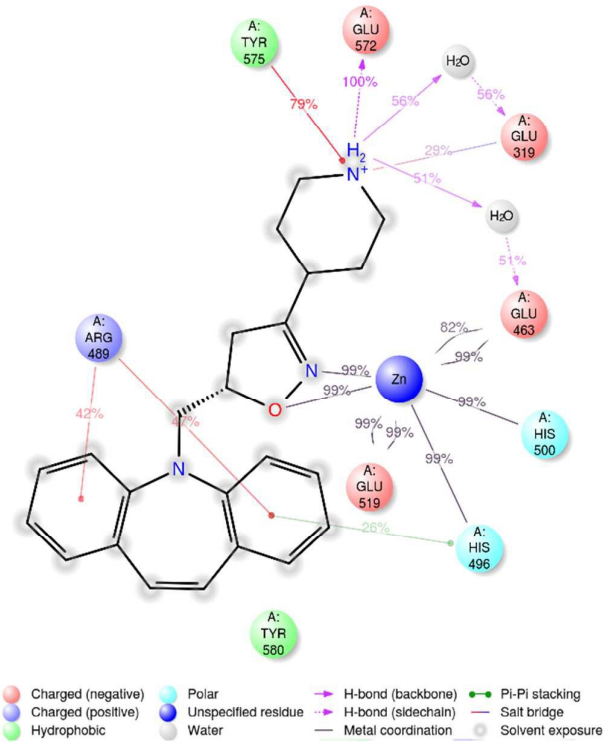

Fig. 4 Schematic diagram of ligand interaction with the amino acid residues of protein evolved during MD simulation. Interactions that occur more than 25% of the simulation time are shown.

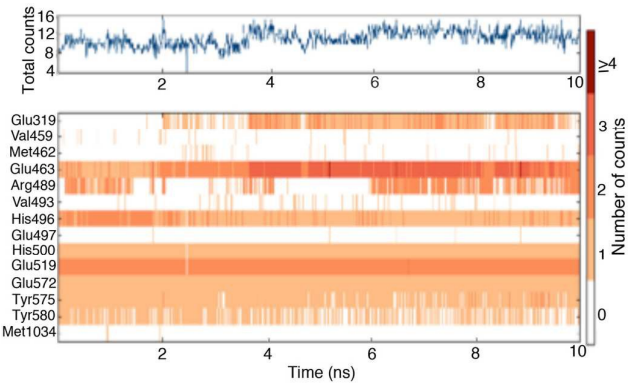

Fig. 5 A timeline representation of the interactions and contacts (H-bonds, and hydrophobic and ionic interactions, and water bridges). The top panel shows the total number of specific contacts the protein makes with ligand over the course of trajectory. The bottom panel shows the residues that interact with the ligand in each trajectory frame. The residues making more than one contact are shown in darker colour shade.

Experimental Section

Materials and methods: The starting chemicals used here were purchased from commercial sources. The solvents were of analytical grade and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated sheets of silica gel 60 (Merck $60F_{254}$, 0.25 mm thickness) and visualization under UV light. The specific molecule purified by preparative HPLC, waters SPD-4245, USA. The melting points were determined on SELACO melting point apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained using Agilent and Bruker NMR spectrometer, respectively. Chemical shifts (δ) are given in parts per million (ppm) using the residue solvent ($CDCl_3$ and DMSO-d_6) peaks as reference relative to TMS. Coupling constant (J) values are given in Hz. Electrospray ionization (ESI) mass spectral analysis was performed using Waters-Synapt G2 mass spectrometer. The C, H, and N analysis were performed using CE-400 CHN analyser. Infrared spectra were recorded on Shimadzu FT-IR model 8300 spectrophotometer.

Experimental procedure for the synthesis of 5-allyl-5*H*-dibenzo[*b,f*]azepine (2)

To 5*H*-dibenzazepine (**1**, 25.8 mmol) in a mixture of toluene and water in the ratio of 1:1 was added sodium hydroxide (51.7 mmol) followed by TBAB (2.5 mmol) at room temperature. After 15 minutes, allyl bromide was added drop wise (38.8 mmol) at room temperature. The reaction mixture was heated at 55 °C for 4 h. After completion of the reaction as monitored by TLC, the reaction mixture was diluted with water (100 mL) and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined ethyl acetate layer was washed with 0.1 N hydrochloric acid (2 x 50 mL), followed by brine solution (2 x 50 mL). The organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure. The crude compound **2**, was purified by column chromatography over silica gel (60-120 mesh) using hexane:ethyl acetate mixture in 9.5:0.5 ratios as eluent. The crude compound, allyl-*5H*-dibenzo[*b,f*]azepine (**2**), was crystallized in ethyl acetate and hexane mixture to obtain yellow crystals.

5-Allyl-5*H*-dibenzo[*b,f*]azepine (2)

Yield 95%; yellow crystal; mp 40-42 °C; FT-IR (cm^{-1}): 3015, 1623, 1515, 1475, 1262; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.40 (d, J = 5.6 Hz, 2H, -*C*=*CH*$_2$), 5.10 (dd, J_{1-2} = 10.4 Hz, J_{1-3} = 1.2 Hz, 1H, N-*CH*), 5.30 (dd, J_{1-2} = 17.2 Hz, J_{1-3} = 1.2 Hz, 1H, N-*CH*), 5.74-5.84 (m, 1H, -*C*=*C*-*H*), 6.75 (s, 2H, Ar*H*), 6.98 (t, J = 6.26 Hz, 4H, Ar*H*), 7.06 (dd, J_{1-2} = 8 Hz, J_{1-3} = 1.6 Hz, 2H, Ar*H*), 7.24 (td, J_{1-2} = 8 Hz, J_{1-3} = 1.2 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.54, 117.5, 120.5, 123.3, 128.6, 129.1, 132.2, 133.7, 135.1, 150.7; MS (ESI): m/z = 234.1 $[M+H]^+$; elemental composition calculated for $C_{17}H_{15}N$: C, 87.52; H, 6.48; N, 6.00; Found: C, 87.56; H, 6.55; N, 6.04.

General procedure for the synthesis of isoxazolines

Preparation of aldoxime (4a-4k)

To a solution of aldehyde (7.1 mmol) in methanol was added hydroxyl amine hydrochloride (7.8 mmol) followed by sodium acetate (7.8 mmol). The mixture was stirred at room temperature. After completion of the reaction as monitored by TLC, the reaction was quenched by adding crushed ice and the white precipitate formed was isolated by filtration, washed with hexane, and dried.

Synthesis of isoxazoline derivatives (6a-6k)

For chlorination of aldoximes (**4a-4k**), their solutions of aldoximes (4.1 mmol) in chloroform at room temperature was added NCS (4.5 mmol) and stirring was continued for 3 h, reaction progress was monitored by TLC. The reaction mixture was cooled to 0 °C and slowly added triethyl amine (10.25 mmol). After 10 minutes, the solution of 5-allyl-5*H*-dibenzazepine **2** in DMF (4.1 mmol) was added dropwise at 0 °C. The stirring was continued for 6-8 h at 0 °C to room temperature. The reaction mixture was diluted with chloroform (2 x 25 mL), the combined organic layer was washed with 0.1 N hydrochloric acid (2 x 20 mL), followed by brine solution (2 x 20 mL). Then, the organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The crude products **(6a-6k),** were purified by column chromatography over silica gel (60-120 mesh) using hexane:ethyl acetate mixture in 8:2 ratios as eluent. The compounds **(6a-6k)** were crystallized in ethyl acetate and hexane mixture.

5-[3-(4-Nitro-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[b,*f*]azepine (6a)

Yield 72%; yellow crystal; mp 166-168 °C; FT-IR (cm^{-1}): 2993, 1727, 1519, 1484, 1269, 1169, 909, 764, 704; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.21 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.46 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 3.58 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 8.8 Hz, 1H, C_4-*H* isoxazoline), 4.32 (dd, J_{1-2} = 13.2 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.94 -5.02 (m, 1H, C_5-*H* isoxazoline), 6.70 (q, J = 11.6 Hz, 2H, Ar*H*), 6.98-7.04 (m, 4H, Ar*H*), 7.09 (d, J = 6.4 Hz, 1H, Ar*H*), 7.18 (d, J = 8.0 Hz, 1H, Ar*H*), 7.23-7.31 (m, 2H, Ar*H*), 7.73 (d, J = 8.8 Hz, 2H, Ar*H*), 8.20 (d, J = 8.8 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.7, 53.5, 79.7, 120.7, 120.9, 123.8, 124.0, 124.1, 127.3, 129.0, 129.2, 129.4, 131.8, 132.1, 133.5, 134.2, 135.7, 148.3, 148.4, 151.1, 154.9; MS: m/z = 397.4 (calculated), m/z = 398.8 $[M+H]^+$ (found); elemental composition calculated for $C_{24}H_{19}N_3O_3$: C, 72.53; H, 4.82; N, 10.57; O, 12.08; found: C, 72.56; H, 4.89; N, 10.59.

5-[3-(4-Fluoro-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6b)

Yield 76%; yellow solid; mp 98-100 °C; FT-IR (cm^{-1}): 2980, 1731, 1523, 1489, 1260, 1153, 901, 764; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.19 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, N-*CH*), 3.83-3.52 (m, 2H, C_4-2*H* isoxazoline), 4.35 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.0 Hz, 1H, N-*CH*), 4.87-4.94 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, J = 2.4 Hz, 2H, Ar*H*), 7.10-6.98 (m, 7H, Ar*H*), 7.18-7.23 (m, 2H, Ar*H*), 7.27-7.32 (m, 1H, Ar*H*), 7.56-7.60 (m, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.3, 54.2, 78.7, 115.9, 116.3, 121.3, 121.4, 124.0, 126.4, 126.5, 129.1, 129.2, 129.4, 129.5, 132.3, 132.5, 133.9, 150.4, 150.7, 155.9, 162.1, 164.6; MS: m/z = 370.4 (calculated), m/z = 371.5 $[M+H]^+$ (found); elemental composition calculated for: $C_{24}H_{19}FN_2O$: C, 77.82; H, 5.17; F, 5.13; N, 7.56; O, 4.32; found: C, 77.84; H, 5.21; N, 7.58.

5-[3-(4-Chloro-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6c)

Yield 82%; Yellow solid; mp 155-157 °C; FT-IR (cm^{-1}): 2925, 1593, 1494, 1485, 1456, 1434, 1355, 1238, 1179; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.18 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, C_4-*H* isoxazoline), 3.40 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 6.0 Hz, 1H, C_4-*H* isoxazoline), 3.51 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 9.2 Hz, 1H, N-*CH*), 4.33 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.87-4.95 (m, 1H, C_5-*H* isoxazoline), 6.73 (q, J = 11.2 Hz, 2H, Ar*H*), 6.98- 7.05 (m, 4H, Ar*H*), 7.09 (d, J = 7.6 Hz, 1H, Ar*H*), 7.18-7.23 (m, 2H, Ar*H*), 7.27-7.33 (m, 3H, Ar*H*), 7.51-7.54 (m, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.1, 54.2, 79.0, 121.3, 124.1, 128.6, 128.8, 129.2, 129.5, 132.2, 132.5, 133.9, 134.9, 156.0; MS: m/z = 386.8 (calculated), m/z = 387.5 $[M+H]^+$ (found); elemental composition calculated for: $C_{24}H_{19}ClN_2O$: C, 74.51; H, 4.95; Cl, 9.16; N, 7.24; O, 4.14; found: C, 74.53; H, 4.99; N, 7.25.

5-[3-(4-Bromo-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6d)

Yield 83%; brown solid; mp 138-140 °C; FT-IR (cm^{-1}): 2923, 1590, 1486, 1457, 1398, 1347, 1235, 1158, 1114; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.17 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.39 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 6.0 Hz, 1H, N-*CH*), 3.50 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 9.2 Hz, 1H, C_4-*H* isoxazoline), 4.31 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.89-4.91 (m, 1H, C_5-*H* isoxazoline), 6.72 (d, J = 3.2 Hz, 2H, Ar*H*), 7.04 (q, J = 15.6 Hz, 3H, Ar*H*), 7.09 (d, J = 6.4 Hz, 1H, Ar*H*), 7.18 (d, J = 8.0 Hz, 1H, Ar*H*), 7.22-7.30 (m, 3H, Ar*H*), 7.43-7.49 (m, 4H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.2, 53.6, 78.8, 120.7, 120.9, 123.9, 124.0, 124.2, 128.1, 128.5, 129.0, 129.1, 129.2, 129.4, 131.7, 131.8, 132.2, 133.5, 134.2, 148.4, 151.4, 155.6; MS: m/z = 431.3 (calculated), m/z = 431.9 $[M+H]^+$ (found); elemental composition calculated for: $C_{24}H_{19}BrN_2O$: C, 66.83; H, 4.44; Br, 18.53; N, 6.49; O, 3.71; found: C, 66.86; H, 4.49; N, 6.71.

5-[3-(2,4-Dimethyl-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6e)

Yield 78%; yellow gummy solid; FT-IR (cm^{-1}): 2935, 1590, 1484, 1481, 1453, 1424, 1359, 1231, 1199; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (S, 3H, *CH*$_3$), 2.37 (S, 3H, *CH*$_3$), 3.18 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, C_4-*H* isoxazoline), 3.36-3.42 (m, 2H, N-*CH* and C_4-*H* isoxazoline), 4.25 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.75 (t, J = 4.8 Hz, 1H, C_5-*H* isoxazoline), 6.65 (S, 2H, Ar*H*), 6.90 -7.02 (m, 7H, Ar*H*), 7.08-7.22 (m, 4H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.1, 22.9, 41.1, 53.5, 77.0, 120.7, 120.9, 123.8, 124.0, 125.7, 126.3, 128.9, 129.02, 129.09, 129.1, 129.4, 131.9, 132.1, 132.3, 133.5, 134.2, 137.8, 139.2, 148.5, 151.7, 157.3; MS: m/z = 380.48 (calculated), m/z = 381.3 $[M+H]^+$ (found); elemental composition calculated for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36; O, 4.21; Found: C, 82.09; H, 6.40; N, 4.23.

5-[3-(4-Methoxy-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6f)

Yield 75%; orange gummy solid; FT-IR (cm^{-1}): 2953, 1575, 1491, 1466, 1376, 1339, 1237, 1160, 1101; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.26 (dd, J_{1-2} = 16.4 Hz, J_{1-1} = 10.0 Hz, 1H, C_4-*H* isoxazoline), 3.44-3.49 (m, 2H, C_4-*H* isoxazoline and N-*CH*), 3.81 (s, 3H, OCH_3), 4.33 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 6.73 (s, 2H, Ar*H*), 4.80-4.85 (m, 1H, C_5-*H* isoxazoline), 6.97-7.03 (m, 7H, Ar*H*), 7.09 (dd, J_{1-2} = 7.6 Hz, J_{1-3} = 1.6 Hz, 1H, Ar*H*), 7.15-7.23 (m, 3H, Ar*H*), 7.26-7.32 (m, 1H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.3, 55.5, 55.6, 79.7, 114.5, 120.3, 120.6, 120.7, 122.0, 123.7, 129.0, 129.1, 130.5, 132.1, 133.5, 146.5, 150.1, 159.6; MS: m/z = 382.4 (calculated), m/z = 383.3 $[M+H]^+$ (found); elemental composition calculated for: $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32; O, 8.37; found: C, 78.54; H, 5.88; N, 7.35.

4-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-phenol (6g)

Yield 70%; buff white solid; mp 169-171 °C; FT-IR (cm^{-1}): 3210, 2924, 1598, 1521, 1485, 1459, 1437, 1360, 1276, 1188, 1128; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.42-3.54 (m, 2H, C_4-*H* isoxazoline and N-*CH*), 4.35 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.89-4.96 (m, 1H, C_5-*H* isoxazoline), 6.74 (d, *J* = 1.2 Hz, 2H, Ar*H*), 6.99-7.06 (m, 4H, Ar*H*), 7.10 (dd, J_{1-2} = 7.6 Hz, J_{1-3} = 1.6 Hz, 1H, Ar*H*), 7.19-7.25 (m, 4H, Ar*H*), 7.28-7.33 (m, 1H, Ar*H*), 7.49-7.53 (m, 2H, Ar*H*), 9.20 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.2, 54.5, 77.2, 120.2, 120.3, 121.5, 123.8, 129.1, 129.2, 129.7, 132.1, 133.5, 134.2, 135.5, 147.0, 149.9; MS: m/z = 368.43 (calculated), m/z = 369.0 $[M+H]^+$ (found); elemental composition calculated for: $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60; O, 8.69; found: C, 78.25; H, 5.49; N, 7.61.

Benzoic acid 4-(5-dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-phenyl ester (6h)

Yield 80%; yellow solid; mp 140-142 °C; FT-IR (cm^{-1}): 2990, 1727, 1484, 1269, 1169, 909, 764, 704; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.30-3.43 (m, 2H, C_4-2*H* isoxazoline), 3.82 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.05 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.75-4.80 (m, 1H, C_5-*H* isoxazoline), 6.77 (d, *J* = 1.6 Hz, 2H, Ar*H*), 7.03 (d, *J* = 5.6 Hz, 2H, Ar*H*), 7.10-7.23 (m, 4H, Ar*H*), 7.30-7.37 (m, 4H, Ar*H*), 7.59-7.69 (m, 4H, Ar*H*), 7.76 (t, *J* = 7.2 Hz, 1H, Ar*H*), 8.14 (d, *J* = 7.2 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.3, 54.2, 78.8, 121.3, 121.4, 122.8, 124.1, 127.7, 128.2, 129.1, 129.4, 129.5, 130.3, 132.3, 132.5, 133.9, 134.6, 150.4, 150.7, 152.1, 156.1, 164; MS: m/z = 472.5 (calculated), m/z = 473.6 $[M+H]^+$ (found); elemental composition calculated for: $C_{31}H_{24}N_2O_3$: C, 78.79; H, 5.12; N, 5.93; O, 10.16; found: C, 78.80; H, 5.17; N, 5.95.

5-[3-(4-Trifluoromethyl-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5H-dibenzo[*b,f*]azepine azepine (6i)

Yield 83%; yellow solid; mp 143-145 °C; FT-IR (cm^{-1}): 2940, 1593, 1485, 1457, 1457, 1410, 1323, 1229, 1163, 1127; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.21 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 10.8 Hz, 1H, N-*CH*), 3.42-3.57 (m, 2H, C_4-2*H* isoxazoline), 4.33 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.92-4.99 (m, 1H, C_5-*H* isoxazoline), 6.72 (q, *J* = 11.2 Hz, 2H, Ar*H*), 6.90-7.05 (m, 4H, Ar*H*), 7.10 (d, *J* = 6.4 Hz, 1H, Ar*H*), 7.19 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.23-7.32 (m, 2H, Ar*H*), 7.61 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.70 (d, *J* = 8.4 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.9, 54.1, 79.4, 121.3, 121.4, 123.1, 124.1, 125.8, 126.03, 126.07, 126.1, 127.6, 129.3, 129.5, 130.0, 130.3, 130.6, 132.2, 132.5, 133.8, 133.9, 150.4, 150.6, 156.0; MS: m/z = 420.4 (calculated), m/z = 421.5 $[M+H]^+$ (found); elemental composition calculated for: $C_{25}H_{19}F_3N_2O$: C, 71.42; H, 4.56; F, 13.56; N, 6.66; O, 3.81; found: C, 71.45; H, 4.59; N, 6.67.

5-[3-(4-Pyridin-3-yl-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (6j)

Yield 76%; pale brown solid; mp 162-164 °C; FT-IR (cm^{-1}): 2922, 2853,1732, 1583, 1583, 1571, 1484, 1433, 1407, 1358, 1334, 1298, 1240, 1225, 1162; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.26 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, N-*CH*), 3.45-3.57 (m, 2H, C_4-2*H* isoxazoline), 4.35 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.4 Hz, 1H, N-*CH*), 4.90-4.97 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 2.8 Hz, 2H, Ar*H*), 6.98-7.05 (m, 4H, Ar*H*), 7.10 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.22 (t, *J* = 8.4 Hz, 2H, Ar*H*), 7.30 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7. 02-7.84 (m, 5H, Ar*H*), 8.00 (d, *J* = 8.4 Hz, 2H, Ar*H*), 8.34 (d, *J* = 4.4 Hz, 1H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.1, 54.2, 78.2, 120.2, 120.3, 120.4, 123.8, 124.0, 127.8, 129.1, 129.2, 132.1, 133.5, 133.6, 141.4, 147.4, 149.6, 149.9; MS: m/z = 429.5 (calculated), m/z = 430.0 $[M+H]^+$ (found); elemental composition calculated for: $C_{29}H_{23}N_3O$: C, 81.09; H, 5.40; N, 9.78; O, 3.73; found: C, 81.10; H, 5.46; N, 9.79.

5-(3-Piperazin-1-yl-4,5-dihydro-isoxazol-5-ylmethyl)-5*H*-dibenzo[*b,f*]azepine (6k)

Yield 67%; buff white solid; mp 126-128 °C; FT-IR (cm^{-1}): 3400, 2712, 1593, 1483, 1458, 1308, 1224, 1160; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85-2.01 (m, 4H, 2CH_2 piperidine), 2.5-2.52 (m, 1H, *CH* piperidine), 2.78 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 2.90-3.01 (m, 3H, CH_2 piperidine and N-*CH*), 3.32 (S, 2H, CH_2 piperidine), 3.41 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 9.2 Hz, 1H, C_4-*H* isoxazoline), 4.22 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 4.0 Hz, 1H, N-*CH*), 4.67-4.73 (m, 1H, C_5-*H* isoxazoline), 5.18 (br, 1H, *NH*), 6.72 (d, *J* = 2.0 Hz, 2H, Ar*H*), 7.04 (t, *J* = 6.4 Hz, 3H, Ar*H*), 7.05-7.07 (m, 2H, Ar*H*), 7.12 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.23-7.28 (m, 2H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.0, 29.6, 33.1, 38.8, 43.0, 53.4, 77.6, 120.7, 121.0, 123.9, 124.0, 129.0, 129.1, 129.2, 129.4, 131.9, 132.1, 133.5, 134.2, 148.5, 151.3, 159.2; MS: m/z = 359.464 (calculated), m/z = 360.189 $[M+H]^+$ (found); elemental composition calculated for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69; O, 4.45; found: C, 76.88; H, 7.05; N, 11.70; O, 4.49.

Experimental procedure for Suzuki coupling reaction

5-[3-(4-Bromo-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine **6d** (1.1 mmol) and aryl boronic acid **(7a-7l)**, (1.1 mmol) were dissolved in a mixture of 1,4-dioxane (6 mL), H_2O (1mL) in a seal tube under nitrogen atmosphere. To the solution was added Pd(dppf)Cl_2.DCM (0.1 mmol) followed by Cs_2CO_3 (3.3mmol). The resulting mixture was heated under reflux at 100 °C for 12h. The reaction was monitored by TLC and the solution was filtered on celite. To the clear filtrate was added ethyl acetate (3 x 25 mL), washed sequentially with water (2 x 25 mL) and brine (2 x 25 mL), dried over anhydrous Na_2SO_4, filtered and distilled under reduced pressure. The crude products **(8a-8l)** were purified by column chromatography on silica gel (60-120 mesh) using hexane:ethyl acetate mixture in 7:3 ratios as eluent.

5-[3-(4'-Chloro-biphenyl-4-yl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (8a)

Yield 82%; yellow solid; mp 165–167 °C; FT-IR (cm^{-1}): 2986, 2873, 1762, 1576, 1573, 1561, 1474, 1433, 1360, 1278, 1230, 1152; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.25 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.37 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.75 (dd, J_{1-2} = 14.0 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.01 (dd, J_{1-2} = 19.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.69-4.74 (m, 1H, C_5-*H* isoxazoline), 6.72 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.97-7.27 (m, 8H, Ar*H*), 7.41(d, *J* = 8.4 Hz, 2H, Ar*H*), 7.61 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.66-7.69 (m, 4H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 54.2, 78.7, 121.4, 124.0, 127.3, 127.5, 128.8, 129.2, 129.4, 129.5, 129.5, 132.2,

133.2, 133.9, 138.4, 140.4, 150.3, 150.7, 156.4; MS: m/z = 462.97 (calculated), m/z = 463.4 $[M+H]^+$ (found); elemental composition calculated for $C_{30}H_{23}ClN_2O$: C, 77.83; H, 5.01; Cl, 7.66; N, 6.05; O, 3.46; found: C, 77.85; H, 5.08; N, 6.06; O, 3.47.

4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-4-ol (8b)

Yield 79%; yellow solid; mp 119–121 °C; FT-IR (cm^{-1}): 2985, 1585, 1482, 1306, 1200, 1047; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.25 (dd, J_{1-2} = 16.0 Hz, J_{1-1} = 5.6 Hz, 1H, C_4-*H* isoxazoline), 3.40 (d, *J* = 3.2 Hz, 1H, C_4-*H* isoxazoline), 3.75 (dd, J_{1-2} = 12.8 Hz, J_{1-1} = 5.6 Hz, 1H, N-*CH*), 4.02 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 5.2 Hz, 1H, N-*CH*), 4.72-4.74 (m, 1H, C_5-*H* isoxazoline), 6.75 (s, 2H, Ar*H*), 6.85 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.00-7.36 (m, 8H, Ar*H*), 7.51 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.60 (d, *J* = 8.1 Hz, 4H, Ar*H*), 9.63 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.3, 54.2, 78.5, 116.0, 116.2, 121.3, 124.0, 126.4, 127.4, 127.7, 128.2, 129.4, 129.5, 130.2, 132.2, 132.4, 133.8, 141.9, 156.5, 158.0; MS: m/z = 444.52 (calculated), m/z = 445.1 $[M+H]^+$ (found); elemental composition calculated for $C_{30}H_{24}N_2O_2$: C, 81.06; H, 5.44; N, 6.30; O, 7.20; found: C, 81.09; H, 5.50; N, 6.32; O, 7.21.

4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-3-ol (8c)

Yield 89%; pale brown solid; mp 108-110 °C; FT-IR (cm^{-1}): 3419, 2988, 2254, 1659, 1481, 1244, 1050, 1023; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.23 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 6.4 Hz, 1H, C_4-*H* isoxazoline), 3.36 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, C_4-*H* isoxazoline), 3.73 (dd, J_{1-2} = 11.2 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.00 (dd, J_{1-2} = 13.2 Hz, J_{1-1} = 6.0 Hz, 1H, N-*CH*), 4.68-4.72 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 1.6 Hz, 2H, Ar*H*), 6.80-6.83 (m, 2H, Ar*H*), 6.98-7.27 (m, 8H, Ar*H*), 7.47-7.50 (m, 12H, Ar*H*), 7.54-7.61 (m, 6H, Ar*H*), 9.32 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.3, 54.2, 78.5, 115.9, 116.2, 121.3, 124.0, 126.4, 127.4, 127.7, 128.1, 129.5, 130.3, 131.6, 132.2, 133.9, 141.9, 150.3, 150.7, 156.5, 156.6, 158.0; MS: m/z = 444.52 (calculated), m/z = 445.1 $[M+H]^+$ (found); elemental composition calculated for $C_{30}H_{24}N_2O_2$: C, 81.06; H, 5.44; N, 6.30; O, 7.20; found: C, 81.09; H, 5.50; N, 6.32; O, 7.21.

[4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-4-yl]-methanol (8d)

Yield 90%; yellow solid; mp 145-147 °C; FT-IR (cm^{-1}): 3445, 2250, 2135, 1672, 1055, 1001; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.28 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 6.4 Hz, 1H, C_4-*H* isoxazoline), 3.40 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.75 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4Hz, 1H, N-*CH*), 4.01 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.54 (d, *J* = 5.6 Hz, 2H, OCH_2), 4.70-4.74 (m, 1H, C_5-*H* isoxazoline), 5.22 (t, *J* = 11.6 Hz, 1H, Ar*H*), 6.73 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.98-7.31 (m, 9H, Ar*H*), 7.39 (t, *J* = 8.4 Hz, 1H, Ar*H*), 7.51 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.62 (d, *J* = 8.4 Hz, 3H, Ar*H*), 7.68 (d, *J* = 8.8 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 54.2, 63.2, 78.6, 121.3, 124.0, 125.0, 125.2, 125.4, 126.4, 127.3, 127.5, 128.8, 129.2, 129.4, 129.5, 132.2, 132.4, 139.4, 133.9, 142.0, 143.8, 150.3, 150.7, 156.4; MS: m/z = 458.55 (calculated), m/z = 459.1 $[M+H]^+$ (found); elemental composition calculated for $C_{31}H_{26}N_2O_2$: C, 81.20; H, 5.72; N, 6.11; O, 6.98; found: C, 81.23; H, 5.79; N, 6.13; O, 6.99.

4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-4-carbaldehyde (8e)

Yield 78%; yellow solid; mp 119–121 °C; FT-IR (cm^{-1}): 2251, 2125, 1661, 1052, 1024, 1005, 821, 758, 624; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.27 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 4.4 Hz, 1H, C_4-*H* isoxazoline), 3.39-3.43 (m, 1H, C_4-*H* isoxazoline), 3.78 (dd, J_{1-2} = 13.2 Hz, J_{1-1} = 6.0 Hz, 1H, N-*CH*), 4.01 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.72-4.76 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 2.8 Hz, 2H, Ar*H*), 6.97-7.31 (m, 8H, Ar*H*), 7.67 (t, *J* = 7.2 Hz, 3H, Ar*H*), 7.79 (dd, J_{1-2} = 6.8 Hz, J_{1-1} = 1.6 Hz, 2H, Ar*H*), 7.87-7.90 (m, 1H, Ar*H*), 8.00-8.03 (m, 1H, Ar*H*), 8.20 (t, *J* = 1.6 Hz, 1H, Ar*H*), 10.06 (s, 1H, *CHO*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 54.2, 78.8, 121.4, 124.1, 127.5, 127.6, 128.3, 128.8, 129.5, 130.3, 132.2, 132.4, 132.9, 133.9, 137.3, 140.4, 140.5, 150.3, 150.6, 156.4, 193.6; MS: m/z = 456.53 (calculated), m/z = 457.0 $[M+H]^+$ (found); elemental composition calculated for $C_{31}H_{24}N_2O_2$: C, 81.56; H, 5.30; N, 6.14; O, 7.01; found: C, 81.58; H, 5.37; N, 6.16; O, 7.02.

5-[3-(4'-Methoxy-biphenyl-4-yl)-4,5-dihydro-isoxazol-5-ylmethyl]-5H-dibenzo[*b,f*]azepine (8f)

Yield 87%; pale yellow solid; mp 187–189 °C; FT-IR (cm^{-1}): 2926, 2853,1777, 1551, 1529, 1424, 1369, 1258, 1222, 1172; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.28 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 6.4 Hz, 1H, C_4-*H* isoxazoline), 3.40 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 10.4 Hz, 1H, C_4-*H* isoxazoline), 3.75-3.80 (m, 4H, N-*CH* and OCH_3), 4.05 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.0 Hz, 1H, N-*CH*), 4.72-4.77 (m, 1H, C_5-*H* isoxazoline), 6.77 (d, *J* = 1.6 Hz, 2H, Ar*H*), 7.03 (d, *J* = 8.8 Hz, 4H, Ar*H*), 7.10-7.32 (m, 6H, Ar*H*), 7.61-7.69 (m, 6H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 54.2, 55.6, 78.5, 114.9, 124.0, 126.7, 127.4, 128.1, 128.2, 129.5, 131.9, 132.2, 132.5, 133.9, 141.5, 156.5, 159.7; MS: m/z = 458.55 (calculated), m/z = 459.10 $[M+H]^+$ (found); elemental composition calculated for $C_{31}H_{26}N_2O_2$: C, 81.20; H, 5.72; N, 6.11; O, 6.98; found: C, 81.23; H, 5.79; N, 6.13; O, 6.99.

5-[3-(4'-Trifluoromethoxy-biphenyl-4-yl)-4,5-dihydro-isoxazol-5-ylmethyl]-5H-dibenzo[*b,f*]azepine (8g)

Yield 80%; yellow solid; mp 165–167 °C; FT-IR (cm^{-1}): 2902, 2260, 2128, 1662, 1075, 1021, 987; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.25 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.37 (dd, J_{1-2} = 17.2 Hz, J_{1-1} = 10.9 Hz, 1H, C_4-*H* isoxazoline), 3.75 (dd, J_{1-2} = 14.0 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.01 (dd, J_{1-2} = 13.6 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.70-4.75 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.96-7.29 (m, 8H, Ar*H*), 7.41 (d, *J* = 8 Hz, 2H, Ar*H*), 7.62 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.70 (d, *J* = 8 Hz, 2H, Ar*H*), 7.77 (d, *J* = 8.8 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 54.1, 78.7, 119.2, 121.3, 121.3, 121.8, 121.9, 124.0, 127.5, 127.6, 129.0, 129.3, 129.4, 129.5, 132.2, 132.4, 133.9, 138.9, 140.3, 148.5, 150.3, 150.7, 156.4; MS: m/z = 512.525 (calculated), m/z = 513.10 $[M+H]^+$ (found); elemental composition calculated for $C_{31}H_{23}F_3N_2O_2$: C, 72.65; H, 4.52; F, 11.12; N, 5.47; O, 6.24; found: C, 72.67; H, 4.57; N, 5.48; O, 6.25.

4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-4-carbonitrile (8h)

Yield 84%; pale yellow solid; mp 190–193 °C; FT-IR (cm^{-1}): 2971, 1483, 1457, 1226, 1052, 907; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.29 (dd, J_{1-2} = 16.8 Hz, J_{1-1} = 6.4 Hz, 1H, C_4-*H* isoxazoline), 3.40 (dd, J_{1-2} = 12.0 Hz, J_{1-1} = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.78 (dd, J_{1-2} = 13.2 Hz, J_{1-1} = 6.0 Hz, 1H, N-*CH*), 4.04 (dd, J_{1-2} = 14.0 Hz, J_{1-1} = 6.4 Hz, 1H, N-*CH*), 4.73 (m, 1H, C_5-*H* isoxazoline), 6.72 (d, *J* = 2.8 Hz, 2H, Ar*H*), 6.99-7.28 (m, 8H, Ar*H*), 7.64-7.76 (m, 2H, Ar*H*), 7.76-7.85 (m, 2H, Ar*H*), 7.85-7.91 (m, 4H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.1, 54.2, 78.9, 110.8, 119.2, 121.3, 124.0, 127.6, 127.8, 127.9, 129.5, 130.1, 132.2, 132.4, 133.3, 133.9, 139.8, 144.0, 150.3, 150.6, 156.3; MS: m/z = 453.53 (calculated), m/z = 454.0 $[M+H]^+$ (found); elemental composition calculated for $C_{31}H_{23}N_3O$: C, 82.10; H, 5.11; N, 9.27; O, 3.53; Found: C, 82.13; H, 5.16; N, 9.29; O, 3.54.

[4'-(5-Dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-biphenyl-4-ylmethyl]-phenyl-amine (8i)

Yield 77%; pale brown solid; mp 173–175 °C; FT-IR (cm^{-1}): 3444, 2251, 1661, 1053, 1005; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.26 (dd, $J_{1\text{-}2}$ = 13.6 Hz, $J_{1\text{-}1}$ = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.36 (dd, $J_{1\text{-}2}$ = 14.8 Hz, $J_{1\text{-}1}$ = 8.4 Hz, 1H, C_4-*H* isoxazoline), 3.76 (dd, $J_{1\text{-}2}$ = 14.0 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 4.01 (dd, $J_{1\text{-}2}$ = 14.0 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 4.30 (d, *J* = 5.6 Hz, 2H, N-CH_2), 4.72 (m, 1H, C_5-*H* isoxazoline), 6.22 (t, *J* = 6.0 Hz, 1H, Ar*H*), 6.47 (t, *J* = 7.2 Hz, 1H, Ar*H*), 6.58 (dd, $J_{1\text{-}2}$ = 8.8 Hz, $J_{1\text{-}1}$ = 0.8 Hz, 2H, Ar*H*), 6.73 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.99-7.02 (m, 4H, Ar*H*), 7.06-7.26 (m, 6H, Ar*H*), 7.33-7.40 (m, 2H, Ar*H*), 7.51 (dd, $J_{1\text{-}1}$ = 7.6 Hz, $J_{1\text{-}2}$ = 1.6 Hz, 1H, Ar*H*), 7.61 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.65-7.67 (m, 3H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.2, 46.9, 54.2, 78.6, 112.8, 116.2, 121.3, 124.0, 125.4, 125.9, 126.0, 127.3, 127.5, 128.8, 129.2, 129.4, 129.5, 132.2, 132.4, 133.9, 139.6, 141.6, 141.9, 149.0, 150.3, 150.7, 156.4; MS: m/z = 533.66 (calculated), m/z = 534.8 $[M+H]^+$ (found); elemental composition calculated for $C_{37}H_{31}ClN_3O$: C, 83.27; H, 5.86; N, 7.87; O, 3.00; found: C, 83.29; H, 5.90; N, 7.88; O, 3.01.

5-[3-(4-Thiophen-2-yl-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (8j)

Yield 87%; pale brown solid; mp 173–175 °C; FT-IR (cm^{-1}): 2958. 1736, 1592, 1483, 1458, 1434, 1360, 1336, 1298, 1246, 1161; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.25 (dd, $J_{1\text{-}2}$ = 17.2 Hz, $J_{1\text{-}1}$ = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.37 (dd, $J_{1\text{-}2}$ = 17.2 Hz, $J_{1\text{-}1}$ = 10.8 Hz, 1H, C_4-*H* isoxazoline), 3.76 (dd, $J_{1\text{-}2}$ = 14.0 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 3.97-4.02 (m, 1H, N-*CH*), 4.69-4.73 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 2.8 Hz, 2H, Ar*H*), 6.98-7.28 (m, 9H, Ar*H*), 7.55-7.56 (m, 4H, Ar*H*), 7.67 (d, *J* = 8.4 Hz, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.1, 54.2, 78.7, 121.3, 124.0, 124.9, 125.9, 126.8, 127.6, 128.7, 129.1, 129.5, 132.2, 132.4, 133.8, 135.4, 142.8, 150.37, 150.70, 156.3; MS: m/z = 434.55 (calculated) m/z = 435.2 $[M+H]^+$ (found); elemental composition calculated for $C_{28}H_{22}N_2OS$: C, 77.39; H, 5.10; N, 6.45; O, 3.68; S, 7.38; found: C, 77.41; H, 5.17; N, 6.47; O, 3.69.

5-[3-(4-Pyridin-4-yl-phenyl)-4,5-dihydro-isoxazol-5-ylmethyl]-5*H*-dibenzo[*b,f*]azepine (8k)

Yield 86%; yellow solid; mp 108-110 °C; FT-IR (cm^{-1}): 2269, 2246, 2177, 1622, 1085, 901; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.25 (dd, $J_{1\text{-}2}$ = 17.2 Hz, $J_{1\text{-}1}$ = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.37 (dd, $J_{1\text{-}2}$ = 17.2 Hz, $J_{1\text{-}1}$ = 10.9 Hz, 1H, C_4-*H* isoxazoline), 3.75 (dd, $J_{1\text{-}2}$ = 14.0 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 4.01 (dd, $J_{1\text{-}2}$ = 13.6 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 4.71-4.76 (m, 1H, C_5-*H* isoxazoline), 6.73 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.98-7.27 (m, 8H, Ar*H*), 7.68 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.83 (d, *J* = 8.4 Hz, 2H, Ar*H*), 8.64 (s, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.1, 54.1, 78.9, 121.3, 124.0, 127.5, 127.6, 129.3, 129.5, 130.6, 132.2, 132.5, 133.9, 138.7, 146.3, 150.3, 150.7, 156.4; MS: m/z = 429.51 (calculated), m/z = 430.1 $[M+H]^+$ (found); elemental composition calculated for $C_{29}H_{23}N_3O$: C, 81.09; H, 5.40; N, 9.78; O, 3.73; found: C, 81.91; H, 5.45; N, 9.79; O, 3.74.

6-Chloro-5-[4-(5-dibenzo[*b,f*]azepin-5-ylmethyl-4,5-dihydro-isoxazol-3-yl)-phenyl]-1,3-dihydro-indol-2-one (8l)

Yield 70%; yellow solid; mp 160–162 °C; FT-IR (cm^{-1}): 3441, 2988, 2251, 2125, 1661, 1052, 1024, 1005; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.29 (dd, $J_{1\text{-}2}$ = 16.8 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, C_4-*H* isoxazoline); 3.40 (dd, $J_{1\text{-}2}$ = 12.0 Hz, $J_{1\text{-}1}$ = 6.8 Hz, 1H, C_4-*H* isoxazoline), 3.45 (s, 2H, CH_2), 3.78 (dd, $J_{1\text{-}2}$ = 13.2 Hz, $J_{1\text{-}1}$ = 6.0 Hz, 1H, N-*CH*), 4.04 (dd, $J_{1\text{-}2}$ = 14.0 Hz, $J_{1\text{-}1}$ = 6.4 Hz, 1H, N-*CH*), 4.74-4.78 (m, 4H, C_5-*H* isoxazoline), 6.77 (d, *J* = 1.6 Hz, 2H, Ar*H*), 6.94 (s, 1H, Ar*H*), 7.02-7.32 (m, 9H, Ar*H*), 7.41-7.44 (m, 2H, Ar*H*), 7.62-7.64 (m, 2H, Ar*H*), 10.58 (s, 1H, *NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.82, 38.25, 54.20, 78.6, 110.4, 121.3, 124.0, 126.0, 126.7, 127.3, 127.4, 128.7, 129.5, 130.1, 130.2, 132.1, 132.2, 132.4, 133.9, 140.9, 145.0, 150.3, 150.7, 156.5, 176.7; MS: m/z = 518.00 (calculated), m/z = 520.2 $[M+2H]^+$ (found); elemental composition calculated for $C_{32}H_{24}ClN_3O_2$: C, 74.20; H, 4.67; Cl, 6.84; N, 8.11; O, 6.18; found: C, 74.23; H, 4.72; N, 8.13; O, 6.19.

Assessment of antimalarial activity

P. falciparum parasites were cultured according to the method of Trager and Jensen.[40] Briefly, 3D7, D6, W2 and 7G8 *P. falciparum* strains were cultured in RPMI 1640 medium (Gibco) supplemented with 25 mM HEPES, 29 mM sodium bicarbonate, 0.005% hypoxanthine, *p*-aminobenzoic acid (2 mg/liter), gentamycin sulfate (50 mg/liter) and 5% AlbuMAX II (Invitrogen) using fresh O-positive human red blood cells at 2% hematocrit. The cultures were maintained at 37 °C under 5% O_2, 5% CO_2, and 90% N_2. The antimalarial activity of isoxazoline compounds were determined by a fluorometric method using SYBR Green I.[41,42] Briefly, stock solutions of isoxazoline were prepared in DMSO (10 mM) and were serially diluted with complete culture medium to give concentrations ranging from 0.2 to 200 µM and the final concentration of DMSO was less than 0.1%. 100 µL of each testing solution was mixed with 100 µL of 0.2% parasitized red blood cells in the ring stage at 2% hematocrit in complete medium and dispensed into 96-well plates. Untreated- and DMSO (0.1%) vehicle-treated parasites were used as controls. The plates were incubated at 37 °C for 72 h and the experiment was performed in triplicate. To each well, 100 µL of lysis buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100) containing 0.2 µL/mL of SYBR Green I (Life Technology) was added. The plates were wrapped with aluminium foil and incubated in the dark at room temperature for 1 h. The fluorescence intensity was measured using multi-well plate fluorescence reader at an excitation and emission wavelengths of 485 and 535 nm, respectively. The values were expressed as relative fluorescence units. The IC_{50} values (the effective concentrations that inhibit parasite growth by 50%) of three independent experiments were plotted using nonlinear regression (Sigmoidal dose response) equation (GraphPad Prism version 4.01, GraphPad Software, La Jolla, CA).

Cytotoxicity assay

The toxicity of 3,5-disubstituted isoxazoline derivaives against human cells was assessed by the MTS assay[43,44] using human embryonic kidney cells (HEK 293 cell line) and human adenocarcinoma epithelial cells (A549 cell line). Briefly, 5×10^3 cells/well in 100 µL of DMEM medium supplemented with 10% bovine fetal serum were plated in 96-well plates. After 24 h, the stock solutions of 3,5-disubstituted isoxazoline in DMSO that were serially diluted to obtain concentrations ranging from 0.5 µM to 200 µM in 100 µl complete medium was added to each well and incubated at 37 °C for 72 h. The cells treated with DMSO (1%) alone were used as vehicle controls. To each well added 20 µl of MTS/PMS reagent (Promega, Madison, WI), incubated for 3 h at 37 °C and the absorbance measured at 490 nm. The assay was performed in triplicate and the values from three independent experiments were used to calculate the IC_{50} values (GraphPad Prism version 4.01).

Molecular modelling methods

The software used for all computational calculations is Schrödinger Software Suite 2015-2 with hardware 2x Intel Xeon 1.9 GHz E5-

2420/ 6C/15MB Cache RAM 6 x 4Gb DDR-3 1333MHz ECC RDIMM 4x 500 Gb Graphics Card NvidiaQuadro 600 machine.

The docking studies of 3,5-disubstituted isoxazoline tethered dibenzazepine derivatives with PfA-M1 were performed by using Schrödinger Glide.[45] The structures were drawn using 2D sketcher of Schrödinger maestro 10.2. The ligands were optimised for the docking using LigPrep[46,47] where the structures were converted to 3D and energy optimised using OPLS2005. Ionisation states were generated for all molecules at pH range 7.0 ± 2 using Epik module, in LigPrep with all other default options. 3D structure of inhibitor bound PfA-M1 was retrieved in form from protein data bank[35] (PDB ID: 4X2U). The downloaded protein structure was refined using Protein preparation wizard where in missing hydrogen atoms, amino acid side chains and missing residues were added and bond order were set. The unnecessary hetero atoms and water molecules were deleted except the Zn^{+2} and co-crystallised inhibitor. The structure was optimized to create H-bond network and finally minimized to remove any steric clashes using OPLS2015 force field.[48] The fully optimized protein structure was used for the receptor grid generation using Glide software.[49] The grid was generated by choosing the centroid of bound ligand as the centroid of the grid. The prepared ligands were docked to protein grid using extra precession (XP) mode. The protein-ligand complex was further analysed and visualized using Maestro.

Molecular dynamics simulation analysis

The molecule (**6k**) that exhibited excellent antimalarial activity and low toxicity to human cells was assessed by molecular dynamics analysis to determine the binding stability and the binding pattern. The protein complex docked with the inhibitor was submitted for 10 ns simulation. The molecular dynamics simulation was run using Desmond.[50] The orthorhombic simulation box was prepared using the system builder panel of Desmond with TIP3P explicit water model so that the minimum distance between the protein surface and the solvent surface is 10 Å. The counter ions were added to neutralise the system and 0.15 M NaCl was used to provide the iso-osmotic salt environment. The system was minimized with 2000 iterations and with convergence criteria of 1 kcal/mol/Å. The minimised system was subjected to the 10 ns molecular dynamic simulation using NPT ensemble at temperature of 300K and atmospheric pressure (1.013 bars) with the default setting of relaxation before simulation. The Nose-Hoover Chain thermostat and Matrtyna-Tobias-Klein barostate was used to maintain the temperature and pressure, respectively. The time step of 2 fs was considered during the simulation and every 10 ps, the energy and structure was recorded and saved in the trajectory. From the trajectory generated, the simulation interaction diagram was generated to analyse the results.

Conclusions

In summary, we have synthesized dibenzazepine tethered 3,5-disubstituted isoxazolines, and evaluated their antimalarial activity against *P. falciparum* drug-sensitive 3D7 strains and toxicity to human lung cancer A549 and kidney HEK 293 cells. Several compounds that showed low cytotoxic activity effectively inhibited the growth of parasite. Compounds (**6j, 6k, 8c, 8k and 8l**) that showed excellent antimalarial activity in initial screening were further assessed using an additional chloroquine-sensitive parasite strain (D6) and two chloroquine-resistant parasite strains (W2 and 7G8). Molecular docking studies revealed the mode of the dibenzazepine derivatives binding to PfA-M1. The molecular dynamics simulation confirmed the stability of the binding mode of the ligand **6k** predicted by docking studies. The co-ordination bond formed between the isoxazoline moiety of the ligand and the enzyme-bound Zn^{+2} ion, π–π interaction between dibenzazepine and His496, and π–cation interactions between piperidine moiety and Tyr575, and hydrophobic interactions with residues Val493, Val523, Tyr580, Tyr575, Val459, Met462, Met1034, Ala320 stabilized the dibenzazepine binding to PfA-M1. Our experimental and theoretical finding data show that, several compounds possess excellent antimalarial activity with minimal or no cytotoxicity. Thus, overall our results suggest that the dibenzazepine tethered 3,5-disubstituted isoxazolines are promising candidates for the development of antimalarial drugs.

Acknowledgements

KSV would like to thank UGC-BSR for financial support and IOE, University of Mysore, Karnataka for NMR, mass spectrometry facility. MPS thank UGC-SAP-DRS-Phase III, New Delhi and VGST-SMYSR, Bangalore, Karnataka for funding. ACV would like to thank CSIR-SRF, New Delhi for financial support. All authors acknowledge Department of Biochemistry and Molecular Biology, Penn State University College of Medicine, Hershey Medical Centre, Hershey, PA, for providing facility for assessing antimalarial activity, and Schrödinger, for providing evaluation licence.

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