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DOI: 10.1039/C7NJ01962A



# Synthesis, characterization, *in silico* molecular docking study and biological evaluation of 5-(phenylthio) pyrazole based polyhydroquinoline core moiety

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## Abstract

A multicomponent cyclocondensation reaction has been developed by incorporating 3-methyl-5-substituted phenylthio-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a-c,** various enaminones **6a-c** and different active methylene compounds **7a-c** (malononitrile **7a** /ethylcaynoacetate**7b** /caynoacetamide**7c**) in presence of piperidine as basic catalyst, to afford combinatorial library of polyhydroquinoline scaffolds *i.e*. **8a-p.** The targeted compounds were synthesized in good to excellent yield (71-84%). All the synthesized compounds have been characterized by $^{1}$H NMR, $^{13}$C NMR, IR, mass spectrometric techniques and elemental analysis. All the synthesized compounds were evaluated *in vitro* for their antibacterial, antitubercular and antimalarial activities. *In silico* molecular docking study as well as *in silico* pharmacokinetics evaluation have been carried out. Many candidates of this new class revealed noticeable activities against first line drugs.

**Key words**: *In silico* molecular docking, *In silico* pharmacokinetics evaluation, 5-(phenylthio) pyrazole, Polyhydroquinoline, Multi-component reaction, Biological screening.

## 1. Introduction

Malaria causes serious social and economic consequences. 300-500 million people are affected annually and about 3 million deaths occur due to this severe global health issue [1, 2]. Amongst the four *Plasmodium* parasites, *Plasmodium falciparum* is considered

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responsible for ~95% of death [3]. Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (MTB) which is a human pathogen. Nearly one-third of the global population is infected by MTB. TB is declared as a global emergency by World Health Organization as it is suspected that about 30 million people will suffer from TB in next two decades [4, 5]. The second most common reason for death is microbial infections after heart attack being the prime one. This is because of their resistance towards the existing antibiotic remedy.

Fluorine plays a crucial role [6-8] in improving pharmacodynamic and pharmacokinetic properties [9, 10] of drugs molecules. Trifluoromethylation is the most significant strategy to modulate physical and biological properties. High lipophilicity of trifluoromethyl group enhances *in vitro* uptake and transport of the candidate [11].

Fluoro-substituted pyrazole and its derivatives are the significant class of heterocycles enjoying a remarkable position in medicinal chemistry. Pyrazole derivatives exhibit variety of pharmacological activities including antimicrobial [12, 13], anti-inflammatory [14, 15], cytotoxic [16], antitumor [17-19], antiviral [20], anticancer [21], analgesic [22-24] and anti-parkinson [25] activities.

Multicomponent condensation reaction, MCR [26, 27] is one of the leading methods to synthesize small molecules. Several condensation reagents react in a single step by simultaneous reactions to yield the desired products. Alteration in the reaction components of MCR can easily lead to the molecular diversity in combinatorial libraries.

From the discovery of the drug molecules, their exposures to the various disease causative agents make them drug-resistant. To overcome that problem, there is an immense need to design fluoro-pyrazole combined polyhydroquinoline nucleus which combats against such pathogens in an effective manner. In this perspective, the current communication describes design and synthesis of polyhydroquinoline core molecules from the 5-substituted phenylthio-1-phenyl-1*H*-pyrazole-4-carbaldehydes, various active methylene and enaminones under conventional conditions. The synthesized compounds were also tested for their diversified activities such as antimalarial, antitubercular and antimicrobial activities. Out of all listed biological activities, synthesized compounds showed comparatively good antimalarial activity. As an outcome of it, we decided to perform molecular docking of synthesized compounds with the dihydrofolate reductase (DHFR) receptor.

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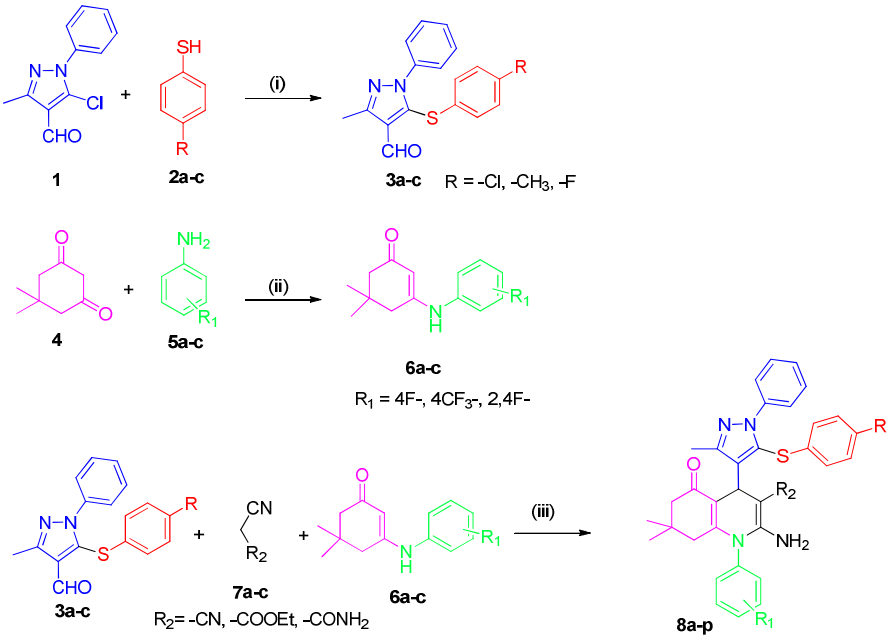
The antimalarial agents target the malaria enzyme, dihydrofolate reductase (DHFR). The incorporation of wild-type and the quadruple mutant DHFR from the *P. falciparum* malarial strain should be considered for development of new antimalarials [28]. The quinolone containing cores (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**) were found active against *P. falciparum* strain. Molecular docking studies of active quinolone compounds and standard drugs (**chloroquine** and **quinine**) were performed to check the possible interactions of synthesized molecules with molecular target wild-type *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (pfdhfr-ts) (PDB ID: **4DPD**).

## 2. Chemistry

The synthesis of targeted polyhydroquinoline clubbed 5-phenylthioxypyrazole derivatives is presented in **Scheme 1**. 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** was prepared according to the reported method [29, 30].




**Scheme 1**. Synthesis of substituted 2-amino-4-(5-((4-substitutedphenyl)thio)-3-methyl-1- phenyl-1*H*-pyrazol-4-yl)-1-(substitutedphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8 hexahydro quinoline-3-carbonitrile **8a-p** (i) DMF, $K_2CO_3$, Reflux 2 h (ii) Methanol, Acetic acid, reflux 0.5-1 h (iii) Piperidine, Ethanol, Reflux for 1-3 hr.

5-((4-substituted-phenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3a-c** was synthesized by refluxing compound **1** and thiophenols **2a-c** in presence of $K_2CO_3$(anhydrous) as a basic catalyst using DMF as a solvent. The required enaminones **6a-c** were synthesized

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by the reaction of dimedone **4** with various amines **5a-c** by refluxing in the presence of catalytic amount of glacial acetic acid in methanol.

**Table 1.** Preliminary characterization of synthesized compounds **8a-p**.

| Comp. | R | $R_1$ | $R_2$ | Yield[a](%) |
|---|---|---|---|---|
| **8a** | 4-Cl | 4-F | -CN | 81 |
| **8b** | 4-Cl | 4-F | -COOEt | 79 |
| **8c** | 4-Cl | 4-F | $-CONH_2$ | 73 |
| **8d** | $4-CH_3$ | 4-F | -CN | 84 |
| **8e** | $4-CH_3$ | 4-F | -COOEt | 76 |
| **8f** | $4-CH_3$ | $4-CF_3$ | -CN | 72 |
| **8g** | $4-CH_3$ | $4-CF_3$ | -COOEt | 80 |
| **8h** | $4-CH_3$ | 2,4-F | -CN | 79 |
| **8i** | $4-CH_3$ | 2,4-F | -COOEt | 73 |
| **8j** | 4-Cl | 2,4-F | -CN | 75 |
| **8k** | 4-Cl | 2,4-F | -COOEt | 78 |
| **8l** | 4-Cl | 2,4-F | $-CONH_2$ | 81 |
| **8m** | 4-F | 4-F | -CN | 83 |
| **8n** | 4-F | 4-F | -COOEt | 71 |
| **8o** | 4-F | $4-CF_3$ | -CN | 72 |
| **8p** | 4-F | $4-CF_3$ | -COOEt | 77 |

[a]Isolated yield

The targeted molecules were synthesized by refluxing the mixture of 5-((4-substituted-phenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3a-c**, various enaminones **6a-c** and different active methylene compounds **7a-c** (malononitrile **7a**, ethylcyanoacetate **7b** and caynoacetamide **7c**) using piperidine in absolute ethanol (**Scheme 1**).

**Scheme 2** represents a plausible mechanism for the reaction. The formation of quinoline derivatives **8a-p** was attempted through an initial *in situ* formation of heterylidenenitrile via Knoevenagel condensation between aldehyde **3a-c** and various active methylene compounds **7a-c**. Finally, Michael addition of enaminones **6a-c** to the heterylidene olefins gave acyclic intermediate **8a'-p'**. Nucleophilic attack of the -NH group on the cyano carbon cyclises this intermediate which then tautomerizes to afford cyclised polyhydroquinoline derivatives **8a-p**.

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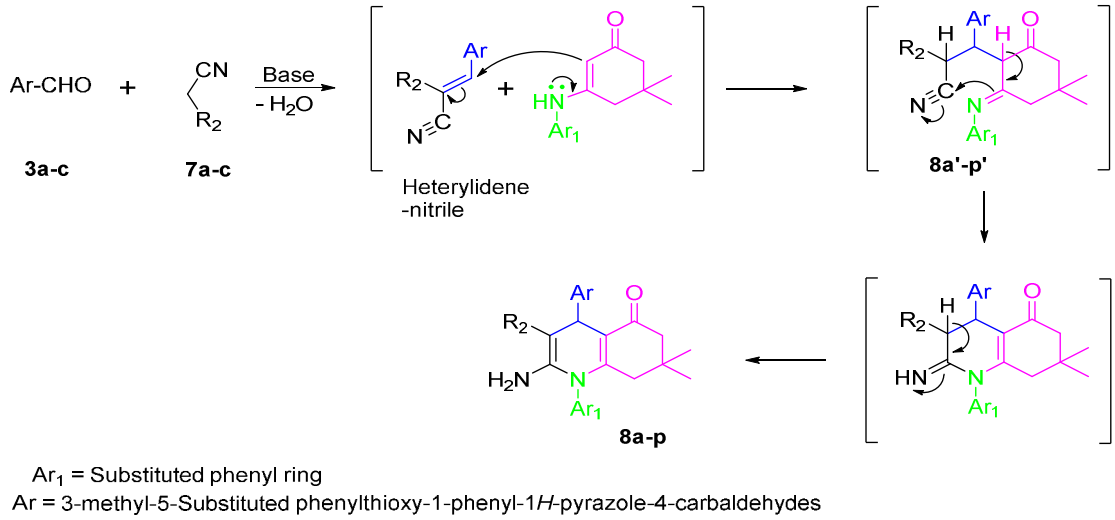


**Scheme 2**. Mechanistic pathway for the synthesis of polyhydroquinoline derivatives **8a-p**.

## 3. Pharmacology

### 3.1. *In vitro* antimicrobial activity

The synthesized compounds **8a-p** were tested for their antimicrobial activity by broth microdilution method according to National Committee for Clinical Laboratory Standards [31-33]. Three Gram-positive (*Bacillus subtilis* MTCC 441, *Clostridium tetani* MTCC 449, and *Streptococcus pneumoniae* MTCC 1936) and three Gram-negative (*Salmonella typhi* MTCC 98, *Escherichia coli* MTCC 443, and *Vibrio cholerae* MTCC 3906) bacteria were chosen for antibacterial screening using **ampicillin**, **ciprofloxacin**, **norfloxacin** and **chloramphenicol** as the standard antibacterial agents. Two fungal species (*Aspergillus fumigats* MTCC 3008 and *Candida albicans* MTCC 227) were screened employing **griseofulvin** and **nystatin** as the standard antifungals. Mueller-Hinton broth was used to dilute the drug suspension for the test. The desired test concentration of compounds was achieved using DMSO as the diluents. The result of antimicrobial testing is depicted in **Table 2**.

### 3.2. *In vitro* antituberculosis activity

Using Lowenstein-Jensen medium as described by Rattan [34] the synthesized compounds **8a-p** were screened for their *in vitro* antituberculosis activity. Screening was conducted at 250 µg/mL of drug concentration against *Mycobacterium tuberculosis* H37Rv strain using **isoniazid** and **rifampicin** as the standard drugs. The results are presented in **Table 3** in form of % inhibition.

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### 3.3. *In vitro* antimalarial activity

**Quinine** and **chloroquine** as the reference compounds were employed for i*n vitro* antimalarial activity of the compounds **8a-p** against *P. falciparum* strain. The result of antimalarial screening is expressed in **Table 4.**

### 3.4. *In silico* study

#### 3.4.1. *In silico* molecular docking study

The mol2 file of active compounds (**8a, 8c, 8d, 8l, 8m, 8o**) and standard drugs (**chloroquine** and **quinine**) were prepared using ChemBio3D Ultra 14.0. The mol2 formats of all ligands were used in the molecular docking study. The 3d structure of wild-type *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase' (PDB ID: **4DPD**) was downloaded from RCSB website (www.rcsb.org/pdb). The protein structure was opened in CLC Drug Discovery Workbench 4.0 software. The water molecules, intramolecular bonds, cofactors and ligands were removed from receptors. The 23/23 binding pockets were found with set up of 25 °A radius size of the protein.

#### 3.4.2. *In silico* pharmacokinetics evaluation

The pharmacokinetic parameters of active compounds indicated earlier were obtained using programs VlifeMDS 4.6 (Vlife Sciences, Pune, India) and ADMET software. These programs computed pharmacokinetic parameters such as logP, molecular weight, PSA, HBA, HBD, BBB, %HIA, Caco2 etc.

## 4. Results and discussion

### 4.2. Analytical results

The structures of the newly synthesized compounds were confirmed by mass spectrometry, $^{1}$H NMR, $^{13}$C NMR, FT-IR and elemental analysis. The IR spectrum of compounds **8a-p** exhibited characteristic absorption band in the range 781-755 $cm^{-1}$. This can be assigned the presence of thioether linkage. The characteristic absorption band around 1680-1652 $cm^{-1}$ which may be due to the presence of carbonyl group ($C_5$ of the polyhydroquinoline ring). All the compounds show -C≡N stretching in the range of 2297-2180 $cm^{-1}$, strong absorption band in the range of 1373-1351 $cm^{-1}$ due to -$CH_3$ stretching and absorption band in the range 3483-3324 $cm^{-1}$ due to asymmetric & symmetric stretching of -$NH_2$. The presence of the -CH proton ($C_4$-H of polyhydroquinoline ring) and –$NH_2$ protons are confirmed by $^{1}$H NMR spectra of compounds **8a-p** as a sharp singlet around δ 5.43-4.63 ppm and a broad singlet at δ 6.69-3.60 ppm respectively. Amide and aromatic protons are found to be resonated as

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multiplets in the range between δ 7.79-6.57 ppm. In $^{13}C$ NMR spectra, the carbonyl carbon ($C_5$ of polyhydroquinoline ring) was displayed at δ 193.88-195.97 ppm. The chemical structures are further confirmed by molecular ion peak ($M^+$) corresponding to their respective molecular weights in mass spectra.

### 4.3. Biological section

#### 4.3.1. *In vitro* antibacterial activity

All the synthesized compounds (**8a-p)** showed moderate to very good antibacterial activity.

**Table 2.** *In vitro* antimicrobial activity expressed in terms of MIC, µg/mL for prepared derivatives **8a-p**

| Compound | Gram-positive bacteria | | | Gram-negative bacteria | | | Fungi | |
|---|---|---|---|---|---|---|---|---|
| | *S.P.* MTCC 1936 | *B.S.* MTCC 441 | *C.T.* MTCC 449 | *E.C.* MTCC 443 | *S.T.* MTCC 98 | *V.C.* MTCC 3906 | *C.A.* MTCC 227 | *A.F.* MTCC 3008 |
| 8a | 500 | 500 | **250** | **100** | 250 | 250 | >1000 | >1000 |
| 8b | **100** | 500 | **62.5** | 200 | 500 | 200 | 1000 | >1000 |
| 8c | 200 | **200** | 500 | 250 | 200 | 200 | 1000 | 500 |
| 8d | **62.5** | **250** | **125** | **100** | 200 | 1000 | **500** | 500 |
| 8e | 500 | **250** | 1000 | 250 | 250 | 1000 | **500** | >1000 |
| 8f | 500 | 500 | 500 | 250 | **100** | **62.5** | 1000 | >1000 |
| 8g | **100** | **100** | 500 | 500 | 200 | 500 | 1000 | 1000 |
| 8h | **100** | **62.5** | **100** | **100** | 500 | 500 | **500** | >1000 |
| 8i | 200 | 500 | **250** | 500 | **100** | **100** | **500** | **100** |
| 8j | 500 | 500 | **200** | **100** | 200 | 200 | **250** | 1000 |
| 8k | **100** | 1000 | **250** | 200 | 200 | **100** | **250** | 1000 |
| 8l | 500 | 500 | **200** | 500 | 500 | 500 | 1000 | **100** |
| 8m | 200 | **100** | **200** | **62.5** | **100** | 500 | 1000 | 1000 |
| 8n | 500 | **100** | 500 | **100** | 200 | 500 | **250** | 1000 |
| 8o | 200 | **200** | **62.5** | **62.5** | **100** | **100** | **500** | 500 |
| 8p | 500 | 1000 | **200** | 500 | 500 | **100** | **500** | 250 |
| A | 100 | 250 | 250 | 100 | 100 | 100 | n. t.[a] | n. t. |
| B | 10 | 100 | 50 | 10 | 10 | 10 | n. t. | n. t. |
| C | 50 | 50 | 50 | 50 | 50 | 50 | n. t. | n. t. |
| D | 25 | 50 | 100 | 25 | 25 | 25 | n. t. | n. t. |
| E | n. t. | n. t. | n. t. | n. t. | n. t. | n. t. | 100 | 100 |
| F | n. t. | n. t. | n. t. | n. t. | n. t. | n. t. | 500 | 100 |

*S.P.= Streptococcus pneumoniae*, *B.S.= Bacillus subtilis*, *C.T.= Clostridium tetani*, *E.C.= Escherichia coli* *S.T.= Salmonella typhi*, *V.C.= Vibrio cholerae*, *C.A.= Candida albicans*, *A.F.= Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Norfloxacin, C: Chloramphenicol, D: Ciprofloxacin, E: Nystatin, F: Griseofulvin, [a] n.t.: not tested.

It has been noticed that against *S. pneumoniae*, compound **8d** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -CN) was found to be more potent *i.e.* 62.5 µg/mL as compared to **ampicillin** *i.e.* 100 µg/mL. The compounds **8b** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -COOEt), **8g** (R = 4-$CH_3$, $R_1$ = 4-$CF_3$, $R_2$ = -

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COOEt), **8h** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), **8k** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -COOEt) showed equivalent activity to that of **ampicillin**. Compound **8h** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt) showed maximum potency *i.e*. 62.5 µg/mL as compared to **ampicillin** *i.e.* 250 µg/mL as well as **norfloxacin** *i.e.* 100 µg/mL against *B. Subtilis*. Compound **8d** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -CN), **8e** (R = 4-$CH_3$, $R_1$ = F, $R_2$ = -COOEt) MIC = 250 µg/mL was found to be equipotent to **ampicillin**. Compounds **8c** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -$CONH_2$) and **8o** (R = 4-F, $R_1$ = 4-$CF_3$, $R_2$ = -CN) *i.e*. 200 µg/mL were noticed to be highly potent as compared to **ampicillin** MIC = 250 µg/mL. Compounds **8b** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -COOEt) and **8o** (R = 4-F, $R_1$ = 4-$CF_3$, $R_2$ = -CN) showed maximum potency *i.e*. MIC = 62.5 µg/mL as compared to **ciprofloxacin** against *C. tetani*. Compounds **8a** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -CN), **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), **8k** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -COOEt), MIC = 250 µg/mL exhibited same influence as compared to **ampicillin**. Compounds **8h** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), MIC = 100 µg/mL and **8d** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -CN), MIC = 125 µg/mL were found to be more potent as compared to **ampicillin** MIC = 250 µg/mL.

Compounds **8m** (R = 4-F, $R_1$ = 4-F, $R_2$ = -CN), and **8o** (R = 4-F, $R_1$ = 4-$CF_3$, $R_2$ = -CN), MIC = 62.5 µg/mL were found to have higher potency against *E. coli* as compared to **ampicillin** against Gram-negative bacteria. Compound **8f** (R = 4-$CH_3$, $R_1$ = 4-$CF_3$, $R_2$ = CN), MIC = 62.5 µg/mL was found to have excellent potency against *V. Cholerae* as compared to that of **ampicillin**. Compounds **8f** (R = 4-$CH_3$, $R_1$ = 4-$CF_3$, $R_2$ = -CN), **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), **8k** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -COOEt), **8m** (R = 4-F, $R_1$ = 4-F, $R_2$ = -CN), **8n** (R = 4-F, $R_1$ = 4-F, $R_2$ = -COOEt) and compounds **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt) **8k** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -COOEt) were noticed to be equipotent as that of **ampicillin** *i.e.* 100 µg/mL respectively against *S. typhi* and *V. cholerae* (**Table 2**).

#### 4.3.2. *In vitro* antifungal activity

**Table 2** depicts antifungal screening data. Compound **8j** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -CN) and **8n** (R = 4-F, $R_1$ = 4-F, $R_2$ = -COOEt) i.e. 250 µg/mL, were found to be more active than **griseofulvin** against *C. albicans*. Also against *C. albicans*, compounds **8d** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -CN), **8e** (R = 4-$CH_3$, $R_1$ = F, $R_2$ = -COOEt), **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), **8o** (R = 4-F, $R_1$ = 4-$CF_3$, $R_2$ = -CN) and **8p** (R = 4-F, $R_1$ = 4-$CF_3$, $R_2$ = -COOEt) showed comparatively similar influence as that of **griseofulvin**. Compounds **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt) and **8l** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -$CONH_2$) exhibited equivalent potency i.e. 100 µg/mL against *A. fumigates*. The results suggest that compound **8o** may be a potent candidate for new class of antimicrobial agent in future.

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#### 4.3.3. *In vitro* antituberculosis activity

The synthesized compounds **8a–p** were screened against *Mycobacterium tuberculosis* H37Rv strain at 250 µg/mL concentration. Compounds **8e** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -COOEt), **8i** (R = 4-$CH_3$, $R_1$ = 2,4-F, $R_2$ = -COOEt), and **8l** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -$CONH_2$) were found to exhibit excellent antituberculosis activity 94%, 95% and 91% respectively.

**Table 3.** *In vitro* antituberculosis activity (% inhibition) of polyhydroquinoline derivatives**8a-p** against *M. tuberculosis* H37Rv (at concentration 250 µg/mL).

| Compound | % Inhibition | Compound | % Inhibition |
|---|---|---|---|
| 8a | 65 | 8j | 79 |
| 8b | 20 | **8k** | **89** |
| 8c | 30 | **8l** | **91** |
| 8d | 46 | 8m | 67 |
| **8e** | **94** | 8n | 80 |
| 8f | 46 | 8o | 65 |
| 8g | 50 | **8p** | **87** |
| 8h | 73 | **Rifampicin** | 98 |
| **8i** | **95** | **Isoniazid** | 99 |

The compounds **8k**(R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -COOEt ) and **8p** (R = 4-f, $R_1$ = 4-$CF_3$, $R_2$ = -COOEt ) were noticed to be quite active. All other remaining compounds showed poor inhibition against *M. tuberculosis* H37Rv growth (**Table 3**).

#### 4.3.4. *In vitro* anti-malarial activity

The synthesized compounds **8a-p** were tested for their anti-malarial screening using **chloroquine** and **quinine** as the reference. Duplicate runs were performed for each experiment and mean values of $IC_{50}$ are summarized in **Table 4**.

**Table 4.** *In vitro* antimalarial activity of polyhydroquinoline derivatives **8a-p.**

| Compound | $IC_{50}$ (µg/mL) | Compound | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| **8a** | **0.065** | 8j | 0.54 |
| 8b | 0.69 | 8k | 1.27 |
| **8c** | **0.085** | **8l** | **0.083** |
| **8d** | **0.076** | **8m** | **0.097** |
| 8e | 0.79 | 8n | 1.32 |
| 8f | 1.25 | **8o** | **0.047** |
| 8g | 0.47 | 8p | 0.54 |
| 8h | 1.10. | **Chloroquine** | 0.020 |
| 8i | 1.40 | **Quinine** | 0.268 |

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The compounds **8a** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -CN), **8c** (R = 4-Cl, $R_1$ = 4-F, $R_2$ = -$CONH_2$), **8d** (R = 4-$CH_3$, $R_1$ = 4-F, $R_2$ = -CN), **8l** (R = 4-Cl, $R_1$ = 2,4-F, $R_2$ = -$CONH_2$), **8m** (R = 4-F, $R_1$ = 4-F, $R_2$ = -CN), **8o** (R = 4-f, $R_1$ = -$CF_3$, $R_2$ = -CN) exhibited $IC_{50}$ in the range of 0.042 to 0.097 which is remarkable against *P. falciparu*m as compared to quinine $IC_{50}$ 0.268.

#### 4.3.4.1. *In silico* molecular docking study

To understand the atomistic level details of plausible protein-ligand interactions and to identify potential ligands against target disease, molecular docking studies was performed. Considering the *in vitro* results against *P. falciparum* strain, it was thought worthy to screen for supportive coordination by *in silico* studies. Thus, molecular docking was performed between ligands (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**, **chloroquine**, **quinine**) and wild-type *P. falciparum* dihydrofolate reductase-thymidylate synthase (pfdhfr-ts) (PDB ID: **4DPD**) using CLC drug discovery workbench 4.0. The docking scores are presented in **Table 5**.

**Table 5.** Molecular Docking Results

| Compound | Score | Hydrogen bond score | Steric Interaction | Ligand Conformation Penalty |
|---|---|---|---|---|
| **8a** | -27.88 | -6.13 | -49.71 | 27.97 |
| **8c** | -28.78 | -9.32 | -48.60 | 29.14 |
| **8d** | -27.82 | -6.07 | -49.73 | 27.98 |
| **8l** | -20.92 | -9.28 | -47.18 | 35.54 |
| **8m** | -27.84 | -6.00 | -49.78 | 27.94 |
| **8o** | -27.10 | -1.72 | -53.51 | 28.13 |
| **Chloroquine** | -49.93 | -2.00 | -50.06 | 2.14 |
| **Quinine** | -45.82 | -2.51 | -44.21 | 0.90 |

A potential interaction was observed between the active molecules (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**, **chloroquine**, **quinine**) and the Pf-DHFR enzyme. The molecules interacted with the active pockets of protein by forming H-bond. The docking scores of molecules (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**) were found to be ranging from -20.92 to -28.78 (**Table 5**) which were not so good as compared to the standard drugs **chloroquine** and **quinine**. Such lower score was due to higher ligand conformation penalty. The -C≡N group of compounds **8a** (**Fig. 1a**), **8d** (**Fig. 1c**) and **8m** (**Fig. 1e**) and -C=O of amide of **8c** (**Fig. 1b**) and **8l** (**Fig. 1d**) interacted with the active pockets of the enzyme forming hydrogen bonds with TYR 365 at different distances

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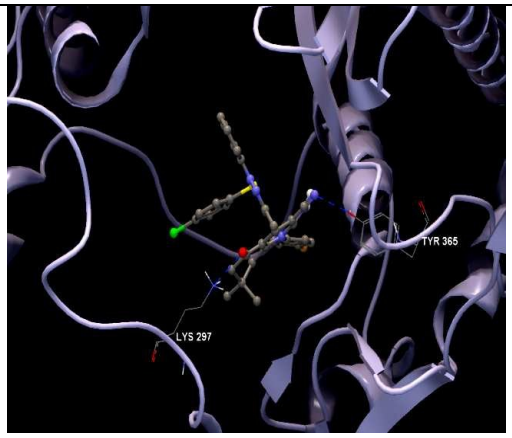
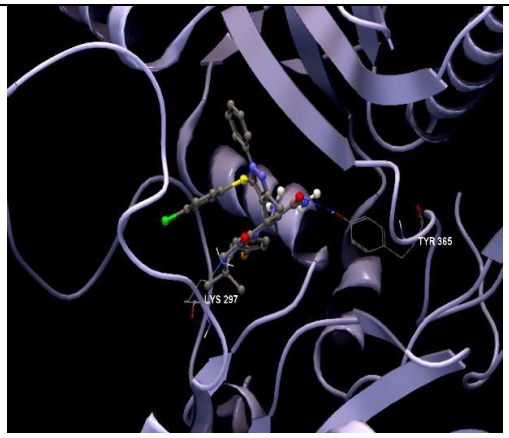
(**Table 6**). It is interesting to note that the -C=O of cyclohexane of these compounds formed a hydrogen bond with LYS 297 also at different distances (**Table 6**). In case of ligand **8o** (**Fig. 1f**), -$NH_2$ group formed H-bond with GLU 287 at a distance of 2.559°A. The standard drug **chloroquine** did not show any H-bond with the receptor **4DPD** (**Fig. 1g**), while -OH group of **quinine** molecule formed H-bond with TYR 322 (3.248 °A) (**Fig. 1h**).

**Table 6.** Hydrogen bonding of compounds (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**) and standard drugs with the receptor wild-type *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PDB ID: **4DPD**).

| Compound | Functional group of ligands | Amino acid of receptor | Bond length (°A) |
|---|---|---|---|
| **8a** | -C≡N | TYR 365 | 2.521 |
| | -C=O of cyclohexene | LYS 297 | 2.942 |
| **8c** | -C=O of cyclohexene | LYS 297 | 3.009 |
| | -C=O of amide | TYR 365 | 2.956 |
| **8d** | -C≡N | TYR 365 | 2.522 |
| | -C=O of cyclohexene | LYS 297 | 2.213 |
| **8l** | -C=O of cyclohexene | LYS 297 | 3.016 |
| | -C=O of amide | TYR 365 | 2.996 |
| **8m** | -C≡N | TYR 365 | 2.493 |
| | -C=O of cyclohexene | LYS 297 | 2.929 |
| **8o** | -$NH_2$ | GLU 287 | 2.559 |
| **Chloroquine** | No H-bonding | - | - |
| **Quinine** | -OH | TYR 322 | 3.248 |




**a**




**b**

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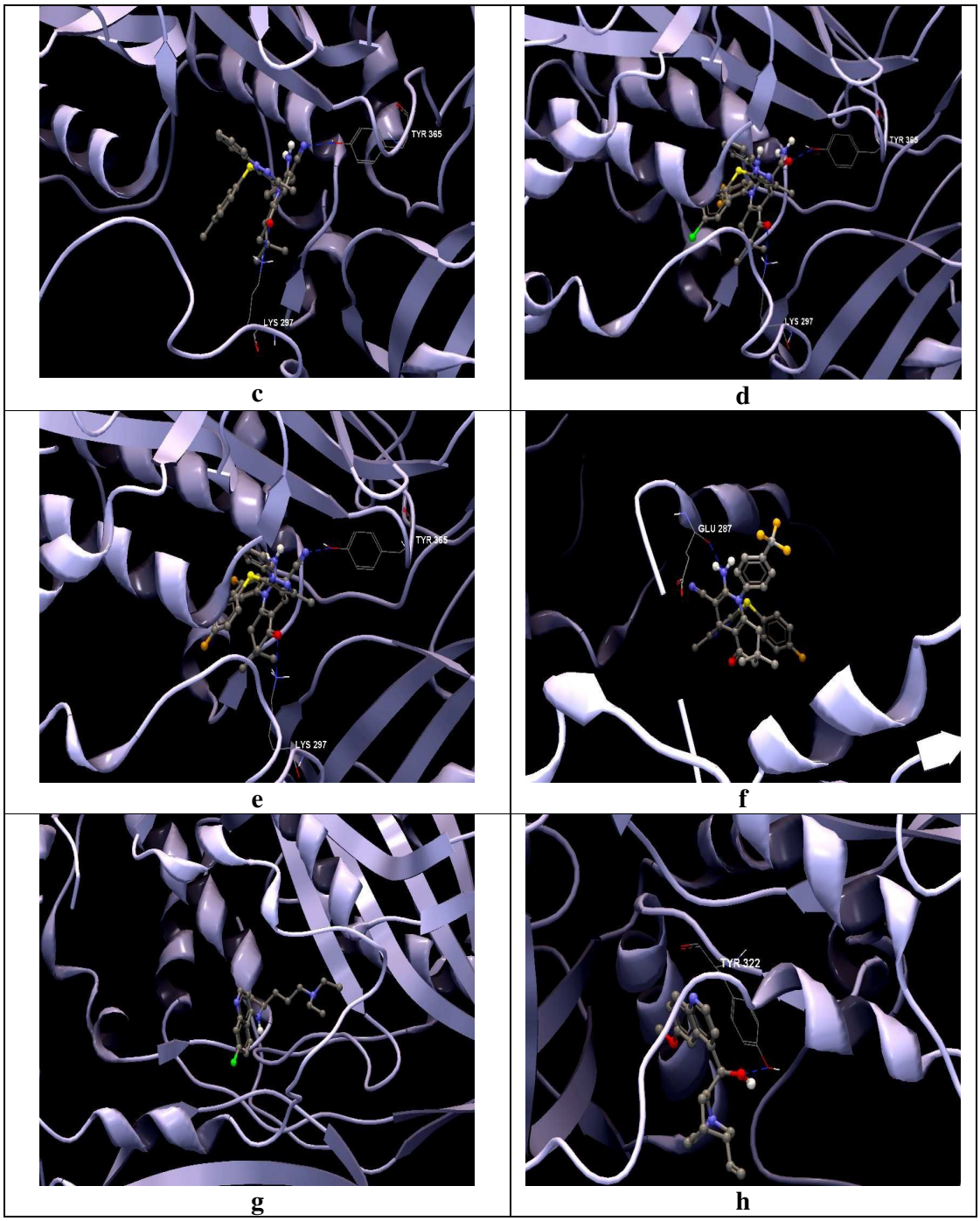


**Fig. 1** shows all ligands docked in best of its conformation with receptor **4DPD**. **(a)** ligand **8a** bound to **4DPD**; **(b)** ligand **8c** bound to **4DPD**; **(c)** ligand **8d** bound to **4DPD**; **(d)** ligand **8l** bound to **4DPD**; **(e)** ligand **8m** bound to **4DPD**; **(f)** ligand **8o** bound to **4DPD**; **(g)** ligand **Chloroquine** bound to **4DPD** and **(h)** ligand **Quinine** bound to **4DPD**

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#### 4.3.4.1. *In silico* pharmacokinetic evaluation

The drug-likeness of the molecules synthesized was identified by predicting the pharmacokinetic properties *in silico*. The potent molecules (**8a**, **8c**, **8d**, **8l**, **8m**, **8o**) were preliminarily screened considering the basic parameters of Lipinski's rule of five, BBB, Caco2, %HIA etc. The pharmacokinetic parameters were obtained by VlifeMDS 4.6 and ADMET. The data found are presented in **Table 7** and **Table 8**. For an orally active compound, two violations of the Lipinski's rule are acceptable. The molecules of the present study were found to follow the rule with maximum violation of two, thus demonstrating their drug-likeness properties.

**Table 7.** Evaluation of pharmacokinetic parameters by VlifeMDS 4.6

| Compound | Mol. Wt.[a] (gm/mol) | HBD[b] | HBA[c] | logP[d] | RotB[e] | PSA[f] (°$A^2$) |
|---|---|---|---|---|---|---|
| **8a** | 610.15 | 2 | 6 | 7.478 | 6 | 66.77 |
| **8c** | 628.16 | 4 | 7 | 6.669 | 6 | 83.78 |
| **8d** | 589.73 | 2 | 6 | 7.214 | 6 | 66.77 |
| **8l** | 646.15 | 4 | 7 | 6.770 | 6 | 83.48 |
| **8m** | 593.70 | 2 | 6 | 6.951 | 6 | 66.77 |
| **8o** | 643.70 | 2 | 6 | 7.734 | 7 | 66.77 |

**a** = Molecular Weight ≤ 500 (gm/mol) [35]; **b** = Hydrogen Bond Acceptor ≤ 10 [35]; **c** = Hydrogen Bond Donor ≤ 5 [35]; **d** = Rotatable Bonds ≤ 10 ; **e** =logP ≤ 5 [36]; **f** = Polar Surface Area ≤ 140°$A^2$ [36]

The logP value and molecular weight of these compounds violated the Rule of Five (RO5) (**Table 7**). The oral bioavailability of the drug molecules are greatly influenced by the optimum values of the descriptors like the polar surface area and rotatable bonds. The important pharmacokinetic parameters with their permissible ranges are delineated in **Table 7**. The active quinolone derivatives showed optimum values of hydrogen bond donor (≤ 5) and hydrogen bond acceptor (≤ 10) and thus owing good bioavailability.

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**Table 8.** Evaluation of pharmacokinetic parameters by ADMET

| Compound | BBB | Caco2 (nm/sec) | % HIA | MDCK (nm/sec) |
|---|---|---|---|---|
| **8a** | 0.0832 | 41.57 | 97.67 | 0.0434176 |
| **8c** | 0.0497 | 25.63 | 96.97 | 0.0434176 |
| **8d** | 0.1542 | 38.54 | 97.42 | 0.0434176 |
| **8l** | 0.0426 | 25.92 | 96.98 | 0.0434158 |
| **8m** | 0.0718 | 39.09 | 97.37 | 0.0434183 |
| **8o** | 0.1350 | 38.31 | 97.44 | 0.0434160 |

**BBB** (Blood Brain Barrier) [37]: High absorption CNS >2.0, Middle absorption CNS 2.0-0.1, Low absorption to CNS <0.1; **Caco2** [38]: High permeability >70, Middle permeability 4-70, Low permeability <4; **%HIA** (Human Intestinal Absorbance) [38]: Well absorbed compounds 70-100%, Moderately absorbed compounds 20-70%, Poorly absorbed compounds 0-20%; **MDCK** [39]: Higher permeability >500, Medium Permeability 25-500, lower permeability <25.

The molecules except **8d** and **8o** showed moderate absorption to central nervous system. Human intestinal absorption is one of the important factors to be studied in relation to the absorption of the drug molecule, which was further confirmed by predicted Caco2 cell permeability. However, the value of %HIA suggested that they had higher absorption to intestine but moderate permeability to Caco2 cell. Moreover, these molecules had lower MDCK permeability. The results showed that these compounds might be good candidates for drug.

## 5. Conclusion

In finding out of new structural motifs looking promising as powerful antimicrobial, antimalarial and antituberculosis agents, we have demonstrated the multicomponent synthesis of **16** new polyhydroquinoline derivatives bearing a polyhydroquinoline nucleus. Many of these new polyhydroquinoline heterocycles displayed noticeable antibacterial activities. Amongst the screened compounds **8e**, **8i**, **8k**, **8l,** and **8p** were found to be superior antituberculosis agents against *M. tuberculosis* H37Rv.Some of the compounds **8a**, **8c**, **8i**, **8l** and **8o** demonstrated more significant antimalarial activity against strains of *P. falciparum* as compared to **quinine**. *In silico* docking study demonstrated the potentiality of these molecules to interact and inhibit the enzyme *P. Falciparum* dihydrofolate reductase (DHFR) by occupying the active binding pocket with great ease. To avoid late stage failure, it is

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important to study the preliminary pharmacokinetic parameters. The results of *in silico* pharmacokinetic data suggested that, molecules may be considered as a drug.

## 6. Experimental section

### 6.1. Chemistry

All the reagents were obtained commercially and used without further purification. Solvents used were of analytical grade. Melting points (°C, uncorrected) were determined in open capillaries on µThermoCal10 melting point apparatus (Analab Scientific Pvt. Ltd, India). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography. Electron impact Mass Spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE programme of DST at Sardar Patel University, Vallabh Vidyanagar, India. The IR spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4000–400 $cm^{-1}$and frequencies of only characteristic peaks are expressed in $cm^{-1}$. The elemental analysis was performed on Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar, India. All the compounds were found to be within ±0.4% of their theoretical values. The reaction mixtures were irradiated by ultrasound at room temperature in a D-compact ultrasonic cleaner with a frequency of 30 kHz and an output power of 250 W. The reaction flask was kept at the maximum energy area in the cleaner and the level of the reactants was kept slightly lower than the level of water in the bath. $^1$H NMR spectra (in DMSO-$d_6$) were recorded on Bruker Avance 400F (MHz) NMR Spectrometer at 400 MHz using TMS as the internal standard.

#### *6.1.1. General procedure for the synthesis of 3-methyl-5-substituted phenylthio-1-phenyl-1H-pyrazole-4-carbaldehydes (3a-c)*

5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde 1(1 mmol), substituted thiophenols **2a–c** (1 mmol) and anhydrous potassium carbonate (2.5 mmol) in dimethyl formamide (10 mL) were charged in a 100 mL round bottom flask equipped with a mechanical stirrer and a condenser. The reaction mixture was heated at 90 °C for 2 h and the progress of the reaction was monitored by TLC. After the completion of reaction as confirmed by the TLC, the reaction mixture was poured in to 100 mL ice water. The solid separated was filtered, washed thoroughly with water, dried and recrystallized from hot ethanol (10 mL) to obtain **3a-c**.

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***6.1.2. General procedure for the synthesis of substituted 3-((substituted)amino)-5,5-dimethylcyclohex-2-enone (6a-c).***

1,3-Dimedone **4** (10 mmol), fluoro substituted amine **5a–c** (10 mmol) and methanol (10 mL) with catalytic amount of acetic acid were charged in a 100 mL round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred at room temperature for 2 h. After the completion of reaction (checked by TLC), the separated substituted enhydrazinoketones **6a–c** were filtered and washed with methanol to obtain the pure solid product.

***6.1.3. General procedure for the synthesis of 2-amino-4-(5-((substituted phenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(phenylamino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8a-p)***

A 50 mL round bottom flask, fitted with a reflux condenser, was charged with a mixture of 3-methyl-5-substituted phenylthio-1-phenyl-*1H*-pyrazole-4-carbaldehydes ***(3a-c)*** (1 mmol), malononitrile **7a** or ethylcynoacetate **7b** or cynoacetamide **7c** (1 mmol), substituted enaminones **6a-c** (1 mmol), and catalytic amount of piperidine (2-3 drops) in ethanol (10 mL). The mixture was heated under reflux for 1-3 h and the progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and stirred magnetically for further 10 min. The solid mass separated was collected by filtration, washed well with ethanol (10 mL) and crystallized from hot chloroform. The physicochemical and spectroscopic characterization data of the synthesized compounds **8a-p** are added in to the **Supplementary Information**.

**Acknowledgment**

We sincerely express our thanks to Head, Department of Chemistry, Sardar Patel University, for providing necessary research facilities. We are also thankful to Dhanji P. Rajani, Microcare Laboratory, Surat for antimicrobial, antituberculosis and antimalarial screening of the compounds reported herein and Sophisticated Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar for FT-IR analysis at concessional rate. BKV, RPT are thankful to UGC meritorious fellowship and NHS specially thanks the UGC, New Delhi, India for providing financial assistance under the UGC-JRF Scheme and also for mass analysis at PURSE central facility at Sardar Patel university sponsored under PURSE program grant vide sanction letter DO. No. SR/59/Z-23/2010/43 dated 16th March 2011.

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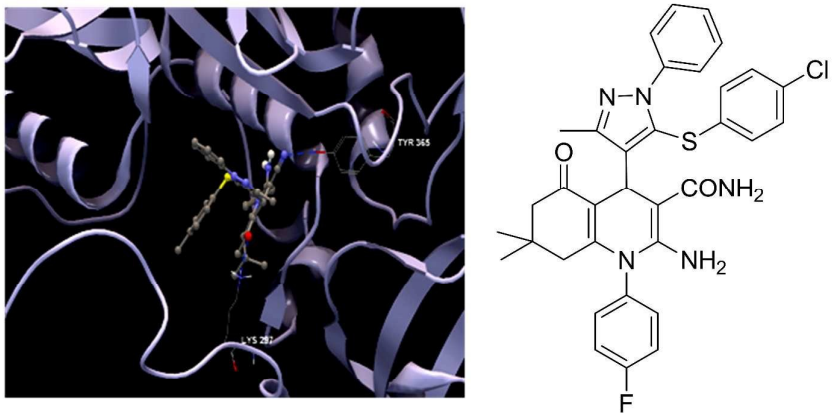


Docking study of compound **8C**

# Graphical Abstract