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Synthesis, docking study and biological evaluation of novel N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide derivatives

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A series of N-(1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido acetamide derivatives have been synthesized by facile and efficient conventional method. The structures of the compounds have been elucidated with the aid of elemental analysis, IR, ESI-MS, and ^{1}H and ^{13}C NMR spectral data. Molecular docking revealed that synthesized derivatives and target proteins are actively involved in the binding pattern and had a significant correlation with biological activity. Molecular dynamics studies have also been performed and ADME parameters for the synthesized compounds determined. Biological evaluation of all synthesized compounds have been carried out *in vitro* for their antibacterial, antituberculosis and antifungal efficacy against various bacterial and fungal strains and $H_{37}Rv$. The different studies indicate that newly synthesized compounds possess moderate to good biological activities.

Keywords: ADME, benzothiazole, docking, *in vitro* study, isoniazid, structure activity relationship

Due to the very high development of drug resistance against the existing antibacterial and antifungal drugs there is an urgent need of synthesis of privileged classes of heterocyclic compounds in the field of synthetic and medicinal chemistry. In spite of many significant progresses in the antimicrobial therapy, diseases which are very infectious caused by bacteria and fungi remain a very big worldwide issue[1,2]. Multi drug resistance (MDR) is an antimicrobial resistance shown by a family of microorganisms to multiple antimicrobial drugs[3]. In particular, the emergence of multidrug resistant strains of gram-positive bacterial pathogens such as methicillin resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and Vancomycin-resistant enterococcus are problem of ever-increasing significance[1,2]. Multi drug resistance tuberculosis is a type of tuberculosis which occurs when the bacteria are resistant to the most powerful anti TB drugs called Isoniazid and Rifampin[4]. *Mycobacterium tuberculosis* is a bacteria which is responsible for the contagious disease called as tuberculosis. Generally it effects the lungs of the body called as a pulmonary TB, but in few case it can also effects other parts of the body also called as extra pulmonary TB. The authoritative symptoms of tuberculosis are fever, night sweats, blood containing sputum, *etc*.[5] Drug resistance emerges when anti-TB medicines are used inappropriately, through incorrect prescription by health care providers, poor quality drugs, and patients stopping treatment prematurely. MDR-TB is treatable and curable by using second-line drugs[6]. Poor chemotherapeutics and inadequate local control programme are the two major reasons that it is not possible to manage TB and thus leads to the emergence of drug resistant strains of MTB. Drug resistant TB is very difficult to cure. It takes 18-20 months if the patient is responsive to the second line treatment regimen in a positive manner. This regimen is very costly to treat in comparison to fully drug sensitive TB. Currently available second line drugs are not very effective and moreover, cause side effects and therefore it is necessary to identify novel drug targets[7].

The calibre treatment for tuberculosis contains different types of drugs such as Isoniazid, Rifampin, Pyrazinamide and Ethambutol[8]. Marketed antituberculosis first line drugs and second line drugs shows in the Figure 1. TB and HIV co-infection occurs when people have both TB as well as HIV and in another scenario when people have active or latent

TB disease. When people have both diseases at that time each disease speeds up the process of the other one. When people have HIV it increases the rate of latent TB to convert in to the active TB. It may happen in a country with a high prevalence of TB[9]. TB is one of the top 10 causes of death worldwide, which is caused by single infectious agent. It was evaluated that, in 2016, 1.30 million deaths among HIV negative people and 3,74,000 deaths among HIV positive people occur due to this disease[10]. Isoniazid also known as isonicotinylhydarazide is an antibiotic which is used for treatment of tuberculosis. An overview of the treatment of tuberculosis shows in the Figure 2[11]. Isoniazid is frequently given with the drugs such as Rifampicin, Pyrazinamide either Ethambutol or Streptomycin[12].

Isoniazid is a prodrug. It is also called as pyridine-4-carbohydrazide. In *Mycobacterium tuberculosis* it is

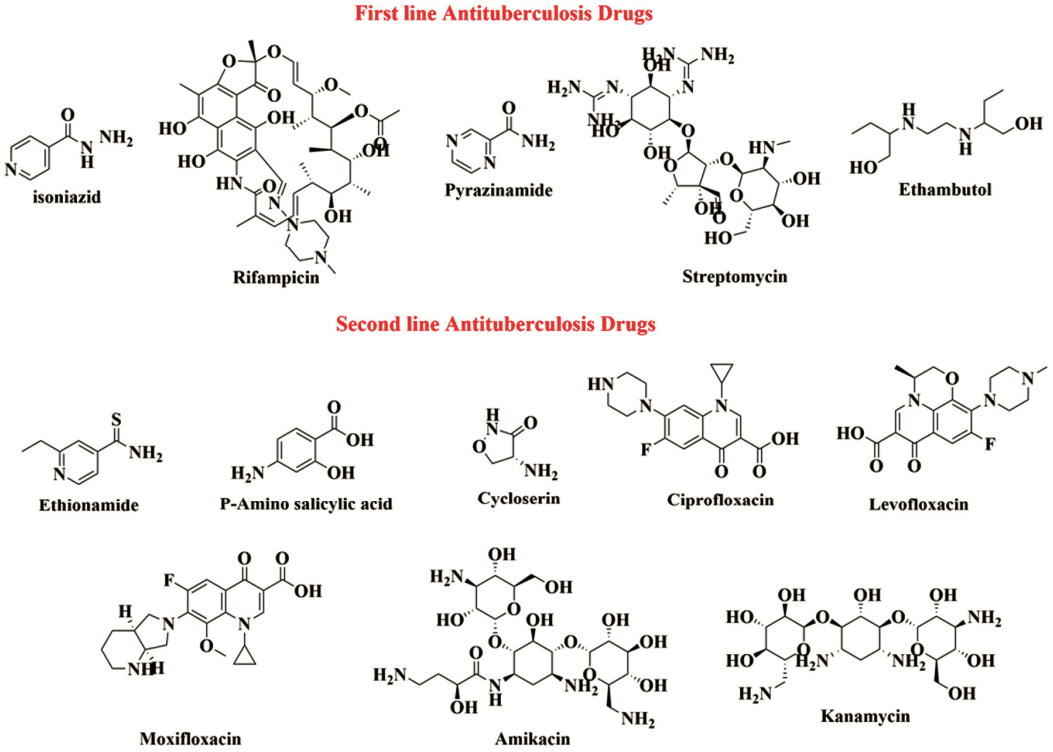


Figure 1 — Analogue marketed first line and second line anti-tuberculosis drugs

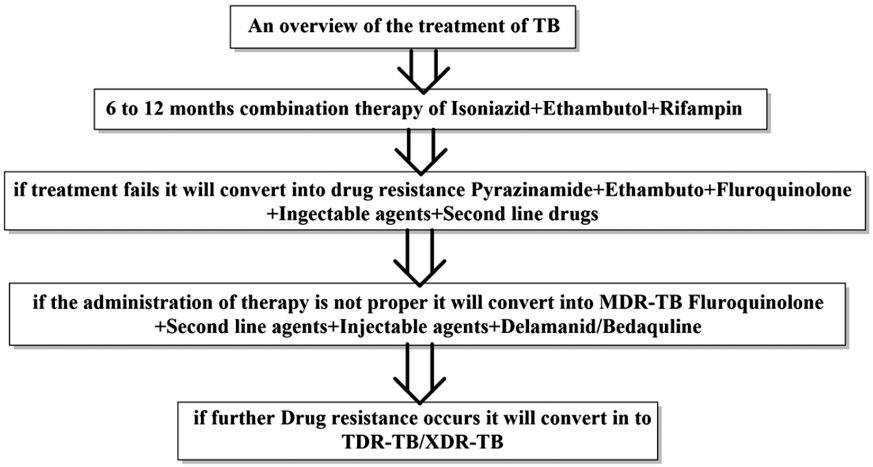


Figure 2 — An overview of the treatment of TB

activated by bacterial catalase- peroxidase enzyme[13]. Kat G catalysis the formation of the Isonicotinic acyl, radical which impulsively combines with NADH to form Nicotinoyl-NAD adducts. This complex binds tightly to the enoyl-acyl carrier protein reductase InhA, thereby blocking the natural enoyl- ACP reductase substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acids, which are required components of the mycobacterial cell wall[14]. Isoniazid may also be used for the nontuberculous mycobacterium such as *M.avium, M.kansasii* and *M.Xenopi*[15]. There is an urgent need to identify the new drug targets for the *Mycobacterium tuberculosis* and gradually develop the new drugs. Although Dots is a best treatment for TB still it takes minimum six months to cure. Due to the high incidence of MDR-TB Dots are failing to control the disease. These all drawbacks suggest that the design of newer and more potent antitubercular drugs with very less toxic effects for improved treatment of drug resistant and drug sensitive TB is indispensable[16]. The present investigation was aimed to find out new derivatives of Nicotinohydrazide (Pridine-3-carbohydrazide) with potent antibacterial, antifungal and antitubercular activities. Nicotine kills the insects very fast within an hour causing the intensive tremors, convulsions and then paralysis[17]. Nicotinic acid containing side chain on third position gives significant effect on toxicity to the insects[18].

Benzothiazole is one of the routinely obtainable heterocyclic compounds, which is basically found in alkaloids. It derived from terrestrial and marine natural products. Benzothiazole are an important class of bioactive and industrially important organic compounds[19]. From the recent literature, these derivatives are also known to possess antitubercular[20], local anesthetic[21], antidiabitic[22], antiulcer[23], antipsychotic[24], antitumor[25], antioxidant[26], analgesic[27], schistosomicidal[28], antileishmanial[29], anticonvulsant[30], antiparasitic[31], diuretic[32], plant growth regulator activities[33], anthelmintic[34], Acaricidal[35].

Inspire by the significant biological activity of the structurally diverse heterocycles with fused heterocyclic systems along with active heterocyclic analogous. A series of N-(1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido acetamide derivatives were synthesized by facile and efficient conventional method (Table I). Biological evaluation of all synthesized compounds was studied In-vitro for their antibacterial, antituberculosis and antifungal efficacy against various bacterial and fungal strains and $H_{37}Rv$. We have carried out molecular docking studies helped in revealing the mode of action of these compounds through their interactions with the active site of enzyme Enoyl-acyl-carrier protein reductase, is a key enzyme of the type II fatty acid synthesis (FAS) system ENR is a target for a narrow spectrum antibacterial drug discovery because the essential role in metabolism and its sequence conservation across many bacterial species. Molecular dynamics (MD) simulations may assistance to explain the binding site and time dynamics of interacting amino acid residues and to extend the information obtained from docking studies. We have also carried out *in silico* ADME prediction and Lipinski rules of 5 of synthesized Compounds.

Results and Discussion

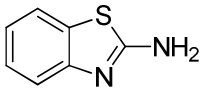
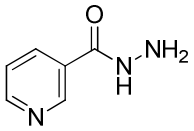
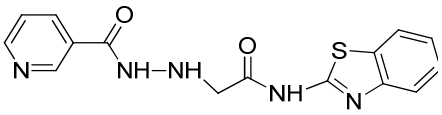
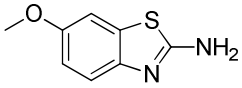
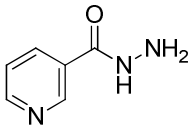
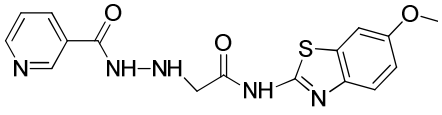
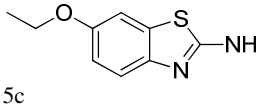
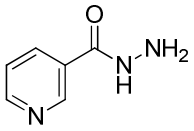
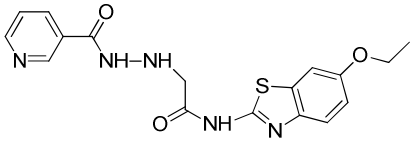
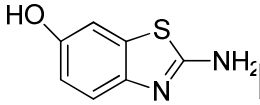
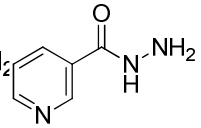
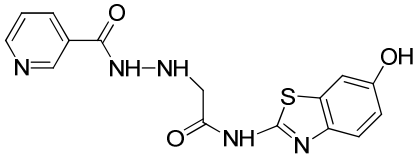
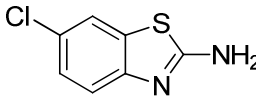
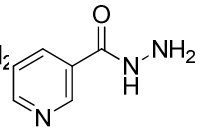
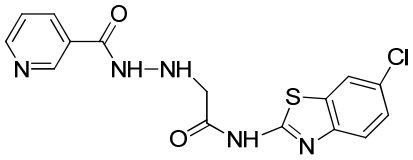
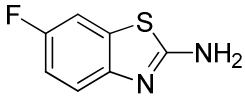
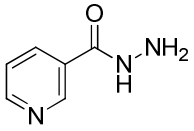
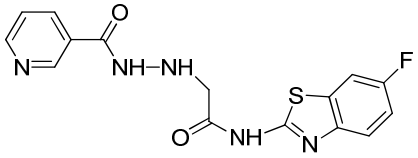
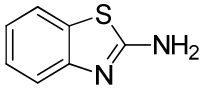
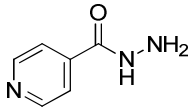
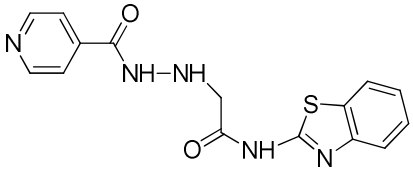
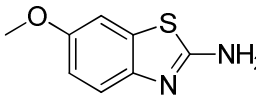
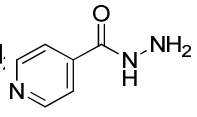
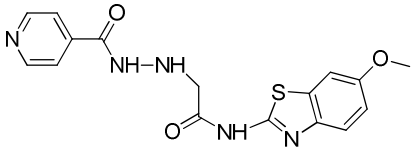
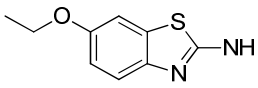
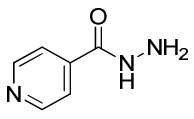
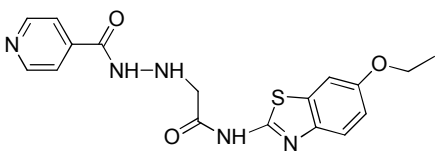
Synthesis and structural characterization

In the present work, novel substituted N-(1,3-benzothiazole-2-yl)-2(pyridine-3-2(pyridine-3-ylformohydrazido) acetamide derivatives were prepared through a plausible conversion way. The synthetic route of N-(1,3-benzothiazole-2-yl)-2(pyridine-3-2(pyridine-3- yl formohydrazido) acetamide derivatives delineated below as Scheme I.

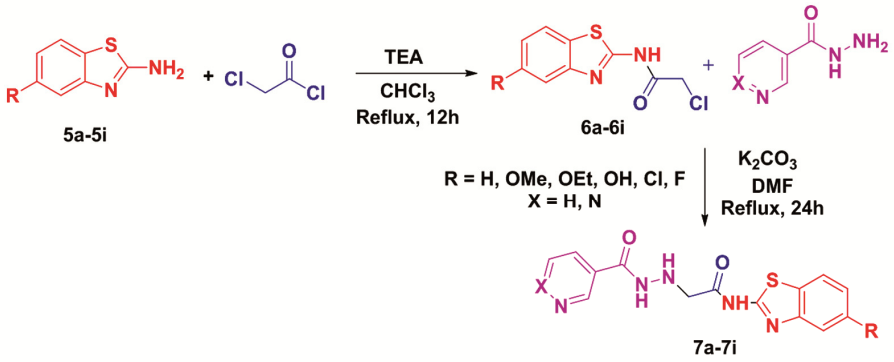
Initial analogue N- (1,3 benzothiazole-2-yl)-2 chloroacetamide was synthesized from 1,3-benzothiazole-2-amine. In the first step nucleophilic addition reaction occurs. The step occurs in two steps. In the first step nucleophilic attack on positive carbon atom by lone pair on Nitrogen atom on amine 1,3-benzothiazole -2-amine reacts with CAC in presence of TEA which acts as a base for the preparation of acyl chloride. In the second step elimination reaction occurs. It happens in two steps first C=O bond reforms and the chloride ion removes from the reaction. It is followed by removal of hydrogen ion from nitrogen from Nicotinohydrazide producing HCl. Due to the mesomeric effect generated on the oxygen atom, the negative charge will be shifted towards CH_3Cl and proton obtained from the reaction absorbed by K_2CO_3. And CH_3Cl will be removed at the end of the reaction by producing final product.

All the synthesized novel substituted N-(1,3-benzothiazole-2-yl)-2(pyridine-3-2(pyridine-3-ylformo-hydrazido) acetamide derivatives were characterized by IR, ^{1}H NMR, mas spectroscopy, melting points and purification by crystallization using appropriate solvents. The IR spectrum of the **7a** showed absorption at 3803 cm^{-1} which is due to the -N-H

Table I — Synthetic route of N-(1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide

Compd	1,3-benzothiazole-2-amine Derivatives	Pyridine-3-carbohydrazide/ Pyridine-4-carbohydrazide	Product	m.p.°C	Yield (%)
7a	 5a			159-162	79
7b	 5b			178.6-180	89
7c	 5c			182-185	72
7d	 5d			191-193	80
7e	 5e			182-185	76
7f	 5f			179-182	70
7g	 5g			160-166	79
7h	 5h			170.5-175	89
7i	 5i			172-175	72

Reaction conditions: Int. (1 eq) Various Benzothiazole derivatives, CAC (1.5 eq), TEA (1 eq), K_2CO_3 (2.5 eq)



Reaction scheme for the benzothiazole derivatives

Scheme I — Synthesis of the designed compounds **7a-i**

stretching of amide. The compound showed absorption at 112 cm^{-1} which is due to C-NH-C stretching. The band appeared at 1670 cm^{-1} is due to -C=O stretching confirmed the structure of the compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.54 (s, 1H), 8.66 (s, 1H), 8.16 (d, J = 14.6 Hz, 2H), 8.09 (s, 1H), 8.02 (s, 1H), 7.55 – 7.43 (m, 3H), 3.74 (d, J = 7.7 Hz, 2H), 3.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.13 (s), 165.32 (s), 153.43 (s), 151.38 (s), 150.91 (s), 149.45 (s), 137.58 (s), 134.41 (s), 132.64 (s), 125.53 (s), 122.77 (d, J = 18.3 Hz), 121.34 (s), 118.71 (s), 53.31 (s); EI-MS: *m/z* 328.01 (M+1); IR (KBr): 3803 (N-H Aromatic), 3549 (C-H), 1745 (C=C Aromatic), 1745 (C=O), 1323 cm^{-1} (C-N).

In vitro biological screening

In vitro antibacterial and antifungal activity

This activity has been done at, Micro care Laboratory and TRC, Surat, India as per reported method[36,37]. All newly synthesized compounds were examined for antimicrobial activity against two gram negative bacterial strains (*Pseudomonas aeruginosa* MTCC 1688, *Escherichia coli* MTCC 443), two gram positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus Niger* MTCC 282) using the agar dilution method. Ampicillin, Ciprofloxacin and Chloramphenicol were used as standard control drugs for antibacterial activity, whereas Nystain and Greseofulvin were used for antifungal activity.

The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2000mg/mL concentration as a stock solution in primary screening 500, 250 and 200mg/mL concentration of the synthesized compounds were taken. The active synthesized compounds found in the primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50 and 25 mg/mL concentrations. The highest dilution showing at least 99% is taken as MIC. The synthesized compounds (Table II) (**7a-7i**) investigated for their *in vitro* antibacterial activity. The bioassay results displays that compounds (**7a-7i**) succeed to show remarkable activity against the follow microorganisms. All novel synthesized compounds were investigated for them in-vitro antibacterial activity. The BioAssay result demonstrated that compounds (**7a-7i**) succeeded to indicate remarkable activity against below mentioned microorganism when compare to standard drugs. Among the gram negative bacterial strain *S. aureus* showed relatively higher sensitivity towards the tested compounds. In the view, analogue compounds **7d** and **7i** (MIC 100 µg /mL) displayed good activity against *S. aureus* as compared to the standard drug Ampicillin (MIC 250 µg/mL). Compounds **7g** and **7f** (MIC 125 µg/mL) also displayed good activity against *S. aureus* as compared to Ampicillin. Compounds **7b, 7g, 7e** and **7h** (MIC 250 µg/mL) were showing equipotent activity against *S.aureus as* compared to Ampicillin. For *Pyogenes9* the best activity was exhibited by compound**7f**. (MIC 62.5 µg/mL) more potent to Ampicillin (MIC100 µg/mL). For *E.Coli* compounds) **7g**, **7e, 7d** and **7h** show good activity compare to (MIC100 µg/mL). All synthesized compounds were tested against *In-vitro* antifungal activity only once strain which is *C.albicans* show certain sensitivity

Table II — Calculated ADME properties

Compd	Percent human oral absorption (>80%,high,<25%poor)	QPlogBB (-3.0-1.5)	QPlog HERG (Below -5)	QPPCaco <25 poor>500 Great	PSA (70-200Å	QPlogS (-6.5-5)
7a	76.796	−1.478	−6.283	187.347	116.463	−3.949
7b	84.444	−1.638	−6.801	272.865	123.955	−5.135
7c	79.382	−1.55	−6.34	222.288	124.332	−4.322
7d	66.483	−1.967	−6.107	76.238	137.825	−3.949
7e	80.444	−1.377	−6.443	194.22	115.912	−4.895
7f	79.787	−1.373	−6.362	194.22	115.912	−4.895
7g	77.95	−1.557	−6.664	198.708	115.596	−4.247
7h	80.951	−1.63	−6.37	226.294	122.997	−4.458
7i	79.742	−1.477	−6.226	244.07	123.574	−4.081
Isoniazid	66.794	−0.843	−6.235	274.207	81.518	−5.235
Ampicillin	17.36	−0.364	−5.369	254.36	152.23	−5.455

Calculated using qikprop v.3.5. Range/recommended values calculated for 95% known drugs.

against some of the tested compounds, the rest of other two fungal strain were inactive to the same compounds. The derivatives were tested by using method as described by S.M.Prajapati[36]. The standard marketed drugs against the synthesized derivatives were Greseofulvin, Nystatin. Compounds **7a, 7c, 7d, 7e, 7f** and **7h** show excellent activity against *C.albicans*as compared to the standard drug Greseofulvin. The graphical representation shows in the Figure 3 and Figure 4.

In vitro Antituberculosis activity

In vitro anti-tuberculosi0s of all the synthesized novel substituted N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide derivatives against mycobacterium tuberculosis H37Rv strain was tested against Lowenstein-Jensen medium (conventional method) as described in A. Rattan37.

Among the entire tested compounds compound **7a** shows good activity against H37Rv strains by measuring the minimum inhibitory concentration (MIC µg/mL).Compounds **7g** and **7i** shows potent activity against the *M.tuberculosis*. Other compounds show negligible activity against *M.tuberculosis*. Biological activity of the compounds shown in the Table III. The graphical representation of all activities shown in the Figure 5. The graphical representation of Antimalarial activity shown in Figure 6.

Structure Activity Relationship

Observing the results, we could deduce valuable data about the structure activity correlations of the tested compounds. We have introduced different electron withdrawing groups and electron donating groups on different ring system such as Benzothiazole ring (6^{th} position group such as -H, -Cl, -OMe, -F, -OH) during the synthesis. The electronic

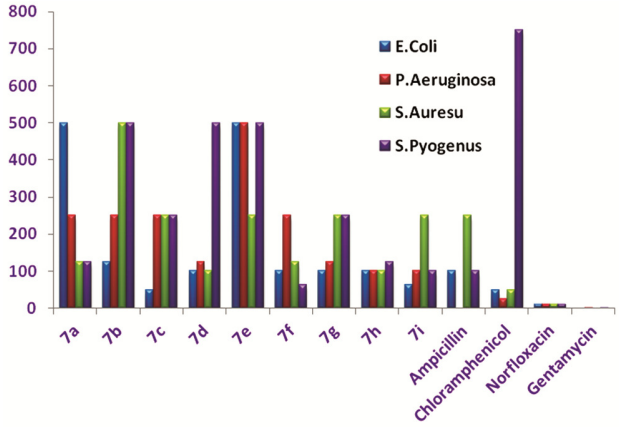


Figure 3 — Graphical representation of the biological activity of the compounds (antibacterial activity)

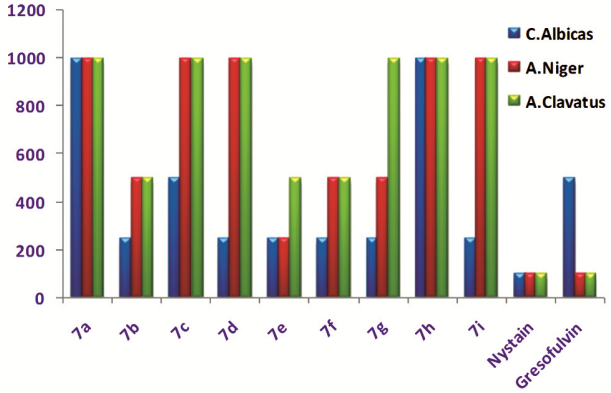


Figure 4 — Graphical representation of the biological activity of the compounds (antifungal activity)

configuration of the various functional groups commanded to promising biological activity. it is our observation that the incorporation of electron withdrawing group like -Cl (sixth position on Benzothiazole) and -F group (*Para* position on benzoyl ring) in compound **7e, 7f** it has yielded an

Table III — Biological results of synthesized compounds

Compd	Antibacterial Activity Minimal Inhibition Concentration [mg/mL]				Antifungal Activity Minimal Inhibition Concentration [mg/mL]			Anti-Malarial Activity [*Plasmodim falciparum*] Minimal Inhibition Concentration [mg/mL]	Anti-Tuberculosis Activity [H37RV] Minimal Inhibition Concentration [mg/mL]
	E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323	Mean IC50 Value	Mean IC50< Value
7a	500	250	125	125	1000	1000	1000	0.53	50
7b	125	250	500	500	250	500	500	1.25	250
7c	50	250	250	250	500	1000	1000	2.09	100
7d	100	125	100	500	250	1000	1000	0.63	250
7e	500	500	250	500	250	250	500	0.25	500
7f	100	250	125	62.5	250	500	500	0.35	250
7g	100	125	250	250	250	500	1000	1.24	62.5
7h	100	100	100	125	1000	1000	1000	1.65	62.5
7i	62.5	100	250	100	250	1000	>1000	1.28	100
INH	–	–	–	–	–	–	–	–	0.20
CHL	50	50	50	750	–	–	–	–	–
AMP	100	–	250	100	–	–	–	–	–
CIP	25	25	50	50	–	–	–	–	–
NOR	10	10	10	10	–	–	–	–	–
GEN	0.05	1	0.25	0.5	–	–	–	–	–
NYS	–	–	–	–	100	100	100	–	–
GRE	–	–	–	–	500	100	100	–	–
Qui	–	–	–	–	–	–	–	0.268	–
Chlo	–	–	–	–	–	–	–	0.020	–

INH=Isoniazid, CHL=Chloramphenicol, AMP=Ampicillin, CIP=Ciprofloxacin, NOR=Norfloxacin, GEN=Gentamycin, NYS= Nystain, GRE= Greseofulvin, Qui=Quinine, Chlo=Chlroquinine

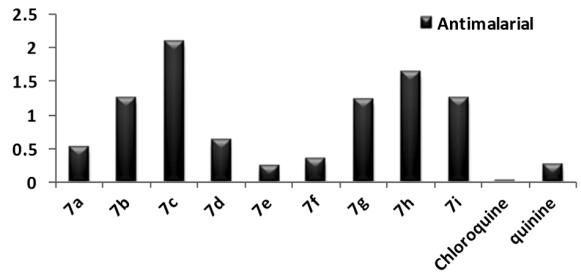


Figure 5 — Graphical representation of the biological activity of the compounds (antimalarial activity)

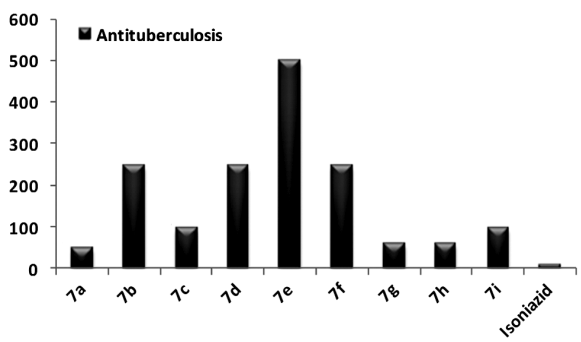


Figure 6 — Graphical representation of the biological activity of the compounds (antituberculosis activity)

excellent activity against gram negative *E. coli.* Bacterial stain. Compounds possessing various functional group different ring system such as (-OH, OC_2H_6) also give good activity in *E.Coli.* Compounds **7a, 7c, 7d, 7e, 7f, 7g, 7h,** and **7i** showed very good activity against the bacterial stain *S.aureus.* When we introduced various group on Benzothiazole, the anti-bacterial activity decreased against *P. Aeruginosa* and *S. Pyogenus* (except compound **7f**) due to function group. Only compound **7f** shows good activity against *S. Pyogenus.*

In case of anti-fungal biological screening, we have observed that the introduction of electron with drawing group like -F and electro donating group like -OH and -OMe at sixth position of compound **7b, 7d, 7g, 7h, 7i** the outstanding activity against *C. albicans* is observed. On the other side the antifungal activity decreased against *A.niger* and *A.claveatus.* In the case of anti-bacterial activity compounds **7a, 7g**, and **7h** contains good activity due to it contains functional groups such as (-H, and $-OC_2H_5$).

***In silico* ADME Prediction and drug likeness study**

The ADME properties of the compounds were predicted by Jorgensen's Method[38] using Qikprop tool (Schrodinger) for novel molecules and the obtained values are provided in supporting data as Table II. Quinine, ampicillin and isoniazid are the reference drugs for the pharmaceutically relevant properties to assess the drug likeness and pharmacokinetic properties.

All molecules have good pharmacokinetics properties (ADME). Most of the molecules found to have depicted decent human oral absorption percentage with in tolerable range (65-100%) and also good blood-brain barrier permeability (QP log BB) values within the suitable range of -3 to 1.5. All the compounds demonstrated great range IC_{50} value for HERG K+ channel blockage (QPlogHERG) below -5. Correspondingly all compounds have great intestinal Absorption Prediction as predicted value of Caco-2cell permeability (QPPCaco) for the all compounds were found between (75-200nm/sec). Å assurances good influence on bioavailability of molecules. So the PSA values of the compounds were also obtained in the range of (70-200Å). The aqua solubility parameter (QPlogS) of the molecules also shows range between (-6.5-5). The indispensable pharmacokinetic parameters are established beside through their tolerated ranges in Table II. ADME prediction may found to expedite assessment of the appropriate molecules. In the present study, to find out the drug-likeness characteristics, molecules were assessed using Lipinski's rule of five showing zero violation of the rule (drug molecule should have molecular weight ≤500, hydrogen bond acceptors, ≤10 and donor ≤5, log P ≤5, polar surface area ≤140 Å)[39]. As rule of five compliance ensures the bioavailability. The values are provided in supporting data as Table IV.

Molecular Docking Study

Docking study was performed on the software called Schrodinger. It was performed on advance scientific programmed glide version 4.5. The program operated under Linux operating system installed on Intel with a 2.8 MHz Processor and 4GB RAM. Ligands were made by the tool which is called Ligprep-v2.1[40].

Before docking process it is necessary to validate your docking tool Schrödinger molecular modelling package) and PDB target which we used. There are a number of methods available in literature[41] for validating docking programs and scoring functions. We have carried out re-docking of cocrystallized native ligands into the active site of 1ENY, 4QRE and 5HUT respectively using Schrodinger software for the validation of the docking process. The RMSD values of the native co-crystallized ligand after docking were 0.199 (1ENY), 0.113 (4QRE) and 0.084(5HUT) Å, respectively which confirms the reliability of Schrodinger for docking compounds under study.

Molecular docking of the synthesized molecules were studied against responsible enzymes *i.e.* enzyme Enoyl-ACP carrier protein (ACP) Reductase, *S.aureus* and *C.albicans* using PDB ID 1ENY, 4QRE, 5HUT correspondingly as respective inhibitors of such enzymes are found accountable for the potency. Enoyl-acyl-carrier protein reductase is a key enzyme of the type II fatty acid synthesis (FAS) system ENR is a target for a narrow spectrum antibacterial drug discovery because their essential role in metabolism and its sequence conservation across many bacterial species. ENR is a target for the Drug Isoniazid and

Table IV — Prediction of Lipinski's 'Rule of 5' for the active test compounds

Compd	Mol_MW (>500)	Donor HB (<5)	Accpt. HB (<10)	QPlog Po/w (<5)	Rule of Five (<4)
7a	327.36	2	8	1.567	0
7b	357.386	2	8.75	1.782	0
7c	371.413	2	8.75	2.374	0
7d	343.359	3	8.75	1.103	0
7e	361.805	2	8	2.142	0
7f	345.35	2	8	1.875	0
7g	327.36	2	8	1.868	0
7h	357.386	2	8.75	1.719	0
7i	371.413	2	8.75	2.026	0
Isoniazid	137.141	3	4.5	–0.647	0
Ampicillin	349.41	2	6	–2.004	0

All values calculated by QikProp v 3.5 and the explanations of the descriptors are given in the text

gene is inhA. Maestro software was hire for the identification of binding sites. All the calculation and numerical data were composed from maestro software, favourable binding poses between protein and simple van der Waals probes were finding at the automated prediction of the software, the top scoring predicted cavities was arranged in active sites directed docking studies. Default docking parameters and flexible space of novel N- (1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide derivatives, were validated by docking parameters, which docked exactly in the cavities present in the different proteins (1ENY, 4QRE, 5HUT) with an affinity of lower energetic compounds. Consequently, small molecule compounds with respect to low energetic molecules were docked using same docking parameters. Docking study was performed on single machine contain 8 GB RAM, 1 TB hard drive, operating system. Proteins were prepared on protein preparation wizard. Glide searches for favourable interactions between ligand molecules and protein receptor using a grid based method. Proteins were downloaded from protein databank few steps were performed before going for docking. The steps are protein preparation, removing the water molecules having less than 3 hydrogen bonds, hydrogen bonding optimization, and adjusting the bond order for ligands protein. The resolutions of the proteins are 2.2Å, 1.7Å, 1.9Å respectively. The protein 1ENY was taken because Enoyl-acyl-carrier protein reductase is a key enzyme of the type II fatty acid synthesis (FAS) system ENR is a target for a narrow spectrum antibacterial drug discovery because the essential role in metabolism and its sequence conservation across many bacterial species. ENR is a target for the Drug Isoniazid and gene is inhA. The Global Stoichiometry of the protein is Homomer. inhA is the drug's primary target. The Global Stoichiometry of the protein 4QRE is Monomer. The Global Stoichiometry of the protein 5HUT is Homomer. Molecular docking score of N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide derivatives with PDB 1ENY (*M. tuberculosis*) shows in the Table IV. Binding poses of the compounds **7a** and **7b** are as described in the Figure 7. Compounds **7a** and shows very good binding score as compared to the standard drug. The docking result shows that some of the synthesized derivatives had good affinity to the active site residue with respect to the standard reference drug Isoniazid.**7a,7b,7d,7h** and **7i** shows better glide score as compared to the reference isoniazid with respect to crystal structure of *M.Tuberculosis* (PDB:1ENY). In this system compound **7b** mainly interact with the amino acids such as GLY:96, LYS:165, PHE:41 and VAL:65.**7a** interact with the amino acids such as PHE:41, GLY:96, SER:94 and THR:196. Binding poses of the compounds having codes **7a** and **7b** shows in the Figure 7.

The results of binding affinity obtained by molecular docking are shown in the Table IV. The binding affinity correlates with their *in vitro* activity. In depth analysis was done for the compounds, which shows the best activity in *in vitro* assay as well as good binding affinity in molecular docking studies. For the antituberculosis activity among all compounds **7a** showed better binding affinity (-8.423 Kcal/mol, Table V) as compared to that of the Isoniazid (-6.33 Kcal/mol, Table V). Similarly compound **7a** showed good *in vitro* antituberculosis activity at the concentration of 50 µg/mL. The interaction of compound **7a** with protein 1ENY (DNA gyrase), the binding site shown in Figure 7.

Under *in vitro* antibacterial activity the compounds **7b, 7c, 7e** and **7i** exhibited minimum zone of inhibition and good binding affinity to Protein 4QRE. The compound **7b** compound has good binding affinity (-9.457Kcal/mol. Table V), which was comparable to Ampicillin (-4.947 K cal/mol Table V). Similarly compound **7b** showed good *in vitro* antibacterial activity at the concentration of 125. The interaction of compounds **7b** and **7c** with protein 4QRE (DNA gyrase), the binding site shows in Figure 8. In this system compound **7b** mainly interact with amino acids such as GLN- 55, ASP-51, TYR-14, ASP-51 and ILE-12. Such as **7c** compound mainly interact with amino acids GLN-55, ILE-12, TYR-14 and ASP-51. The binding sites shown in the Figure 8.

Under *in vitro* antifungal activity the compounds **7a, 7c, 7e** and **7f** and 7iexhibited minimum zone of inhibition and good binding affinity to Protein 5HUT. The compound **7c** compound has good binding affinity (-5.717 Kcal/mol, Table V), which was comparable to Nystain (-4.585K cal/mol Table V). Similarly compound **7c** showed good *in vitro* antifungal activity at the concentration of 250. The interaction of compounds **7a** and **7c** with protein 5HUT (DNA gyrase), the binding site shows in Figure 9 the interaction of compounds **7b** and **7a**with protein 5HUT (DNA gyrase), the binding site shows in Figure 9. In this system compound **7a** mainly

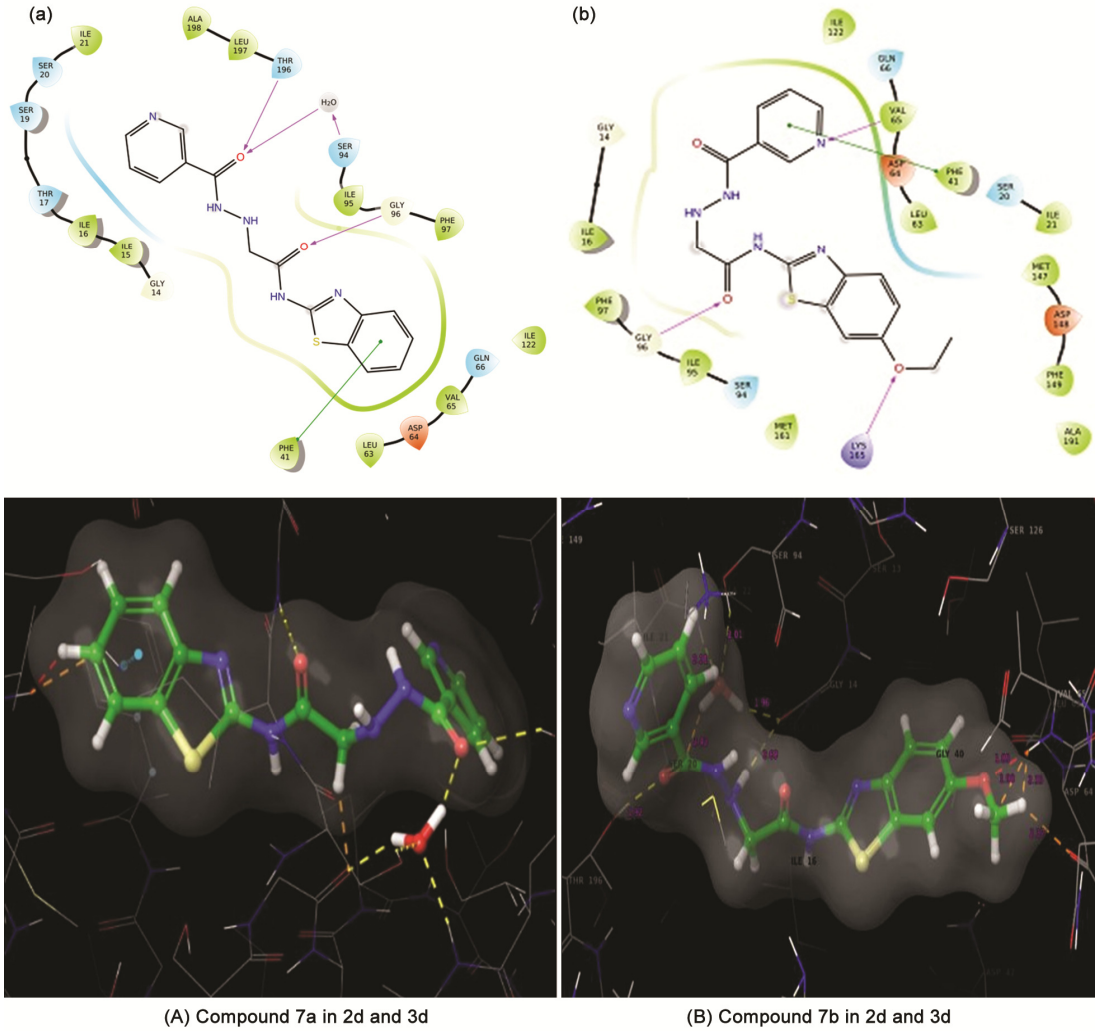


Figure 7 — Binding interaction of ligand **7a** (A = 3D and 2D) and **7b** (B = 3D and 2D) with *Mycobacterium tuberculosis* DNA complex (PDB: 1ENY)

Table V — Docking score of the derivatives of novel N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide

		1ENY (Anti-tuberculosis)		4QRE (Antibacterial)		5HUT (Antifungal)	
Entry	Compd	G-score	XP HBond	G-score	XP HBond	G-score	XP HBond
1	**7a**	−7.126	−1.195	−8.342	−1.139	−4.768	−1.225
2	**7b**	−9.055	−1.029	−9.457	−0.35	−5.156	−0.998
3	**7c**	−6.351	−1.179	−9.817	−0.914	−5.717	−2.086
4	**7d**	−8.423	−1.160	−5.68	−0.932	−4.299	−0.998
5	**7e**	−6.541	−0.348	−8.987	−0.855	−5.177	−1.399
6	**7f**	−6.664	−0.749	−5.6	−0.17	−5.376	−1.821
7	**7g**	−6.903	−0.989	−6.8	−0.273	−3.958	−2.114
8	**7h**	−7.209	−1.133	−6.38	−0.665	−4.729	−1.187
9	**7i**	−7.994	−1.143	−8.191	−0.665	−5.981	−1.462
10	**INH**	−6.223	−0.993	N.T	N.T	N.T	N.T
12	**AMP**	N.T	N.T	−4.947	−0.7	N.T	N.T
13	**NYS**	N.T	N.T	N.T	N.T	−4.585	−1.93
14	**GRE**	N.T	N.T	N.T	N.T	N.T	N.T

INH=Isoniazid, AMP=Ampicillin, NYS= Nystain, GRE= Greseofulvin, N.T=NOT tested

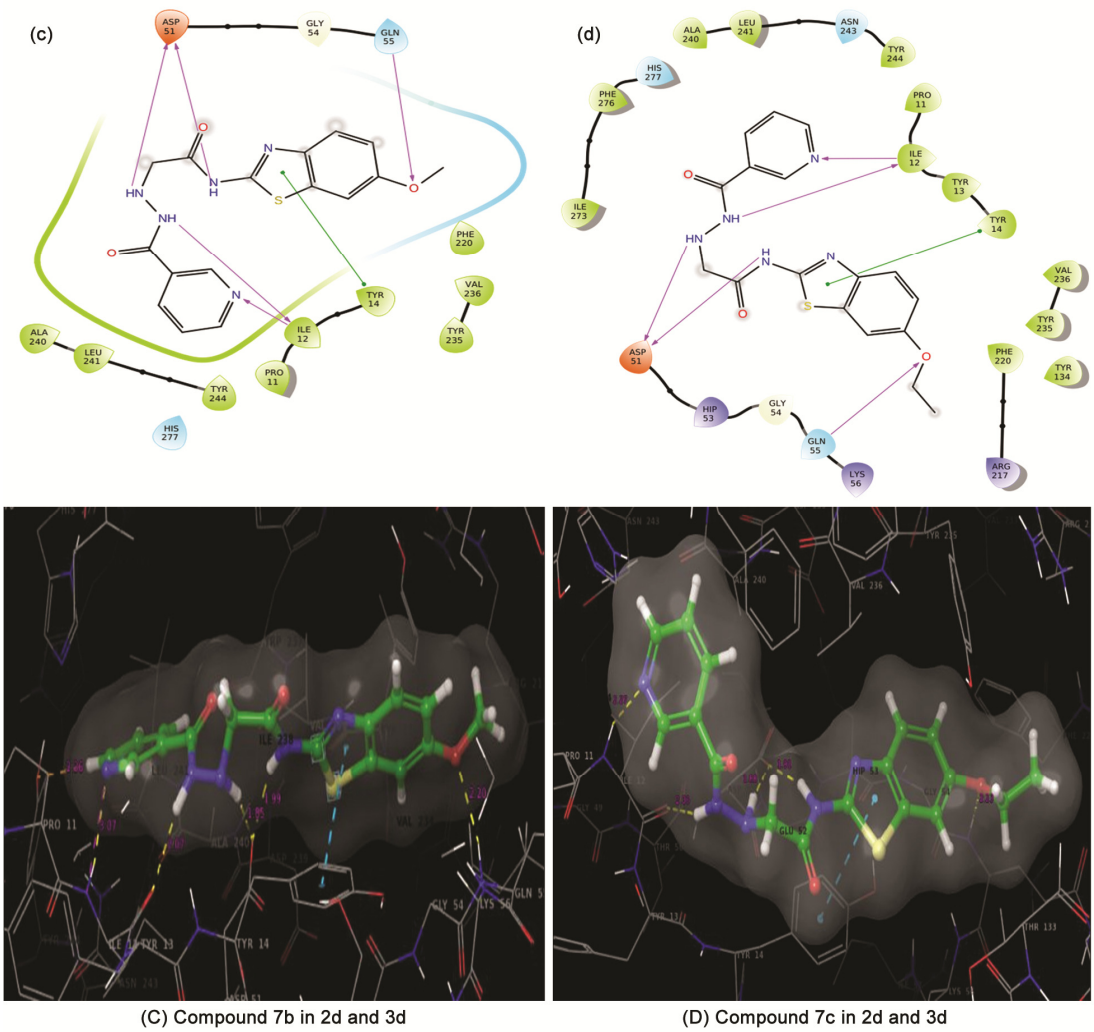


Figure 8 —Binding interaction of ligand: **7b** (C = 3D and 2D) and **7c** (D = 3D and 2D) with antibacterial (*S. aureus*) DNA complex (PDB: 4QRE cal representation of the biological activity of the compounds (antifungal activity)

interact with amino acids GLY-96, THR-196 and PHE-41. **7c** compound mainly interact with amino acids PHE-41, GLY-96 and LYS-165. The binding sites shown in the Figure 9.

Materials and Methods

All the required reagents and crude materials were procured from marketed sources and were utilized without any refinement. Completion of reaction and purity of all compounds were checked on coated thin layer chromatographic plates (TLC) plates 60245 (Merck) used as stationary phase and (MDC: Methanol) (9.5:0.5) utilized as mobile phase and visualized under ultraviolet light. Melting points were taken from Optimelt MPA 100 melting point apparatus. FT-IR spectra were recorded on a Perkin Elmer FT-IR 377 spectrometer using KBr powder. ^{1}H NMR was recorded on a Bruker AV400 MHz spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as the internal reference. Mass spectra was recorded at Advion epression CMS, USA. Molecular study was performed using Glide on Schrodinger Maestro. ADME prediction was done on the tool called Qikprop.

Experimental Section

Synthetic Protocol of Targeted Molecules

Synthetic procedure of N-(1, 3 benzothiazole-2-yl)-2 chloroacetamide) (Intermediate), 6a-i

In a 50 mL round bottomed flask (RBF), 1,3-benzothiazole-2-amine (1.0mmol) was dissolved in 25 mL of chloroform. In the same RBF add catalytic

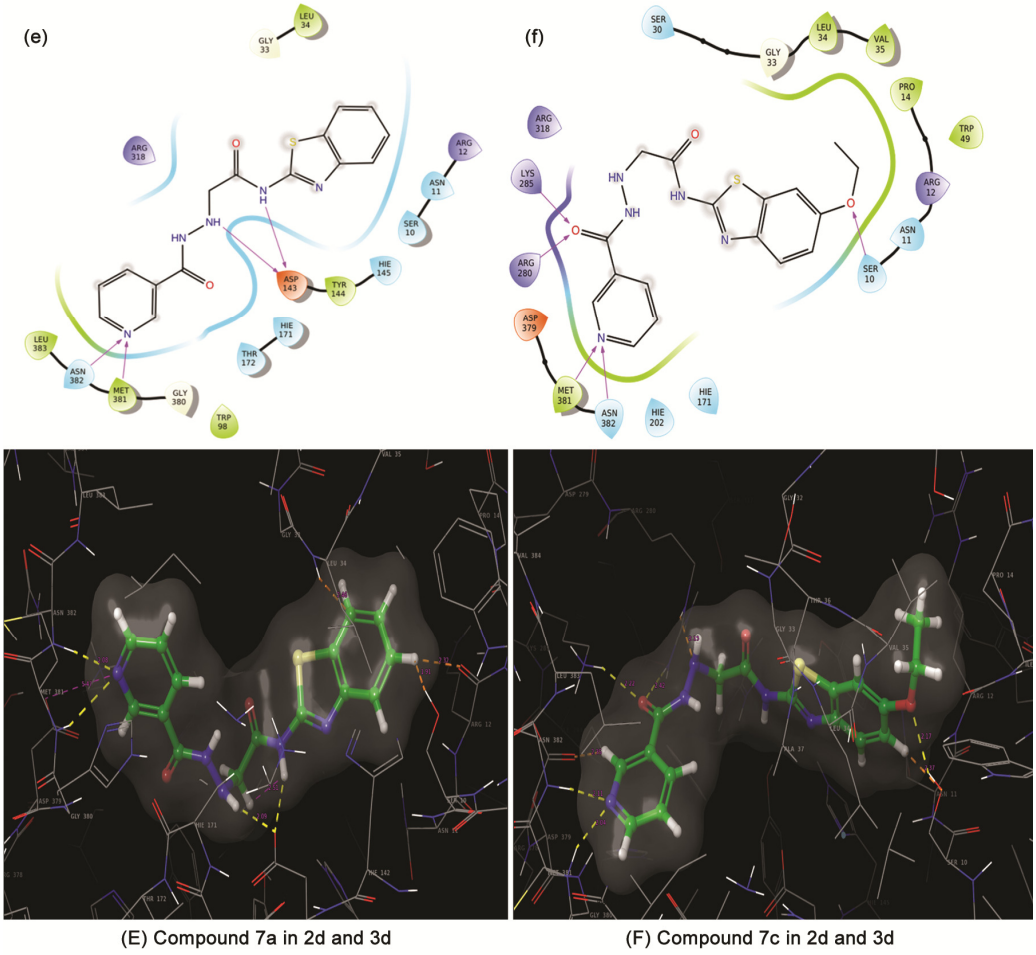


Figure 9 — Binding interaction of ligand) **7a** (E = 3D and 2D) and **7c** (F = 3D and 2D) with Antifungal (*C. albicans*) DNA complex (PDB: 5HUT)

amount of triethyl amine (1.0mmol) (TEA). Chloroacetyl chloride (CAC) (1.5mmol) was added drop by drop in to the solution. After the addition of all components it was stirred for 15 minutes at 0°C. After 15 minutes the reaction was stirred under a reflux condition for 6 h. The reaction progress was monitored on TLC plate MDC: Methanol (9.5:0.5). After completion of the reaction, solvent was distilled out from the solution and the dark yellow precipitates of N-(1,3 benzothiazole-2-yl)-2 chloroacetamide thus obtained was used for further reaction. The reaction product was identified by comparing their spectral data. Product in good yield (80%).

Dark yellow, mp 188-192°C; IR (KBr): 3361 (C-H Aromatic), 3498 (N-H), 1745 (C=C Aromatic), 1598 (C=O), 1323 (C-N), 869 cm^{-1} (C-Cl); ^1H NMR (400 Hz, DMSO-d_6): δ 8.01 (d, 1H), 8.18 (t, 1H), 7.53 (t, 1H), 7.22 (d, 1H), 9.17 (s, 1H), 4.21 (s, 2H); EI-MS: *m/z* 227.01 (M+2). Anal. Calcd for $C_9H_7ClN_2OS$: C, 47.60; H, 3.11; Cl, 15.64; N, 11.29; O, 7.06; S, 14.15. Found C, 47.52; H, 3.25; N,11.29; S, 14.12%.

Synthetic procedure of novel N-(1,3-benzothaizol-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide derivatives [Step2], 7a-i

In a 50 mL round bottom flask (RBF) N- (1, 3 benzothiazole-2-yl)-2 chloroacetamide (1.0mmol) was dissolved in 25 mL of DMF and add K_2CO_3 in to the flask. After the addition of K_2CO_3 Pyridine-3-carbohydrazide was added slowly in to the flask. After 15 minutes the reaction was stirred under the reflux condition 12 h. The reaction progress was monitored on TLC plate Ethyl Acetate: Hexane (5:5). After completion of the reaction, solvent was distilled out from the solution and the dark brown precipitates of novel N-(1,3-benzothiazole-2-yl)-2(pyridine-3-2(pyridine-3-yl-formohydrazido) acetamide obtained.

The reaction product was identified by comparing their spectral data. Further products were confirmed by Spectral data (^{1}H NMR, ESI-MS and IR).

N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide, 7a: Dark red solid, mp 159-162°C; IR (KBr) cm^{-1}: 3803 (N-H Aromatic), 3549 (C-H), 1745 (C=C Aromatic), 1745 (C=O), 1323 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.66 (s, 1H), 8.16 (d, *J* = 14.6 Hz, 2H), 8.09 (s, 1H), 8.02 (s, 1H), 7.55 – 7.43 (m, 3H), 3.74 (d, *J* = 7.7 Hz, 2H), 3.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 130.7 (C_1), 135.3 (C_2), 125.1 (C_3), 148.1 (C_4), 148.7 (C_6), 164.8 (C_7), 54.5 (C_{11}), 168.5 (C_{12}), 174.5 (C_{15}), 153.2 (C_{17}), 130.8 (C_{18}), 118.3 (C_{20}), 125.3 (C_{21}), 124.5 (C_{22}), 121.8 (C_{23} EI-MS *m/z* 328.01 (M+1). Anal. Calcd for $C_{15}H_{13}N_5O_2S$: C, 54.02; H, 3.00; N, 22.39; O, 9.76; S, 10.11. Found C, 54.14; H, 3.00; N,21.39; S, 10.09%.

N-(6, methoxy-1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide, 7b: Dark red, mp 178.6-180°C; IR (KBr) cm^{-1}: 3661 (N-H Aromatic), 3336 (C-H), 2856 (C=C Aromatic), 1680 (C=O), 1388 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.66 (s, 1H), 8.16 (d, *J* = 13.7 Hz, 2H), 8.00 (s, 1H), 7.79 (s, 1H), 7.53 (s, 1H), 7.11 (s, 1H), 3.82 – 3.77 (m, 3H), 3.74 (d, *J* = 8.6 Hz, 2H), 3.33 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 130.7 (C_1), 135.3 (C_2), 125.1 (C_3), 148.1 (C_4), 148.7 (C_6), 164.8 (C_7), 54.5 (C_{11}), 168.5 (C_{12}), 174.5 (C_{15}), 145.5 (C_{17}), 131.9 (C_{18}), 118.3 (C_{20}), 114.5 (C_{21}), 156.7 (C_{22}), 104.9 (C_{23}), 55.8 (C_{24}). EI-MS *m/z* 357.01 (M+1). Anal. Calcd for $C_{16}H_{17}N_5O_3S$: C, 53.77; H, 4.24; N, 19.60; O, 13.44; S, 8.99. Found C, 54.60; H, 3.23; N, 18.60; S, 8.70%.

N-(6, ethoxy-1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide, 7c: Dark brown, mp 182-185°C; IR (KBr) cm^{-1}: 3659 (N-H Aromatic), 3050 (C-H), 1469 (C=C Aromatic), 1653 (C=O), 1479 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.63 (d, *J* = 13.3 Hz, 2H), 8.19 (d, *J* = 10.8 Hz, 2H), 8.02 (s, 1H), 7.81 (s, 1H), 7.50 (s, 1H), 7.13 (s, 1H), 4.12 – 3.97 (m, 2H), 3.94 (s, 1H), 3.63 (s, 1H), 2.80 (s, 1H), 1.42 – 1.37 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 132.5 (C_1), 137.3 (C_2), 127.5 (C_3), 146.8 (C_4), 148.7 (C_6), 164.8 (C_7), 54.5 (C_{11}), 168.5 (C_{12}), 174.5 (C_{15}), 144.8 (C_{17}), 131.5 (C_{18}), 117.8 (C_{20}), 114.7 (C_{21}), 153.5 (C_{22}), 105.0 (C_{23}), 64.6 (C_{24}), 14.8 (C_{25}). EI-MS *m/z* 372 (M+1). Anal. Calcd for $C_{17}H_{17}N_5O_3S$: C, 54.97; H, 4.61; N, 18.95; O, 12.11; S, 8.90. Found C, 53.80; H, 4.50; N, 18.55; S, 8.22%.

N-(6, hydroxy-1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide, 7d: Dark brown, m.p 191-193°C; IR (KBr) cm^{-1}: 3659 (N-H Aromatic), 3067 (C-H), 1450 (C=C Aromatic), 1653 (C=O), 1379 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.63 (d, *J* = 13.5 Hz, 2H), 8.19 (d, *J* = 12.0 Hz, 2H), 7.90 (s, 1H), 7.69 (s, 1H), 7.50 (s, 1H), 7.03 (s, 1H), 4.95 (s, 1H), 3.94 (s, 1H), 3.63 (s, 1H), 2.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 132.5 (C_1), 135.3 (C_2), 124.5 (C_3), 144.8 (C_4), 149.7 (C_6), 168.8 (C_7), 58.5 (C_{11}), 168.5 (C_{12}), 177.3 (C_{15}), 147.1 (C_{17}), 135.5 (C_{18}), 120.8 (C_{20}), 114.7 (C_{21}), 163.5 (C_{22}), 106.1 (C_{23}). EI-MS *m/z* 345 (M+1). Anal. Calcd for $C_{15}H_{13}N_5O_3S$: C, 52.47; H, 3.82; N, 20.40; O, 13.99; S, 8.34. Found C, 52.44; H, 3.70; N, 20.22; S, 7.34%.

N-(6,chloro-1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido)acetamide, 7e: Dark brown, mp.:182-185°C; IR (KBr) cm^{-1}: 3644 (N-H Aromatic), 3055 (C-H), 1700 (C=C Aromatic), 1680 (C=O), 1336 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.63 (d, *J* = 14.3 Hz, 2H), 8.27 – 8.15 (m, 3H), 7.99 (s, 1H), 7.51 (d, *J* = 6.1 Hz, 2H), 3.93 (s, 1H), 3.63 (s, 1H), 2.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 130.7 (C_1), 135.3 (C_2), 125.1 (C_3), 148.1 (C_4), 148.7 (C_6), 164.8 (C_7), 54.5 (C_{11}), 168.5 (C_{12}), 175.3 (C_{15}), 151.3 (C_{17}), 132.3 (C_{18}), 118.3 (C_{20}), 125.8 (C_{21}), 129.8 (C_{22}), 121.2 (C_{23}). EI-MS *m/z* 328.1 (M+2). Anal. Calcd for $C_{15}H_{12}ClN_5O_2S$: C, 40.79; H, 3.34; Cl, 9.80; N, 19.36; O, 8.70; S, 8.16. Found C, 39.72; H, 3.20; Cl, 9.90; N, 19.20; S, 8.15%.

N-(6,fluoro-1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido)acetamide, 7f: White solid, mp 160-166°C; IR (KBr) cm^{-1}: 3644 (N-H Aromatic), 3055 (C-H), 1700 (C=C Aromatic), 1680 (C=O), 1336 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H), 8.63 (d, *J* = 13.8 Hz, 2H), 8.19 (d, *J* = 7.3 Hz, 2H), 8.00 (d, *J* = 34.6 Hz, 2H), 7.50 (s, 1H), 7.27 (s, 1H), 3.93 (s, 1H), 3.63 (s, 1H), 2.80 (s, 1H);); ^{13}C NMR (100 MHz, DMSO-d_6): δ 135.7 (C_1), 140.3 (C_2), 131.1 (C_3), 144.6 (C_4), 152.7 (C_6), 169.4 (C_7), 64.5 (C_{11}), 162.5 (C_{12}), 170.3 (C_{15}), 148.3 (C_{17}), 136.2 (C_{18}), 116.4 (C_{20}), 123.1 (C_{21}), 158.5 (C_{22}), 108.0 (C_{23}). EI-MS *m/z* 345 (M+1). Anal. Calcd for $C_{15}H_{12}FN_5O_2S$: C, 52.17; H, 3.50; F, 5.60; N, 20.28; O, 9.25; S, 9.28. Found C, 51.17; H, 3.52; F, 5.55; N, 20.14; S, 8.18%.

N-(1,3-benzothiazole-2-yl)-2-(pyridine-4-ylformohydrazido) acetamide, 7g: Dark red mp 159-162°C; IR (KBr) cm^{-1}: 3803 (N-H Aromatic), 3549 (C-H), 1745 (C=C Aromatic), 1745 (C=O), 1323 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.91 – 8.72 (m, 2H), 8.23 (s, 1H), 8.11 (s, 1H), 8.02 (s, 1H), 7.89 – 7.70 (m, 2H), 7.49 (d, J = 1.1 Hz, 2H), 4.25 (s, 1H), 3.55 (s, 1H), 2.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 140.8 (C_1), 121.7 (C_2), 149.7 (C_3), 149.7 (C_4), 121.7 (C_6), 167.7 (C_7), 67.4 (C_{11}), 168.5 (C_{12}), 174.3 (C_{15}), 158.3 (C_{17}), 128.2 (C_{18}), 119.1 (C_{20}), 125.3 (C_{21}), 130.5 (C_{22}), 121.8 (C_{23}). EI-MS *m/z* 328.01 (M+1). Anal. Calcd for $C_{15}H_{13}N_5O_2S$: C, 54.02; H, 3.00; N, 22.39; O, 9.76; S, 10.11. Found C, 54.14; H, 3.00; N, 21.39; S, 10.09%.

N-(6, methoxy-1,3-benzothiazole-2-yl)-2(pyridine-4-ylformohydrazido) acetamide, 7h: Dark red, mp 170.5-175°C; IR (KBr) cm^{-1}: 3661 (N-H Aromatic), 3336 (C-H), 2856 (C=C Aromatic), 1680 (C=O), 1388 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.91 – 8.72 (m, 2H), 8.24 (s, 1H), 8.00 (s, 1H), 7.84 – 7.70 (m, 3H), 7.12 (s, 1H), 4.25 (s, 1H), 3.83 – 3.78 (m, 3H), 3.55 (s, 1H), 2.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 144.8 (C_1), 129.2 (C_2), 152.8 (C_3), 152.8 (C_4), 129.2 (C_6), 162.1 (C_7), 58.4 (C_{11}), 169.5 (C_{12}), 177.3 (C_{15}), 145.5 (C_{17}), 131.9 (C_{18}), 118.2 (C_{20}), 114.6 (C_{21}), 156.7 (C_{22}), 104.9 (C_{23}), 55.8 (C_{24}). EI-MS *m/z* 357.01 (M+1). Anal. Calcd for $C_{16}H_{17}N_5O_3S$: C, 53.77; H, 4.24; N, 19.60; O, 13.44; S, 8.99. Found C, 54.60; H, 3.23; N, 18.60; S, 8.70%.

N-(6, ethoxy-1,3-benzothiazole-2-yl)-2(pyridine-4-ylformohydrazido)acetamide, 7i: Dark brown, mp 172-175°C; IR (KBr) cm^{-1}: 3659 (N-H Aromatic), 3050 (C-H), 1469 (C=C Aromatic), 1653 (C=O), 1479 cm^{-1} (C-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.90 – 8.72 (m, 2H), 8.24 (s, 1H), 8.00 (s, 1H), 7.85 – 7.70 (m, 3H), 7.12 (s, 1H), 4.25 (s, 1H), 4.12 – 3.97 (m, 2H), 3.55 (s, 1H), 2.66 (s, 1H), 1.42 – 1.37 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 141.8 (C_1), 123.7 (C_2), 150.1 (C_3), 150.1 (C_4), 123.7 (C_6), 169.4 (C_7), 64.4 (C_{11}), 162.5 (C_{12}), 178.3 (C_{15}), 148.5 (C_{17}), 131.1 (C_{18}), 118.2 (C_{20}), 112.6 (C_{21}), 158.7 (C_{22}), 107.2 (C_{23}), 64.6 (C_{24}), 14.8 (C_{25}). EI-MS *m/z* 372 (M+1). Anal. Calcd for $C_{17}H_{17}N_5O_3S$: C, 54.97; H, 4.61; N, 18.95; O, 12.11; S, 8.90. Found C, 53.80; H, 4.50; N, 18.55; S, 8.22%.Synthetic route of N-(1,3-benzothiazole-2-yl)-2(pyridine-3-ylformohydrazido) acetamide derivatives is shown in the Table I.

In vitro biological Screening

We have carried out biological screening at Microcare Laboratory and TRC, Surat, India as per earlier reported methods[36,37]. All synthesized compounds were evaluated in-vitro for their antimicrobial efficacy against two gram positive bacterial strains (*Staphylococcusaureus* MTCC 96, *Streptococcus pyogenes* MTCC 442), two gram negative bacterial strains(*Escherichia coli.* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus Niger* MTCC 282), anti-tubercular activity against H_{37}Rv and anti-malarial efficacy against *Plasmodium falciparum* respectively. In antibacterial evaluation, ampicillin, was used whereas in antifungal evaluation, Greseofulvin and Nystain were used as standard control drugs. Isoniazid was used as a standard drug for antitubercular.

Computational Study

In-silico ADMET prediction and drug likeness study

The pharmacokinetic parameters such as absorption, distribution, metabolism and excretion (ADME) were evaluated by using Qikprop tool of Schrodinger software for forecasting the drug-likeness properties of all synthesised molecules. This software package used for the exact possessions of molecules as compared with 95% of known drugs. It also assesses the fittingness of the compounds based on Lipinski's rule of five for checking the drug like properties. Some other significant properties such as Lipinski's rule of 5 (molecular weight, hydrogen bond donor (Donor-HB), hydrogen bond acceptor (Accept-HB),octanol/water partition coefficient (QlogPo/w), aqueous solubility (QPlogS)) and ADMET. Parameter such as brain and blood partition co-efficient (QlogBB), percent human oral absorption were reflected for our present study to evaluate the potentiality of the drug.

Molecular docking Study

From the RCSB Protein Data Bank, protein structure coordinates (PDB Code 1ENY, 4QRE & 5HUT were procured. The Glide score is calculated by using the following equation[38]:

GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB +Site

Where, vdW denotes the van der Waals energy; Coul is the coulomb energy; Lipo denotes the lipophilic contact term; Hbond is the hydrogen-bonding term; Metal is the metal binding term; BuryP represents the penalty for buried polar groups; RotB is

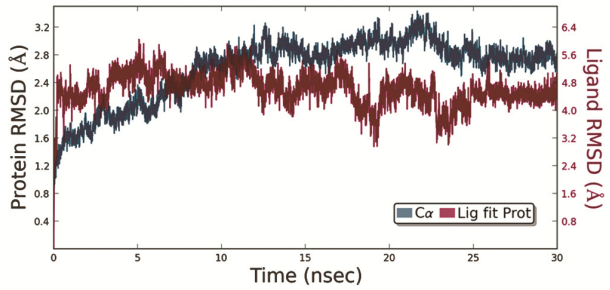


Figure 10 — The Root Mean Square Deviations (RMSD) of backbone atoms relative to the starting complexes during 30 ns MD simulation of 1PFZ-12c. Each plot shows the RMSD of protein on left Y-axis whereas ligand RMSD is presented on right Y-axis

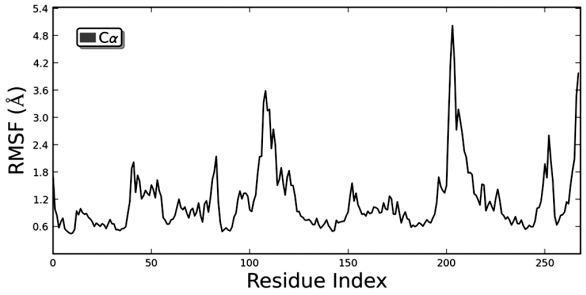


Figure 11 — The Root Mean Square Fluctuation (RMSF) of complexes during 50 ns MD simulation of protein-1ENY

the penalty for freezing rotatable bonds and Site is the polar interactions at the active site.

Molecular Dynamics Simulation

The coordinates of the best docking configurations of selected 1ENY-7a complexes were subjected to molecular dynamics simulation using Desmond simulation package of Schrödinger Materials Science Suite 2015-4. Molecular dynamics is a one type of modern tool to explore the interaction between inhibitor and protein. The simulation were applies on the molecule which had the best docking score which is **7a**. MD simulations supported to have correct synchronizes for the molecular docking studies and structural variation have been found between the bound and unbound systems docked cavity of present ligand. **7a** formed inhibitor bound and catalytic active site of PHE 41 and GLY 96. **7a** show more flexibility during the simulation. Molecular dynamics was performed to find the best equilibrium confirmation. Ligand with the best docking score was selected for the analysis. Protein-ligand interactions (or 'contacts') are categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. According to the interaction and forces **7a** ligand fluctuated at some level yielding a RMSD value of 1.4Å. The analysis results of simulation are shown in the Figure 10,

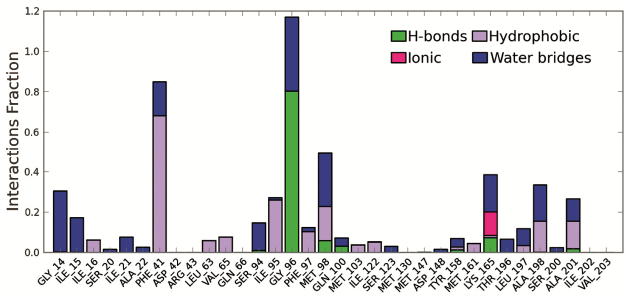


Figure 12 — — Per-residues analysis of the representative **7a** in complex with 1ENY. The analysis was based throughout the 30-ns MD simulations. Hydrogen bond, hydrophobic, ionic and water bridge Protein-ligand interactions are illustrated by green, purple, and blue color, respectively

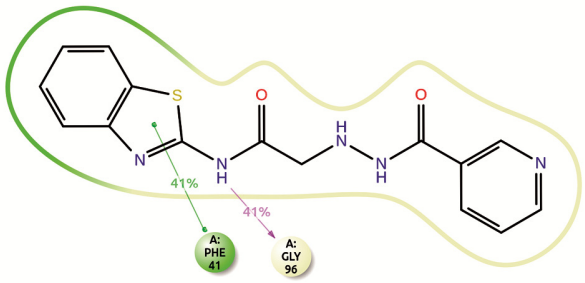


Figure 13 — Desmond MD calculated protein (1ENY) – ligand **7a** contacts at the allosteric site region

Figure 11, Figure 12, and Figure 13. (A-G).The input as well as output files were operated by Maestro graphical user interface application for Schrödinger Materials Science Suite 2015-2016.

Conclusions

In the summary novel series of novel substituted N-(1,3-benzothiazole-2-yl)-2-(pyridine-3-ylformohydrazido) acetamide derivatives were synthesized in very good yields. All the newly synthesized compounds **7a-7i** were screened for antibacterial, antifungal, anti-tuberculosis activity and the results showed good activity in antibacterial and antifungal activities when compared with the standard drugs. *In-vitro* antibacterial data indicated that all compounds illustrated with appreciable antibacterial activity (MIC µg/mL) against *Staphylococcus aureus* (MTCC 96), and antifungal activity (MIC µg/mL) against *Candida albicans* (MTCC 227). Among the entire tested compounds compound **7a** shows good activity against H37Rv strains by measuring the minimum inhibitory concentration (MIC µg/mL). Compounds **7g** and **7i** shows potent activity against the *M.tuberculosis*. Other compounds show negligible activity against *M.tuberculosis*. Among the gram

negative bacterial strain *S. aureus* showed relatively higher sensitivity towards the tested compounds. In the view, analogue compounds **7d** and **7i** (MIC 100 µg /mL) displayed good activity against *S. aureus* as compared to the standard drug Ampicillin (MIC 250 µg/mL). Compounds **7g** and **7f** (MIC 125 µg/mL) also displayed good activity against *S.aureus* as compared to Ampicillin. Compounds **7b, 7g, 7e** and **7h** (MIC 250 µg/mL) were showing equipotent activity against *S.aureus as* compared to Ampicillin. All synthesized compounds were tested against *In-vitro* antifungal activity only once strain which is *C.albicans* show certain sensitivity against some of the tested compounds, the rest of other two fungal strain were inactive to the same compounds. The standard marketed drugs against the synthesized derivatives were Greseofulvin, Nystatin. Compounds **7a 7c, 7d, 7e, 7f** and **7h** show excellent activity against *C.albicans* compared to the standard drug Greseofulvin. We have also done molecular docking studies with protein (PDB: 1ENY (*M.tuberculosis*), 4QRE (*S.aureus*) and (5HUT) (*C.albicans*). In this study we designated interaction between ligand and protein. Biological activity and docking results of derivatives creates fascinating lead in drug development. The deliberate ADME parameters recommend good pharmacokinetic properties. Molecular dynamics simulation can be concluded that MD can be successfully fulfilled for new drug development. Overall this study discloses that active molecules are used as term plate for the development of active biological agents.

Conflicts of interest

Authors have declared that there is not any conflict of interest.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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