Design, Synthesis and Biological Investigation of Some Novel Quinazolin-4(3H)-One Tethered 1, 3, 4-Thiadiazole-Thiol Motifs as Direct Enoyl Acyl Carrier Protein Reductase Inhibitors

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AD designed the study, performed the computational studies and the bench work, synthesized the derivatives, and prepared the first manuscript draft. Author SD guided throughout the research and confirmed the final result analyses. Authors SB and CB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47A33052
Editorial:
(1) Dr. Giuseppe Murdaca, University of Genoa, Italy.
(2) Hassan H. Abdallah, Salahaddin University – Erbil, Iraq.
Reviewers:
(1) Ali N. Sabbar, Al-Muthanna University, Iraq.
Complete Peer review History: https://www.sdiarticle4.com-review-history/76063

Received 11 August 2021
Accepted 23 October 2021
Published 29 October 2021

ABSTRACT

Aims: In this study two noteworthy pharmacophores quinazolin-4(3H)-one and 1,3,4-thiadiazole through methylene bridge were utilized to design, synthesize and characterize some novel 2-methyl quinazolin-4(3H)-one and 6-chloro-2-methyl quinazolin-4(3H)-one tethered S-substituted-1,3,4-
thiadiazole-thiol structural analogs respectively as direct *Mycobacterium Tuberculosis* (MTB) enoyl acyl carrier protein reductase (InhA) inhibitors.

**Study Design:** Design of structural analogs of quinazolin-4(3H)-one tethered 1,3,4-thiadiazole-thiol through methylene bridge by functional group modifications in core scaffold followed by computational studies to select promising compounds. Synthesis of some novel compounds, structural characterization and screening of biological activity of the same.

**Methodology:** The molecular docking of designed compounds was carried out using schrodinger Glide XP into the active site of MTB InhA with protein data bank code (PDB ID: 2H7M). The interactions were evaluated based on the glide G score compared with reference standard isoniazid. Ten new compounds 7(A1-A10) were synthesized, characterized and screened for their *in-vitro* antitubercular activity by Microplate Almar Blue Assay (MABA) method followed by cytotoxicity evaluation of compounds 7A4 and 7A10 using Vero cell line.

**Results:** All the designed compounds of series 7(A1-A10) had drug-like characteristics and were non-toxic to normal cells. In the molecular docking studies, compounds 7A4, 7A5, and 7A10 demonstrated strong binding affinity in the active region of MTB InhA protein and retained necessary amino acid interaction, similar to co-crystal 2H7M. Synthesized compounds 7(A1-A10) were found to have good antitubercular activity. Out of the series the compounds 7A4 and 7A10 were found to possess excellent antitubercular activity equipotent to reference standard streptomycin with minimum inhibitory concentration (MIC) value of 6.25µg/ml. The cytotoxic potential of compounds 7A4 and 7A10 showed remarkable selectivity index against Vero cell line.

**Conclusion:** The findings of this study highlights the importance of tethering two pharmacophoric motifs in one compound to develop novel antitubercular agents that can be exploited as promising leads as direct InhA inhibitors.

**Keywords:** Quinazoline-4(3H)-one; enoyl ACP reductase; antitubercular activity; cytotoxicity.

1. **INTRODUCTION**

Tuberculosis is one of the most dangerous infections, claiming the lives of around 1.21 million people worldwide in the year 2019. The long duration of therapy, combined with the microorganism's resistance, has resulted in tuberculosis recurrence, particularly in multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. Currently, very few drugs are effective against MDR and XDR strains of *Mycobacterium tuberculosis*. Nonetheless, second-line drugs have been used for many decades are contributing to a rise in emergence of drug-resistant TB. As a result, new drugs are urgently needed to reduce the length of treatment and treat drug-resistant strains. The development of compounds that can serve as enzyme inhibitors is now the focus of the quest for new entities [1-4].

![Quinazoline-4(3H)-one clubbed S-substituted 1,3,4-thiadiazole thiol derivatives 7(A1-A10)](image-url)

**Fig. 1.** Designed quinazoline-4(3H)-one tethered S-substituted 1,3,4-thiadiazole thiol derivatives 7(A1-A10)
The biosynthesis of mycolic acid requires several enzymes. The InhA gene encodes a NADH-dependent enoyl-acyl carrier protein (ACP) reductase that is involved in fatty acid prolongation in cell wall formation. Moreover, the first-line anti-TB drug isoniazid (INH) is reported to be a potent enoyl ACP reductase inhibitor but needs direct activation by KatG, a catalase-peroxidase enzyme. The fundamental issue, which is responsible for INH resistance, is mutations in KatG. As a result, the current investigation focuses on developing a new antitubercular medication that does not require KatG activation prior to acting as a direct InhA inhibitor [5,6].

Literature survey revealed that several quinazoline and quinazolinones have gained extensive importance due to the diverse pharmacological activities that they display such as antimicrobial, anticonvulsant, antitumor, anti-inflammatory, anticancer, and antitubercular [7-10]. Recently, 2-styrylquinazolines, N-phenyl acetamides, thiazolidin-4-one based quinazolines, 2, 4-diaminoquinazolines, 2-(4-oxoquinazolin-3(4H)-yl)-acetamide and new series of direct InhA inhibitors, have been discovered and their minimum inhibitory concentrations (MIC) against *Mycobacterium tuberculosis* are being initiated in search of potent antitubercular drugs [11,12]. Among the structural modifications used to introduce different functional residues into the main skeleton of quinazoline and quinazolin-4(3H)-one, substitution at position-3 has been linked to antitubercular activity. This hypothesis was confirmed for many compounds containing quinazolin-4(3H)-one as structural moiety with phenyl rings, 1,2,4-triazoles, 1,3-thiadiazidine, 1,3,4-thiadiazole, 1,3,4-oxadiazole rings, and alkyl/aryl/allyl and phenacyl groups. Furthermore, diverse chemotherapeutic agents containing pharmacophores like 2-alkylthiobenzothiazoles, 1-aryl-5-alkylthio-1,2,3,4-tetrazoles, and 2-alkylthiopyridine-4-carbothioamides are described to possess antitubercular activity [13-19]. A new class of azole anti-mycobacterial, quinazolin-4(3H)-one clubbed 1,3,4-thiadiazoles have proved to be highly active both in-vivo and in-vitro. Since 1,3,4-thiadiazole is relatively inert to electrophilic substitution but susceptible to nucleophilic attack, substitutions at positions-2 and/or 5 readily react to yield marked derivatives with a wide range of pharmacological activities such as anticonvulsant, anti-inflammatory, antimycobacterial, antihypertensive, antidepressant, anxiolytic, and cytotoxic [20-23].

Despite the wide range of biological activities demonstrated by quinazolines and 1,3,4-thiadiazole derivatives, there is scarcity of literature reported on the development of potential antitubercular agents based on the combination of these heterocycles in a single molecule which we have attempted in present study [24,25]. In the present study, with the help of computational drug discovery tools, we planned to explore quinazolin-4(3H)-one tethered 1,3,4-thiadiazole-thiol through methylene bridge having the general structure as represented in (Fig.1). The goal of this study was to design and synthesize a library of 2-methyl-quinazolin-4(3H)-one and 6-chloro-2-methyl-quinazolin-4(3H)-one tethered S-substituted-1,3,4-thiadiazole thiol compounds respectively as *Mycobacterium tuberculosis* enoyl acyl carrier protein reductase (MTB InhA) inhibitors. The insertion of methyl tail at position-2, chloro group at position-6 and various phenacyl bromides at position-3 on right hand side through thiadiazole thiol methylene bridge were taken in the quinazolin-4(3H)-one core to establish the early structure-activity relationship (SAR) of the same. Moreover the SAR and computational studies gave an insight of synergistic impact within the two rings to obtain the novel antitubercular agents.

2. MATERIALS AND METHODS

All the reagents and chemicals used were of synthetic grade and were obtained from E. Merck and Sigma-Aldrich (Mumbai, India). The open tube capillary method was used to determine the melting points of synthesized compounds, and the results are uncorrected. Hexane:ethyl acetate (1:1) was used as the mobile phase for thin-layer chromatography (TLC) to examine the purity by observing a single spot on Merck silica gel 60 F254 coated alumina plates (0.25mm) and visualization was done by iodine chamber. All the solvents used for silica gel column chromatography were distilled before use. Fourier-transform infrared spectroscopy (FT-IR) absorption spectra were recorded in KBr pellets on Shimadzu FT-IR spectrometer 8400, vmax in cm⁻¹. ¹H and ¹³C NMR spectra were recorded on a Bruker AscendTM FT-NMR spectrometer 500 using TMS as the internal standard in DMSO-d₆ solvent. All the chemical shift values were reported in ppm (δ) downfield from TMS. Data was reported as s= singlet, d= doublet, t= triplet, dd= doublet of doublets and m= multiplet. The
mass spectra were recorded on Bruker Daltonik GmbH, Mass spectrometer ESI. Satisfactory analysis for CHN on EuroVector E3000 elemental analyzer was obtained for the compounds within ± 0.4 % of the theoretical values.

2.1 Computational Study for the Framed Scheme

2.1.1 Lipinski’s rule and ADMET prediction

Before docking pre-validation of the ligand was done by computation of molecular properties, drug-likeness and prediction of pharmacokinetic parameters (ADME). All designed compounds were subjected to ADME prediction and Lipinski’s rule using Qikprop module 3.2 available in Schrodinger [26,27]. Further these compounds were subjected to in silico toxicity prediction using OSIRIS molecular property explorer to indicate the mutagenic, irritating, tumorigenic, or reproductive effects of these compounds [28].

2.1.2 Molecular docking studies

The molecular docking tool Glide, version 6.7, Schrodinger, LLC, New York, NY, 2015 served the purpose of molecular docking studies. The ligand structures were created in MOL 2 format with ChemDraw and then transformed to 3D conformations with Schrodinger’s LigPrep module. The geometry optimization for all the molecules was carried out using OPLS-2005 (optimized potential liquid simulations) force field until the root mean square deviation (RMSD) reached the value of 1.4Å or less. The EPIK module, which generates ionization states in the pH range of 7 ± 2, was used to create all probable tautomers and stereoisomers.

The X-ray crystal structure of (InhA) enoyl acyl carrier protein (ACP) reductase of MTB complexed with 1-cyclohexyl-N-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide (PDB ID: 2HTM) inhibitor having a resolution of 1.62 Å, retrieved from identifier protein data bank. Before calculations, the protein was optimized for docking using the protein preparation wizard available in Schrodinger’s Maestro. In the first step, the water molecules beyond 5Å and cofactors from the proteins were removed, hydrogen bonds were optimized and ligand present in the crystal structure was deleted. The receptor grid was generated using the co-ordinates of the X-ray ligand with the standard settings using glide. Glide energy grids were generated for each prepared protein complex. A rectangular box surrounded the X-ray ligand marking the binding site. The ligand’s of lowest energy conformation were chosen and docked into the grid created by the protein structure. The best docked compounds are selected based on Glide gscore. Extra precision (XP) visualizer of Glide module was utilized to analyze the results based on the active site amino acid interactions. Glide gscore is a modified and extended version of the empirically based chemscore function [29-31].

2.2 Synthetic Procedures

In this study, construction of the target quinazolin-4(3H)-one tethered S-substituted 1,3,4-thiadiazole-thiol 7(A1-A10) compounds was achieved by using a facile synthetic route as depicted in (Fig. 2). In step 1 & 2, methyl tail at the position-2 of the quinazolin-4(3H)-one core was introduced by condensing anthranilic acid 1a and 6-chloro anthranilic acid 1b with acetic anhydride followed by dehydration with formamide respectively [32,33]. The desired products, 2-methylquinazolin-4(3H)-one 3a and 6-chloro-2-methylquinazolin-4(3H)-one 3b were obtained in high yields. In step 3, the acetoxydiazine linker was introduced by alkylation of 3a,b with chloro ethyl acetate (ClCH2COOC2H5) to obtain the products 2-methyl-4-oxoquinazolin-3(4H)-yl) acetate 4a and 6-chloro-2-methyl-4-oxoquinazolin-3(4H)-yl) acetate 4b [34]. In step 4, intermediates 4a,b were refluxed with hydrazine hydrate 99% in ethanol to yield 5a,b respectively [35]. In step 5, intermediates 5a,b were refluxed in ethanol with equimolar quantity of carbon disulfide by adding few drops of conc. sulphuric acid to yield 2-methyl-3-[[5-sulfanyl-1,3,4-thiadiazol-2-y] methyl]quinazolin-4(3H)-one 6a and 6-chloro-2-methyl-3-[[5-sulfanyl-1,3,4-thiadiazol-2-y]methyl]quinazolin-4(3H)-one 6b [36]. The purity of final products was tested by melting point determination and TLC.

2.2.1 General procedure for the synthesis of quinazolin-4(3H)-one tethered S-substituted-1, 3, 4-thiadiazole thiol derivatives 7(A1-A10)

2-methyl-3-[[5-sulfanyl-1,3,4-thiadiazol-2-y]methyl]quinazolin-4(3H)-one and (6a) and 6-chloro-2-methyl-3-[[5-sulfanyl-1,3,4-thiadiazol-2-y]methyl]quinazolin-4(3H)-one (6b) were synthesized by modification of a previously
reported method. Anhydrous potassium carbonate (0.006 mol) dissolved in absolute ethanol (25 ml), was added to intermediates 6a and 6b (0.004 mol each), followed by part wise addition of substituted phenacyl bromides (0.004 mol) dissolved in (15 ml) absolute ethanol respectively. The mixture in each case was then refluxed and stirred for 10 h. The completion of reaction was monitored at an appropriate time interval, and then the reaction mixtures were poured into ice and neutralized in glacial acetic acid. The solids were filtered; dried and crude products so obtained were purified by recrystallization with a suitable solvent to afford corresponding compounds. Purification of some compounds was carried out by silica gel column chromatography using hexane:ethyl acetate (3:1) as eluent [37,38].

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\begin{align*}
&\text{Fig. 2. Representative Scheme for the synthesis of target compounds 7(A1-A10)}
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2.3 In-vitro Antitubercular Activity

The in-vitro antitubercular activity of the compounds 7(A1-A10) was assessed against Mycobacterium tuberculosis H37Rv, ATCC 27294 [39] using the microplate alamar blue assay method [40]. The methodology used was nontoxic, involving the use of a thermostable substance, and revealed an excellent correlation between radiometric and proportional methods. Medium evaporation in the test wells was reduced by adding 200μl of sterile deionized water in sterile 96 well plates at outer-perimeter wells during incubation. This was followed by the addition of 100μl Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) to 96 plates. Initial serial dilutions for test compounds of series were prepared and added to the plate. The final drug concentrations in the range of 0.4-100μg/ml in DMSO were used for further testing. Plates were incubated at 37°C beforehand and sealed with parafilm. On the seventh day of incubation, a freshly prepared 1:1 mixture of alamar blue dye solution (25μl) and 10% tween 80 (12.5ml) were added to the wells, and the plates were re-incubated for 24 h. Bacterial growth was interpreted by observing the colour change from blue to pink. The reference drugs selected for comparison were pyrazinamide, ciprofloxacin, streptomycin and isoniazid. The lowest drug concentration that prevented a colour change was identified as the MIC [41,42].

2.4 In-vitro Cytotoxicity by MTT Assay

The selected compounds from series 7(A1-A10) were further assayed in-vitro for cytotoxic activity against Vero (kidney normal cell line of African monkey) colorimetrically [43]. This assay is based on the capacity of the mitochondrial succinate dehydrogenase enzyme to convert soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product. The intensity was estimated by a spectrophotometer [44,45]. This type of MTT reduction can only be seen in metabolically active cells. The number of viable cells determines the level of activity. Cell viability of selected compounds was determined using MTT assay in presence of 10% and 0.2% FBS respectively. Solution of compounds under test were prepared in DMSO at 10 mM concentration and stored at −20℃. The dilutions were made in a culture medium before treatment. For the MTT assay, six concentrations in μg/ml were employed for cell lines: 10, 20, 25, 30, and 50. Test compound solutions with targeted cells were incubated for 72 h at 37°C with 5% CO₂. After the exposure, the test solutions were replaced with 500μg/ml MTT solution. The absorbance at 570nm was measured for mixtures in each plate with an ELISA reader. For each test compound, the tumor cell inhibitory concentration (IC) was calculated and the cytotoxicity of compounds was listed as IC50 (μg/ml).

The selectivity index (SI) was calculated by taking the ratio of IC₅₀ and the MIC values; the compounds with value of SI ≥10, were investigated further [46-48].

3. RESULTS AND DISCUSSION

The study was aimed to synthesize new compounds by tethering quinazolin-4(3H)-one and 1,3,4-thiadiazole-thiol as one entity with improved antitubercular potential against direct InhA inhibitors. The computational study was carried out first for determination of drug-like molecules, all the designed compounds presented values within an acceptable range for the properties analyzed indicating their potential as drug-like molecules (Table 1) based on Lipinski’s rule of 5. This rule in general is considered to influence good membrane permeability and oral bioavailability associated with filters namely, molecular weight ≤ 500, QlogP ≤ 5, number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5. As the cutoffs for each of the four parameters were all close to 5 this simple mnemonic was called the Lipinski’s rule of 5. Drug candidate that comply with the Lipinski rule have less failure rate during clinical trial. Drug like physicochemical parameters of compounds affect drug pharmacokinetics (ADME) in human body.

An in silico evaluation of ADME profile of synthesized derivatives is necessary to understand pharmacological behavior of the lead. The ADME properties of the majority of designed compounds were within acceptable limits. However, it can be noted that PSA values predicted a poor bioavailability for the reference standards, and a good bioavailability for the compounds selected for synthesis. It was observed that the low hydrogen bonding ability of designed compounds could enhance the biological membrane permeating capacity. The toxicity profile evaluation of designed compounds was performed employing the Osiris Molecular Property explorer software, none of the
compounds were found to be toxic. A low in silico toxicity risk profile, ADME predictions, and Lipinski’s parameters made these compounds 7(A1-A10) a better lead for development of safe and efficient antitubercular agents.

Docking analysis of the crystal structure of 2H7M revealed that the reference inhibitor in the MTB InhA active site formed a hydrogen-bonding network with Lys165. The predicted bound conformation of the lead molecule 6a,b within the active site of MTB InhA protein showed that the oxygen atom of the carbonyl group of quinazolinone formed a hydrogen bonding network with the side chain of Tyr158. It served as the key feature to explain the orientation of the compounds within the active site. The docking results of compounds 7(A1-A10) showed the highest Glide gscore in kcal/mol and were more tightly bound to the active site of MTB InhA than other designed compounds. All these compounds showed π-stacking interactions but were weak as compared to observed hydrogen bonding interactions. The amino acid residues like Lys-165, Ile-21, Asn-247, Thr-28, Trp-32 and Phe-304 were involved in these interactions. These observations provided a good basis for the estimation of the inhibitor activity and indicated high binding affinity of these selected compounds to MTB InhA than the lead molecule (Table 2).

| Comp Code | PSA | H | BD | MW | QPlogP | QPlogS | QPlogBB | Violation of rule of 5 | QPP MDCK | Human oral absorption |
|-----------|-----|---|----|----|--------|--------|---------|-----------------------|----------|----------------------|
| Lead      | 69.53 | 0.8 | 6 | - | 2.114 | -3.403 | -0.272 | 0 | 211.68 | 3 |
| 7A1       | 94.31 | 0 | 8 | 487.38 | 3.767 | -5.468 | -0.879 | 0 | 1590.44 | 3 |
| 7A2       | 94.31 | 0 | 8 | 422.51 | 3.507 | -5.192 | -1.072 | 0 | 598.33 | 3 |
| 7A3       | 94.31 | 0 | 8 | 456.96 | 4.013 | -5.959 | -0.924 | 0 | 1477.45 | 3 |
| 7A4       | 94.31 | 0 | 8 | 426.48 | 3.422 | -4.955 | -0.93 | 0 | 1084.09 | 3 |
| 7A5       | 94.30 | 0 | 8 | 460.92 | 3.927 | -5.722 | -0.782 | 0 | 2675.55 | 3 |
| 7A6       | 120.10 | 0 | 9.5 | 433.50 | 2.339 | -5.354 | -1.94 | 0 | 109.33 | 3 |
| 7A7       | 120.10 | 0 | 9.5 | 467.94 | 2.845 | -6.121 | -1.807 | 0 | 269.94 | 3 |
| 7A8       | 139.13 | 0 | 9 | 453.49 | 2.425 | -4.616 | -2.198 | 0 | 60.44 | 3 |
| 7A9       | 139.12 | 0 | 9 | 487.93 | 2.93 | -5.382 | -2.071 | 0 | 149.14 | 3 |
| 7A10      | 98.70 | 8.75 | 3 | 438.51 | 3.332 | -4.693 | -1.046 | 0 | 696.85 | 3 |
| INH       | 81.35 | 3.25 | 4.5 | 137.14 | -0.646 | -0.052 | -0.843 | 0 | 123.74 | 2 |
| PYZ       | 78.71 | 2 | 5 | 123.11 | -0.65 | -0.541 | -0.735 | 0 | 126.06 | 2 |
| STM       | 330.64 | 16 | 25.25 | 581.57 | -6.043 | -0.196 | -5.113 | 3 | 0.014 | 1 |

PSA: Polar surface area, HBD: Hydrogen bond donors, HBA: Hydrogen bond acceptor, MW: Molecular weight, QPlogP: Octanol/water partition coefficient, QPlogS: Aqueous solubility, QPlogBB: Brain/blood partition coefficient, QPP MDCK: Apparent MDCK cell permeability

Table 2. Docking results of selected compounds 7(A1-A10) based on Glide gscore

| Compound Code | Glide g score (kcal/mol) |
|---------------|-------------------------|
| 7A1           | -8.335                  |
| 7A2           | -7.579                  |
| 7A3           | -8.543                  |
| 7A4           | -8.302                  |
| 7A5           | -8.533                  |
| 7A6           | -8.309                  |
| 7A7           | -8.335                  |
| 7A8           | -8.038                  |
| 7A9           | -8.560                  |
| 7A10          | -8.876                  |
Based on the outcomes of computational studies, the new series of compounds 7(A1-A10) were synthesized in good yield by condensing 6a,b with various phenacyl bromides in absolute ethyl alcohol. The reaction time was greatly shortened, and the reflux temperature was lowered to get compounds 7(A1-A10). All the synthesized compounds 7(A1-A10) were accurately analyzed by spectroscopic techniques, and results were in full accordance with the proposed structures.

3.1 3-[[5-[[2-(4-bromophenyl)-2-oxoethyl]sulfanyl]-1,3,4-thiadiazol-2-yl]methyl]-2-methyl-3,4-dihydroquinazolin-4-one (7A1)

Yield: 56%, m.p. (C): 164-166°C, Rf 0.58. 1H NMR (500 MHz, DMSO-d6) δ ppm: 2.35 (s, 3H, -CH3), 3.36 (s, 2H, -S-CH2-CO), 4.74 (s, 2H, -CH2), 7.20-8.13 (m, 4H, quinazoline-H), 7.74-7.90 (m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δ ppm: 23.36 (-CH3), 30.15 (-S-CH2-CO), 51.13 (-CH2), quinazolinone-C [119.9 (C), 126.9 (CH), 128.9 (CH), 133.8 (CH), 135.1 (CH), 147.6 (C), 155.5 (C=CH)], Phenyl-C [124.3 (CH), 128.9 (CH), 126.6(CH), 127.1(C-Br), 130.7(CH)], 132.5(O)], Thiadiazole-C [164.3(C), 154.2(C)], 164.3 (C=O), 193.1(-S-CH2-C=O); HRMS(ESI): m/z (pos): 488.0626 (M+H); FT-IR (KBr, cm-1): 3037.99 (Ar C-H str.), 2978.11(C-H str., -CH3), 1732.64 (C=O str.), 1670.41(C=O str.), 1609.23(C=O str.), 1593.25(C=N str.), 1475.69, 1338.64 (C-H bend in -CH2 and CH2), 1226.77 (C-S-C str.), 702.97(C-Br str.); Anal. calcd. for C20H13BrN5O2S2: C, 49.29, H, 3.10, N, 11.50. Found: C, 49.39, H, 3.15, N, 11.42 (C20H13BrN5O2S2; calc. 487.3927).

3.2 2-methyl-3-[[5-[[2-(4-methylphenyl)-2-oxoethyl]sulfanyl]-1,3,4-thiadiazol-2-yl]methyl]-3,4-dihydroquinazolin-4-one (7A2)

Yield: 59%, m.p.(C): 266-268°C, Rf 0.45. 1H NMR (500 MHz, DMSO-d6) δ ppm: 2.40 (d, 3H, -CH3), 2.53 (d, 3H, -CH3 of quinazoline), 3.73 (s, 2H, -S-CH2-CO), 4.74 (s, 2H, -CH2), 7.27-8.16 (m, 4H, quinazoline-H), 7.70-7.86 (m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δ ppm: 20.00(-CH3), 22.45 (-CH3 of phenyl), 39.98 (-S-CH2-CO), 50.67 (-CH2), quinazolinone-C[120.0 (C),122.1(CH), 126.9(CH), 128.2(CH), 132.2(CH), 147.1(C), 154.2(C=CH)], phenyl-C[134.4(CH), 137.1(CH), 138.2(CH), 139.7(C), 141.2(CH), 144.4(C=CH)], thiadiazole-C [158.9 (C),142.8(C)], 160.6 (>C=O), 191.0 (-S=CH-)

3.3 6-chloro-2-methyl-3-[[5-[[2-(4-methylphenyl)-2-oxoethyl]sulfanyl]-1, 3, 4-thiadiazol-2-yl]methyl]-3,4-dihydroquinazolin-4-one (7A3)

Yield: 51%, m.p.(C): 184-186°C, Rf 0.31. 1H NMR (500 MHz, DMSO-d6) δ ppm: 2.43 (d, 3H, -CH3), 2.57 (d, 3H, -CH3 of quinazoline), 3.63 (s, 2H, -S-CH2-CO), 4.62 (s, 2H, -CH2), 7.25-8.37 (m, 3H, quinazoline-H), 7.60-7.95 (m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δ ppm: 21.40 (-CH3), 24.35(-CH3 of phenyl), 40.98 (-S-CH2-CO), 51.58 (-CH2), quinazolinone-C[121.9 (C), 131.0(C), 128.3(CH), 128.9 (CH), 129.2(CH), 132.1(C=Cl), 153.4(C=CH2)], phenyl-C[126.0(CH), 127.1(CH), 131.4(CH), 131.8(CH), 134.3(C), 145.7(C=CH3)], thiadiazole-C[157.1(C), 132.1(C)], 151.6 (>C=O), 194.1(-S-CH2-C=O); HRMS(ESI): m/z (pos): 457.3639 (M+H); FT-IR (KBr, cm-1): 3082.35(=C=O str.), 2972.40(C=H str., -CH3), 1724.42(C=O str.), 1672.34(C=O str.), 1610.61(C=O str.), 1577.82(C=N str.), 1471.14, 1350.22(CH bend -CH3 and CH2), 1269.20(N-N str.), 1107.18(C=S-C str.), 834.90(C=Cl str.); Anal. calcd. for C21H17ClN5O2S2: C, 55.20, H, 3.75, N, 12.26. Found: C, 55.33, H, 3.64, N, 12.13(C21H17ClN5O2S2; calc. 456.9682).

3.4 6-fluoro-2-methyl-3-[[5-[[2-(4-fluorophenyl)-2-oxoethyl]sulfanyl]-1,3,4-thiadiazol-2-yl]methyl]-2-methyl-3,4-dihydroquinazolin-4-one (7A4)

Yield: 60%, m.p.(C): 234-236°C, Rf 0.47. 1H NMR (500 MHz,DMSO-d6) δ ppm: 2.66 (s, 3H, -CH3), 3.73 (s, 2H, -S-CH2-CO), 4.77 (s, 2H, -CH2), 7.46-8.15 (m, 4H, quinazoline-H), 7.75-8.02(m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δ ppm: 23.15 (-CH3), 40.24(-S-CH2-CO), 51.17(-CH2), quinazolinone-C[120.0(C), 146.1(C), 126.7(CH), 128.1(CH), 127.1(CH), 131.8(CH), 156.1(C=CH3)], phenyl-C[122.2(CH), 126.9(CH), 131.9(CH), 135.3(CH), 136.1(C), 164.3(C=CH), thiadiazole-C[158.1(C), 143.8(C)], 160.6 (>C=O), 192.5(-S-CH2-C=O); HRMS(ESI): m/z (pos): 427.1361(M+H); FT-IR(KBr, cm-1):
3.5 6-chloro-3-[[5-[[2-(4-fluorophenyl)-2-oxoethyl]sulfanyl]-1,3,4-thiadiazol-2-yl]methyl]-2-methyl-1,3,4-dihydroquinazolin-4-one (7A5)

Yield: 50%, m.p. (C): 177-179°, Rf: 0.38, 1H-NMR (500 MHz, DMSO-d6) δppm: 2.46 (s, 3H, -CH3), 3.71 (s, 2H, S-CH2-CO), 4.63 (s, 2H, -CH2), 7.37-8.34 (m, 3H, quinazoline-H), 7.65-8.12 (phenyl-H); 13C-NMR (DMSO-d6) δppm: 23.07-CH3, 40.76-(S-CH2-CO), 50.23-(CH3), quinazoline-C(121.2(C), 128.1(CH2), 129.8(CH), 129(CH), 131.9(CH), 133.2(C-Cl), 145.8(C), 154.6(CH3)), phenyl-C(121.7(CH), 132.7(CH), 131.4(C), 167.2(C-F), thiadiazolone-C[144.6(C), 156.4(C)], 161.5(>C=O), 193.8(S-CH2-C=O); HRMS (ESI) m/z (pos): 461.1200 (M+H+); FT-IR(KBr, cm−1): 3072.71 (Ar C-H str.), 2979.19(C-H str., -CH3), 1737.41(C=O str., CO), 1687.77(C=O str.), 1602.90(C=C str.), 1537.35(C=N str.), 1475.89, 1361.79(C-H bend in -CH3 and CH2), 1014.59(C-S-C str.), 851.15(C-Cl str.), 779.27(C-F str.); Anal. calcd. for C21H16ClF2N4O2S2: C, 52.11, H, 3.06, N, 12.16. Found: C, 52.01, H 3.14, N, 12.05 (C21H16ClF2N4O2S2) calcd, 460.9321.

3.6 4-[2-[[5-[[2-methyl-4-oxo-3,4-dihydroquinazolin-3-yl]methyl]-1,3,4-thiadiazol-2-yl]sulfanyl]acetyl]benzotriazole (7A6)

Yield: 62%, m.p. (C): 256-257°, Rf: 0.28, 1H-NMR (500 MHz, DMSO-d6) δppm: 2.57 (s, 3H, -CH3), 3.73 (s, 2H, -S-CH2-CO), 4.67 (s, 2H, -CH2), 7.56-8.16 (m, 4H, quinazoline-H), 7.78-7.98 (m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δppm: 20.90-(CH3), 40.14 (1C, -S-CH2-CO), 51.26(CH3), quinazoline-C[121.7(C), 147.2(C), 126.7(CH), 126.9(CH), 128.2(CH), 133.1(CH), 154.3(C=CH2), 113.8(-C=), phenyl-C[116.7(C-CN), 120.8(CH2-C), 130.3(CH2-C), 136.2(C)], thiadiazolone-C[143.8(C), 159.0(C)], 160.5(>C=O), 194.1(S=CH2-C=O); HRMS (ESI) m/z (pos): 432.1316(M+H+); FT-IR (KBr, cm−1): 3037.99(Ar C-H str.), 2972.40(C-H str., -CH3), 2231.71(C=C=N str.), 1723.20(C=O str.), 1664.98(C=O str.), 1608.69(C=C str.), 1577.82(C=N str.), 1477.52, 1388.79(C-H bend in -CH3 and CH2), 1294.28 (C-N str.); Anal. calcd. for C21H16N4O2S2: C, 58.18, H, 3.49, N, 16.16. Found: C, 58.25, H, 3.39, N, 16.02 (C21H16N4O2S2) calcd. 432.5061.

3.7 4-[2-[[5-[[6-chloro-2-methyl-4-oxo-3,4-dihydroquinazolin-3-yl]methyl]-1,3,4-thiadiazol-2-yl]sulfanyl]acetyl]benzotriazole (7A7)

Yield: 55%, m.p. (C): 196-198°, Rf: 0.63, 1H-NMR (500 MHz, DMSO-d6) δppm: 2.51 (s, 3H, -CH3), 3.68 (s, 2H, S-CH2-CO), 4.63 (s, 2H, -CH2), 7.52-8.45 (m, 3H, quinazoline-H), 7.70-8.24 (m, 4H, phenyl-H); 13C-NMR (DMSO-d6) δppm: 23.01-(CH3), 40.61-(S-CH2-CO), 52.03-(CH3), quinazoline-C[121.9(C), 127.1(C), 128.9(C), 131.1(CH), 132.0(Cl), 145.4(C), 153.3(C=CH3)], 117.4(C=CN), phenyl-C[116.6(C-CN), 129.3(CH2-C), 133.3(CH2-C), 135.8(C)], thiadiazolone-C[144.1(C), 157.7(C)], 191.5(>C=O), 193.1(S=CH2-C=O); HRMS (ESI) m/z (pos): 468.4725(M+H)+; FT-IR(KBr, cm−1): 3082.35(Ar C-H str.), 2974.33(C-H str., -CH3), 2227.86,(C=N str.), 1730.21(C=O str.), 1674.27(C=O str.), 1608.69(C=O str.), 1579.75(C=N str.), 1497.81, 1409.57(C-H bend in -CH3 and CH2), 1276.27(C-N str.), 839.06 (C-Cl str.); Anal. calcd. for C21H16ClN4O2S2: C, 53.90, H, 3.02, N, 14.97. Found: C, 53.78, H, 3.13, N, 14.89 (C21H16ClN4O2S2) calcd. 467.9511.

3.8 2-methyl-3-[[5-[[2-(3-nitrophenyl)-2-oxoethyl]sulfanyl]-1,3,4-thiadiazol-2-yl]methyl]-3,4-dihydroquinazolin-4-one (7A8)

Yield: 67%, m.p. (C): 240-241°, Rf: 0.28, 1H-NMR (500 MHz, DMSO-d6) δppm: 2.63 (s, 3H, -CH3), 3.73(2H, s, -S-CH2-CO), 4.89(2H, s, -CH2), 7.67-8.47(4H, m, quinazoline-H), 7.80-8.12(4H, m, phenyl-H); 13C-NMR(DMSO-d6) δppm: 22.45 (-CH3), 40.48 (-S-CH2-C=O), 51.56 (-CH3), quinazoline-H[120.8(C), 124.1(CH), 127.1(CH), 128.6(CH), 134.0(CH), 145.4(C), 153.2(C=CH3)], phenyl-C[127.7(CH2, 2C), 129.8(CH, 2C), 136.6(C), 150.0(C-NO2), thiadiazolone-C[(140.2(C), 145.2(C)], phenyl-C[116.7(C-CN), 120.8(CH2-C), 130.3(CH2-C), 136.2(C)], thiadiazolone-C[143.8(C), 159.0(C)], 160.5(>C=O), 194.1(S-CH2-C=O); HRMS (ESI) m/z (pos): 454.4413(M+H)+; FT-IR (KBr, cm−1): 158.0(C), 160.1(>C=O), 193.5(S=CH2-C=O); 3034.13(Ar C-H str.), 2953.12(C-H stretch, -CH3), 1718.63(C=O str.), 1684.98(C=O str.), 1568.18(C=C str.), 1531.53(C=NO2 aromatic), 1477.52, 1386.86(-CH3 bend quinazoline),
1294.28 (N-N str. in ring);  Anal. calcd. for C_{20}H_{15}N_{2}O_{5}S_{2}: C, 52.97, H, 3.33, N, 15.44. Found: C, 53.06, H, 3.21, N, 15.39 (C_{20}H_{15}N_{2}O_{5}S_{2} calc. 453.49). 3.9 6-chloro-2-methyl-3-[[5-[[2-(3-nitrophenyl)-2-oxoethyl] sulfanyl]-1,3,4-thiadiazol-2-yl] methyl]-3,4-dihydroquinazolin-4-one (7A9)

Yield: 55%, m.p. (6°C): 205-207°C; Rf: 0.50; 1H-NMR (500 MHz, DMSO-d_6) δ ppm: 2.35 (s, 3H, -CH_3), 3.84 (s, 2H, -S-CH_2-CO), 4.58 (s, 2H, -H_2), 7.37-8.21 (m, 3H, quinazolinoine-H); 7.64-7.90 (m, 4H, phenyl-H); 13C-NMR (DMSO-d_6) δ ppm: 22.10 (-CH_3), 41.62 (-S-CH_2-C=O), 50.41 (-CH_), quinazolinone-C[121.6(C), 146.6(C), 128.1(CH), 128.8(CH), 129.4(CH), 131.1(C-Cl), 153.6(C=CH)], phenyl-C[148.9(C-NO_2), 123.0(CH)], 127.4(CH), 131.8(CH), 134.2(CH), 135.3(C)], thiadiazole-C[144.7(C), 157.5(C)], 162.5(>C=O), 193.7(-S-CH_2-C=O); HRMS (ESI), m/z (pos): 488.2485 (M+H); FT-IR (KBr, cm⁻¹): 3078.49 (Ar), 3078.49; 3049.48 (Ar), 2845.10 (C=CH), 2784.30 (C=CH), 1734.06(C=O str.), 1670.41(C=O str.), 1610.61, (C=C str. aromatic), 1575.89 (C=N str. in ring), 1467.88, 1384.94 (C-H, -CH_2 and CH_3 bend); Anal. calcd. for C_{21}H_{18}N_{4}O_{4}S_{2}: C, 57.52, H, 4.14, N, 12.78. Found: C, 57.43, H, 4.19, N, 12.67 (C_{21}H_{18}N_{4}O_{4}S_{2} calc. 438.5226).

Based on the analytical support and docking studies these newly synthesized compounds were further screened for in-vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (ATCC 27294) for the estimation of MIC values with the reference standards. Based on the results of MABA (Table 3), The compounds 7A4 bearing fluoro group (R_1=4-F) and 7A10 possessing electron-withdrawing substituent methoxy (R_1=4-OCH_3) showed the highest inhibition at a MIC value of 6.25µg/ml against Mycobacterium tuberculosis H37Rv, which wasasequopotent to the clinically successful drug streptomycin. On the other hand, insertion of lipophilic halogen (R=Cl) in the compound 7A5 at C-6 of quinazolinoine and further substituent into the position-4 of phenyl ring (R_1=4-F) exhibited promising activity at MIC, 12.5µg/ml. It was interesting to note that the remaining compounds with electron releasing substituents: 7A2 and 7A3 as well as electron-withdrawing substituents: 7A1, 7A6, 7A7, 7A8, and 7A9 exhibited promising activity against Mycobacterium tuberculosis H37Rv with MIC ranging from 25-50 µg/ml.

Out of the ten only two compounds 7A4 and 7A10 were further examined for cytotoxicity in the African green monkey kidney normal cell line (Vero) at various concentrations. The % inhibitory cytotoxicity data along with IC_{50} µg/ml of respective compounds is given in (Table 3) and graphically represented in (Fig. 3) whose columns reflect the viable cells in each treatment, DMSO denotes an experimental control. The most active MTB InhA inhibitors 7A4 and 7A10 showed no toxicity with % cell inhibition of 42.62 and 47.10 at IC_{50} of 50µg/ml and 30µg/ml respectively (Fig. 4). By comparing in-vitro antitubercular and cytotoxicity data it was found that most of the active compounds 7A4 and 7A10 were non-toxic (≥50% inhibition). Especially the compounds 7A4 and 7A10 showed an outstanding selectivity index of 8 and 4.8 respectively. The results confirmed that variation in selectivity over the Vero cell line was an outcome of variations in the substitution pattern.
Fig. 3. Graphical representation of percentage cytotoxicity data for compounds 7A4 and 7A10 in Vero cell line

Fig. 4. Photomicrograph of inhibition percentage with regard to cytotoxicity with IC\textsubscript{50} of Vero cell line: Control, 7A4 (50 µg/ml) and 7A10 (30 µg/ml)

Table 3. Summary of \textit{in-vitro} antitubercular activity against MTB \textit{H37Rv} strain and \textit{in-vitro} cytotoxicity in Vero cell

| Compound Code | R  | R'   | MIC | % Cell Inhibition\textsuperscript{a} | IC\textsubscript{50} | SI | IC50/MIC |
|---------------|----|------|-----|--------------------------------------|---------------------|----|----------|
| 7A1           | H  | 4-Br | 25  | 51.29 Nd                             | -                   | -  | -        |
| 7A2           | H  | CH\textsubscript{3} | 50  | 102.58 Nd                            | -                   | -  | -        |
| 7A3           | Cl | 4-CH\textsubscript{3} | 25  | 54.7 Nd                              | -                   | -  | -        |
| 7A4           | H  | 4-F  | 6.25| 14.65 42.62                         | 50 117.23           | 8  |          |
| 7A5           | Cl | 4-F  | 12.5| 57.67 Nd                             | -                   | -  | -        |
| 7A6           | H  | 4-CN | 25  | 51.73 Nd                             | -                   | -  | -        |
| 7A7           | Cl | 4-CN | 25  | 53.42 Nd                             | -                   | -  | -        |
| 7A8           | H  | 3-NO\textsubscript{2} | 25  | 55.12 Nd                             | -                   | -  | -        |
| 7A9           | Cl | 3-NO\textsubscript{2} | 25  | 51.33 Nd                             | -                   | -  | -        |
| 7A10          | H  | 4-OCH\textsubscript{3} | 6.25| 14.25 47.1                          | 30 68.41            | 4.8|          |
| PYZ           | -  |      | 3.125 | 25.38 Nd                             | -                   | -  | -        |
| CFX           | -  |      | 3.125 | 9.43 Nd                              | -                   | -  | -        |
| STM.          | -  |      | 6.25 | 10.74 Nd                             | -                   | -  | -        |
| INH           | -  |      | 1.56 | 11.37 Nd                             | -                   | -  | -        |

\textsuperscript{a} % Inhibition at IC\textsubscript{50} concentration µg/ml determined against vero cell lines. ND: Not determined, PYZ: Pyrazinamide, CFX: Ciprofloxacin, STM: Streptomycin, INH: Isoniazid, MIC: Minimum inhibitory concentration
A complete SAR can be drawn considering the MIC values and cytotoxicity data as above. The systematic variations on the structural framework (Fig.1) with methyl tail at position-2, side-chain at position-3 with electron-withdrawing and electron-donating substituents on phenyl ring have confirmed that the presence and position of the substituents have a great impact on their antitubercular activity. The compounds 7A4 (R=H, R¹= 4-F) and 7A5 (R=Cl, R¹= 4-F) due to the presence of halogens, and 7A10 with methoxy moiety (R=H, R¹= 4-OCH₃) exhibited the highest antitubercular activity and were found to be important fragments for hydrogen bonding that could be formed at the receptor site. Thus, low lipophilicity, less steric hindrance, electron-withdrawing groups, and electron-donating groups are summarized to be preferential for design and synthesis of novel quinazolin-4(3H)-one tethered S-substituted-1,3,4-thiadiazole-thiol derivatives as antitubercular leads. According to the Glide gscore and experimentally determined MIC values against MTB InhA, compound 7A5 (MIC 12.5 µg/ml) exhibited good binding affinities with a Glide gscore (kcal/mol) of -8.533. The most active compounds 7A4 (Fig. 5) and 7A10 (Fig. 6) showed Glide gscore of -8.302 and -8.676 respectively (Table 2) both with MIC, 6.25 µg/ml have displayed hydrogen bonding interactions, one with the side chain of Lys165 and with similar orientation to that of crystal ligand respectively. This was considered to be critical for bioactivity, making these compounds more potent.

The insufficient binding of compounds 7A2 (Fig. 7), 7A6 (Fig. 8), and 7A7 into the active site of MTB InhA rationalizes their lower antitubercular potential, though they have higher Glide gscore. From the docking results, the presence of hydrogen bonds with Lys165, Tyr158, and Asn247 along with hydrophobic interactions with the active site were predicted as the most pivotal factors affecting the inhibitory potency of these compounds.

Fig. 5. Docked pose of compound 7A4 in the active site of InhA protein

Fig. 6. Docked pose of compound 7A10 in the active site of InhA protein
An analysis of the enzyme inhibition activity revealed that the compounds containing substitutions with \( R=H, R'=4-F \) (7A4), \( R=Cl, R'=4-F \) (7A5), and \( R=H, R'=4-OCH_3 \) (7A10), moieties were more favored. Based on the structure of MTB InhA receptor and the structure activity relationship study, the results further supported the molecular design of these compounds as MTB InhA inhibitors using an Extra precision module.

4. CONCLUSION

The present investigation concludes that the newly synthesized compounds 7 (A1-A10) showed superior inhibitions as direct MTB InhA inhibitors. Compounds 7A4 and 7A10 emerged as highly active agents against *Mycobacterium tuberculosis* H37Rv and inhibited drug-sensitive MTB with MIC value of 6.25\( \mu \)g/ml, which was equipotent to the reference standard streptomycin. Compounds 7A4 and 7A10 were found to be non-cytotoxic with superior potency against the Vero cell line. The ADME profiling, virtual toxicity and Lipinski’s parameters of compounds were found to be as effective as the reference standards. The molecular docking results were in good agreement with the outcomes of biological activity. Therefore, taking into account the results presented herein, it can
be inferred that these novel MTB InhA inhibitors are promising candidates for the effective treatment of TB.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

ACKNOWLEDGEMENTS
The authors would like to express their gratitude to Dr. K. G. Bhat, Maratha Mandal's Dental College, Hospital and Research Centre, Belgaum, India, for permitting the screening of antitubercular activity of synthesized compounds. Our sincere acknowledgments to Principal, Government College of Pharmacy, Karad, and NMIMS, School of Pharmacy, Shirpur for providing experimental support for the work and permission to use the software packages.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. Fukunaga R, Philippe G, Jennifer BH, Date A, Katherine F, Tereza K. Epidemiology of Tuberculosis and Progress Toward Meeting Global Targets- Worldwide, 2019. MMWR Morb Mortal Wkly Rep. 2021;70(12):427-430.
2. Bloom BR, Murray CJ. Tuberculosis: commentary on a reemerging killer. Science. 1992;257:1055-1064.
3. Odingo J, Malley TO, Kesicki EA, Alling T, Bailey MA, Early J, Ollinger J, Dalai S, Kumar N, et al. Synthesis and evaluation of the 2,4-diaminoquinazoline series as antitubercular agents. Bioorg Med Chem. 2014;22(24):6965-6979.
4. Bhilare NV, Dhaneshwar SS, Mahadik KR, Dasgupta A. Co-drug of isoniazid and sulphur containing antioxidant for attenuation of hepatotoxicity and treatment of tuberculosis. Drug Chem Toxicol. 2020;15:1-1.
5. Banerjee A, Dubnau E, Quemard A, Balasubramaniam V, et al. InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263(5144):227-230.
6. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature. 1992;358(6387):591-593.
7. Deshpande AN, Dhwale SC. Design, synthesis, characterization and antimicrobial evaluation of novel 2,4-disubstituted quinazoline derivatives. J Chem Pharm Res. 2017;9(2):74-84.
8. El-Azab AS, Kamal EH, Tahir E. Design and synthesis of novel 7-aminoquinazoline derivatives: Antitumor and anticonvulsant activities. Bioorg Med Chem Lett. 2012;22(5):1879-1885.
9. Khan I, Ibraa A, Naeem A, Saeed A. Recent advances in the structural library of functionalized quinazoline and quinazoline scaffolds: synthetic approaches and multifarious applications. Eur J Med Chem. 2014;76:193-244.
10. Bhilare NV, Dhaneshwar SS, Mahadik KR, Dasgupta A, Zende T, Kapoor S. Hepatoprotective Bile acid co-drug of isoniazid: Synthesis, Kinetics and Investigation of Antimycobacterial Potential. Pharm Chem J. 2020;54(7):678-88.
11. Patel AB, Kumari P, Chikhalia KH. Exploring antimicrobial and antitubercular potential of novel quinazoline based thiazolidin-4-ones. Ind J Chem Sec. B. 2015;54(2):260-271.
12. Pedgaonkar GS, Jonnalagadda PS, Jeankumar VU, Saxena S, et al. Development of 2-(4-oxoquinazolin-3(4H)-yl)acetamide derivatives as novel enoyl-acyl carrier protein reductase (InhA) inhibitors for the treatment of tuberculosis. Eur J Med Chem. 2014;86:613-627.
13. Asif M. Chemical characteristics, synthetic methods, and biological potential of quinazoline and quinazolinone derivatives. Int J Med Chem 2014;1-27:395637. Available: http://dx.doi.org/10.1155/2014/395637.
14. Ouyang G, Peiquan P, Gangfang Xu, Baoan S, et al. Synthesis and antifungal bioactivities of 3-alkylquinazolin-4-one derivatives. Molecules. 2006;11(6):383-392.
15. Mosaad SM, Mohsen M, Kamel B, et al. Novel 6, 8-dibromo-4 (3H) quinazolinone
derivatives of anti-bacterial and anti-fungal activities. Eur J Med Chem. 2010;45(8):3311-3319.
16. Kovalenko SI, Lyudmyla MA, Andriy KB, et al. Synthesis and anticancer activity of 2-(alkyl, alkaryl-aryl hetaryl-)-[1,2,4]triazolo[1,5-c]quinazolines. Scientia Pharmaceutica. 2013;81(2):359-391.
17. Desai NC, Dodiya A, Shihory N. Synthesis and antimicrobial activity of novel quinazolinone-thiazolidine-quinoline compounds. J Saudi Chem Soc. 2013;17(3):259-267.
18. Patel NB, Patel JC. Synthesis and Antimicrobial Activity of 3-(1,3,4-Oxadiazol-2-yl)quinazolin-4(3H)-ones. Scientia Pharmaceutica. 2010;78(2):171-193.
19. Deshpande AN, Dhawale SC, Bari SB, Bonde CG. Synthesis, Molecular docking and SAR study of isoniazid incorporated 2-sulfanyquinazolines as novel inhibitors of protein kinase B. Int J Adv Sci Tech. 2019;28(03):300-319.
20. Antypenko OM, Lyudmyla MA, Sergiy IK, Andrey MK, Olena MA. Potential of N-aryl (benzyl, heteryl)-2-(tetrazolo [1,5-c]quinolin-5-ylthio) acetamides as anticancer and antimicrobial agents. Arabian J Chem 2016;9(6):792-805.
21. Kolavi G, Hegde V, Khazi IA, Gadad P. Synthesis and evaluation of antitubercular activity of imidazo[2,1-b][1,3,4]thiadiazole derivatives. Bioorg Med Chem 2006;14(9):3069-3080.
22. Yang Hu, Cui-Yun Li, Xiao-Ming W, Yong-Hua Y, Hai-Liang Z. 1,3,4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry. Chem Rev. 2014;114(10):5572-610.
23. Jatav V, Mishra P, Kashaw S, Stables JP. CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-steryl quinazoline-4(3H)-ones. Eur J Med Chem. 2008;43(9):1945-1951.
24. Sheng-Li C, Yu-Ping F, Yu-Yang J, Shi-Ying L, Guo-Yu D, Run-Tao Li. Synthesis and in vitro antitumor activity of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains. Bioorg Med Chem Lett. 2005;15(7):1915-1917.
25. Dixit R, Mehta H, Dixit B. Synthesis and biological evaluation of novel phenanthridinyl piperazine triazoles via click chemistry as anti-proliferative agents. Med Chem Res. 2015;24(2):773-786.
26. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev. 2001;46(1-3):3-26.
27. Tripathi S, Selvaraj C, Singh S, Reddy KK. Molecular docking, QPLD and ADME prediction studies on HIV-I Integrase leads. Med Chem Res. 2012;21:4239-4252.
28. Nalini CN, Deepthi SR, Ramalakshmi N, Uma G. Toxicity risk assessment of isatins. Rasayan J Chem. 2011;4(4):829-833.
29. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, et al. Glide: A new approach for rapid, accurate docking and scoring 1. Method and Assessment of docking Accuracy. J Med Chem. 2004;47(7):1739-1749.
30. Jorgensen WL, Maxwell DS, Tirado-Rives J. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am Chem Soc. 1996;118(45):11225-36.
31. Friesner RA, Banks JL, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, et al. Extra precision Glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem. 2006;49(21):6177-96.
32. Weiwel N, Ju Zhu, Canhui Z, Xuefei Liu, et al. Fragment-based design of novel quinazolinone derivatives as human acrosin inhibitors. Chem Biol Drug Des. 2013;81(4):437-441.
33. Shashidhar K, Kallanagouda A, Hailiang Z, Fakkirappa M. Synthesis, characterization and molecular docking studies of novel S-substituted phenacyl-1,3,4-thiadiazole-thiol derivatives as antimicrobial agents. Eur J Med Chem. 2012;3(3):293-297.
34. Kunes J, Bazant J, Pour M, Waissker S, Slosarek M, Janota J. Synthesis of quinazoline derivatives with antitubercular activity. IL Farmaco. 2000;55:725-729.
35. Algarsamy V, Agendr V, Poongavanam V, Rajapann R. Synthesis, analgesic and anti-inflammatory activities of some novel 2,3-disubstituted quinazolin-4(3H)-ones. Bio Pharm Bull. 2003;26(4):557-559.
36. Kumar D, Kumar NM, Chang KH, Shah K. Synthesis and anticancer activity of 5-(3-
indolyl)-1,3,4-thiadiazoles. Eur J Med Chem. 2010;45:4664-4668.

37. Joshi SD, Vagdevi HM, Vaidya VP, Gadaginamath GS. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular agents. Eur J Med Chem 2008;43(9):1989-1996.

38. Stefania-Felicia B, Gabriela LA, Loanna S, Constantin D, Ana IT, Gabriela B. Synthesis and antimicrobial evaluation of some fused heterocyclic[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives. Eur J Med Chem. 2012;44:752-757.

39. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, et al. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate alamar blue assay. J Clin Microbiol 1998;36(2):362-366.

40. Lourenco MCS, Ferreira ML et al. Synthesis and anti-mycobacterial activity of (E)-N′-(monosubstituted-benzylidene) is on icotinohydrazide derivatives. Eur J Med Chem. 2007;43(6):1344-1347.

41. Roberto SR, Ivan N Jr, Lourenco SLS, et al. Comparison of flow cytometric and Alamar blue tests with the proportional method for testing susceptibility of Mycobacterium tuberculosis to rifampicin and isoniazid. J Clin Microbiol 2004;42(5):2247-2248.

42. Lourenco MCS, De Souza MVN, Pinheiro AC, Ferreira ML, et al. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. Arkivoc. 2007:xv:181-191.

43. Al-Deeb OA, Ahmed MA. Synthesis of some new 3H-quinazolin-4-one derivatives as potential antitubercular agents. World Applied Sciences Journal. 2008;5(1):94-99.

44. Florento L, Matias R, Tauno E, Santiago K, Frederick DC, Tuazon A. Comparison of cytotoxic activity of anticancer drugs against various human tumor cell lines using in-vitro cell-based approach. Int J Biomed Sci. 2012;8(1):76-80.

45. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Method 1989;119(2):203-210.

46. Maurya HK, Verma R, Alam S, Pandey S, Pathak V, Sharma S, Srivastava KK, et al. Studies on substituted benzo[H]quinazolines, benzo[g]indazoles, pyrazoles, 2,6-diarylp yridines as anti-tubercular agents. Bioorg Med Chem Lett. 2013;23(21):5844-5849.

47. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.

48. Fernando RP, Pedro I da SM, Sergio RAL, Victor MD, et al. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti-mycobacterium tuberculosis activity and cytotoxicity. Eur J Med Chem. 2010;45(5):1898-1905.

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