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Design, synthesis and docking studies of some novel (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol derivatives as antitubercular agents

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PII: S0223-5234(14)00538-8

DOI: 10.1016/j.ejmech.2014.06.019

Reference: EJMECH 7062

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 24 February 2014

Revised Date: 5 June 2014

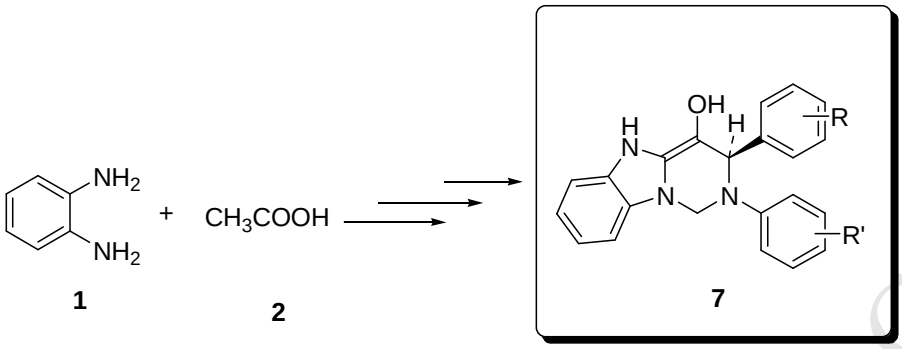
Accepted Date: 10 June 2014

Please cite this article as: K.P. Barot, S.V. Jain, N. Gupta, L. Kremer, S. Singh, V.B. Takale, K. Joshi, M.D. Ghate, Design, synthesis and docking studies of some novel (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol derivatives as antitubercular agents, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.06.019.

Highlights

- Novel imidazo [1,2-*c*] pyrimidin-4-ol derivatives are designed.
- Molecular docking study was carried out.
- Synthetic methodologies leading to targeted molecules have been reported.
- X-ray crystallographic study (ORTEP) of compound **7g** was carried out.
- Compounds are subjected to screening for antitubercular activity and cytotoxicity assay.

Graphical Abstract



1 **2** **7**

where

R = 4-NO_2, 3-NO_2, 4-Cl, 2-Cl, 4-OH, 2-OCH_3, 3-OH, 3-Br, 4-OH, etc.

R' = 4-Cl, 4-NO_2, 4-Br, 4-OCH_3, 2-OCH_3, 4-COOH, 4-OH, 2-OH, 4-NO_2, etc.

Design, synthesis and docking studies of some novel (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol derivatives as antitubercular agents

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Abstract: Filamenting temperature-sensitive mutant (FtsZ) is a novel target for the treatment of tuberculosis. A series of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5] imidazo[1,2-*c*]pyrimidin-4-ol derivatives were designed and docked on the FtsZ protein crystal structure (PDB Id: 1RLU, resolution 2.08 Å). Compound **7t** showed the highest docking score and H-bond interaction with Arg140 and Gly19. Our strategy for synthesis of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol derivatives from *o*-phenylenediamine as illustrated in scheme. All the synthesized compounds were characterized by FTIR, Mass spectra, ^{1}H NMR, ^{13}C NMR, elemental analysis and purity was confirmed by HPLC and LCMS. Compound **7g** was also confirmed by single crystal X-ray analysis. The *in silico* results are also validated with *in vitro* antitubercular activity of compound **7t**. Compound **7b** exhibited *in vitro* antitubercular activity 3.13 µg/mL and 4.7 µg/mL whereas compound **7t** exhibited *in vitro* antitubercular activity 6.25 µg/mL and 9.4 µg/mL using GAST/Fe medium after week 1 and week 2 respectively against *M. tuberculosis* H_{37}Rv. Medium 7H9/ADC/Tween was found to be very less effective for *in vitro* antitubercular activity of all the benzimidazole derivatives. Assays for *in vitro* cytotoxicity against VERO cells of all the synthesized compounds was found to be very less cytotoxic.

Keywords: Tuberculosis, Docking study, Benzimidazole derivatives, *In vitro* antitubercular activity, Cytotoxicity assay, Structure-activity relationship (SAR).

1. Introduction

Tuberculosis (TB) is a foremost communicable disease and we have not yet been able to eradicate the malady. It is primarily caused by *Mycobacterium tuberculosis* (*M. tb*) of the ‘‘Tuberculosis Complex’’ [1]. The chemical composition of *M. tb* cell wall includes peptidoglycans and a vast array of lipids, including mycolic acids, which are significant determinant of its virulence. The unique structure of the cell wall of *M. tb* allow it to lie dormant for many years as a latent infection, particularly as it can reside inside macrophages, hiding it from the host immune system [2]. TB is a contagious and deadly disease that has reached pandemic proportions in the world. According to the World Health Organization (WHO), 10-12 million new cases of TB are diagnosed each year and making *M. tb* a leading cause of death in adults (2-3 million/year) due to an infectious agent [3]. A high proportion of these new cases and deaths occur in HIV-positive people with a significant number of AIDS deaths in developing countries being attributed to TB infections. Global population growth is increasing the disease burden, posing a continuing health and financial burden in various parts of the world, particularly Asia and Africa [4].

The continuous rise in multi-drug resistant strains of *M. tb* (MDR-TB) has further contributed to the urgent need for new antitubercular drugs, as no new TB drugs have been introduced into clinical use in the past 4 decades. Drugs active against resistant forms of TB are less potent, more toxic and need to be taken for an extended period of time (≥18 months) [5]. Current emergences of virtually untreatable extensively-drug resistant TB (XDR-TB) posses a new threat to TB control world-wide. Recent findings by WHO from 2000 to 2004 suggested that 4% of MDR-TB cases meet the criteria for XDR-TB. Effective treatment of TB in persons co-infected with HIV is complicated because of drug-drug interactions [6]. Therefore, shorter and simpler regimens are safe and effective against drug-susceptible and drug-resistant TB, which are appropriate for joint HIV-TB treatment and amenable to routine clinical settings, are required. New chemotherapeutic agents to combat the emergence of the resistance and strategies which can effectively shorten the duration of chemotherapy are urgently needed [7].

Isoniazid (INH), a well-known antitubercular drug, is believed to kill mycobacteria by inhibiting the biosynthesis of mycolic acids and critical components of the mycobacterial cell wall. The catalase and peroxidase activities are thought to participate in the drug sensitivity mechanism by converting INH *in vivo* into its biologically active form, which then acts on its intracellular target [8]. Substituted benzimidazole derivatives have been reported to possess antitubercular activity against *M. tb* H_{37}Rv. These compounds, after penetration of the mycobacterial cell wall, could be bio-transformed by esterases or peroxidase-catalases. They are more active than the unmodified

polar isosteres of isonicotinic acid, which may be due to better penetration of these agents into the cell wall of the mycobacteria [9,10].

In the last 40 years, few drugs have been approved by the Food and Drug Administration (FDA) to treat TB, reflecting the inherent difficulties in drug discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area [11]. In addition to the current drugs approved by the FDA for the treatment of TB and to the drugs that commonly are recommended for the treatment of TB but are not FDA approved, many compounds are under investigation as potential antitubercular drugs [12]. Among them, benzimidazole derivatives were first reported in 1998 as antimycobacterial agents. The most potent derivative showed minimum inhibitory concentration (MIC) values ranging from 0.7 to 1.5 µg/mL against *M. tb*. Activity toward drug-resistant strains of *M. tb* was similar to that found toward sensitive strains. Finally, it was also active toward non-tuberculosis mycobacteria with MICs higher that against *M. tb* [13].

Filamenting temperature-sensitive mutant Z (FtsZ), a tubulin homologue, is a highly conserved and ubiquitous bacterial cell division protein. Similar to the process of microtubule formation by tubulin, FtsZ polymerizes in a GTP-dependent manner, forming a highly dynamic cytokinetic structure, designated as the Z-ring, at the mid-point of the cell. The recruitment of the other cell division proteins leads to Z-ring contraction and results in septum formation [14]. Because of the requirement of FtsZ in mycobacterial cytokinesis, inhibition of FtsZ is a promising target for antitubercular drug discovery. While tubulin and FtsZ share structural and functional homology and because tubulin inhibitors are known to affect FtsZ assembly, the limited sequence homology at the protein level affords an opportunity to discover FtsZ-specific compounds with limited cytotoxicity to eukaryotic cells. Since FtsZ is a proposed novel drug target, compounds targeting FtsZ are expected to be active against drug resistant *M. tb* strains. Furthermore, the validation of FtsZ as a novel antitubercular drug target has been confirmed by the work of various groups [15].

Southern Research Institute researchers have screened known tubulin inhibitors against *M. tb* and identified several benzimidazole, pyridopyrazine and pteridine based FtsZ inhibitors with potent antituberular activity [16,17]. They have concluded that substituted benzimidazole derivatives interfered and delayed the *M. tb* cell division processes. Benzimidazole derivatives are also reported as potent antitubercular agents. They are polar and ionisable aromatic compounds and used as a remedy to optimize solubility and bio-availability parameters of proposed molecules. Clubbed-[1,2,3] triazole by fluorine benzimidazoles are also very potent antitubercular agents

against *M. tb* H_{37}Rv. It has broadened the scope in remedying various dispositions in clinical medicines and play vital role in the field of medicinal chemistry which is an important pharmacophore and exhibits potent *in vivo* and *in vitro* antitubercular activities [18,19]. Extensive research works are also reported on benzimidazole but relatively very little is known so far about substituted benzimidazoles. Ordinary organic synthesis has been widely employed for the synthesis of diverse heterocycles. In bacteria, the main cytoskeletal element involved in cell division is the Z-ring, a membrane associated structure consisting of polymers of the essential tubulin-related protein FtsZ. The Z-ring then reduces in diameter during cell division, drawing the cytoplasmic membrane inwards. A number of accessory proteins that are essential for cell division require being located to the cell centre during cell division. The localization of these proteins is achieved through recruitment to Z-ring by either direct or indirect interaction with FtsZ [20,21]. Threrefore, docking studies were performed on FtsZ target to understand the basis of the mechanism of antitubercular activity of the benzimidazole derivatives synthesized in this study. The binding domain identified to accommodate GTP (Guanosine diphosphate monothiophosphate) appears large enough to hold the benzimidazole derivatives synthesized in this study.

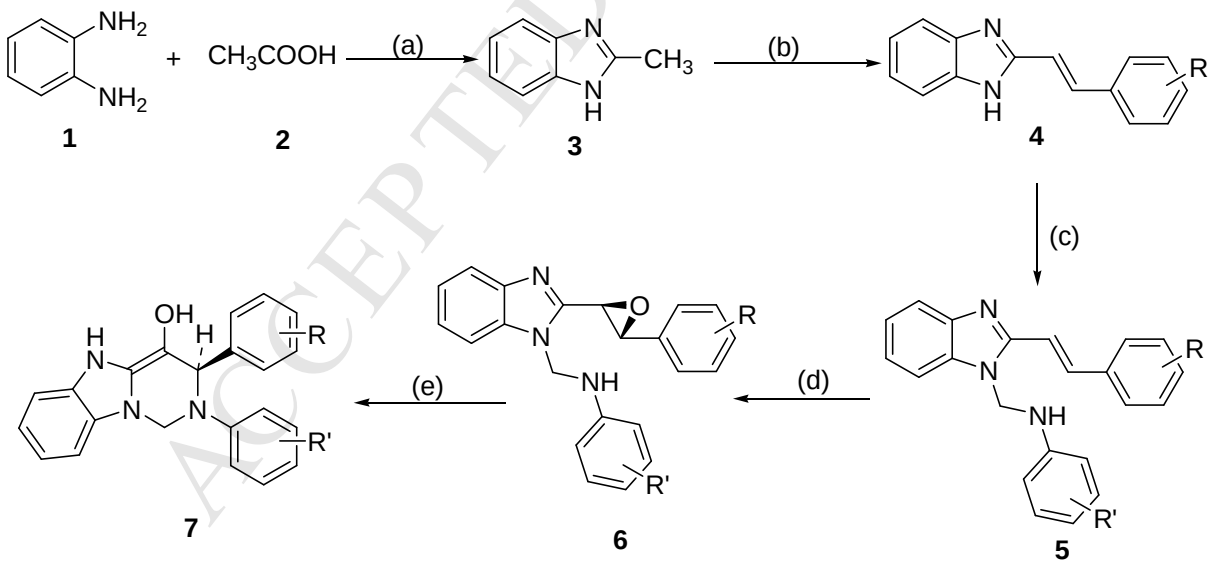
Taking into account the structural similarity of the pyridopyrazine moiety, pteridine moiety, albendazole, and thiabendazole, they envisioned that the benzimidazole scaffold would be a good starting point for the development of novel FtsZ inhibitors, which have activity against both drug-sensitive and drug-resistant *M. tb*. Therefore, substituted benzimidazoles were investigated for potent antitubercular activity [17]. Specifically, we report the activity of 1,2,3,4-tetrahydrobenzo [4,5] imidazo [1,2-*c*]pyrimidin-4-ol derivatives against *M. tb* H_{37}Rv. Importantly, it substantiates disubstituted benzimidazoles for the development of next generation mycobacterial inhibitors with activity against difficult to treat clinical strains.

2. Results and discussion

2.1. Chemistry

Since benzimidazoles are well known for their *in vivo* and *in vitro* antitubercular activities and their mode of action has been characterized [22]. Our work has focused on the synthesis of novel molecules based on the benzimidazole skeleton and the synthesized compounds were tested for their *in vitro* antitubercular activity. The synthetic pathways are illustrated in scheme. Molecular modeling of their binding to ENR was also performed to study whether this target was the ideal site to exhibit their mechanism of antitubercular activity or not. Our strategy for synthesis of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol **7a**

derivatives of benzimidazole is very simple as illustrated in scheme. As per prescribed procedure, compound **3** was synthesized by the condensation of *o*-phenylenediamine with acetic acid and water by refluxing at 80-82^{o}C for 4-4.5 h to obtain the intermediate derivative **3** in good yields [23]. Compounds **3** was refluxed with aromatic aldehyde derivatives at 100-110^{o}C for 1.0 h and then 160-170^{o}C for 30 min to obtain the corresponding alkene derivatives **4** [24]. Compound **4** was refluxed with aniline derivatives with formaldehyde and triethylamine in ethanol at 70-72^{o}C for 3.5-4.0 h to obtain corresponding aniline substituted benzimidazole derivative **5** [25]. Epoxide was formed by the condensation of compound **5** with sodium hypochlorite and (*R*,*R*)-Jacobsen's catalyst with dichloromethane as solvent at 50-52^{o}C for 2.5-3.0 h [26]. Cyclization of the compound **6** was take place by refluxing in aluminum trichloride with methanol as solvent at 75-77^{o}C to afford the target molecules **7a-7t** [27]. Synthesized compounds were purified by column chromatography and structures of the compounds were confirmed by FTIR, Mass spectra, ^{1}H NMR, ^{13}C NMR and elemental analysis. Structure of compound **7g** was also confirmed by single crystal X-ray analysis. Purity of all the final synthesized compounds was confirmed by HPLC and purity of compound **7g** and **7j** was also confirmed by LCMS. A series of cyclic substituted benzimidazole derivatives were synthesized using *o*-, *m*- or *p*-substituted benzaldehydes and substituted aniline derivatives with different reagents to form the desired heterocyclic benzimidazole derivatives. Percentage yield of the intermediate and final compounds were in the range of 68-87% and 60-67% respectively.



Scheme. Synthesis of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5] imidazo[1,2-*c*]pyrimidin-4-ol derivatives **7a**−**7t**. Reagents and conditions: (a) H_2O, 80-82^{o}C, 4 h; (b) 100-110^{o}C for 1.0 h and then 160-170^{o}C for 30 min, Ar-CHO; (c) C_2H_5OH, HCHO, TEA, Ar-NH_2, 70-72^{o}C, 3.5-4.0 h; (d) NaOCl, DCM, (*R*,*R*)-Jacobsen's catalyst, 50-52^{o}C, 2.5-3.0 h; (e) $AlCl_3$, CH_3OH, 75-77^{o}C.

The IR spectrum of compound **7a** illustrates broad stretching band around 2930.31 cm^{-1} and 3598.52 cm^{-1} due to aromatic CH and OH respectively and strong stretching band at 1090.55 cm^{-1} accounting for C-N stretch. Strong stretching band of NO_2 and Cl was observed at 1381.75 cm^{-1} and 746.31cm^{-1} respectively. ^{1}H NMR of compound **7a** shows sharp singlets at δ 2.1 accountable for OH and which was absent on D_2O exchange. The two protons of benzimidazole ring was appeared as doublet at δ 7.49-7.51 with J value 8.6 Hz and other doublet at δ 7.31-7.31 with J value 2.0 Hz for four aromatic hydrogen. Proton of CH_2 and CH was appeared as doublet at δ 4.48 and 3.90 respectively. ^{13}C NMR shows a peak at δ 145.54 because of Ar-C of benzimidazole ring system. Peak of CH and CH_2 was appeared at δ 76.74 and 49.29 respectively. Further, molecular ion peak (M^+, 96%), (M+2, 30%) and (M-1, 94%) were found at m/z 420.9, 422.8 and 419.0 respectively which confirm its molecular fragmentation patterns. Elemental analysis of C, H and N were within ±0.4% of the predicted values. HPLC purity at retention time 9.747 min was found as 98.39% where 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade ACN selected as mobile phase.

Broad stretching band around 2932.23 cm^{-1} and 3611.05 cm^{-1} due to aromatic CH and OH respectively were found of the IR spectrum of compound **7b**. Strong stretching band of NO_2 was observed at 1383.68 cm^{-1}. ^{1}H NMR of compound **7b** shows sharp singlets at δ 2.1 accountable for OH and which was absent on D_2O exchange. The two protons of benzimidazole ring was appeared as singlet at δ 7.87 and multiplet at δ 7.06-7.11 with four aromatic hydrogen. Proton of CH_2 and CH was appeared as doublet at δ 4.68 and 3.49 respectively. ^{13}C NMR shows a peak at δ 175.32 due to Ar-C of aromatic ring system. Peak of CH and CH_2 was appeared at δ 76.74 and δ 50.53 respectively. Further, molecular ion peak (M+1, 96%) and (M-1, 97%) were found at m/z 432.1 and 430.0 respectively confirms its molecular fragments. Elemental analysis of C, H and N are within ±0.4% of the predicted values.

Strong stretching band of NH was observed at 3492.45 cm^{-1} and broad stretching band around 3076.87, 2948.63 cm^{-1} and 3775.94 cm^{-1} due to aromatic CH and OH respectively was found of the IR spectrum of compound **7g**. ^{1}H NMR of compound **7g** shows sharp singlets at δ 2.02 accountable for OH and which was absent on D_2O exchange. Two protons of aromatic ring were appeared as doublet at δ 7.66 and multiplet at δ 6.68-6.75 with two aromatic hydrogens. Proton of CH_2 and CH was appeared as doublet at δ 5.68 and 4.52 respectively. ^{13}C NMR of the compound **7g** confirms resonance of CH_3 and C-N at δ 21.7 and δ 170.04 respectively. It shows a peak at δ 148.47 because of Ar-C of aromatic ring system. Peak of CH and CH_2 was appeared at δ 77.37 and 62.54 respectively. Further, molecular ion peak (M+1, 98%) and (M-1, 97%) was found at m/z 412.8 and

411.0 respectively which confirms its molecular fragmentation. Elemental analysis of C, H and N are within ±0.4% of the predicted values. HPLC purity at retention time 5.598 min was found as 97.67% where 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade ACN selected as mobile phase. LCMS purity was found as 100% at retention time 3.015 min which was acquired by UPLC-02 using the method of Formic_Normal at 230.0 nm.

The IR spectrum of compound **7j** illustrates strong stretching band of NH at 3329.50 cm^{-1} and broad stretching band around 2781.81, 2978.52 cm^{-1} and 3550.31 cm^{-1} due to aromatic CH and OH respectively. ^{1}H NMR of compound **7j** shows sharp singlets at δ 1.87 and δ 3.91 accountable for OH and disappeared on D_2O exchange. The two protons of aromatic ring was appeared as multiplet at δ 7.47 and singlet at δ 7.51 for one aromatic hydrogen. Proton of CH_2 and CH was appeared as doublet at δ 6.86, 5.12 and δ 3.93 respectively. ^{13}C NMR shows a peak at δ 156.62 because of Ar-C of aromatic ring system. Peak of CH and CH_2 was appeared at δ 108.51 and 76.29 respectively. Further, molecular ion peak (M^+, 97%), (M+2, 30%), (M+4, 10%) and (M-1, 74%) were found at m/z 426.8, 428.5, 430.7 and 425.0 respectively confirms its fragments. Elemental analysis of C, H and N were within ±0.4% of the predicted values. HPLC purity at retention time 4.216 min found as 98.25% where 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade ACN selected as mobile phase. LCMS purity was found as 99.5% at retention time 1.903 min which was acquired by UPLC-01 using the method of Formic_Normal at 309.0 nm. Single isomer identification of compound **7g** was also confirmed by chiral HPLC methods of analysis.

2.2. Analogue design

Our impetus to investigate the benzimidazole subclasses, exemplified by analogues **7a**-**7t**, was driven by its ease of synthesis and the likelihood that compounds with lowered or absent enzymatic induction effects would be generated. This latter expectation was based on the known diminution of FtsZ induction with the progression from isoniazid. Within this same order of structural subclasses, there is also a trend of isoniazid possessing increased potency against drug susceptible isolates of slow-growing mycobacteria and better *in vivo* efficacy in mice. Our analysis of the recently published structures has revealed an approach for the chemical elaboration of the benzimidazole core in a novel way that should lead to enhanced binding affinity to both wild-type and resistant strains [28].

2.3. Molecular docking study

The X-ray crystal co-ordinates of *M. tb* FtsZ in complex with GTP- gamma-S at a resolution of 2.08 Å were obtained from the Protein Data Bank under the accession code 1RLU.pdb. *M. tb*

FtsZ crystallized as a tight, laterally oriented dimer distinct from the longitudinal polymer observed for αβ-tubulin. FtsZ and tubulin are known to pass through cycles of polymerization and depolymerisation, but the structural mechanisms underlying this cycle remain to be determined. This protein play an essential role in bacterial cell division and interruption of this process is a bactericidal event [29]. Ramachandran plot of the backbone torsion angles PHI and PSI for local geometry and the location of buried polar residues or exposed non-polar residues were examined. Energy refinement procedure was performed using these structures. Kollman charges were used for the protein while Gasteigere Huckel charges were calculated for the ligand. Then, this model was subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Kollman united force field with non bonding cut off set at 109.0 and the dielectric constant set at 4.5. While FtsZ retains only limited sequence similarity to tubulin, the three-dimensional X-ray structure shows a high degree of similarity to the structure of α and β tubulin. 3-D structure of GTP was obtained from its X-ray crystal co-ordinate which was found in 1RLU.pdb file [30].

The binding site of the benzimidazole derivatives was estimated using a variety of scoring functions that have been compiled into the single consensus score (Total Score). The Surflex-Dock scoring function (originally used within Hammerhead9) was tuned to predict the binding affinities of 34 protein-ligand complexes, with its output being represented in units of -log(*K*d). Prior to start of the docking operation, essential hydrogens and Gasteiger charges were added to the macromolecule [31]. Molecular docking of inhibitors into the active site of protein identifies the binding orientations and the protein–inhibitor interactions responsible for the observed activity. The benzimidazole derivatives were docked to FtsZ using Surflex Dock in Sybyl X1.2 [32]. Molecular docking is used to determine the binding orientation of inhibitors to their protein targets to predict their affinity and activity when bound to the protein. Surflex is a fully automatic flexible molecular docking algorithm that combines the scoring function from the Hammerhead docking system with a search engine that relies on a surface based molecular similarity method as a mean to rapidly generate suitable putative poses for molecular fragments [33]. The dominant terms are the hydrophobic contact term and a polar contact term that has a directional component and is scaled by formal charges on the protein and ligand atoms. These functional terms are characterized on the basis of distances between Vander Waals surfaces, with negative values indicating interpenetration [34]. The docking study reveals compound **7t** as highest active compound having docking score 7.1272.

Twenty poses were obtained from all compounds. Out of them all, second pose of compound **7t** obtains highest occupancy and highest docking score of 7.1272 (Table **A** of supplementary file). Compound **7t** shows various hydrogen bonding and hydrophobic interaction with the aminoacids. Hydrogen bonding of compound **7t** with Arg140, Gly19, Gly101 and Glu102 takes within the distance of less than 2.5 Å take place (Fig. **1**). The terminal -NH of the Gly19 make a hydrogen bond with oxygen of the hydroxyl group of the linker heterocyclic ring of the **7t** (N-H---O-H, 2.48 Å). Another hydrogen bonding is observed between hydrogen of the same hydroxyl group and carboxyl oxygen of the amide linkage of the Gly101 (C-O---H-O, 2.05 Å). Two more hydrogen bonding is observed with the hydroxyl group of the terminal aryl substitution of compound **7t**. Carboxyl oxygen of the Glu102 make bonding with the hydrogen of the hydroxyl group (C-O---H-O, 2.14 Å) and oxygen of the same hydroxyl group make another hydrogen bonding with guanidino amine hydrogen of Arg140 (N-H---OH, 2.20 Å). The occupancy of the molecule in the binding pocket of the enzyme is the other reason of the potent activity. Compound **7t** occupies the whole cavity in the receptor site for the inhibition of the FtsZ (Fig. **2a**). The terminal aryl substitution is oriented near positively charged Arg139 and Arg140, because the negatively charged benzene ring cloud is attracted towards the positively charged amino acid residues (Fig. **2b**). Most of the amino acids are oriented in the same manner as per the GTP interaction with the receptor. In the receptor, the active site is in the V-shaped cavity and the most active compounds perfectly fit into the pocket, hence validate the *in silico* results (Fig. **3**). The proposed docking hypothesis is proposed to become the reason of inhibitory action on *Mtb* H_{37}Rv strain.

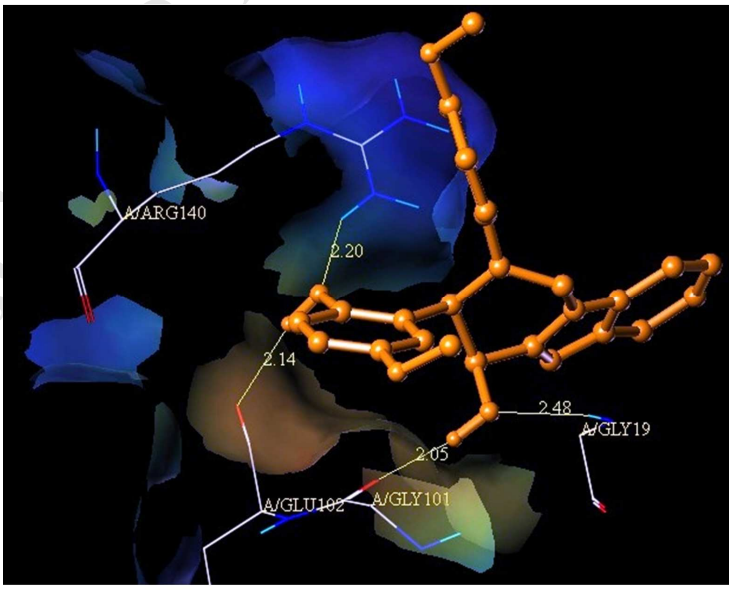


Fig. 1. Hydrogen bond interaction of compound **7t** and compound **7t** is in the orange coloured ball and stick model.

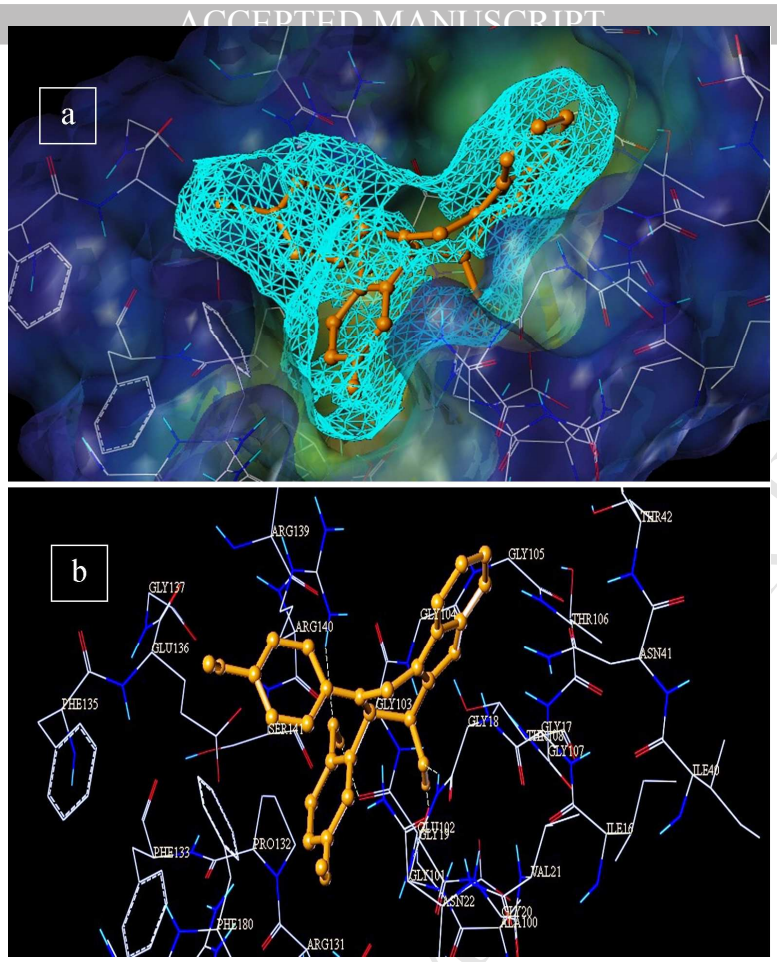


Fig. 2. (a) The docking pose of compound **7t** in the receptor site and cyan coloured area indicated the excluded volume of the compound **7t**. (b) Interacting amino acids of binding site and compound **7t** in the field as orange coloured ball and stick model.

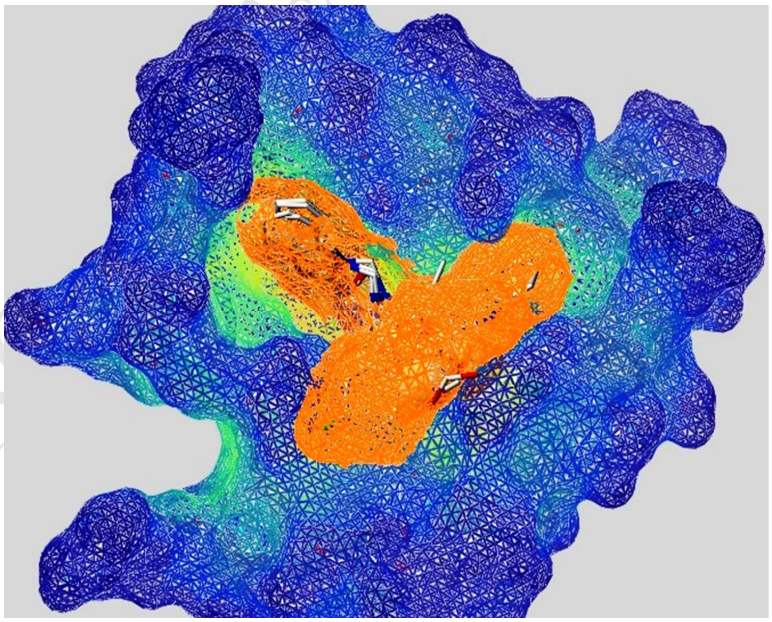


Fig. 3. The occupancy of most potent compounds in the binding pocket and the orange colour cloud indicates the excluded volume of the compounds in the lines format. The colour of binding domain is as per cavity depth.

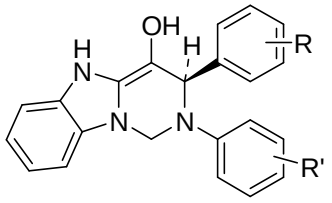
2.3. Pharmacology

2.3.1. In vitro antitubercular activity

Benzimidazoles exhibits various types of pharmacological activities including antimicrobial, antitubercular, anticancer etc [22,35]. All the final synthesized substituted benzimidazole derivatives **7a-7t** were evaluated for *in vitro* antitubercular activity against *M. tb* H_{37}Rv. MICs were determined in quadruplicate in iron-supplemented GAST or in Middlebrook 7H9 broth base supplemented with 0.2% glycerol, 0.4% glucose, 0.5% BSA fraction V, 0.08% NaCl and 0.05% Tween 80 in 96w plates according to the broth microdilution method using drugs from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. Briefly, two-fold serial dilutions of compounds in DMSO were added to 50 µL of medium in round-bottom 96-well plates (Nunc). An equal volume of *M. tb* H_{37}Rv ATCC27294 diluted to 10,000 cells/mL in the respective medium was added to each well. Positive controls included isoniazid. Plates were incubated for 1 and 2 weeks at 37 °C after which growth was determined using an inverted enlarging mirror. The MIC was determined as the lowest concentration of compound that completely inhibited growth of the bacterium. The MIC for isoniazid was 0.02 µg/mL [36]. The result of MICs of all the final targeted synthesized compounds and standard drug are reported in Table **1.** Thus, all the final synthesized substituted benzimidazole derivatives were evaluated for *in vitro* antitubercular activity against *M. tb* H_{37}Rv. Compounds **7b, 7j** and **7t** are active against H_{37}Rv strain of *M. tb*. MIC value of compound **7b**, **7j** and **7t** was found to be 3.13 µg/mL, 6.25 µg/mL and 6.25 µg/mL respectively.

Table 1

In vitro antitubercular activity of benzimidazole derivatives against *Mycobacterium tuberculosis* H_{37}RV.



Compd.	R	R'	1-week MIC[a] in µg/mL (GAST/Fe)	2-week MIC[a] in µg/mL (GAST/Fe)	1-week MIC[a] in µg/mL (7H9/ADC/Tween)	2-week MIC[a] in µg/mL (7H9/ADC/Tween)
7a	4-NO_2	4-Cl	100	>100	>100	>100
7b	3-NO_2	4-NO_2	3.13	4.7	25	37
7c	4-Cl	3,5-Cl	37	50	100	>=100

7d	2-Cl	3,5-Cl	>100	>100	>=100	>100
7e	3,4-OCH_3	3,5-Cl	100	>100	>100	>100
7f	4-OH	4-Br	75	75	>=100	>100
7g	2-NH_2, 3,4-CH_3	2,6-CH_3	37	75	100	>100
7h	2-OCH_3	3,5-Cl	50	100	100	100
7i	2-OCH_3	2,6-CH_3	100	100	50	>100
7j	3-OH	2,6-Cl	6.25	9.4	37	50
7k	2-NO_2	4-NO_2	37	37	37	50
7l	3-Br	4-OCH_3	>100	>100	>100	>100
7p	Pyridine	4-OCH_3	12.5	19	>=100	>100
7q	4-OCH_3	4-OCH_3	50	75	100	>=100
7r	2-OH	2-OCH_3	19	25	50	75
7s	2-OH, 5-OCH_3	4-COOH	>100	>100	>100	>100
7t	4-OH	2,6-CH_3	6.25	9.4	12.5	19
Isoniazid			0.02	0.02	0.02	0.02

[a]Minimum Inhibitory Concentration (MICs) against *Mycobacterium tuberculosis* $H_{37}Rv$

2.3.2. Assays for in vitro cytotoxicity against VERO cells

In vitro cytotoxicity of the active compounds was measured using Vero cells grown in DMEM medium containing 10% fetal bovine serum. The cell suspensions were plated in 96-well tissue culture plates (20,000 cells/well) and incubated overnight at 37 °C in the presence of 5% CO_2 to allow cells to adhere. The medium was removed and replaced by 180 µl of fresh medium containing the test compounds at two different concentrations (25 µg/ml and 50 µg/ml). Cells were then incubated for a further 48 h. 20 µl of Cell-Titer Blue Reagent (Promega) were then added to each well and incubated for a further 2 h at 37 °C with 5% CO_2. Optical density was recorded at 560 nm using a microplate reader. Percentage of cell survival was calculated considering the control wells (cells incubated in DMSO-containing medium). Table **2** shows that most compounds are non toxic for the Vero cells, as a compound is considered toxic if it causes over 50% inhibition at concentration 10 fold higher than its MIC. The most active compounds **7j** and **7t** only showed moderate cytotoxicity (% Survival = Optical density of the test compound/Optical density of the control *100).

Table 2

In vitro cytotoxicity assay against VERO cells.

Compound	% Survival of Vero cells (50 µg/ml)	% Survival of Vero cells (25 µg/ml)
7a	ND	ND
7b	69	66
7c	69	63
7d	ND	ND
7e	ND	ND
7f	ND	ND
7g	73	64
7h	ND	ND
7i	ND	ND
7j	61	63
7k	61	53
7l	ND	ND
7p	56	66
7q	ND	ND
7r	69	63
7s	ND	ND
7t	63	66
Isoniazid	100	100

ND, not determined

2.4. Crystallography

Suitable needle shape crystals of compound **7g** was formed by evaporation of solution of compound in dichloromethane and the crystal structure was measured by "Single crystal X-ray analysis". Bruker Smart Apex CCD area detector was used for the collection of diffraction data using a graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 20 ^{o}C. The structure of compound was solved using the program SHELXL and Fourier difference techniques and was refined by full-matrix least-squares method on F^2. All hydrogen atoms were added theoretically and crystal structure of compound **7g** is shown in Fig. **4**.

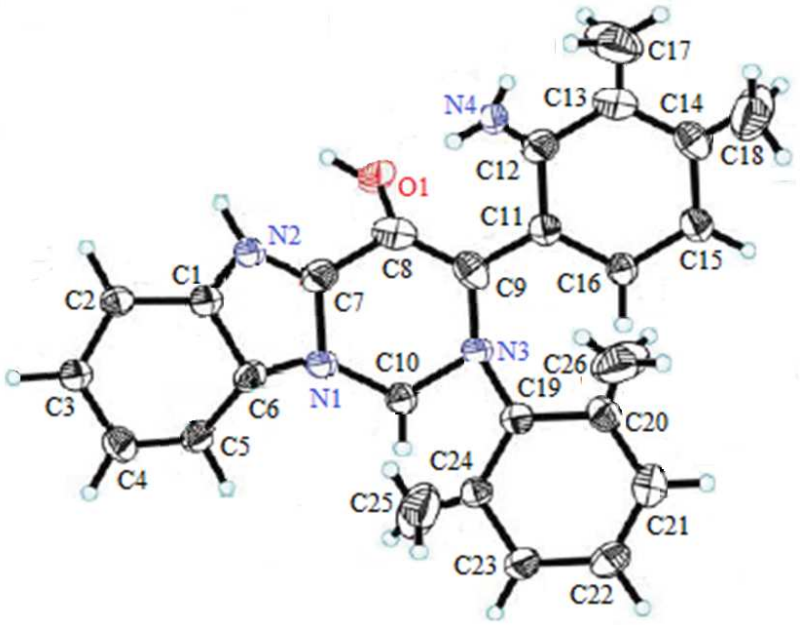


Fig. 4. X-ray crystal structure (ORTEP) of compound **7g**.

2.5. Structure-activity relationship (SAR) study

The structure-activity relationship study demonstrated that (*R*)-2-(4'-nitrophenyl)-3-(3'-nitrophenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-*c*]pyrimidin-4-ol **7b**, (*R*)-2-(2',6'-dicholrophenyl)-3-(3'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5]imidazo[1,2-*c*]pyrimidin-4-ol **7j** and (*R*)-2-(2',6'-dimethylphenyl)-3-(4'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-4-ol **7t** were exhibited good *in vitro* antitubercular activity against *M. tb* H_{37}Rv. But, 3-nitrophenyl and 4-nitrophenyl containing compound **7b** is cytotoxic up to certain level due to the presence of nitro group. Therefore, compound **7b** is not favorable for therapeutic potency against *M. tb* H_{37}Rv. The cyclic substituted benzimidazole ring is more favorable for the potent antitubercular activity. Optimum *in vitro* antitubercular activity was found by these compounds may be due to formation of free substituted benzimidazole-NAD^+ complex. It may be responsible for the inhibition of *Mycobacterium* cell wall bio-synthesis. 3-Hydroxyphenyl; 2,6-dichlorophenyl; 4-hydroxyphenyl and 2,6-dimethylphenyl are more favorable for the formation of substituted benzimidazole-NAD^+ complex. Benzimidazole containing 3,4-dimethylaminophenyl and 2,6-dimethylphenyl or 3-hydroxyphenyl and 3,4-dichlorophenyl are highly favorable moieties for potent *in vitro* antitubercular activity against *M. tb* H_{37}Rv.

3. Conclusion

The present study describes the docking, synthesis, structure ellucidations, *in vitro* antitubercular activity and cytotoxicity assay against VERO cells of benzimidazole derivatives. We have synthesized and evaluated a series of cyclic substituted benzimidazole derivatives in good

yields. All the synthesized compounds were evaluated for *in vitro* antitubercular activity against *M. tb* $H_{37}Rv$. Compound **7b**, **7j** and **7t** showed good *in vitro* antitubercular activity and other compounds showed moderate to significant *in vitro* antitubercular activity against *M. tb* $H_{37}Rv$. All the synthesized compounds were found to be very less cytotoxic using *in vitro* cytotoxicity assay against VERO cells. The potency, selectivity and low cytotoxicity of these compounds make them valid leads for improved antitubercular developments.

4. Experimental protocols

4.1. Technical details

All the reagents were purchased from Sigma-Aldrich Chemicals and were used without further purification. All solvents were distilled and dried using dry sieves as the usual manner. TLC analysis was carried out on aluminum foil pre-coated with silica gel 60 F254 (Sigma-Aldrich, Bangalore dealer). Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. FTIR spectra were determined as KBr solid disc method on a Shimadzu model 470 spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded using Bruker Avance II 400 MHz and 100 MHz spectrometer using DMSO as NMR solvent and are reported in ppm down field from the residual DMSO. Elemental analysis was performed on a Elementar Vario Micro Cube analyzer and results were within ±0.4% of the predicted values for all compounds. FTIR (KBr) spectrum of all the synthesized compounds has strong O-H absorptions at about 3500-3700 cm^{-1}. The medium strong C=C band in IR spectra of all the compounds appeared at 1450-1575 cm^{-1}, which is similar as that of the ordinary C=C absorption (1400-1600 cm^{-1}). The formation of H-bond leads to an increase of their polarity, so the strength of their double bond decreased and absorption moved to lower wave number. The medium strong C-H band and C-N band in IR spectra of all the compounds appeared at 3000-3050 cm^{-1} and 1160-1350 cm^{-1} respectively which is similar as that of the ordinary C-H absorption (3000-3100 cm^{-1}) and C-N absorption (1080-1360 cm^{-1}). The ^{1}H NMR spectrum exhibited different signals at different ppm which were assigned to the different types of protons. ^{13}C NMR showed various δ values at different ppm for all the target synthesized compounds. Compound **7g** is also confirmed by single crystal X-ray analysis (ORTEP). Purity of the final compounds were determined by HPLC (HP-03 Offline) instrument using 0.1% TFA in HPLC Grade water and 0.1% TFA in HPLC Grade ACN. LCMS purity was acquired using UPLC-02 and UPLC-01 by Formic_Normal at 230.0 nm and 309.0 nm. Single isomer identification was observed by chiral SFC and chiral HPLC methods of analysis.

*4.1.1. General procedure for synthesis of 2-methyl-1H-benzo[d]imidazole (**3**)*

5.43g (0.03 mmol) *o*-Phenylenediamine **1** was mixed with 20 mL water and 5.4 g (0.09 mmol) acetic acid **2**. Resulting reaction mixture was allowed to reflux at 80-85 ^{c}C for 3.5-4.0 h. After completion of the reaction, it was cooled and basified by the gradual addition of concentrated ammonia solution. Precipitated crude product was filtered by vacuum filtration and recrystalized from 10% aqueous ethanol. Mobile phase: Tolune: Acetonitrile = 2 : 1. Yield 87.15%. mp 130-132 ^{o}C. IR (KBr) cm^{-1}: 1465.63 (C=C, Ar), 3496.31 (N-H), 1087.66 (C-N), 1457.36 (C=N), 2950.55 (-CH_3). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.52-7.58 (m, 2H), 7.22-7.27 (m, 2H), 5.0 (s, 1H), 2.51(s, 3H). ^{13}C NMR (DMSO, 100 MHz) δ: 153.25, 138.37, 124.51, 115.20, 18.16. Anal. Calcd for $C_8H_8N_2$: C, 72.70; H, 6.10; N, 21.20; Found: C, 71.16; H, 5.37; N, 20.78. MS (ESI, m/z): 133.2 (M+1, 98%), 131.0 (M-1, 63%).

*4.1.2. General procedure for the synthesis of (E)-2-(4-nitrostyryl)-1H-benzo[d]imidazole (**4**)*

2-Methyl-1*H*-benzo[*d*]imidazole **3** (0.05 mmol) was mixed with 4-nitro benzaldehyde (0.8 mmol). Resulting reaction mixture was allowed to reflux on silicon bath. When temperature reached at 100 °C, mixture melted, and at 110 °C, water elimination was observed by water drops appearing on the neck of flask. Reaction mixture became viscous and water elimination ceased after 1 h. Temperature increased to 160-170 °C and held for 0.5 h. After completion of the reaction, it was purified by dissolving in pyridine, precipitation with water and resolving by heating. Methanol treatment was done to remove any soluble impurities. Further purification was accomplished by column chromatography. Mobile phase: Ethyl acetate: Hexane = 6 : 4. Yield 81.20%. mp 151-153 ^{o}C. IR (KBr) cm^{-1}: 1602.56 (C=C, Ar), 3320.82 (N-H), 1307.50 (C=N), 1698.98 (CH=CH, alkene), 1446.35 (N-O, NO_2). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.21-8.24 (d, J = 7.6 Hz, 2H), 8.01-8.03 (d, J = 7.6 Hz, 2H), 7.52-7.58 (m, 2H), 7.22-7.28 (m, 3H), 7.07-7.09 (d, J = 15.6 Hz, 1H), 5.0 (s, 1H). ^{13}C NMR (DMSO, 100 MHz) δ: 157.31, 142.04, 137.62, 133.27, 126.38, 124.09, 121.82, 114.30, 111.56. Anal. Calcd for $C_{15}H_{11}N_3O_2$: C, 67.92; H, 4.18; N, 15.84; O, 12.06; Found: C, 66.52; H, 3.72; N, 16.26; O, 11.63. MS (ESI, m/z): 266.1 (M+1, 97%), 264.0 (M-1, 45%).

*4.1.3. General procedure for synthesis of (E)-4-chloro-N-((2-(4-nitrostyryl)-1H-benzo [d] imidazol-1-yl)methyl)aniline **(5)**.*

(*E*)-2-(4-Nitrostyryl)-1*H*-benzo[*d*]imidazole **4** (0.03 mmol) was dissolved in ethanol (10 vol). It was allowed to stirred at room temperature for 20 min. Formaldehyde (0.02 mmol) was added drop wise to the above reaction mixture. Then, 4-nitro aniline (0.04 mmol) was added to above reaction mixture. After 15 min, triethylamine (0.01 mmol) was added drop wise to above reaction mixture. Resulting reaction mixture was allowed to reflux at 70-72^{o}C for 3.5-4.0 h. After

completion of reaction, it was distilled off to remove ethanol from reaction mixture. Then, it was quinched with ice cold water and extracted with dichloromethane. Dichloromethane layer was distilled off to get crude product and further purification was accomplished by column chromatography. Mobile phase: Ethyl acetate: Hexane = 8 : 2. Yield 70.26%. mp 180-182 ^{o}C. IR (KBr) cm^{-1}: 1440.56 (C=C, Ar), 2952.48 (-CH_2), 3361.32, (N-H), 1052.94 (C-N), 1401.28 (C=N), 1691.27 (CH=CH, alkene), 1238.08 (-NO_2), 763.67 (-Cl). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.21-8.24 (d, J = 7.6 Hz, 2H), 8.01-8.03(d, J = 7.6 Hz, 2H), 7.52-7.58 (m, 2H), 7.22-7.28 (m, 5H), 7.07-7.09 (d, J = 15.6 Hz, 1H), 6.51-6.53 (d, J = 7.4 Hz, 2H), 5.68 (s, 2H), 4.0 (s, 1H). ^{13}C NMR (DMSO, 100 MHz) δ: 158.63, 147.82, 143.54, 138.37, 136.52, 134.76, 129.73, 123.64, 121.86, 112.08, 111.94, 65.30. Anal. Calcd for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; Cl, 8.76; N, 13.84; O, 7.90; Found: C, 64.86; H, 3.81; Cl, 8.34; N, 14.25; O, 7.49. MS (ESI, m/z): 404.9 (M^+, 94%), 406.7 (M+2, 33%), 403.0 (M-1, 61%).

4.1.4. General procedure for synthesis of 4-chloro-N-((2-((2S,3S)-3-(4-nitrophenyl)oxiran-2-yl)-1H-benzo[d]imidazol-1-yl)methyl)aniline ***(6)***.

(*E*)-4-Chloro-*N*-((2-(4-nitrostyryl)-1*H*-benzo [*d*] imidazol-1-yl)methyl)aniline **5** (0.05 mmol) was dissolved in dichloromethane (10 vol). Sodium hypochlorite (1.01 mmol) was added to above reaction mixture at rt. After 30 min, *R,R*-Jacobsen's catalyst (0.01 mmol) was added portion wise to above reaction mixture. It was allowed to reflux at 50-52^{o}C for 2.5-3.0 h. After completion of reaction, it was allowed to cool at rt and quinched in water followed by extracting with dichloromethane. Dichloromethane layer was distilled off to get crude product. Further purification was accomplished by Combiflash chromatography to get pure product. Mobile phase: Methanol: Dichloromethane = 1 : 9. Yield 68.26%. mp 214-216 ^{o}C. IR (KBr) cm^{-1}: 1585.20 (C=C, Ar), 2991.05 (-CH_2), 3333.36 (N-H), 1151.29 (C-N), 1417.42 (C=N), 1374.03 (-NO_2), 765.01 (-Cl). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.19-8.21 (d, J = 7.6 Hz, 2H), 7.59-7.62 (m, 4H), 7.22-7.27 (m, 4H), 6.52-6.54 (d, J = 7.6 Hz, 2H), 5.5 (s, 2H), 4.2 (s, 2H), 4.0 (s, 1H). ^{13}C NMR (DMSO, 100 MHz) δ: 167.31, 158.76, 145.84, 141.33, 139.09, 134.78, 131.67, 125.39, 121.66, 115.38, 66.37, 63.86, 55.03. Anal. Calcd for $C_{22}H_{I7}ClN_4O_3$: C, 62.79; H, 4.07; Cl, 8.42; N, 13.31; O, 11.41; Found: C, 62.38; H, 3.65; Cl, 8.02; N, 13.73; O, 10.99. MS (ESI, m/z): 420.8 (M^+, 96%), 422.5 (M+2, 30%), 419.4 (M-1, 54%).

4.1.5. General procedure for synthesis of (R)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-c]pyrimidin-4-ol ***(7a)***.

4-Chloro-*N*-((2-((2*S*,3*S*)-3-(4-nitrophenyl)oxiran-2-yl)-1*H*-benzo[*d*] imidazol-1-yl) methyl) aniline **6** (0.05 mmol) was dissolved in methanol (10 vol). It was allowed to stir for 15 min at rt.

Then, Aluminum trichloride (1.05 mmol) was added to it. Resulting reaction mixture was allowed to reflux at 75-77^{o}C for 2.0-2.5 h. After completion of reaction, it was distilled off to remove methanol from the reaction mixture. Then, it was neutralized by sodium bicarbonate solution and extracted with dichloromethane and dichloromethane layer was distilled off to get crude product. Further purification was accomplished by column chromatography to get pure product. Mobile phase: Methanol: Dichloromethane = 0.5 : 9.5. Yield 65.25%. mp 285-287 °C. IR (KBr) cm^{-1}: 1583.27, 1664.27 (C=C, Ar), 3439.42 (N-H), 2930.31 (-CH_2), 1090.55 (C-N), 3598.52 (-OH), 746.31 (-Cl), 1381.75 (-NO_2). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.49-7.51 (d, J = 8.6 Hz, 1H), 7.31-7.32 (d, J = 4.0 Hz, 1H), 6.98-7.0 (d, J = 5.0 Hz, 1H), 6.84 (s, 3H), 6.76-6.78 (dd, J = 8.60, 2.28 Hz, 2H), 6.65 (s, 4H), 4.48 (s, 1H), 3.90 (s, 1H), 3.79 (s, 1H), 3.53 (s, 1H), 2.1 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 176.75, 145.54, 139.92, 139.31, 138.21, 128.92, 127.62, 127.56, 127.15, 127.12, 126.33, 121.54, 119.73, 77.37, 77.26, 77.05, 76.74, 63.52, 55.84, 49.29, 43.63, 39.14. Anal. Calcd for $C_{22}H_{17}ClN_4O_3$: C, 62.79; H, 4.07; Cl, 8.42; N, 13.31; O, 11.41; Found: C, 62.39; H, 3.72 ; Cl, 8.13; N, 12.94; O, 10.95. MS (ESI, m/z): 420.9 (M^+, 96%), 422.8 (M+2, 30%), 419.0 (M-1, 94%), 299.7 ($C_{16}H_{14}ClN_3O$, 11%), 310.0 ($C_{16}H_{14}N_4O_3$, 15%), 332.7 ($C_{17}H_{17}ClN_2O_3$, 14%). HPLC purity (RT 9.747 min, 98.39%) where mobile phase 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade acetonitrile.

4.1.6. (R)-2-(4'-Nitrophenyl)-3-(3'-nitrophenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo[1,2-c] pyrimidin-4-ol ***(7b)***.

Yield 67.23%. mp 290-292 °C. IR (KBr) cm^{-1}: 1667.16, 1583.27 (C=C, Ar), 2932.23 (-CH_2), 3213.79 (-NH), 1084.76 (C-N), 3611.05 (-OH), 1383.68 (-NO_2). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.87 (s, 2H), 7.62-7.66 (m, 3H), 7.50-7.56 (m, 3H), 7.06-7.11 (m, 4H), 4.68 (s, 2H), 3.49 (s, 1H), 2.27 (s, 1H), 2.1 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 176.75, 175.32, 148.54, 145.06, 128.78, 127.44, 126.57, 77.37, 77.26, 77.05, 76.74, 58.66, 50.53, 48.71, 43.90, 40.57, 40.36, 39.94, 39.32. Anal. Calcd for $C_{22}H_{17}N_5O_5$: C, 61.25; H, 3.97; N, 16.23; O, 18.54; Found: C, 60.80; H, 3.57; N, 15.86; O, 18.23. MS (ESI, m/z): 432.1 (M+1, 96%), 430.0 (M-1, 94%), 309.3 ($C_{16}H_{14}N_4O_3$, 11%), 341.2 ($C_{17}H_{15}N_3O_5$, 20%), 343.5 ($C_{17}H_{17}N_3O_5$, 7%), 379.3 ($C_{17}H_{13}N_5O_5$, 23%), 415.8 ($C_{22}H_{17}N_5O_4$, 34%).

4.1.7. (R)-2-(3',5'-Dichlorophenyl)-3-(4'-chlorophenyl)-1,2,3,5-tetrahydrobenzo [4,5]imidazo [1,2-c]pyrimidin-4-ol ***(7c)***.

Yield 63.28%. mp 295-297 °C. IR (KBr) cm^{-1}: 1582.31 (C=C, Ar), 2951.52 (-CH_2), 3202.22 (-NH), 1156.12 (C-N), 3597.56 (-OH), 749.20, 677.85 (-Cl). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.10-8.12 (m, 1H), 7.65 (s, 1H), 7.46-7.50 (m, 2H), 7.41-7.43 (m, 1H), 7.34-7.38 (m, 1H), 7.28-7.30

(d, J = 7.5 Hz, 1H), 7.15-7.19 (m, 2H), 7.08-7.10 (d, J = 7.8 Hz, 1H), 6.83 (s, 1H), 5.12 (s, 1H), 3.97 (s, 2H), 3.34 (s, 1H), 1.70 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 170.15, 148.61, 139.29, 130.15, 129.56, 128.52, 127.63, 126.71, 119.96, 117.97, 77.37, 63.35, 52.33, 50.12, 46.52, 44.12, 40.51, 36.17, 34.58, 29.28, 22.04, 21.70. Anal. Calcd for $C_{22}H_{16}Cl_3N_3O$: C, 59.41; H, 3.63; Cl, 23.91; N, 9.45; O, 3.60; Found: C, 58.96; H, 3.35; Cl, 22.12; N, 9.07; O, 3.17. MS (ESI, m/z): 444.9 (M^+, 97%), 446.7 (M+2, 31%), 448.4 (M+4, 15%), 443.0 (M-1, 48%), 300.0 ($C_{16}H_{14}ClN_3O$, 10%), 334.0 ($C_{16}H_{13}Cl_2N_3O$, 29%), 356.8 ($C_{17}H_{16}Cl_3NO$, 41%), 397.0 ($C_{18}H_{16}Cl_3N_3O$, 26%), 341.5 ($C_{22}H_{19}N_3O$, 18%).

4.1.8. (R)-2-(3',5'-Dichlorophenyl)-3-(2'-chlorophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo [1,2-c] pyrimidin-4-ol ***(7d)***.

Yield 61.36%. mp 290-292 °C. IR (KBr) cm^{-1}: 1509.03 (C=C, Ar), 2969.84 (-CH_2), 3338.18 (-NH), 1036.55 (C-N), 3624.55 (-OH), 665.32, 770.42 (-Cl). ^1H NMR (400 MHz, DMSO) δ ppm 7.56-7.63 (m, 4H), 7.37-7.52 (m, 2H), 7.12-7.18 (m, 1H), 6.96-7.0 (m, 4H), 5.67 (s, 2H), 4.57-4.62 (q, 1H), 3.50 (s, 1H), 2.52-2.54 (m, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 170.15, 145.61, 139.20, 138.98, 130.15, 129.95, 128.52, 127.63, 127.40, 126.71, 124.01, 119.74, 117.97, 117.56, 58.66, 50.53, 48.71, 43.50, 40.57, 40.38, 33.73, 33.32. Anal. Calcd for $C_{22}H_{16}Cl_3N_3O$: C, 59.41; H, 3.63; Cl, 23.91; N, 9.45; O, 3.60; Found: C, 58.43; H, 3.30; Cl, 22.48; N, 9.17; O, 3.21. 443. MS (ESI, m/z): 444.8 (M^+, 95%), 446.7 (M+2, 32%), 448.4 (M+4, 11%), 443.3 (M-1, 60%), 299.0 ($C_{16}H_{14}ClN_3O$, 14%), 334.2 ($C_{16}H_{13}Cl_2N_3O$, 32%), 428.8 ($C_{22}H_{16}Cl_3N_3$, 5%), 341.4 ($C_{22}H_{19}N_3O$, 23%), 325.4 ($C_{22}H_{19}N_3$, 20%).

4.1.9. (R)-2-(3',5'-Dichlorophenyl)-3-(3',4'-dimethoxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7e)***.

Yield 63.41%. mp 287-289 °C. IR (KBr) cm^{-1}: 1576.52 (C=C, Ar), 3037.34, 2928.38, 2852.20 (C-H), 3325.64 (-NH), 1088.62 (C-N), 3578.27 (-OH), 648.92 (-Cl), 1001.25 (C-O), 1440.56 (C-H, OCH_3). ^1H NMR (400 MHz, DMSO) δ ppm 7.49-7.51 (m, 3H), 7.31-7.32 (d, J = 4.0 Hz, 2H), 6.98-7.0 (d, J = 5.0 Hz, 1H), 6.84 (s, 2H), 6.76-6.78 (dd, J = 8.6, 4.2 Hz, 2H), 4.57-4.60 (d, J = 12.8 Hz, 1H), 4.45-4.48 (d, J = 12.8 Hz, 1H), 3.90 (s, 1H), 3.79 (s, 6H), 3.53 (s, 1H), 2.52 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 163.42, 158.25, 146.40, 145.64, 144.46, 144.18, 119.73, 117.19, 114.64, 111.47, 107.58, 107.51, 60.81, 57.43, 55.63, 40.16, 39.74, 39.11. Anal. Calcd for $C_{24}H_{21}Cl_2N_3O_3$: C, 61.29; H, 4.50; Cl, 15.08; N, 8.93; O, 10.20; Found: C, 60.84; H, 4.09; Cl, 14.71; N, 8.52; O, 9.82. MS (ESI, m/z): 470.6 (M^+, 96%), 472.8 (M+2, 31%), 474.5 (M+4, 14%), 469.1 (M-1, 82%), 440.2 ($C_{23}H_{19}Cl_2N_3O_2$, 20%), 407.1 ($C_{23}H_{20}ClN_3O_2$, 4%).

4.1.10. (R)-2-(4'-Bromophenyl)-3-(4'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo[1,2-c] pyrimidin-4-ol ***(7f)***.

Yield 60.28%. mp 293-295 °C. IR (KBr) cm^{-1}: 1605.45, 1507.10 (C=C, Ar), 2880.17 ($-CH_2$), 3428.81 (-NH), 1171.54, 1252.54 (C-N), 3677.59 (-OH), 1056.78 (C-O), 537.07 (-Br). ^1H NMR (400 MHz, DMSO) δ ppm 8.11 (s, 4H), 7.62-7.68 (m, 2H), 7.50-7.55 (m, 2H), 7.06-7.14 (m, 4H), 4.66 (s, 2H), 3.44 (s, 1H), 2.52 (s, 1H), 2.27-2.31 (t, J = 7.5 Hz, 1H), 1.86-1.91 (m, 1H), 1.70-1.72 (m, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 185.61, 160.99, 154.95, 140.93, 138.40, 135.97, 130.39, 129.14, 127.50, 126.06, 123.4, 75.72, 42.31, 39.38, 27.69, 26.68, 25.47, 21.56, 13.50. Anal. Calcd for $C_{22}H_{18}BrN_3O_2$: C, 60.56; H, 4.16; Br, 18.31; N, 9.63; O, 7.33; Found: C, 60.15; H, 3.76; Br 17.90; N, 9.23; O, 6.92. MS (ESI, m/z): 436.8 (M^+, 99%), 438.9 (M+2, 94%), 435.0 (M-1, 65%), 281.0 ($C_{16}H_{15}N_3O_2$, 20%), 343.8 ($C_{16}H_{14}BrN_3O$, 43%), 421.2 ($C_{22}H_{18}BrN_3O$, 17%), 357.3 ($C_{22}H_{19}N_3O_2$, 14%), 349.0 ($C_{17}H_{18}BrO_2$, 30%). HPLC purity (RT 11.68 min, 95.14%) where mobile phase 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade acetonitrile.

4.1.11. (R)-2-(2',6'-Dimethylphenyl)-3-(2'-amino,3,4-dimethylphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo[1,2-c]pyrimidin-4-ol ***(7g)***.

Yield 61.29%. mp 289-291 °C. IR (KBr) cm^{-1}: 1509.99, 1588.09, 1650.77 (C=C, Ar), 3076.87 ($-CH_3$), 2948.63 (C-H), 1148.4, 1226.5, 1274.72 (C-N), 3775.94 (-OH), 3492.45 (-NH). ^1H NMR (400 MHz, DMSO) δ ppm 7.66 (s, 2H), 7.42 (s, 1H), 7.29 (s, 1H), 7.0 (s, 1H), 6.86 (s, 2H), 6.68-6.75 (m, 2H), 5.69 (m, 1H), 5.68 (s, 1H), 5.48 (s, 1H), 4.52 (s, 1H), 2.99 (s, 6H), 2.31 (s, 6H), 2.02 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 170.4, 148.47, 139.06, 138.98, 130.15, 129.88, 128.65, 127.61, 126.71, 120.09, 119.74, 117.58, 62.54, 52.02, 50.14, 46.62, 46.12, 42.05, 40.81, 36.17, 34.95, 22.04, 21.7. Anal. Calcd for $C_{26}H_{28}N_4O$: C, 75.70; H, 6.84; N, 13.58; O, 3.88; Found: C, 75.24; H, 6.47; N, 13.11; O, 3.48. MS (ESI, m/z): 412.8 (M^+, 99%), 411.0 (M-1, 97%), 307.3 ($C_{18}H_{20}N_4O$, 34%), 294.3 ($C_{18}H_{19}N_3O$, 36%), 396.5 ($C_{26}H_{28}N_4$, 32%), 324.4 ($C_{22}H_{19}N_3$, 49%). HPLC purity (RT 5.598 min, 97.67%) where mobile phase 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade acetonitrile. LCMS (RT 3.015 min, 99%) acquired using UPLC-02 by Formic_Normal at 230.0 nm.

4.1.12. (R)-2-(3',5'-Dichlorophenyl)-3-(2'-methoxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7h)***.

Yield 63.78%. mp 286-288 °C. IR (KBr) cm^{-1}: 1469.49 ($-OCH_3$), 1586.16 (C=C, Ar), 2970.80 ($-CH_2$), 2780.85 (C-H), 1088.62 (C-N), 3475.1 (-NH), 3623.59 (-OH), 777.17 (-Cl), 2970.8, 2780.85 ($-CH_2$). ^1H NMR (400 MHz, DMSO) δ ppm 7.74 (m, 1H), 7.46-7.52 (m, 1H), 7.38-7.42 (m, 2H), 7.22-7.33 (m, 5H), 7.15-7.17 (d, J = 8.1 Hz, 2H), 5.55 (s, 2H), 3.8 (s, 1H), 3.35

(s, 1H), 2.95-2.97 (d, J = 7.8 Hz, 3H), 2.53-2.55 (m, 1H), 1.85-1.91 (m, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 169.53, 156.03, 153.98, 142.73, 141.99, 140.54, 136.19, 135.04, 134.48, 132.18, 129.92, 128.60, 126.05, 123.12, 121.8, 118.58, 109.22, 46.58, 31.58, 29.02, 21.03, 16.38. Anal. Calcd for $C_{23}H_{19}Cl_2N_3O_2$: C, 62.74; H, 4.35; Cl, 16.10; N, 9.54; O, 7.27; Found: C, 62.33; H, 3.93; Cl, 15.68; N, 9.95; O, 6.85. MS (ESI, m/z): 440.8 (M^+, 96%), 442.9 (M+2, 31%), 444.6 (M+4, 12%), 439.1 (M-1, 71%), 295.0 ($C_{17}H_{17}N_3O_2$, 25%), 334.3 ($C_{16}H_{13}Cl_2N_3O$, 32%), 371.4 ($C_{23}H_{21}N_3O_2$, 15%), 425.0 ($C_{23}H_{19}Cl_2N_3O$, 14%), 406.0 ($C_{23}H_{20}ClN_3O_2$, 9%).

4.1.13. (R)-2-(2',6'-Dimethylphenyl)-3-(2'-methoxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7i)***.

Yield 65.28%. mp 293-295 °C. IR (KBr) cm^{-1}: 1587.13 (C=C), 3322.75 (-NH), 1229.4, 1267.97 (C-N), 1469.49 (-OCH_3), 2965.02, 2924.52 (-CH_3), 2782.78 (C-H), 3500.17 (-OH). ^1H NMR (400 MHz, DMSO) δ ppm 7.60-7.66 (m, 2H), 7.49-7.50 (m, 2H), 7.06-7.11 (m, 6H), 6.96-7.02 (m, 1H), 5.67 (s, 1H), 4.68 (s, 2H), 4.10 (s, 1H), 2.54 (s, 3H), 2.27-2.31 (t, J = 7.5 Hz, 6H), 1.85-1.92 (m, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 185.65, 161.0, 140.9, 138.44, 135.87, 130.29, 128.80, 127.44, 126.02, 123.47, 78.83, 78.17, 75.73, 42.35, 36.78, 27.75, 26.73, 25.48, 21.58, 13.47. Anal. Calcd for $C_{25}H_{25}N_3O_2$: C, 75.16; H, 6.31; N, 10.52; O, 8.01; Found: C, 74.73; H, 5.91; N, 10.11; O, 8.41. MS (ESI, m/z): 400.0 (M+1, 95%), 398.2 (M-1, 71%), 294.0 ($C_{18}H_{19}N_3O$, 14%), 295.0 ($C_{17}H_{17}N_3O_2$, 21%), 372.0 ($C_{23}H_{21}N_3O_2$, 12%), 384.1 ($C_{25}H_{25}N_3O$, 20%), 341.2 ($C_{22}H_{19}N_3O$, 14%), 386.0 ($C_{24}H_{23}N_3O_2$, 27%).

4.1.14. (R)-2-(2',6'-Dicholrophenyl)-3-(3'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7j)***.

Yield 61.27%. mp 287-289 °C. IR (KBr) cm^{-1}: 1586.16, 1468.53 (C=C), 2978.52, 2781.81 (-CH_2), 3329.50 (-NH), 1088.62, 1228.43 (C-N), 3550.31 (-OH), 775.24 (-Cl). ^1H NMR (400 MHz, DMSO) δ ppm 8.10-8.12 (m, 1H), 7.51 (s, 1H), 7.47 (m, 2H), 7.41-7.43 (m, 1H), 7.34-7.38 (m, 1H), 7.28-7.30 (m, 1H), 7.13-7.19 (m, 2H), 7.00-7.10 (d, J = 7.0 Hz, 1H), 6.86 (s, 1H), 5.12 (s, 1H), 3.93 (s, 1H), 3.91 (s, 1H), 3.65 (s, 1H), 3.34 (s, 1H), 1.87 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 164.87, 156.62, 153.83, 137.47, 135.28, 133.22, 131.98, 129.95, 127.80, 125.68, 120.53, 114.65, 109.71, 76.29, 55.33, 39.34, 32.24, 4.70, 23.25, 19.72. Anal. Calcd for $C_{22}H_{17}Cl_2N_3O_2$: C, 61.98; H, 4.02; Cl, 16.63; N, 9.86; O, 7.51; Found: C, 61.57; H, 3.61; Cl, 16.21; N, 10.28; O, 7.92. MS (ESI, m/z): 426.8 (M^+, 98%), 428.5 (M+2, 30%), 430.7 (M+4, 10%), 425.0 (M-1, 74%), 281.0 ($C_{16}H_{15}N_3O_2$, 7%), 335.0 ($C_{16}H_{13}Cl_2N_3O$, 21%), 358.1 ($C_{22}H_{19}N_3O_2$, 14%), 410.3 ($C_{22}H_{17}Cl_2N_3O$, 35%). HPLC purity (RT 4.216 min, 98.25%) where mobile phase 0.1% TFA in HPLC grade water and 0.1% TFA in HPLC grade acetonitrile. LCMS (RT 1.903 min,

99.5%) acquired using UPLC-01 by Formic_Normal at 309.0 nm.

4.1.15. (R)-2-(4'-Nitrophenyl)-3-(2'-nitrophenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo[1,2-c] pyrimidin-4-ol ***(7k)***.

Yield 64.92%. mp 293-295 °C. IR (KBr) cm^{-1}: 1595.81, 1436.71 (C=C), 2944.77 ($-CH_2$), 1087.66, 1161.90 (C-N), 3273.57 (-NH), 3696.87 (-OH), 1349.93 ($-NO_2$). ^1H NMR (400 MHz, DMSO) δ ppm 7.45 (m, 5H), 7.12-7.14 (m, 2H), 6.94-7.02 (m, 5H), 4.07-4.15 (m, 1H), 3.86-3.89 (t, J = 6.66 Hz, 2H), 3.41 (s, 1H), 2.20-2.29 (m, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 154.31153.66, 130.42, 129.50, 127.95, 124.82, 120.25, 119.88, 109.63, 108.56, 107.51, 79.17, 78.51, 54.76, 52.62, 50.28, 40.14, 39.51, 38.89, 38.28, 28.58, 25.24. Anal. Calcd for $C_{22}H_{17}N_5O_5$: C, 61.25; H, 3.97; N, 16.23; O, 18.54; Found: C, 60.84; H, 3.55; N, 16.65; O, 18.13. MS (ESI, m/z): 432.1 (M+1, 98%), 430.2 (M-1, 95%), 309.7 ($C_{16}H_{14}N_4O_3$, 31%), 343.5 ($C_{17}H_{17}N_3O_5$, 33%).

4.1.16. (R)-2-(4'-Methoxyphenyl)-3-(3'-bromophenyl)-1,2,3,5-tetrahydrobenzo[4,5]imidazo [1,2-c] pyrimidin-4-ol ***(7l)***.

Yield 60.28%. mp 288-290 °C. IR (KBr) cm^{-1}: 568.89 (-Br), 1494.56 ($-OCH_3$), 3500.06 (-OH), 1585.20 (C=C), 2982.37, 2775.06 ($-CH_2$), 3256.22 (-NH), 1194.69, 1375.96 (C-N). ^1H NMR (400 MHz, DMSO) δ ppm 7.88-7.91 (m, 2H), 7.32-734 (m, 4H), 7.25-7.29 (m, 4H), 7.17-7.22 (m, 2H), 5.50 (s, 2H), 3.39 (s, 1H), 2.92-2.96 (t, J = 7.16 Hz, 1H), 2.71 (s, 1H, D_2O exchangeable, OH), 2.33-2.36 (t, J = 7.16 Hz, 3H). ^{13}C NMR (DMSO, 100 MHz) δ: 199.86, 156.53, 142.99, 134.70, 128.37, 128.14, 127.35, 127.17, 125.52, 80.07, 77.57, 77.25, 76.93, 72.75, 57.78, 51.19, 36.31, 35.12, 31.53, 31.21, 22.13. Anal. Calcd for $C_{23}H_{20}BrN_3O_2$: C, 61.34; H, 4.48; Br, 17.74; N, 9.33; O, 7.11; Found: C, 61.52; H, 3.64; Br, 16.85; N, 8.18; O, 2.85. MS (ESI, m/z): 450.7 (M^+, 99%), 452.5 (M+2, 97%), 449.1 (M-1, 73%).

4.1.17. (R)-2-(2',6'-Dimethylphenyl)-3-(3'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c] pyrimidin-4-ol ***(7n)***.

Yield 61.27%. mp 289-291 °C. IR (KBr) cm^{-1}: 1491.67, 1528.31, 1651.73 (C=C), 1110.8, 1216.86 (C-N), 3102.9 ($-CH_2$), 3322.75 (-NH), 3788.47 (-OH), 2965.02 ($-CH_3$). ^1H NMR (400 MHz, DMSO) δ ppm 7.67-7.71 (t, J = 9.3 Hz, 3H), 7.50-7.57 (m, 1H), 7.40-7.46 (m, 1H), 7.22-7.33 (m, 4H), 7.16-7.20 (m, 2H), 5.61 (s, 2H), 3.81 (s, 1H), 3.35 (s, 1H), 2.91 (s, 1H, D_2O exchangeable, OH), 2.63 (s, 1H, D_2O exchangeable, OH), 2.50 (d, J = 6.4 Hz, 6H). ^{13}C NMR (DMSO, 100 MHz) δ: 172.11, 169.50, 156.11, 154.0, 14.67, 140.49, 136.5, 134.65, 130.25, 126.31, 123.06, 121.77, 118.60, 110.29, 99.49, 79.23, 78.57, 46.18, 39.51, 37.71, 28.77, 20.76, 16.43, 13.82. Anal. Calcd for

$C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90; O, 8.30; Found: C, 74.37; H, 5.61; N, 11.32; O, 7.89. MS (ESI, m/z): 386.2 (M+1, 97%), 384.0 (M-1, 94%).

4.1.18. (R)-2-(4'-Methoxyphenyl)-3-(3'-pyridinylphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7p)***.

Yield 52.37%. mp 281-283 °C. IR (KBr) cm^{-1}: 3657.34 (-OH), 1490.70 ($-OCH_3$), 1652.70, 1529.27 (C=C), 2960.20 ($-CH_2$), 3322.75, (-NH), 1216.66, 1346.07 (C-N). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.81-7.82 (d, J = 4.0 Hz, 1H), 7.75-7.77 (d, J = 8.6 Hz, 1H), 7.38-7.45 (m, 2H), 7.29-7.32 (d, J = 8.6 Hz, 1H), 7.22-7.24 (d, J = 7.2 Hz, 1H), 7.04-7.19 (m, 4H), 6.81-6.90 (m, 2H), 4.57 (s, 2H), 3.81 (s, 1H), 3.10 (s, 1H), 2.55 (s, 1H, D_2O exchangeable, OH), 2.06-2.11 (m, 3 H). ^{13}C NMR (DMSO, 100 MHz) δ: 180.55, 156.82, 148.47, 145.34, 143.87, 140.53, 136.42, 131.56, 128.04, 125.98, 119.48, 77.18, 73.49, 49.98, 43.83, 40.30, 39.77, 32.37, 31.84, 17.42, 13.37, 12.18. Anal. Calcd for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04; O, 8.59; Found: C, 69.88; H, 4.55; N, 16.60; O, 7.37. MS (ESI, m/z): 373.0 (M+1, 98%), 371.1 (M-1, 88%).

4.1.19. (R)-2-(4'-Methoxyphenyl)-3-(4'-methoxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7q)***.

Yield 58.39%. mp 274-27 °C. IR (KBr) cm^{-1}: 3548.38 (-OH), 1428.99 ($-OCH_3$), 1649.80, 1509.03 (C=C), 2955.38 ($-CH_2$), 3291.87 (-NH), 1230.36, 1326.79 (C-N). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.61-7.67 (m, 3H), 7.50-7.55 (m, 3H), 7.07-7.12 (s, 6H), 4.68 (s, 2H), 4.10 (s, 1H), 3.52-3.56 (m, 3H), 3.33-3.35 (m, 3H), 1.92 (s, 1H), 1.72 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 185.61, 161.06, 154.99, 140.92, 138.43, 135.9, 130.67, 130.33, 129.15, 127.49, 126.06, 123.47, 78.92, 78.27, 75.73, 42.33, 40.17, 39.34, 27.72, 25.48, 21.58, 13.49. Anal. Calcd for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47; O, 11.96; Found: C, 70.37; H, 5.67; N, 11.31; O, 10.83. MS (ESI, m/z): 402.0 (M+1, 98%), 400.0 (M-1, 96%).

4.1.20. (R)-2-(2'-Methoxyphenyl)-3-(2'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7r)***.

Yield 58.37%. mp 277-279 °C. IR (KBr) cm^{-1}: 3759.48 (-OH), 1439.6 ($-OCH_3$), 1509.03, 1561.09, 1649.8 (C=C), 2795.31 ($-CH_2$), 3195.31 (-NH), 1113.69, 1235.18 (C-N). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.72-7.75 (m, 2H), 7.43-7.46 (m, 2H), 7.06-7.10 (m, 2H), 6.99-7.03 (m, 4H), 6.84-6.88 (m, 2H), 4.48-4.61 (m, 2H), 3.84 (s, 1H), 3.39-3.47 (m, 1H), 3.11 (s, 3H), 2.88-2.89 (d, J = 4.4 Hz, 2H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 189.22, 147.09, 141.68, 134.13, 132.03, 130.08, 129.45, 129.41, 126.60, 123.11, 119.62, 77.05, 76.73, 53.38, 53.7, 46.91 41.01, 32.03, 22.68. Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85; O, 12.39; Found: C,

70.52; H, 4.68; N, 10.22; O, 11.96. MS (ESI, m/z): 388.0 (M+1, 96%), 386.2 (M-1, 85%).

4.1.21. (R)-2-(4'-Carboxylphenyl)-3-(2'-hydroxy,5-methoxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7s)***.

Yield 60.25%. mp 279-281 °C. IR (KBr) cm^{-1}: 3657.34 (-OH), 1420.32 ($-OCH_3$), 1509.03 (C=C), 2953.45 ($-CH_2$), 3356.22 (-NH), 1177.33, 1326.79 (C-N), 1714.41 (C=O of COOH), 1227.47 (C-O of COOH). ^{1}H NMR (400 MHz, DMSO) δ ppm 12.19 (s, 1H), 7.50-7.51 (d, J = 7.64 Hz, 2H), 7.24-7.34 (m, 7H), 7.11-7.15 (t, J = 7.3 Hz, 2H), 5.54 (s, 2H), 5.32 (s, 1H), 4.55 (s, 1H), 3.39 (s, 2H, D_2O exchangeable, OH), 2.77 (s, 3H). ^{13}C NMR (DMSO, 100 MHz) δ: 177.63, 146.15, 143.58, 143.48, 127.71, 125.48, 125.13, 78.95, 78.62, 78.30, 77.97, 71.34, 56.30, 51.87, 45.39, 41.0, 40.18, 38.92, 35.79, 26.78, 23.81. Anal. Calcd for $C_{24}H_{21}N_3O_5$: C, 66.81; H, 4.91; N, 9.74; O, 18.54; Found: C, 65.48; H, 4.16; N, 8.56; O, 17.28. MS (ESI, m/z): 432.1 (M+1, 99%), 430.0 (M-1, 83%).

4.1.22. (R)-2-(2',6'-Dimethylphenyl)-3-(4'-hydroxyphenyl)-1,2,3,5-tetrahydrobenzo [4,5] imidazo [1,2-c]pyrimidin-4-ol ***(7t)***.

Yield 52.81%. mp 274-276 °C. IR (KBr) cm^{-1}: 3696.87 (-OH), 2959.23 ($-CH_3$), 1469.49 (C=C), 3349.34 (-NH), 1166.76, 1267.34 (C-N). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.56-7.63 (m, 4H), 7.37-7.52 (m, 2H), 7.12-7.18 (m, 1H), 6.96-7.03 (m, 4H), 5.67 (s, 2H), 5.44 (s, 1H), 4.57-4.62 (q, J = 7.0 Hz, 1H), 4.20 (s, 1H), 2.52-2.54 (m, 6H), 2.10 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO, 100 MHz) δ: 167.49, 158.10, 141.51, 140.86, 138.05, 136.68, 131.23, 130.44, 130.35, 128.82, 127.40, 126.46, 123.48, 121.21, 120.34, 116.34, 78.91, 78.58, 78.25, 66.23, 46.27, 39.53, 38.90, 14.30. Anal. Calcd for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90; O, 8.30; Found: C, 73.20; H, 5.45; N, 11.16; O, 7.25. MS (ESI, m/z): 386.0 (M+1, 99%), 384.2 (M-1, 94%).

Acknowledgements

Authors would like to thank Institute of Pharmacy, Nirma University for providing facilities for experimental work. We are also thankful to Piramal Discovery Solutions for providing spectral analysis of compounds. Authors would also like to thank Dr. Helena Boshoff, Tuberculosis Research Section, LCID, NIAID, NIH, USA for *in vitro* antitubercular activity. Shubhra Singh is supported by a Raman-Charpak Fellowship and is also thankful to IFTM University, Moradabad for registering her in their university. Authors are also thankful to Laboratoire de Dynamique des Interactions Normales et Pathologiques, France for *in vitro* cytotoxicity assay against vero cells. We are also thankful to Department of Science & Technology (DST), Govt. of India for providing INSPIRE fellowship which helps as financial support for research work.

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Captions to Scheme, Figures and Tables

Scheme. Synthesis of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5] imidazo[1,2-*c*]pyrimidin-4-ol derivatives **7a**−**7t**. Reagents and conditions: (a) H_2O, 80-82^oC, 4 h; (b) 100-110^oC for 1.0 h and then 160-170^oC for 30 min, Ar-CHO; (c) C_2H_5OH, HCHO, TEA, Ar-NH_2, 70-72^oC, 3.5-4.0 h; (d) NaOCl, DCM, (*R*,*R*)-Jacobsen's catalyst, 50-52^oC, 2.5-3.0 h; (e) $AlCl_3$, CH_3OH, 75-77^oC.

Fig. 1. Hydrogen bond interaction of compound **7t** and compound **7t** is in the orange colored ball and stick model.

Fig. 2. (a) The docking pose of compound **7t** in the receptor site and cyan colored area indicated the excluded volume of the compound **7t**. (b) Interacting amino acids of binding site and compound **7t** in the field as orange colored ball and stick model.

Fig. 3. The occupancy of most potent compounds in the binding pocket and the orange color cloud indicates the excluded volume of the compounds in the lines format. The color of binding domain is as per cavity depth.

Fig. 4. X-ray crystal structure (ORTEP) of compound **7g**.

Table 1

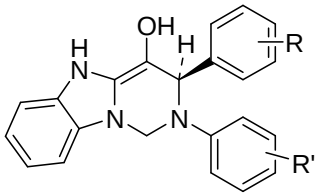
In vitro antitubercular activity of benzimidazole derivatives against *Mycobacterium tuberculosis* $H_{37}RV$

Table 2

In vitro cytotoxicity assay against VERO cells

Table 1

In vitro antitubercular activity of benzimidazole derivatives against *Mycobacterium tuberculosis* $H_{37}RV$.



Compd.	R	R'	1-week MIC[a] in µg/mL (GAST/Fe)	2-week MIC[a] in µg/mL (GAST/Fe)	1-week MIC[a] in µg/mL (7H9/ADC/Tween)	2-week MIC[a] in µg/mL (7H9/ADC/Tween)
7a	4-NO_2	4-Cl	100	>100	>100	>100
7b	3-NO_2	4-NO_2	3.13	4.7	25	37
7c	4-Cl	3,5-Cl	37	50	100	>=100
7d	2-Cl	3,5-Cl	>100	>100	>=100	>100
7e	3,4-OCH_3	3,5-Cl	100	>100	>100	>100
7f	4-OH	4-Br	75	75	>=100	>100
7g	2-NH_2, 3,4-CH_3	2,6-CH_3	37	75	100	>100
7h	2-OCH_3	3,5-Cl	50	100	100	100
7i	2-OCH_3	2,6-CH_3	100	100	50	>100
7j	3-OH	2,6-Cl	6.25	9.4	37	50
7k	2-NO_2	4-NO_2	37	37	37	50
7l	3-Br	4-OCH_3	>100	>100	>100	>100
7p	Pyridine	4-OCH_3	12.5	19	>=100	>100
7q	4-OCH_3	4-OCH_3	50	75	100	>=100
7r	2-OH	2-OCH_3	19	25	50	75
7s	2-OH, 5-OCH_3	4-COOH	>100	>100	>100	>100
7t	4-OH	2,6-CH_3	6.25	9.4	12.5	19
Isoniazid			0.02	0.02	0.02	0.02

[a]Minimum Inhibitory Concentration (MICs) against *Mycobacterium tuberculosis* $H_{37}Rv$

Table 2

In vitro cytotoxicity assay against VERO cells.

Compound	% Survival of Vero cells (50 µg/ml)	% Survival of Vero cells (25 µg/ml)
7a	ND	ND
7b	69	66
7c	69	63
7d	ND	ND
7e	ND	ND
7f	ND	ND
7g	73	64
7h	ND	ND
7i	ND	ND
7j	61	63
7k	61	53
7l	ND	ND
7p	56	66
7q	ND	ND
7r	69	63
7s	ND	ND
7t	63	66
Isoniazid	100	100

ND, not determined

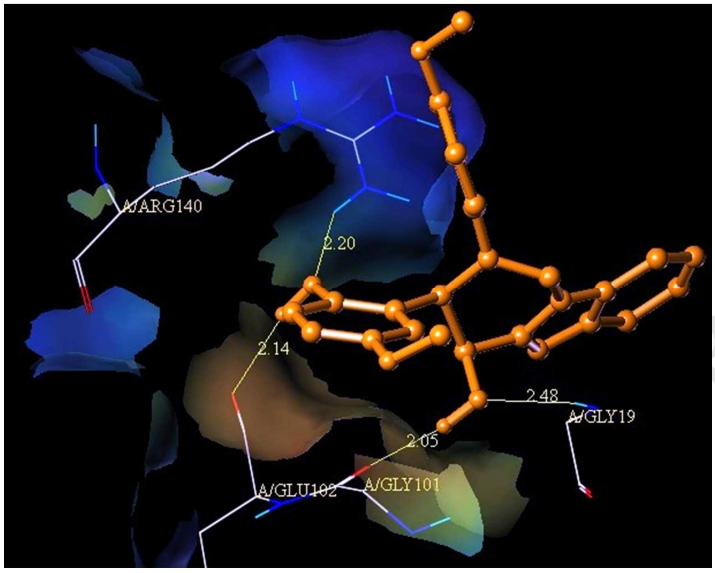


Fig. 1. Hydrogen bond interaction of compound **7t** and compound **7t** is in the orange coloured ball and stick model.

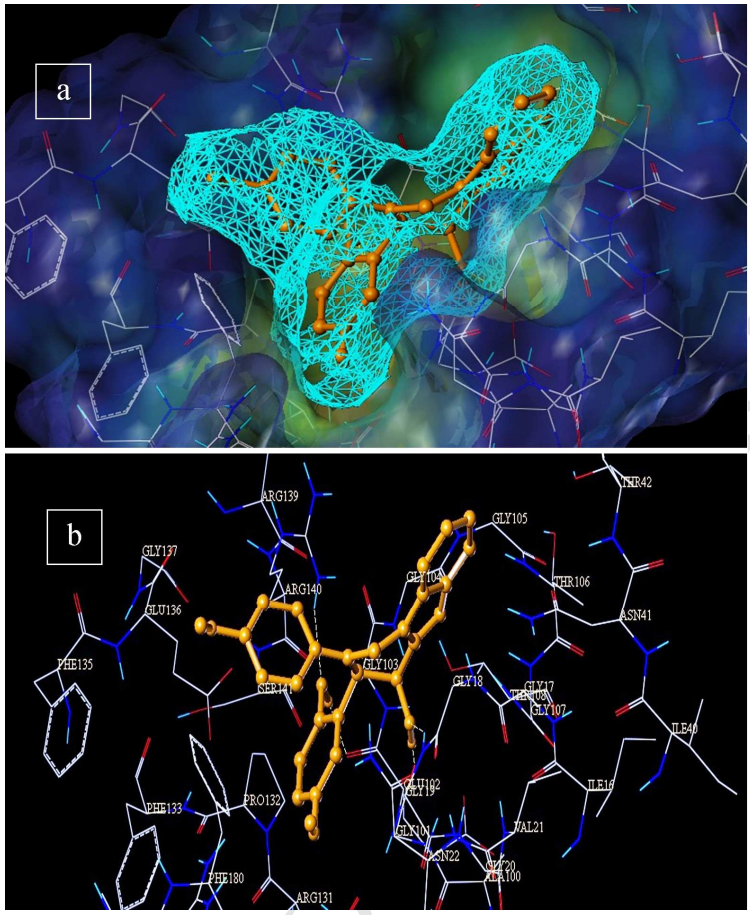
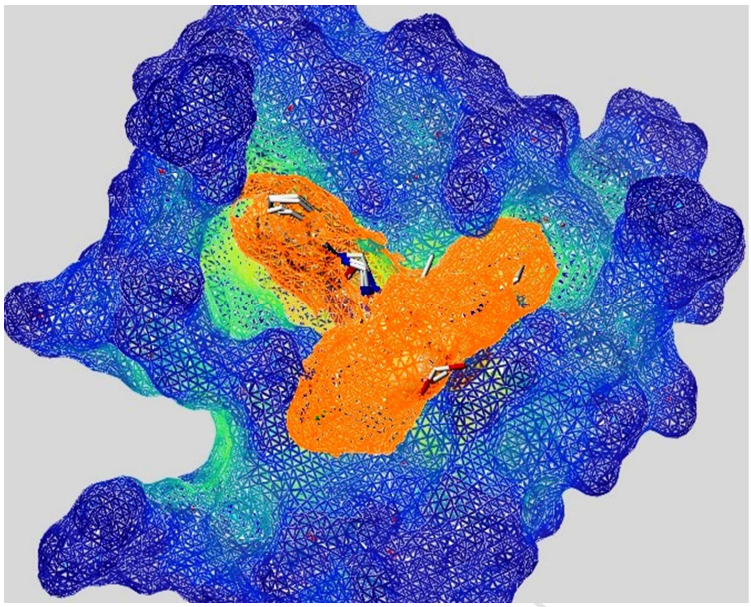


Fig. 2. (a) The docking pose of compound **7t** in the receptor site and cyan coloured area indicated the excluded volume of the compound **7t**. (b) Interacting amino acids of binding site and compound **7t** in the field as orange coloured ball and stick model.



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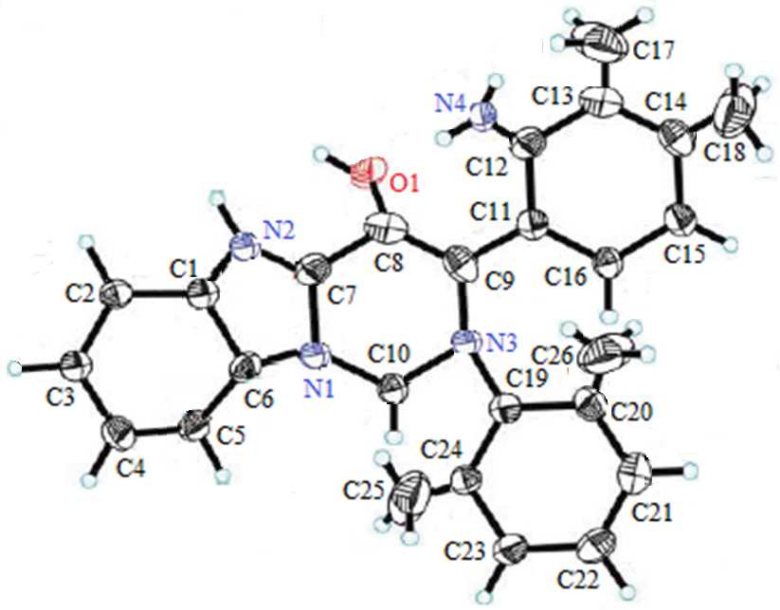
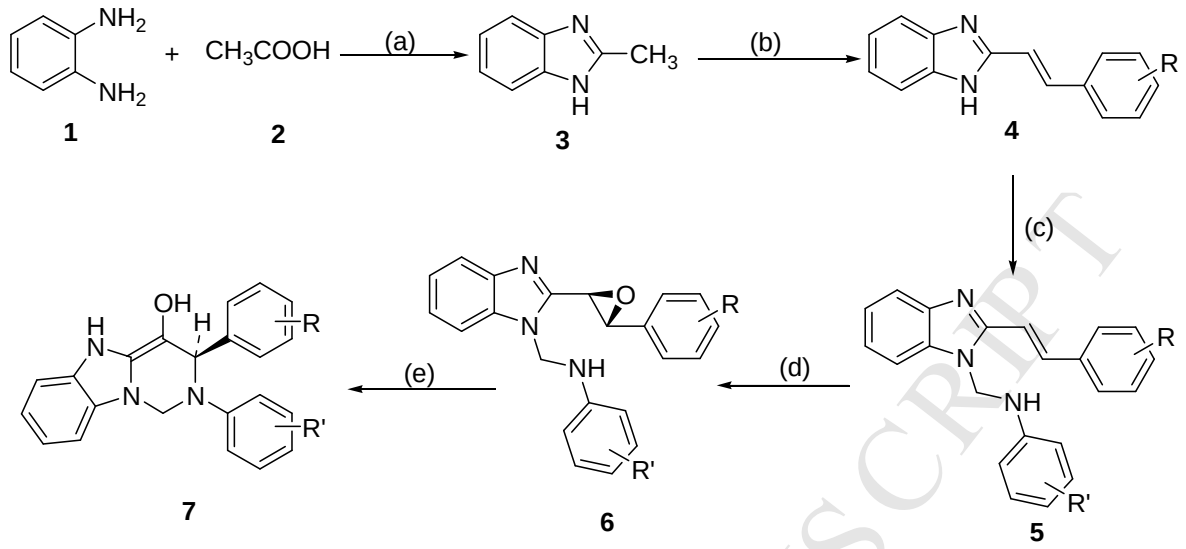


Fig. 4. X-ray crystal structure (ORTEP) of compound **7g**.



Scheme. Synthesis of (*R*)-2-(4'-chlorophenyl)-3-(4'-nitrophenyl)-1,2,3,5-tetrahydrobenzo[4,5] imidazo[1,2-*c*]pyrimidin-4-ol derivatives **7a**−**7t**. Reagents and conditions: (a) H_2O, 80-82^oC, 4 h; (b) 100-110^oC for 1.0 h and then 160-170^oC for 30 min, Ar-CHO; (c) C_2H_5OH, HCHO, TEA, Ar-NH_2, 70-72^oC, 3.5-4.0 h; (d) NaOCl, DCM, (*R*,*R*)-Jacobsen's catalyst, 50-52^oC, 2.5-3.0 h; (e) $AlCl_3$, CH_3OH, 75-77^oC.