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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis and antibacterial, antimycobacterial and docking studies of novel *N*-piperazinyl fluoroquinolones

**Kapil M. Agrawal · Gokul S. Talele**

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**Abstract** The present study deals with the synthesis of some novel fluoroquinolone derivatives as antibacterial and antitubercular agents. The titled compounds **7a–g** and **8a–g** were found to possess comparable or more potent activity than the reference compounds ciprofloxacin, norfloxacin, isoniazid and rifampicin. The synthesized compounds showed activity against *S. aureus* and *C. bacterium*, whereas poor activity was observed against *P. aeruginosa* and *E. coli*. These compounds were subjected to in vitro cytotoxicity study by MTT assay, and their selectivity index was calculated. Compound **7d** was found to be the most efficient antimycobacterial agent amongst the series. Molecular docking revealed that synthesized derivatives and target proteins were actively involved in a binding pattern and had significant correlation with biological activity.

**Keywords** Fluoroquinolone (FQ) · Antibacterial · Antimycobacterial · Cytotoxicity · Docking study

## Introduction

Tuberculosis (TB) is the oldest documented, chronic necrotizing bacterial infection with a wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has plagued humans throughout the recorded and archaeological history (Dutt and Stead, 1999). The number of individuals succumbing to this disease has been increasing vastly because of the HIV/AIDS pandemic (Cambau *et al.*, 1994; Grosset, 1992; Tsukamura *et al.*, 1985). Treatment outcomes of MDR-TB are poor with low cure rates of around 60 % and high recurrence rates close to 30 % after standard short-course TB treatment (Becerra *et al.*, 2000; Espinal *et al.*, 2000; Migliori *et al.*, 2002). Besides being increasingly popular in treatment of TB, complicated by intolerance or relative contraindication for first-line drugs, fluoroquinolones (FQs) are important for improving treatment outcomes of MDR-TB as second-line drugs (Yew *et al.*, 2000; Migliori *et al.*, 2008a, b). Fluoroquinolones, such as ciprofloxacin, gatifloxacin, sparfloxacin, moxifloxacin, etc. have demonstrated potential for shortening treatment duration, (Rustomjee *et al.*, 2008; Conde *et al.*, 2009; Dorman *et al.*, 2009) and have been recommended in the treatment of XDR-TB (WHO/HTM/TB/, 2008). During the past few decades, several fluoroquinolone (FQ) antibacterial drugs have been used to treat *M. tuberculosis* and bacterial infections because of their favourable pharmacokinetic profiles.

In the present scenario, much of the quinolone antibacterial research indicates that functionality at C-7 position of 6-fluoroquinolone can be enhanced with the entry of bulkier substituents. Moreover, C-7 substitution is the most adaptable site for chemical change and is an area that determines potency and target preferences (Foroumadi *et al.*, 2005; Domagala *et al.*, 1988; Chu *et al.*, 1985; Shen *et al.*, 1989). Hence, there is the need for the development of novel antimycobacterial agents to combat mycobacterial and bacterial infections, with reduced toxicity profile and resistance (Rattan *et al.*, 1998).

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At present, we synthesized the substituted C-5 benzoylmethylenethio-1,3,4-thiadiazole which was fused with C-7 and N-1 piperazinyl FQs. They could be considered as hybrid compounds (Gadad and Talath, 2006; Manzo *et al*., 1992). The titled compounds were subjected to in vitro antibacterial, antimycobacterial and cytotoxicity studies to examine their relationship between structural modifications and biological activities with reduced toxicity profile. Finally, these biological activity findings and dock score have helped in predicting a potent/efficient agent against bacteria and *M. tuberculosis* from the synthesized derivatives; these objectives of our study were really achieved with the help of molecular docking study in which the binding patterns of the synthesized derivatives with targeted protein, DNA gyrase (subunit A), was significantly compared (Ghosh and Bagchi, 2010).

## Result and discussion

### Chemistry

In the present study, we synthesized few reported substituted phenacyl bromides and 2-amino-5-mercapto-1,3,4-thiadiazole which in turn were used to prepare the newly synthesized *N*-piperazinyl fluoroquinolonic derivatives, preceded by chlorobenzoylmethylenethio-1,3,4-thiadiazole **6a**–**g**. The synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole **2** involved the method of direct cyclization through thiosemicarbazide **1** in the presence of carbon-di-sulphide and dry ethanol (Foroumadi *et al.*, 1999; Freund and Imgart, 1895). The synthesis of phenacyl bromide/α-bromoketones **4a**–**g** was based on free radical reaction mechanism followed by side-chain halogenation of alkylbenzene via homolytic fission of bromide ion in the presence of lewis acid (anhydrous aluminium chloride) that easily abstracted the benzoylic hydrogen ion from the substituted acetophenones **3a**–**g**, with hydrogen bromide getting released and the substituted phenacyl bromides **4a**–**g** being formed (Morrison and Boyd, 2004). The synthesis of 2-amino-5-benzoylmethylenethio-1,3,4-thiadiazoles **5a**–**g** was carried out by reacting α-bromoketones **4a**–**g** and 2-amino-5-mercapto-1,3,4-thiadiazole **2** via dehydrobromination mechanism, in the presence of ethanolic potassium hydroxide which abstracted the hydrogen bromide. The synthesized compounds **5a**–**g** were directly converted to 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazole **6a**–**g** by diazotization of amines followed by chlorination with concentrated hydrochloric acid in the presence of $NaNO_2$ and copper powder. The synthesis of 1-cyclopropyl/ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-*p*(substituted)-phenylethylthio)-1,3,4-thiadiazol-2-yl} piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid **7a**–**g** and **8a**–**g** was based on aromatic nucleophilic substitution mechanism involving substituted 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazole **6a**–**g** and piperazinyl fluoroquinolones with weak base sodium-bi-carbonate in the presence of dimethylformamide, which seemed to be a convenient route to fulfil our objectives. The structure of novel synthesized derivatives **7a**–**g** and **8a**–**g** were elucidated on the basis of physical constants, spectral data (FTIR, $^1$H-NMR, $^{13}$C-NMR and FAB MASS) and elemental analysis.

In general, FTIR spectra of compounds **7a**–**g** and **8a**–**g** gave representative broad absorption bands ranging around 3,457–3,400 $cm^{-1}$ for –OH *str*., indicating carboxylic acid group in the final structure; and 3,120–2,910 $cm^{-1}$ for aromatic C–H *str*., indicating the presence of 2-oxo-2-*p*(substituted)phenylethylthio moiety. Aliphatic $CH_2$-stretching vibrations appeared between 2,850 and 2,822 $cm^{-1}$ for methylene bridge, which confirmed the 2-oxo-2-*p*(substituted)phenylethylthio-1,3,4-thiadiazole linker with *N*-piperazinyl FQ derivatives. 1750–1710, 1694–1660 and 1645–1620 $cm^{-1}$ indicated the presence of three C=O *str*. groups in titled compounds (The C=O as carboxylic, mono aryl and 4-oxo quinolonic ketonic group, respectively). Absorption peaks appeared around 705–610 $cm^{-1}$ for aliphatic C–S *str*. vibration, justifying the presence of 1,3,4-thiadiazole group. $^1$HNMR spectra for **7a**–**g** and **8a**–**g** showed broad singlet around $\delta$ 14.49–15.09 ppm, confirming the presence of acid proton of –COOH. The characteristic singlet was arrested in the final compounds around $\delta$ 4.44–4.82 ppm for $-CH_2$ methylene proton, which confirmed the reaction between α-bromoketones **4a**–**g** and 2-amino-5-mercapto-1,3,4-thiadiazole **2**. Quinolone protons splitted and appeared as singlet for 2nd proton of quinolone at $\delta$ 8.46–8.68 ppm, and doublets appeared between the ranges at $\delta$ 7.80–7.98 and 7.51–7.68 for 5th and 8th quinolone proton, respectively. Aryl protons appeared at $\delta$ 7.22–7.54 as multiplets for 5H (**7a** and **8a**) protons, but when the substitutions (–Cl, –Br, $-NO_2$, $-CH_3$, $-OCH_3$ and –F) occured at para position in aryl group (**7b**–**g** and **8b**–**g**), the remaining four aryl protons (4H) splitted into two protons each and appeared as multiplets at different $\delta$ positions which seemed to be at $\delta$ 8.10–8.78 and 6.82–8.18 ppm for four protons. Eight piperazinyl protons for **7a**–**g** were characterized as multiplets and splitted into set of four protons at $\delta$ 3.67–3.73 and 3.50–3.61 ppm and at $\delta$ 3.41–3.58 and 3.18–3.32 ppm for compounds **8a**–**g**, respectively. Compounds **7a**–**g** for four cyclopropylic protons appeared as multiplets at $\delta$ 1.22–1.43 ppm, and remaining one proton of cyclopropyl appeared as multiplets at $\delta$ 3.41–3.58 ppm along with piperazinyl protons. Compounds **8a**–**g** for N-bonded five ethylic ($CH_2CH_3$) protons splitted as quartet (2H, $\delta$ 4.20–4.54) and triplet (3H, $\delta$ 1.18–1.42 ppm). N-bonded both cyclopropylic and ethylic protons **7a**–**g** and **8a**–**g** showed characteristic peaks indicating that both the FQs are distinguishably synthesized. The final derivatives **7e** and

**7f** were also confirmed by characteristic peaks as singlets around $\delta$ 2.35 for methylic and $\delta$ 3.33 ppm for methoxyl proton and $\delta$ 2.38 and $\delta$ 3.82 for (**8e** and **8f**), respectively.

In $^{13}$C-NMR, the higher $\delta$ values appeared at around 194.1–195.2, 177.5–178.5 and 165–167 for three carbonyl carbons which are present in compounds **7a–g** and **8a–g**. The characteristic peak as singlet for C–S was observed at 164–165 $\delta$ ppm, confirming the formation of 2-oxo-2-*p*(substituted)phenylethylthio linker in final derivatives. Nitro-substituted **7d** and **8d** appeared at higher $\delta$ values 152.8 ppm as compared to other substitutions because of electron-withdrawing nature of nitro group. On the contrary, the electron-releasing nature of methyl and methoxyl group **7e**, **7f**, **8e,** and **8f** shifted the $\delta$ values at upfield position at 24.3, 24.4, 55.8 and 56.1 ppm, respectively. Aliphatic $CH_2$ methylene carbon showed the characteristic peak at $\delta$ 37.5–38.5 ppm in the final derivatives. Four piperazinyl carbons of FQs appeared at around $\delta$ 49.6 and 49.3 ppm. Cyclopropyl and ethylic group in **7a–g** and **8a–g** represented characteristic peaks at around at $\delta$ 36, 5.6, 49 and 13 ppm, respectively. The FAB Mass spectra showed molecular ion peak data for the selected compounds. All the compounds produced satisfactory elemental analysis (±0.4 %).

## Antibacterial activity

Antibacterial activity of **7a–g** and **8a–g** was performed by broth microdilution method. Ciprofloxacin and norfloxacin were used as reference. Amongst all the synthesized compounds, **7d** (MIC = 0.5) was found to be more potent than reference drugs, whereas, compounds **7f**, **8d** and **8f**, (MIC = 1–2) possessed activity comparable to the reference, against Gram-positive bacterial strains, but all these compounds were not able to produced enhanced antibacterial activity against Gram-negative organisms. These observed antibacterial activity suggests that; all these compounds were not able to exert their effects against comparatively tough (double layered and excessive lipids containing) cell wall of Gram-negative organisms; however, these compounds were effective against single cell wall layer containing Gram-positive organisms. Besides the possibility of resistance development against Gram-negative organisms cannot be ruled out. The results of antibacterial studies are presented in Table 1.

## Antimycobacterial activity

The activity was performed against *M. tuberculosis* $H_{37}$Rv strain by broth dilution assay using Mueller–Hinton agar medium. Results revealed that **7a** and **8a** did not produce significant results, whereas moderate activity was observed in case of **7e**, **7f**, **8e** and **8f**. On the contrary **7b–7d**, **8b–8d** resulted in encouraging MIC values from 0.5 to 2.5 μg/mL. Compounds **7d**, **8d**, **7b** and **8b** (MIC = 0.5, 1.0, 1.0, 1.5 μg/mL respectively) exhibited a prominent activity as compared to first-line anti-TB drugs isoniazid (INH, MIC = 1.5 μg/mL) and rifampicin (RIP, MIC = 1.0 μg/mL). The results are depicted in Table 1.

## Cytotoxicity study

Selected compounds **7b–7d**, **8b–8d** were examined for in vitro cytotoxicity study ($IC_{50}$) by MTT assay in mammalian vero cell lines from the kidney of African green monkey, organism *Cercopethicus aethiops*, followed by determination of selectivity index (SI), ($IC_{50}$/MIC). The results were expressed in μM, but for determination of SI, these values were converted into μg/mL. Compounds having high SI are categorized as non-toxic agent (Sriram *et al.*, 2006). Compound **7d** was found to be least toxic SI > 12.2 as compared to reference RIP, (SI = 10) INH, (SI = 8), compounds **8d**, **7b**, **7c**, **8b**, and **8c** were near to non-toxic, as per the SI i.e. >10.2, 3.0, 3.0, 2.67 and 2.0 respectively. The presence of domain as 2-oxo-2-*p*-phenylethylthio-1,3,4-thiadiazole with nitro as *p*-substituted in ciprofloxacin/norfloxacin analogue, makes it the most efficient antimycobacterial agent. The results of cytotoxicity studies are depicted in Table 1.

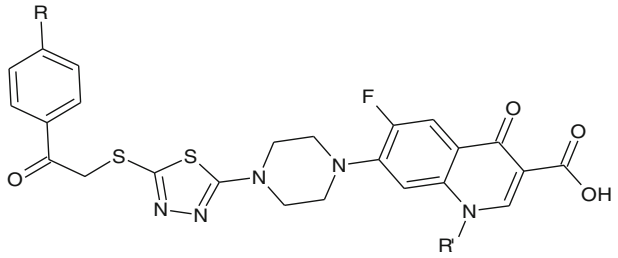
## SAR and docking study

1- Introduction of 1,3,4-thiadiazole carrying benzoylmethylenethio moiety at N-4 position on the piperazine ring was well tolerated in terms of Gram-positive activity, exemplified by the potency of ciprofloxacin and norfloxacin analogues. The absence of substitutions at R position in FQ derivatives **7a** and **8a** did not produce enhanced activity.
2- The presence of chloro, bromo, or nitro substituents at 2-oxo-2-*p*-phenylethylthio-1,3,4-thiadiazole linked to N-4 piperazinyl quinolone **7b–7d**, **8b–8d**, resulted in prominent activity with MIC = 0.5–2.5 μg/mL.
3- The presence of methyl and methoxyl substituent **7e**, **7f**, **8e** and **8f**, showed moderate activity while compound **7a** and **8a** which were unsubstituted did not show enhanced activity.

This indicates that electron withdrawing group increases activity while electron releasing group slightly raise in activity while unsubstituted derivatives lacks the activity.

To explore further, all the synthesized compounds were subjected to Grid based molecular docking studies. DNA gyrase-A is one of the few thoroughly characterized and well validated targets in anti TB and antibacterial inhibitors

**Table 1** Antibacterial, antimycobacterial, cytotoxicity and SI data for **7a–g** and **8a–g**




| Compounds | R | R′ | Gram-positive organisms[a] | | Gram-negative organisms[b] | | M. TB MIC[c] | $IC_{50}^{d}$ | SI[e] |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sa | Cb | Pa | Ec | | | |
| **7a** | H |  | 16 | 16 | >64 | 32 | Resist | – | – |
| **7b** | –Cl |  | 4 | 2 | >64 | 16 | 1 | 3 | 3 |
| **7c** | –Br |  | 8 | 4 | >64 | 16 | 2 | 6 | 3 |
| **7d** | $–NO_2$ |  | 1 | 0.5 | >64 | 32 | 0.5 | >6.1 | >12.2 |
| **7e** | $–CH_3$ |  | 8 | 4 | >64 | 16 | Resist | – | – |
| **7f** | $–OCH_3$ |  | 2 | 1 | >64 | 32 | 10 | – | – |
| **7g** | –F |  | 8 | 4 | 32 | 16 | 5 | – | – |
| **8a** | H | $–C_2H_5$ | 32 | 16 | >64 | >64 | Resist | – | – |
| **8b** | –Cl | $–C_2H_5$ | 4 | 2 | >64 | 32 | 1.5 | 4 | 2.67 |
| **8c** | –Br | $–C_2H_5$ | 8 | 4 | >64 | 32 | 2.5 | 5 | 2 |
| **8d** | $–NO_2$ | $–C_2H_5$ | 2 | 1 | >64 | 16 | 1 | >10.2 | >10.2 |
| **8e** | $–CH_3$ | $–C_2H_5$ | 8 | 4 | >64 | >64 | Resist | – | – |
| **8f** | $–OCH_3$ | $–C_2H_5$ | 2 | 1 | >64 | >64 | 10 | – | – |
| **8g** | –F | $–C_2H_5$ | 4 | 2 | 32 | >64 | 5 | – | – |
| Ciprofloxacin* | | | 2 | 1 | 4 | 2 | – | – | – |
| Norfloxacin* | | | 2 | 2 | 4 | 2 | – | – | – |
| Rifampicin* | | | – | – | – | – | 1 | >10 | >10 |
| Isoniazid* | | | – | – | – | – | 1.5 | >12 | 8 |

* Reference drugs

[a] Values expressed in μg/mL, *Corynebacterium* (Cb) ATCC 29355, *Staphylococcus aureus* (Sa) ATCC25365

[b] Values expressed in μg/mL, *Pseudomonas aeruginosa* (Pa) ATCC 25619, *Escherichia coli* (Ec) MTCC1089

[c] *Mycobacterium tuberculosis* $H_{37}Rv$ strain: (values expressed in μg/mL)

[d] $IC_{50}$ inhibition concentration (inhibited 50 % of total cells in μM and converted to μg/mL for SI calculation)

[e] *SI* selectivity index (ratio between $IC_{50}$ and *M. tuberculosis* MIC value)

like fluoroquinolones (Ghosh and Bagchi, 2010). The docking results revealed that some of the synthesized fluoroquinolone derivatives had good affinity to the active site residue with respect to reference drugs ciprofloxacin, norfloxacin and isoniazid (Table 2). The compounds **7a–7d**, **7f**, **7g**, **8d–8f** and **8g** showed better Glide score (G-score) as compared to reference ciprofloxacin and norfloxacin with respect to crystal structure of *Staphylococcus aureus*. Similarly compounds **7e**, **7g** and **8g** showed better G-score than reference isoniazid with respect to crystal structure of *M. tuberculosis* (Fig. 1).

In case of *M. tuberculosis*, the carboxyl group at 3rd position in quinolone ring interacts mainly with Arg120 and Gly161 residue, which participated in hydrogen bonding, whereas Phe172, Ala173 and Gln168 participated in electrostatic interactions. It was also found to interact on the same position, with Gly459, Asp437, Arg458 and DG9 residues of *S. aureus*, which participated in hydrogen bonding and electrostatic interaction, which are essential characteristics for drug receptor binding. Amongst the series, compound **7d** possessed higher H-bonding and electrostatic score i.e. −1.49 and −0.79 than isoniazid. Besides H-bonding and electrostatic interaction, hydrophobic interactions are also one of the major interactive forces in ligand recognition and binding pattern (Ghosh and Bagchi, 2010). As per Fig. 2, substituted derivatives at N-4 position of benzoylmethylenethio-1,3,4-thiadiazole group showed the hydrophobic interaction with Gly, Val and Ala residues which impart the effective binding affinity to the synthesized fluoroquinolone derivatives. Hence, compound **7d** showed better biological activities despite low G-score.

## Highly active compounds

Compound **7b** produced significant G-score of −9.37 indicating high binding affinity of the ligand towards *DNA gyrase* for *S. aureus.* The chloro group held at C-5 benzoylmethylenethio-1,3,4-thiadiazole of **7b**, produced strong hydrophobic interactions with Ala1118, Glu1088, Gly1117, the carboxyl group at C-3 and C-4 position in quinolone ring of **7b**, showed hydrogen bonding interaction with Gly459 and Asp437 residue, whereas Phe172, Ala173 and Gln168 participated in electrostatic interactions, which are essential characteristics for drug receptor interaction. However, **7b** showed significant antimycobacterial activity despite of moderate G-score (−2.43) as compared to standard isoniazid (−3.2).

## Moderately active compounds

Compounds **7e**, **7f**, **8e** and **8f** produced moderate activity with moderate G-scores of −5.95, −8.97, −7.53 and −8.53

**Table 2** Docking results of synthesized compounds **7a–g** and **8a–g**

| S. no. | Ligands | PDB: 2XCT (*S. aureus*) | | | | PDB: 2BM7 (*M. tuberculosis*) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G-score | Lipophilic EvdW | HBond | Electrostatic score | G-score | Lipophilic EvdW | HBond | Electrostatic score |
| 1 | **7a** | −9.24 | −6.7 | −0.91 | −1.62 | −2.93 | −3.84 | 0 | −0.01 |
| 2 | **7b** | **−9.37** | −6.8 | −0.92 | **−1.65** | −2.43 | −3.98 | 0 | −0.05 |
| 3 | **7c** | −9.24 | −6.78 | −0.81 | −1.64 | −3.11 | −2.86 | 0 | 0.02 |
| 4 | **7d** | −7.96 | −5.96 | −0.71 | −1.30 | −2.57 | −1.72 | **−1.49** | **−0.79** |
| 5 | **7e** | −5.95 | −5.06 | −0.54 | −0.35 | −3.65 | −3.14 | 0 | −0.54 |
| 6 | **7f** | −8.97 | −6.53 | −0.92 | −1.53 | −2.22 | −2.7 | 0 | −0.46 |
| 7 | **7g** | −9.1 | −6.55 | −0.96 | −1.59 | −3.74 | −3.82 | 0 | 0.14 |
| 8 | **8a** | −7.17 | −6.83 | −0.96 | −0.39 | −2.54 | −3.04 | 0 | −0.25 |
| 9 | **8b** | −5.15 | −6.91 | −0.91 | −1.33 | −3.05 | −3.01 | −0.7 | −0.58 |
| 10 | **8c** | −2.85 | −4.96 | −1.34 | −0.62 | −2.5 | −3.13 | −0.22 | −0.37 |
| 11 | **8d** | −8.76 | −6.28 | −0.96 | −1.52 | −2.22 | −4.06 | 0 | 0.28 |
| 12 | **8e** | −7.53 | −6.08 | −1.85 | −0.76 | −3.06 | −3.22 | −0.7 | −0.44 |
| 13 | **8f** | −8.53 | −6.89 | −0.99 | −0.64 | −2.22 | −4.06 | 0 | −0.49 |
| 14 | **8g** | −8.2 | −6.73 | −0.63 | −0.84 | −3.66 | −3.79 | 0 | −0.33 |
| 15 | Ciprofloxacin | −7.58 | −5.95 | −1.02 | −0.43 | −2.12 | −0.52 | **−2.12** | −0.41 |
| 16 | Norfloxacin | −7.49 | −5.12 | −1.67 | −0.41 | – | – | – | |
| 17 | Isoniazid | – | – | – | – | −3.2 | −1.2 | −0.7 | −0.56 |

Bold value indicates high G-score amongst the series

*G-score* total GlideScore, *Lipophilic EvdW* lipophilic term derived from hydrophobic grid potential and fraction of the total protein ligand vdW energy, *HBond* ChemScore H-bond pair term, *Electrostatic score* electrostatic reward

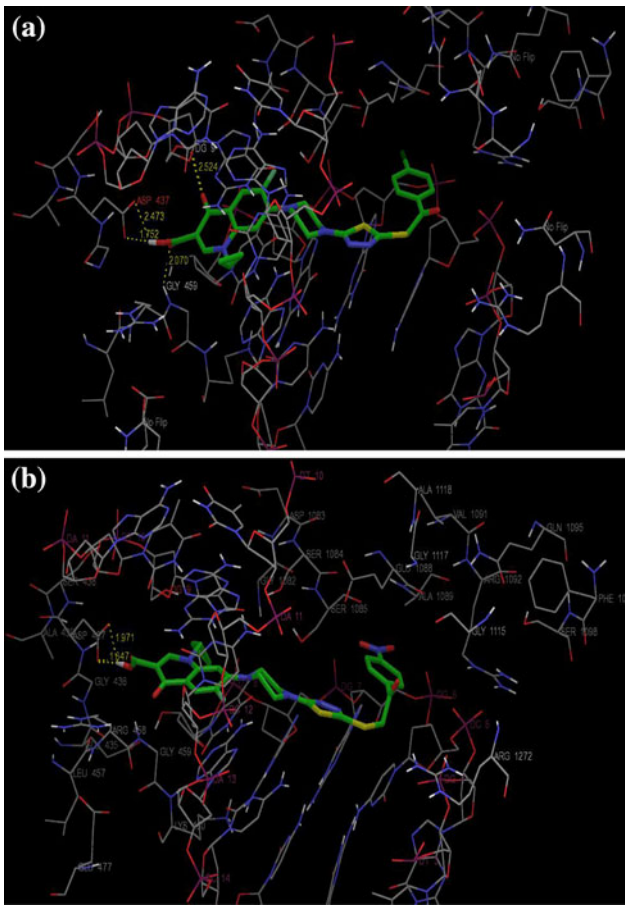



**Fig. 1** Binding interaction of synthesize ligands with *S. aureus* DNA gyrase complex *(PDB: 2XCT)* domain. **a** Binding pose of highest scoring compound **7b** (*green stick*). **b** Binding pose of the most active compound **7d** (*green stick*). *Dotted yellow bond* showing H-bond interaction with binding site residue (Color figure online)

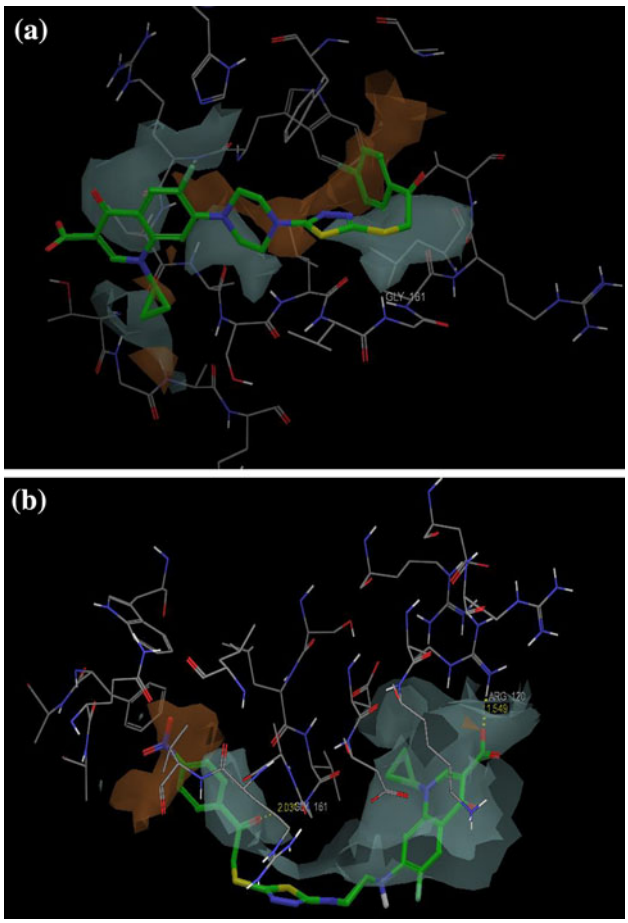



**Fig. 2** Binding interaction of synthesize ligands with *M. tuberculosis* DNA complex *(PDB: 2BM7)* domain. **a** Binding pose of highest scoring compound **7g** (*green stick*). (**b**) Binding pose of the most active compound **7d** (*green stick*). *Dotted yellow bond* showing H-bond interaction with binding site residue. *Grey surface* showing lipophilic area and *brown colour* showing electrostatic area (Color figure online)

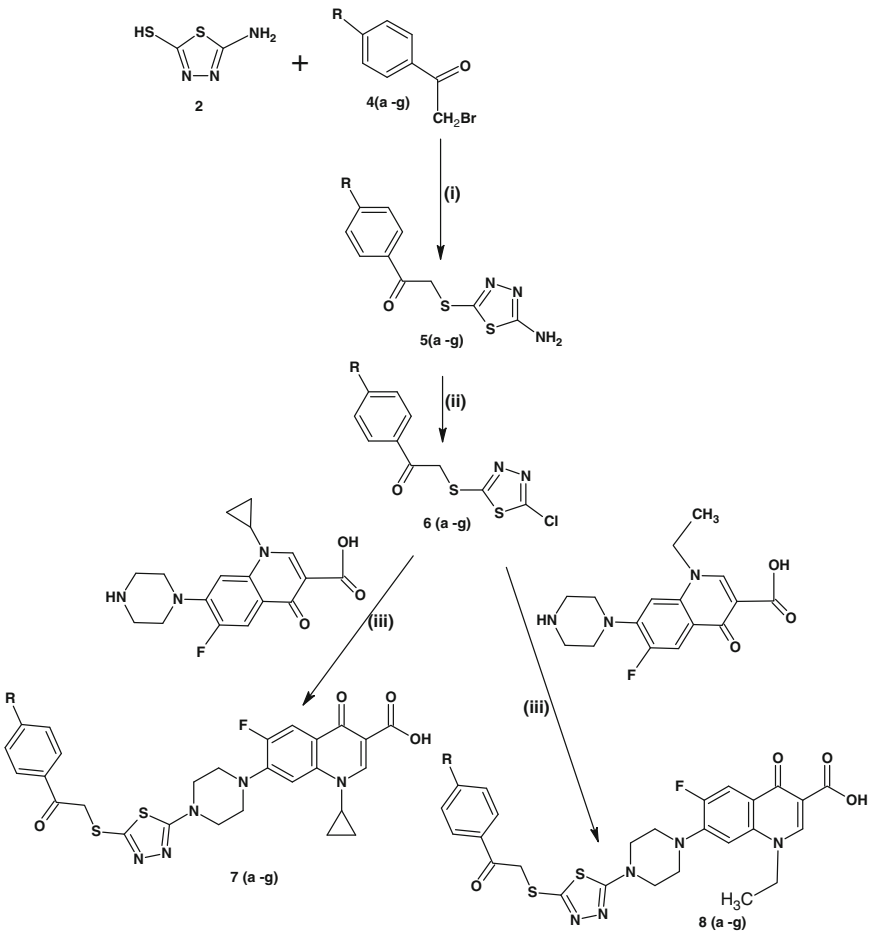
against DNA gyrase from *S. aureus* and −3.65, −2.22, −3.06 and −2.22 against *M. tuberculosis DNA gyrase* respectively. However, **7f** and **8f** produced higher G-scores than **7e** and **8e**. Here once again our assumption proved that electron withdrawing group has significant impact over the biological activity, as compared to electron releasing substituents (the presence of O with $–CH_3$ in **7f** and **8f** compared with $–CH_3$ group in **7e** and **8e**) as shown in Tables 1 and 2.

Least active compound

Unsubstituted analogues **7a** and **8a** produced good G-score [−9.24 and −7.17 (DNA gyrase from *S. aureus*)] and [−2.93 and −2.54 (DNA gyrase from *M. tuberculosis*)] but minimal anti-bacterial and anti-mycobacterial activity, because of −0.91 and −0.96 and 0.00 hydrogen bonding energy for both **7a** and **8a** with the respective receptors.

## Conclusions

In brief, a series of *N*-piperazinyl fluoroquinolone derivatives **7a–g** and **8a–g** in which N-4 hydrogen of piperazinyl group of ciprofloxacin and norfloxacin is substituted with various chlorobenzoylmethylenethio-1,3,4-thiadiazol moieties has been synthesized. These compounds showed prominent in vitro antibacterial activity against Gram-positive organisms, whereas poor activity was observed against Gram-negative organisms than ciprofloxacin and norfloxacin. The characteristics of the substituted functional groups at C-7 position of quinolone ring system have strong influence on the spectrum and extent of antimycobacterial activity. Electron withdrawing groups such as –Cl, –Br, $–NO_2$ substituents, produced greater/comparable antimycobacterial activity than the electron releasing groups as substituents (R in Scheme 1). Especially, nitro




**Scheme 1** R: (a) = H, (b) = –Cl, (c) = –Br, (d) = $–NO_2$, (e) = $–CH_3$, (f) = $–OCH_3$ and (g) = –F. (i) EtOH, KOH/4 h stirring, rt; (ii) $NaNO_2$, HCl, 0–5 °C/stirring; and (iii) DMF, $NaHCO_3$,/18–20 h reflux/ 120 °C. Note: (**4a–g**). *1* Reaction should be carried out in fuming hood with strong alkali trap and the reaction assembly to be surrounded by strong ammonia vapour which masks the bromine fumes. *2* Improved yield may be obtained by exposing the flask to the light of two 300-watt tungsten lamps during the bromination (Coleman and Honeywell, 1943)

substituted analogues **7d** and **8d** were more active than other compounds. Interestingly, fluoro substitution **7g** and **8g** were not able to produce enhanced antimycobacterial activity than chloro and bromo substituted analogues. On the contrary, the presence of electron releasing groups like methyl, methoxyl **7e**, **7f**, **8e** and **8f** resulted in declined activity. Encouraging antimycobacterial agents were selected for in vitro cytotoxicity studies, in which **7d** and **8d** were found to be the most efficient (potent with least cytotoxicity) antimycobacterial agents. As per docking studies, compounds did not show any clear correlation between G-score and biological activities; however, compound **7d** showed strong (MIC = 0.5 μg/mL) biological activity and low G-score as compared to reference. This might be due to high score of H-bonding and electrostatic force, which is key factor for drug receptor binding pattern. However, the role of substitutions in the titled compounds is unclear to explain the exact mechanism of action, for giving variations in results, as all the substitutions (R), were held at the same para position. Therefore, our studies could provide tremendous space for chemists and pharmacists to further explore in the same region and search for efficient antimycobacterial agents.

## Experimental

### Materials and methods

The pure FQ drugs used in the synthesis were procured as gift samples from Mcleods Pharmaceuticals Ltd., Mumbai and IPCA laboratories Ratlam, India. Reagents, starting materials and solvents were purchased from common commercial suppliers (S.D. Fine chemicals, Rankem, E.merck, Himedia, Spectrochem). Melting points were determined using Thiels tube (paraffin bath) and are uncorrected. The purification of synthesized compounds was achieved by passage through column chromatography on silica gel (mesh 230–400) with the indicated solvent system. Thin layer chromatography was performed on precoated silica gel 60 F254 plates (E. Merck Co. Darmstadt, Germany). Infra red spectra was recorded using KBr disc on a Nicolet MX-1 FTIR spectrophotometer, $^{1}H$ NMR spectra were recorded at 400 MHz and $^{13}C$ NMR spectra were recorded at 100 MHz on a Bruker AM spectrometer, IISc Bangalore, India and their chemical shifts are reported in $\delta$ ppm units with respect to TMS as internal standard. Microanalysis for C, H, N was performed

in a Heraeus CHN Rapid Analyzer. The FAB Mass spectra were recorded on Autospec Mass spectrometer, IICT, Hyderabad, India data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within ±0.4 % of the theoretical values.

*Synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole* ***2*** *(Foroumadi et al., 1999; Freund and Imgart, 1895)*

A mixture of thiosemicarbazide **1** 0.1 mol and carbon-di-sulphide 0.1 mol were refluxed gently for half an hour. After cooling, 90 mL water was added and the mixture was refluxed for 4 h and filtered. The solution was neutralized with potassium hydroxide. The precipitate was filtered and crystallized from ethanol and recrystallized from aqueous ethanol to get white crystalline mass **2**. M.p. 230–232 °C dec. (75–80 %).

*Synthesis of α-bromoketones/phenacyl bromide* ***4a–g*** *(Cowper and Davidson, 1943; Naidir and Khim, 1982)*

Equimolar quantity of substituted acetophenones **3a–g** and appropriate anhydrous solvent such as ether, acetone, carbon-di-sulphide, methanol, chloroform or glacial acetic acid were taken into two necked flask with magnetic bead, and fitted with bromine dropping funnel and reflux condenser. The reaction condition was maintained either in cold or at room temperature, anhydrous aluminium chloride 0.1 g was introduced, bromine (0.084 mol) was added gradually to the flask with stirring. After addition of bromine, appropriate solvent was added to the reaction mixture. Compounds **4a–g** were obtained as brownish yellow to colourless crystalline mass, washed twice with solvents, and recrystallised from methanol to get colourless shining lachrymatory crystals.

Melting point ranges of **4a–g**; R = H, Cl, Br, $NO_2$, $CH_3$, $OCH_3$, F; 50–52°, 90–92°, 103–105°, 94–96°, 52–54°, 68–70°, 48–50° respectively (56–61 %, Scheme 1).

*General procedure for 2-amino-5-benzoylmethylenethio-1,3,4-thiadiazole* ***5a–g***

Addition of 0.1 mol of 85 % potassium hydroxide to a slurry of 0.1 mol of 2-amino-1,3,4-thiadiazole-5-mercapto **2** in 10 mL of water produced a brownish solution. Solution was clarified with activated charcoal and diluted with 28.5 mL of ethanol. 0.1 mol of various substituted α-bromoketones **4a–g** were added rapidly with stirring. Thick reaction mixture was formed, stirred vigorously and cooled for 30 min, and then diluted with 200 mL of cold water. The solid was removed by filtration, washed with water and ether. **5a–g** was obtained, m.p. 88–91 °C (60–65 %).

*General procedure for 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazoles* ***6a–g***

5-Benzoylmethylenethio-2-amino-1,3,4-thiadiazole **5a–g** (10 mmol) was ground with an excess of sodium nitrite (30 mmol) and the mixture was introduced in small portions with continuous stirring, into an ice cooled solution of 30 mL conc. hydrochloric acid and 15 mL water, maintained at 0–5 °C, containing copper powder (0.1 times). The reaction mixture was allowed to cool at room temperature, and heated to 75 °C for 1 h. The reaction mixture was cooled again and extracted with chloroform (50 mL × 3). The combined extracts were washed with sodium bicarbonate solution, dried over anhydrous sodium sulphate for overnight and chloroform was evaporated under reduced pressure and recrystallized from ethanol to yield **6a–g**. The reaction was monitored through TLC, purification was achieved by column chromatography with chloroform: methanol 9:1 as mobile phase, m.p. 90–110 °C (55–60 %).

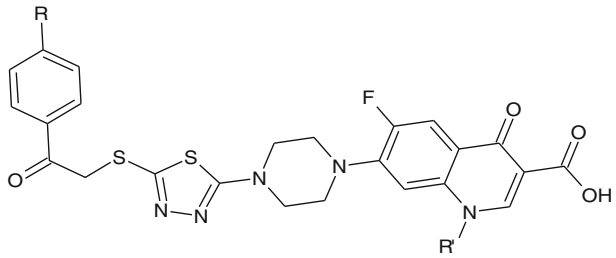
*General procedure for N-piperazinyl quinolone derivatives* ***7a–g*** *and* ***8a–g***

A mixture of equimolar quantities of 2-chloro-5-benzoylmethylenethio-1,3,4-thiadiazole **6a–g**, and substituted piperazinyl quinolone, along with sodium-bi-carbonate in 5 mL dimethylformamide was heated on oil bath and refluxed at 120 °C for 18–20 h. After cooling 10 mL of cold water was added to the reaction mixture and the precipitate was filtered, washed with water twice and recrystallized from dimethylformamide–water to yield the titled compounds. For physical constants please refer Table 3.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-phenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (****7a****)*

IR (KBr) $cm^{-1}$: 3420 (O–H *str.*), 3020 (Ar. C–H *str.*), 2830 (Ali. C–H *str.*), 1730 (Carboxylic C=O *str.*), 1680 (Ar., C=O *str.*), 1620 (4-Oxo quinolone, C=O *str.*), 742 (out of plane, Ar. C–H *def.*), 680 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$), $\delta$ ppm: 15.03 (s, 1H, COOH), 8.52 (s, 1H, H2-quinoline), 7.94 (d, 1H, H5-quinoline, $J$ = 13.04 Hz), 7.63 (d, 1H, H8-quinoline, $J$ = 7.44 Hz), 7.54 (m, 5H, Ar.), 4.80 (s, 2H, Ali. $CH_2$), 3.81–3.87 (m, 4H, piperazine), 3.35–3.77 (m, 4H, piperazine and 1H, CH, cyclopropyl), 1.21–1.42 (m, 4H, cyclopropyl). $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.1, 177.3, 166.2, 163.8, 148, 144.4, 143.5, 136.7, 133.2, 128.5, 116.4, 110.2, 100.5, 49.4, 48.9, 36.4, 5.4. MS (FAB) *m/z*:

**Table 3** Physical constants for **7a–g** and **8a–g**



| Compounds | R | R′ | Yield | Colour | M.P. (°C) | $R_f$ |
|---|---|---|---|---|---|---|
| **7a** | H | | 63 | Brown | 248–250 | 0.42 |
| **7b** | –Cl | | 53 | Brown | 242–246 | 0.44 |
| **7c** | –Br | | 58 | Brown | 241–244 | 0.62 |
| **7d** | $-NO_2$ | | 60 | Brownish yellow | 244–46 | 0.32 |
| **7e** | $-CH_3$ | | 62 | Yellowish brown | 244–247 | 0.40 |
| **7f** | $-OCH_3$ | | 54 | Brown | 240–242 | 0.34 |
| **7g** | –F | | 50 | Colourless | 168–170 | 0.52 |
| **8a** | H | $-C_2H_5$ | 62 | Brownish yellow | 234–238 | 0.46 |
| **8b** | –Cl | $-C_2H_5$ | 52 | Light brown | 236–240 | 0.37 |
| **8c** | –Br | $-C_2H_5$ | 53 | Brown | 238–241 | 0.56 |
| **8d** | $-NO_2$ | $-C_2H_5$ | 54 | Yellow | 231–232 | 0.48 |
| **8e** | $-CH_3$ | $-C_2H_5$ | 57 | Brown | 239–242 | 0.32 |
| **8f** | $-OCH_3$ | $-C_2H_5$ | 62 | Brown | 229–232 | 0.36 |
| **8g** | –F | $-C_2H_5$ | 50 | Off-white | 162–165 | 0.52 |

$M^+$ 584. Anal. Calcd. for $C_{27}H_{24}FN_5O_4S_2$: C 57.33, H 4.28, N 12.38. Found: C 57.31, H 4.30, N 12.36.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-chlorophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7b**)*

IR (KBr) $cm^{-1}$: 3448 (O–H *str.*), 3120 (Ar.C–H *str.*), 2822 (Ali. C–H *str.*) 1720 (Carboxylic C=O *str.*), 1690 (Ar. C=O *str.*), 1622 (4-Oxo quinolone C=O *str.*), 780 (Ar. C–Cl *str.*), 744 (out of plane, Ar. C–H *def.*), 684 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.04 (s, 1H, OH), 8.54 (s, 1H, H2-quinoline), 8.12 (m, 2H, Ar.), 7.92 (d, 1H, H5-quinoline, $J = 13.0$ Hz), 7.60 (d, 1H, H8-quinoline, $J = 7.42$ Hz), 7.24 (m, 2H, Ar.), 4.54 (s, 2H, $CH_2$), 3.78–3.84 (m, 4H, piperazine), 3.54–3.60 (m, 4H, piperazine and 1H, CH, cyclopropyl), 1.22–1.34 (m, $CH_2$, 4H, cyclopropyl) ppm.$^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm:194.5, 177.3, 166.5, 164.5, 149.2, 144.8, 143.9, 139.2, 135.2, 130.4, 128.7, 117.7, 111.5, 101.4, 49.7, 49.6, 36.2, 5.3 ppm. Anal. Calcd. for $C_{27}H_{23}ClFN_5O_4S_2$: C 54.04, H 3.86, N 11.67. Found: C 54.02, H 3.85, N 11.69.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-bromophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7c**)*

IR (KBr) $cm^{-1}$: 3408 (O–H *str.*), 2990 (Ar. C–H *str.*), 2845 (Ali. C–H *str.*) 1710 (Carboxylic C=O *str.*), 1686 (Aryl C=O *str.*), 1628 (4-Oxo quinolone C=O *str.*), 750 (out of plane, Ar. C–H *def.*), 686 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 14.90 (s, 1H, OH), 8.50 (s, 1H, H2-quinoline), 8.10 (m, 2H, Ar.), 7.80 (d, 1H, H5-quinoline, $J$ = 13.02 Hz), 7.68 (d, 1H, H8-quinoline, $J$ = 7.44 Hz), 7.22 (m, 2H, Ar.), 4.65 (s, 2H, $CH_2$), 3.76–3.82 (m, 4H, piperazine), 3.58–3.64 (m, 4H, $CH_2$, piperazine and 1H, CH, cyclopropyl), 1.22–1.35 (m, $CH_2$, 4H, cyclopropyl) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.3, 177.2, 167.2, 164.1, 148.7, 144.6, 144.9, 135.7, 131.6, 127.5, 116.8, 109.2, 100.7, 49.3, 49.4, 36.4, 5.3 ppm. MS (FAB) *m/z*: $M^+$ 654. Anal. Calcd. for $C_{27}H_{23}BrFN_5O_4S_2$: C 50.31, H 3.60, N 10.87. Found: C 50.33, H 3.62, N 10.89.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-nitrophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7d**)*

IR (KBr) $cm^{-1}$: 3400.53 (O–H *str.*), 2910.00 (Ar.C–H *str.*), 2848 (Ali. C–H *str.*) 1750 (Carboxylic C=O *str.*), 1690 (Aryl C=O *str.*), 1635 (4-Oxo quinolone C=O *str.*), 1348 (Ar. –N=O *str.*), 748 (out of plane, Ar. C–H *def.*), 705 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.09 (s, H, OH), 8.68 (m, 2H, Ar.), 8.51 (s, 1H, H2-quinoline), 8.11 (m, 2H, Ar.), 7.81 (d, 1H, H5-quinoline, $J$ = 13.04 Hz), 7.59 (d, 1H, H8-quinoline, $J$ = 7.48 Hz), 4.53 (s, 2H, $CH_2$), 3.74–3.80 (m, 4H, piperazine), 3.56–3.64 (m, 4H, $CH_2$ piperazine and 1H, CH, cyclopropyl), 1.58–1.62 (m, 4H, $CH_2$, cyclopropyl) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 195.2, 177.7, 167.9, 165.2, 152.8, 149.4, 145.3, 142.9, 136.5, 130.5, 121.5, 117.8, 110.7, 101.4, 49.7, 50.3, 37.3, 5.6 ppm. MS (FAB) *m/z*: $M^+$ 611.08. Anal. Calcd. for $C_{27}H_{23}FN_6O_6S_2$: C 53.11, H 3.80, N 13.76. Found: C 53.10, H 3.82, N 13.78.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-tolylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7e**)*

IR (KBr) $cm^{-1}$: 3440 (O–H *str.*), 2911 (Ar. C–H *str.*), 2840 (Ali. C–H *str.*), 1740 (Carboxylic C=O *str.*), 1665 (Aryl C=O *str.*), 1626 (4-Oxo quinolone C=O *str.*), 744 (out of plane, Ar. C–H *def.*), 660 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.06 (s, 1H, OH), 8.56 (s, 1H, H2-quinoline), 8.10 (m, 2H, Ar.), 7.98 (d, 1H, H5-quinoline, $J$ = 13.08 Hz), 7.62 (d, 1H, H8-quinoline, $J$ = 7.50 Hz), 7.02 (m, 2H, Ar.), 4.48 (s, 2H, $CH_2$), 3.78–3.84 (m, 4H, $CH_2$, piperazine), 3.48–3.56 (m, 4H, $CH_2$, piperazine and 1H, CH, cyclopropyl), 2.35 (s, 3H, Ar.$CH_3$, methyl), 1.21–1.44 (m, $CH_2$, 4H, cyclopropyl) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.5, 176.4, 166.4, 164.5, 149.6, 144.3, 141.7, 133.7, 129.5, 128.4, 116.5, 109.4, 100.5, 49.2, 51.4, 36.2, 24.4, 5.2 ppm. Anal. Calcd. for $C_{28}H_{26}FN_5O_4S_2$: C58.02, H 4.52, N12.08. Found: C 58.00, H 4.50, N 12.10.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-methoxyphenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7f**)*

IR (KBr) $cm^{-1}$: 3457 (O–H *str.*), 2920 (Ar. C–H *str.*), 2838 (Ali. C–H *str.*), 1740 (Carboxylic C=O *str.*), 1674 (Ar. C=O *str.*), 1636 (4-Oxo quinolone C=O *str.*), 1029 (C–O–C str.) 747 (out of plane, Ar. C–H *def.*), 650 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.07 (s, 1H, OH), 8.58 (s, 1H, H2-quinoline), 8.14 (m, 2H, Ar.), 7.93 (d, 1H, H5-quinoline, $J$ = 13.03 Hz), 7.58 (d, 1H, H8-quinoline, $J$ = 7.48 Hz), 6.84 (m, 2H, Ar.), 4.54 (s, 2H, $CH_2$), 3.71–3.77 (m, 4H, $CH_2$, piperazine), 3.51–3.58 (m, 4H, $CH_2$, piperazine and 1H, CH, cyclopropyl), 3.33 (s, 3H, Ar. –$OCH_3$, methoxyl), 0.92–1.42 (m, 4H, $CH_2$, cyclopropyl), $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm:194.6, 176.7, 166.3, 165.2, 149.2, 144.3, 141.2, 133.4, 129.8, 114.2, 116.2, 108.6, 100.1, 56.1, 49.3, 36.2, 5.2 ppm. MS (FAB) *m/z*: $M^+$ 595.6. Anal. Calcd. for $C_{28}H_{26}FN_5O_5S_2$: C 56.46, H 4.40, N 11.76. Found: C 56.44, H 4.42, N 11.74.

*1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-fluorophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7g**)*

IR (KBr) $cm^{-1}$: 3446 (O–H *str.*), 2912 (Ar. C–H *str.*), 2840 (Ali. C–H *str.*) 1745 (Carboxylic C=O *str.*), 1688 (Ar. C=O *str.*), 1642 (4-Oxo quinolone C=O *str.*), 740 (out of plane, Ar. C–H *def.*), 610 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.07 (s, 1H, OH), 8.55 (s, 1H, H2-quinoline), 8.12 (m, 2H, Ar.), 7.90 (d, 1H, H5-quinoline, $J$ = 13.01 Hz), 7.54 (d, 1H, H8-quinoline, $J$ = 7.40 Hz), 7.37 (m, 2H, Ar.), 4.44 (s, 2H, $CH_2$), 3.68–3.72 (m, 4H, $CH_2$, piperazine), 3.48–3.54 (m, 4H, $CH_2$, piperazine and 1H, CH, cyclopropyl), 1.22–1.42 (m, 4H, $CH_2$, cyclopropyl) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 195.1, 177.7, 167.5, 166.6, 165.4, 148.4, 144.6, 141.5, 132.7, 130.4, 115.5, 109.5, 100.5, 56.3, 49.4, 36.3, 5.6 ppm. Anal. Calcd. for $C_{27}H_{23}F_2N_5O_4S_2$: C 56.56, H 3.97, N 12.00. Found: C 56.54, H 3.99, N 12.02.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-phenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8a**)*

IR (KBr) $cm^{-1}$: 3435 (O–H *str.*), 3122 (Ar. C–H *str.*), 2825 (Ali. C–H *str.*) 1742 (Carboxylic C=O *str.*), 1667 (Ar. C=O

*str.*), 1622 (4-Oxo quinolone C=O *str.*), 752 (out of plane, Ar. C–H *def.*), 640 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 14.70 (s, 1H, OH), 8.48 (s, 1H, H2-quinoline), 7.88 (d, 1H, H5-quinoline, $J = 13.04$ Hz), 7.56 (d, 1H, H8-quinoline, $J = 7.42$ Hz), 7.22 (m, 5H, Ar.), 4.54 (q, 2H, $NCH_2CH_3$, $J = 6.94$ Hz), 4.42 (s, 2H, $CH_2$), 3.58 (m, 4H, piperazine), 3.10–3.30 (m, 4H, piperazine), 1.42 (*t*, 3H, $NCH_2CH_3$, $J = 6.92$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.2, 177.7, 166.5, 164.2, 148.2, 144.5, 143.7, 140.5, 136.7, 133.2, 128.9, 118.4, 116.5, 109.4, 100.1, 49.8, 49.3, 38.5, 13.1 ppm. MS (FAB) *m/z*: $M^+$ 553. Anal. Calcd. for $C_{26}H_{24}FN_5O_4S_2$: C 56.41, H 4.37, N 12.63. Found: C 56.44, H 4.38, N 12.65.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-chlorophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8b**)*

IR (KBr) cm$^{-1}$: 3436 (O–H *str.*), 2916 (Ar. C–H *str.*), 2850 (Ali. C–H *str.*) 1738 (Carboxylic C=O *str.*), 1690 (Ar. C=O *str.*), 1624 (4-Oxo quinolone C=O *str.*), 864 (Ar.C–Cl *str.*), 737 (out of plane, Ar. C–H *def.*), 690 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 14.52 (br, s, 1H, OH), 8.46 (s, 1H, H2-quinoline), 8.30 (m, 2H, Ar.), 7.84 (d, 1H, H5-quinoline, $J = 13.00$ Hz), 7.52 (d, 1H, H8-quinoline, $J = 7.40$ Hz), 7.24 (m, 2H, Ar.), 4.64 (s, 2H, $CH_2$), 4.52 (q, 2H, $NCH_2CH_3$, $J = 6.94$ Hz), 3.55 (m, 4H, piperazine), 3.15–3.24 (m, 4H, piperazine), 1.40 (*t*, 3H, $NCH_2CH_3$, $J = 6.92$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.3, 178.3, 167.5, 164.5, 148.3, 144.7, 144.2, 140.5, 138.7, 136.5, 134.9, 130.2, 128.8, 116.7, 109.7, 100.5, 49.7, 49.8, 38.6, 13.3 ppm. MS (FAB) *m/z*: $[M+1]^+$ 588.42. Anal. Calcd. for $C_{26}H_{23}ClFN_5O_4S_2$: C 53.10, H 3.94, N 11.91. Found: C 53.12, H 3.96, N11.89.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-bromophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8c**)*

IR (KBr) cm$^{-1}$: 3437 (O–H *str.*), 2975 (Ar. C–H *str.*), 2828 (Ali. C–H *str.*) 1747 (Carboxylic C=O *str.*), 1660 (Ar. C=O *str.*), 1628 (4-Oxo quinolone C=O *str.*), 729 (out of plane, Ar. C–H *def.*), 670 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.09 (s, 1H, OH), 8.50 (s, 1H, H2-quinoline), 8.12 (m, 2H, Ar.), 7.81 (d, 1H, H5-quinoline, $J = 13.08$ Hz), 7.57 (d, 1H, H8-quinoline, $J = 7.41$ Hz), 7.20 (m, 2H, Ar.), 4.50 (s, 2H, $CH_2$), 4.22 (q, 2H, $NCH_2CH_3$, $J = 6.90$ Hz), 3.45 (m, 4H, piperazine), 3.10–3.20 (m, 4H, piperazine), 1.38 (*t*, 3H, $NCH_2CH_3$, $J = 6.92$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.2, 178.4, 167.7, 164.5, 147.8, 143.8, 143.2, 139.9, 135.8, 134.7, 131.6, 131.0, 129.8, 127.4, 109.2, 100.2, 50.4, 49.6, 38.2, 13.1 ppm. Anal. Calcd. for $C_{27}H_{23}BrFN_5O_4S_2$ : C 50.31, H 3.60, N 10.87. Found: C 50.33, H 3.62, N 10.84.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-nitrophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8d**)*

IR (KBr) cm$^{-1}$: 3438 (O–H *str.*), 2932 (Ar. C–H *str.*), 2837 (Ali. C–H *str.*) 1742 (Carboxylic C=O *str.*), 1672 (Ar. C=O *str.*), 1629 (4-Oxo quinolone C=O *str.*), 1354 (Ar. –N=O *str.*), 760 (out of plane, Ar. C–H *def.*), 672 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.07 (s, 1H, OH), 8.78 (m, 2H, Ar.), 8.46 (s, 1H, H2-quinoline), 8.14 (m, 2H, Ar.), 7.82 (d, 1H, H5-quinoline, $J = 13.09$ Hz), 7.60 (d, 1H, H8-quinoline, $J = 7.44$ Hz), 4.55 (s, 2H, $CH_2$), 4.25 (q, 2H, $NCH_2CH_3$, $J = 6.97$ Hz), 3.41 (m, 4H, piperazine), 3.14–3.24 (m, 4H, piperazine), 1.20 (*t*, 3H, $NCH_2CH_3$, $J = 6.90$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.7, 178.6, 168.7, 165.2, 152.8, 148.1, 144.3, 143.7, 142.9, 140.1, 134.9, 130.2, 129.7, 121.0, 109.5, 100.7, 50.9, 50.1, 38.7, 13.5 ppm. MS (FAB) *m/z*: $M^+$ 598.12. Anal. Calcd. for $C_{26}H_{23}FN_6O_6S_2$: C 52.17, H 3.87, N 14.04. Found: C 52.20, H 3.89, N 14.06.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-tolylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8e**)*

IR (KBr) cm$^{-1}$: 3434 (O–H *str.*), 3011 (Ar. C–H *str.*), 2908 (Ar. C–$CH_3$ *str.*), 2842 (Ali. C–H *str.*) 1740 (Carboxylic C=O *str.*), 1694 (Ar. C=O *str.*), 1638 (4-Oxo quinolone C=O *str.*), 744 (out of plane, Ar. C–H *def.*), 647 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 14.78 (br, s, 1H, OH), 8.57 (s, 1H, H2-quinoline), 8.24 (m, 2H, Ar.), 7.98 (d, 1H, H5-quinoline, $J = 13.07$ Hz), 7.60 (d, 1H, H8-quinoline, $J = 7.44$ Hz), 6.98 (m, 2H, Ar.), 4.50 (s, 2H, $CH_2$), 4.22 (q, 2H, $NCH_2CH_3$, $J = 6.98$ Hz), 3.47 (m, 4H, piperazine), 3.12–3.22 (m, 4H, piperazine), 2.38 (s, 3H, Ar.$CH_3$) 1.28 (*t*, 3H, $NCH_2CH_3$, $J = 6.94$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 193.8, 177.1, 167.2, 164.6, 147.3, 144.1, 142.8, 142.2, 139.1, 133.8, 129.5, 129.0, 128.7, 108.7, 100.5, 49.5, 50.1, 38.3, 24.3, 13.1 ppm. Anal. Calcd. For $C_{27}H_{26}FN_5O_4S_2$ : C 57.13, H 4.62, N 12.34. Found: C 57.15, H 4.61, N 12.32.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-methoxyphenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8f**)*

IR (KBr) cm$^{-1}$: 3433 (O–H *str.*), 2934 (Ar. C–H *str.*), 2830 (Ali. C–H *str.*) 1735 (Carboxylic C=O *str.*), 1690 (Ar. C=O *str.*), 1645 (4-Oxo quinolone C=O *str.*), 1031 (Ar.C–O–C *str.*), 740 (out of plane, Ar. C–H *def.*), 674 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 15.05 (s, 1H, OH), 8.58 (s, 1H, H2-quinoline), 8.18 (m, 2H, Ar.), 7.94 (d, 1H, H5-quinoline, $J = 12.98$ Hz), 7.54 (d, 1H, H8-quinoline,

$J = 7.38$ Hz), 6.82 (m, 2H, Ar.), 4.82 (s, 2H, $CH_2$), 4.20 (q, 2H, $NCH_2CH_3$, $J = 6.94$ Hz), 3.82 (s, 3H, Ar. –$OCH_3$, methoxyl), 3.45 (m, 4H, piperazine), 3.10–3.20 (m, 4H, piperazine), 1.30 (*t*, 3H, $NCH_2CH_3$, $J = 6.96$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.2, 177.5, 168.5, 165.1, 164.9, 148.2, 144.5, 142.5, 139.5, 130.2, 129.8, 129.1, 114.2, 109.3, 100.7, 55.9, 50.2, 49.7, 38.5, 13.2 ppm. MS (FAB) *m/z*: $M^+$ 583.49. Anal. Calcd. for $C_{27}H_{26}FN_5O_5S_2$: C 55.56, H 4.49, N 12.00. Found: C 55.54, H 4.47, N 12.12.

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-p-fluorophenylethylthio)-1,3,4-thiadiazol-2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8g**)*

IR (KBr) $cm^{-1}$: 3440 (O–H *str.*), 2889 (Ar. C–H *str.*), 2847 (Ali. C–H *str.*) 1742 (Carboxylic C=O *str.*), 1688 (Ar. C=O *str.*), 1652 (4-Oxo quinolone C=O *str.*), 762 (out of plane, Ar. C–H *def.*), 665 (Ali. C–S *str.*) $^1$H NMR (DMSO-$d_6$) $\delta$ ppm: 14.49 (s, 1H, OH), 8.56 (s, 1H, H2-quinoline), 8.22 (m, 2H, Ar.), 7.91 (d, 1H, H5-quinoline, $J = 13.04$ Hz), 7.51 (d, 1H, H8-quinoline, $J = 7.35$ Hz), 7.35 (m, 2H, Ar.), 4.74 (s, 2H, $CH_2$), 4.24 (q, 2H, $NCH_2CH_3$, $J = 6.92$ Hz), 3.42 (m, 4H, piperazine), 3.14–3.32 (m, 4H, piperazine), 1.18 (*t*, 3H, $NCH_2CH_3$, $J = 6.98$ Hz) ppm. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 194.7, 177.2, 168.9, 167.3, 165.7, 148.2, 144.7, 143.2, 139.7, 132.1, 130.4, 115.4, 109.3, 100.2, 50.5, 49.6, 38.2, 13.0 ppm. Anal. Calcd. for $C_{26}H_{23}F_2N_5O_4S_2$: C 54.63, H 4.06, N 12.25. Found: C 54.65, H 4.10, N 12.26.

## Biological assay

### *In vitro antibacterial activity*

Broth microdilution method was employed for preliminary in vitro antibacterial activity. Activity was investigated against Gram-positive organisms, *S. aureus* and *Corynebacterium,* (ATCC-25365 and ATCC-29355 strain); and Gram-negative organisms, *Pseudomonas aeruginosa* and *Escherichia coli*, (ATCC 25619 and MTCC-1089 strain). The activity of the test compounds was compared with ciprofloxacin and norfloxacin as standards.

Twofold serial dilutions of the test compounds and reference drugs were prepared in Mueller–Hinton agar medium. Test compounds and standard drugs (6.4 mg) were dissolved in dimethylsulphoxide (DMSO, 1 mL) and diluted with distilled water (9 mL). Progressive double dilutions with melted Mueller–Hinton agar were performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/mL. Petri dishes were inoculated with 1–5 $\times$ $10^4$ colony-forming units (cfu/mL) and incubated at 37 °C for 18 h. A control was performed with the medium supplemented with DMSO at the same dilutions as used in the experiments (Goto *et al.*, 1982).

### *In vitro antitubercular activity*

In vitro antitubercular screening was performed using *M. tuberculosis* virulent $H_{37}$Rv strain. The MIC of each drug was determined by broth dilution assay. A frozen culture of Middlebrook 7H9 broth supplemented with 10 % ADC (albumin dextrose catalase) and 0.2 % glycerol was thawed and diluted in broth to 2 $\times$ $10^5$ cfu/mL and used as inoculum. In the assay, U-tubes were used to accommodate compounds in 0.1–25 μg/mL dilutions i.e. (25, 20, 15, 10, 05, 2.5, 1.5, 1.0, 0.5 and 0.1). Each test compound was dissolved in DMSO and then diluted in broth at twice the desired concentration. The final concentration of DMSO in the assay medium was 1.3 %. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. The growth in U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and standard isoniazid and rifampicin (Suling *et al.*, 2000; Yajko *et al.*, 1995).

### In vitro cytotoxic activity

MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra sodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amount of MTT. Thus, the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal, (MP) India using the standard operating procedures. The test compounds were dissolved in DMSO and serially diluted with complete medium to get the range of test concentration. DMSO concentration was kept <0.1 % in all the test compounds. Cell lines maintained in appropriate conditions were seeded in 96-well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5 % $CO_2$ for 96 h. MTT reagent was added to the wells and incubated for 4 h; the dark blue formazan product formed by the cells was dissolved in DMSO in a safety cabinet and read at 550 nm. Percentage inhibitions were calculated and plotted with the concentrations and used to calculate the $IC_{50}$ values in micromolar (Sriram *et al.*, 2006).

### *Docking study*

Degree of affection between the synthesized novel molecules and target in terms of structural and chemical complementation was explored by advanced scientific

programme 'Glide 4.5' (Glide version 4.5 Schrödinger, 2007; Protein Preparation Wizard Schrödinger, 2007) module of Schrödinger Molecular Modeling Interface. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule were made using a grid based method.

In the present study, X-ray crystal structure of the twinned 3.35 Å structure of *S. aureus* gyrase complexed with ciprofloxacin and DNA gyrase (*PDB entry: 2XCT)* for antibacterial activity and structure of *M. tuberculosis* complexed with isoniazid and DNA gyrase *(PDB entry: 2BM7)* was taken from PDB (www.rscb.org). Before docking, the proteins were prepared by means of the protein preparation wizard (Maestro-v8.0 Schrödinger, 2007) by removing the water molecule and cofactors from the proteins, optimizing hydrogen bonding and deleting the ligand present in crystal structure. Solvent molecules were deleted, and bond order for crystal ligands and proteins were adjusted. The structures were minimized up to 0.30 Å RMSD. The ligands were built using Maestro v-8.0 (LigPrep-v2.1 Schrödinger, 2007) build panel and prepared by LigPrep-v2.1 (Metropolis *et al.*, 1953) by means of the OPLS-2005 force field using extra precision (XP) mode of Glide v-4.5. All the molecules were docked into active site of target molecule, and final scoring was carried out in terms of G-score multi-ligand scoring function.

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