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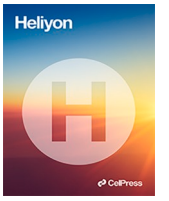

Research article

Glycerol based carbon sulfonic acid catalyzed synthesis, in silico studies and in vitro biological evaluation of isonicotinohydrazide derivatives as potent antimicrobial and anti-tubercular agents

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ABSTRACT

The present pathway involves synthesis of isonicotinohydrazide derivatives using isoniazid and diversely substituted aldehydes in the presence of EtOH and catalytic amount of glycerol based carbon sulfonic acid catalyst. The developed pathway has so many merits like excellent yields (91–98%), short reaction time (4–10 min), easy reaction set up, no need of column chromatography, large substrate scope, easily recyclable and reusable catalyst. The synthesized compounds were screened for antimicrobial and anti-tubercular activity and it was observed that compounds possessed high biological potency against the Gram positive and Gram negative bacterial and fungal strains. Regarding anti-tubercular activity, compound **3m** exhibited high % inhibition against *Mycobacterium tuberculosis* $H_{37}RV$ strain. Based on the outcome of in vitro studies, all the synthesized compounds were docked against *E. coli* (1KZN), *C. albicans* (1IYL), and *M. tuberculosis $H_{37}Rv$* strain (2NSD). The synthesized derivatives were docked within the binding site of 1KZN, and 1IYL. However, with 2NSD, apart from **3h**, all the derivatives displayed interaction within the binding cavity of the protein. All the crucial interactions with Asn46, Asp73, and Arg136 in 1KZN, His227, Leu451 in 1IYL, and Tyr158 in 2NSD were witnessed in the top-scored docked candidates. Molecular docking studies revealed the importance of the substitution at R position on isonicotinohydrazide scaffold. The nitrogen atoms of hydrazide moiety were involved in forming hydrogen bonding with the active site amino acids, and the substitution at the R position occupy the hydrophobic position in the binding pocket. Also, the functional groups present on the substituted R position were involved in forming hydrogen bonding with the crucial active site residues.

1. Introduction

Tuberculosis (TB) is a highly contagious and foremost deadliest disease all over the world that primarily affects the lungs. It is

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originated from bacteria *Mycobacterium tuberculosis (Mtb)*. As per WHO report 2020, it is the 13th primary reason behind the mortality of people all over the globe and after corona virus, it is the next infectious killer. Approximately 2 billion US dollar have been invested for TB research globally from 2018 to 2022.

For the treatment of TB, several heterocyclic scaffolds based drugs are market available and have been used. Among these drugs, isoniazid has gained much attention from the researchers for the treatment of patients suffering from TB. Numerous analogs of isoniazid have been synthesized and possessed anti-tubercular activity [1,2]. It has been used as a building block for the synthesis of several therapeutic potent molecules like azetidinone, lactam, oxadiazole [3,4,5], etc. Its analogs possess miscellaneous applications like anti-depressant, and nootropic agent [6], antimicrobial [7,8], anti-inflammatory and anti-diabetic [9], anti-cancer [10], anti-hyperlipidemic agents [11] and anti-malarial [12], act as ligand to form metal complexes [13,14], and so on [15,16]. They are also used in tunable photoswitches [17].

Schiff bases (imines/azomethines) are synthesized from primary amines and aldehydes/ketones. They have several applications in synthetic organic chemistry like intermediates, ligands to form complexes, pigments, dyes, and many more [18,19]. They also possess biological applications like anti-fungal, anti-malarial, anti-proliferative, anti-bacterial, anti-viral, anti-inflammatory, antipyretic, and many more [20,21]. Schiff bases of isoniazid (Isonicotinoylhydrazones) are active pharmacophores and possess promising biological applications like anti-tubercular [22], anti-convulsant [23,24], anti-depressant [6], xanthine oxidase inhibitory activity [25], anti-microbial [26], anti-oxidant, anti-Alzheimer's [27], and many more [28].

The designing of eco-friendly procedure in synthetic chemistry field is inevitable. Nowadays, carbon based solid acid catalysts play a vital role in organic reactions. Various organic compounds like chitosan, cellulose, starch, caffeine, glycerol, and many more are sulfonated and carbonized to fabricate a robust heterogeneous solid acid catalyst [29]. Among such catalysts, glycerol based solid acid catalyst is very renown and was first come into limelight by Devi and co-workers [30]. The authors synthesized the catalyst by *in situ* carbonization and sulfonation of glycerol and used it in the synthesis of bio-diesel formation. This catalyst also has several applications in heterocyclic chemistry [31,32].

Encouraged by the promising catalytic activity of our previously synthesized and characterized glycerol based carbon sulfonic catalyst [33,34,35], a facile protocol for the synthesis of isonicotinohydrazide analogs using glycerol based sulfonic acid as a catalyst has been demonstrated (Scheme 1).

Furthermore, the synthesized compounds (**3a-o**) were tested against using different gram-positive bacteria *S. aureus* (ATCC No. 25923), *B. subtilis* (ATCC No. 29212), gram-negative bacteria *E. coli* (ATCC No. 25922), *B. shigella* (ATCC No. 27853) and fungi *P. chrysogenum* (ATCC No. 25922), *M. furfur* (ATCC No. 25922), *A. niger* (ATCC No. 25922), *P. notatum* (ATCC No. 25922).

Regarding molecular docking studies, they were docked against *E. coli* (Gram-negative bacteria) 1KZN, *C. albicans* (fungal pathogen) 1IYL, and *M. tuberculosis* H_{37}Rv strain (tuberculosis) 2NSD proteins.

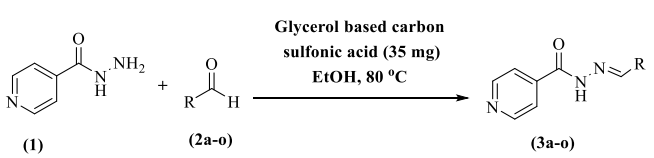
2. Results and discussion

Firstly, the catalyst glycerol based carbon sulfonic acid was prepared by earlier published methodology [30,33]. Its synthesis was confirmed by different techniques such as FT-IR, FE-SEM, XRD, TGA, DTA, and all the results were in accordance with the previously reported articles. In addition to the investigation on catalytic efficiency of glycerol based carbon sulfonic acid catalyst towards heterocyclic compounds [33–35], a highly efficient pathway for the synthesis of Schiff bases of isoniazid with diversely substituted aldehydes was developed.

The pilot reaction was initiated with isoniazid (0.5 mmol) and 4-CN benzaldehyde (0.5 mmol) in ethanol using 20 mg of glycerol based carbon sulfonic acid at room temperature (Table 1, Entry 1). The progression in reaction was predicted by a change in the color of reaction mixture as monitored by TLC. It is interesting to observe that after 18 min, 88% yield of the desired compound was obtained (Table 1, Entry 1). With positive results in hand, the reaction mixture was refluxed and there was an increase in the yield of product and reaction time also decreased (Table 1, Entry 2). Furthermore, the effect of catalyst loading on the pilot reaction was also studied by successive increase in the amount of catalyst (Table 1, Entry 4–7). It was interesting to note that 35 mg of catalyst was found to be adequate for the formation of desired product.

With optimized conditions of catalyst amount and temperature, the effect of solvent on the model reaction was also scrutinized. For this, solvents like H_2O, EtOH, H_2O:EtOH (1:1), and methanol were examined (Table 2, Entry 1–4). It was noteworthy that the highest yield was obtained with EtOH as a solvent. So for further reactions, 35 mg of catalyst, EtOH, 80 °C were the best optimized reaction condition.

Afterward, with optimized reaction conditions, a library of isonicotinohydrazide analogs were synthesized by taking diversely substituted aromatic aldehydes (with electron donating, electron-withdrawing substituents, heterocyclic and polycyclic) (Table 3). All aldehydes gave excellent yields (91–98%) of desired products in a short period of time (4–10 min). Furthermore the efficacy of



Scheme 1. Synthesis of isonicotinohydrazide analogs using isoniazid and aldehydes.

Table 1
Effect of different amount of catalyst and different temperature on model reaction.

S.No.	Catalyst loading	Time	Temperature	Solvent	Yield (%)
1	20 mg	18 min	RT	EtOH	88
2	20 mg	12 min	80 °C	EtOH	90
4	25 mg	8 min	80 °C	EtOH	92
5	30 mg	8 min	80 °C	EtOH	94
6	35 mg	4.5 min	80 °C	EtOH	97
7	40 mg	4.5 min	80 °C	EtOH	97

*Conditions: 1 mmol of each isoniazid, 4-CN benzaldehyde were taken.

Table 2
Optimization of solvents for the preparation of Schiff base of isoniazid.

Entry	Solvent	Time	Yield (%)
1	H_2O	25 min	80
2	C_2H_5OH	4.5 min	97
3	H_2O: EtOH	15 min	85
4	MeOH	10 min	90

Conditions: 1 mmol of isoniazid and 4-CN benzaldehyde at reflux temperature with 35 mg of catalyst.

designed protocol was compared with the previously published procedure (Table 4).

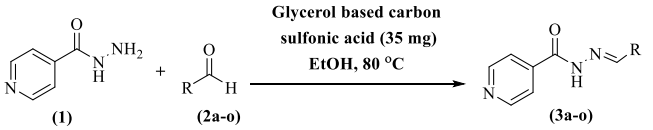
Inspite of less reaction time in some previously reported methods [24,36–38], the present protocol is more fruitful because earlier reported protocols include harsh reaction conditions, metal-contamination, tiresome process, need work-up for purification, no recyclability of catalyst, less substrate scope, etc. Whereas, the designed pathway has several benefits like reusability of catalyst up to five runs, cost-effective, excellent yields, large substrate scope. All the synthesized compounds (3a-o) (Fig. 1) were well characterized by melting point and spectroscopy.

The suggested mechanism for the synthesis of isonicotinohydrazide derivatives involved a simple nucleophilic addition reaction. Initially, catalyst activated the aldehyde and this activated aldehyde attacked on isoniazid and liberated H_2O to form a Schiff base.

2.1. Screening and reusability of catalyst

To recover the catalyst, methanol was added in the reaction mixture. As the product is soluble in methanol and catalyst being insoluble, can be easily recovered by simple filtration. The recovered catalyst was washed with ethanol to remove any other impurities, and then put in hot-air oven for approximately 2 h and now it could be reused further for a new reaction. The recovered catalyst could be reused further for up to fifth runs devoid of any momentous changes in the yield of desired product. For first 2 runs of reused catalyst, no change in the yield of product was observed in assessment with the yield of fresh catalyst. On the other hand for successive

Table 3
Synthesis of isonicotinohydrazide analogs in the presence of glycerol based carbon sulfonic acid.



Entry	R	Symbol for product	Time (min)	Yield (%)	M.P. (°C) Observed literature	
1	4-$NMe_2C_6H_4$	3a	8.5	95	193–195	192-194 [24]
2	4-CN C_6H_4	3b	4.5	97	270	[14]
3	4-OH C_6H_4	3c	5	94	250–252	242-245 [24]
4	3-Br C_6H_4	3d	5	92	245–247	248-250 [39]
5	2,3,4-$(OMe)_3$ C_6H_2	3e	5	91	178–180	–
6	4-Methylthiazole	3f	8	95	254–256	[10]
7	2-Benzyloxy	3g	10	92	169–170	–
8	1-Pyrene	3h	8	93	200–202	[10]
9	Furfural	3i	10	94	188–190	[10]
10	4-NO_2 C_6H_4	3j	4.5	96.5	191–193	190-192 [38]
11	4-F C_6H_4	3k	4	98	257–259	258-260 [38]
12	3,4-$(OMe)_2$ C_6H_3	3l	9	91.5	168–170	167-169 [24]
13	4-OH-3-OMe C_6H_3	3 m	4.5	96	210–212	212-214 [38]
14	3,4,5-$(OMe)_3$ C_6H_2	3n	8	94	198–201	205 [40]
15	2-Me C_6H_4	3o	5	94	178–180	[41]

Table 4
A comparative study of designed protocol with different catalyst for the synthesis of isonicotinohydrazide analogs.

Entry	Catalyst	Reaction conditions	Yield (%)	Recyclability of catalyst	Ref.
1.	Glacial acetic acid	Ethanol, 3 h, reflux (12 derivatives)	65–75	–	[24]
2.	–	H_2O, MWI, Power level 3 (240 W, 35% irradiation), 6–8 min (5 derivatives)	90.7–98.1	–	[5]
3.	p-toluene sulfonic acid	(0.5 mol/mol of catalyst) vibrating ball mill, 50 Hz, 2.0 mm, 2 h, RT (10 derivatives)	80–99	–	[2]
4.	$NiCl_2$. $6H_2O$	(0.5 mol/mol of catalyst), pH = 5, 1–2 h, reflux, methanol, few drops of acetic acid. (4 derivatives)	79–89	–	[11]
5.	Modified Fly Ash zeotype catalyst	(10% w/w of catalyst) EtOH, RT, 5–15 min (8 derivatives)	84–93	Up to three runs	[36]
6.	Ceric ammonium nitrate	(0.25 mmol) of catalyst, reflux, EtOH, 30 min (7 derivatives)	77–89	–	[37]
7.	Citric acid	(10 mol% of catalyst) EtOH:H_2O (8:2), reflux, 5–15 min (15 derivatives)	88–97	–	[38]
8.	Glycerol based carbon sulfonic acid catalyst (35 mg)	35 mg of catalyst, EtOH, reflux, 4–10 min (15 derivatives)	91–98	Up to five runs	Present work

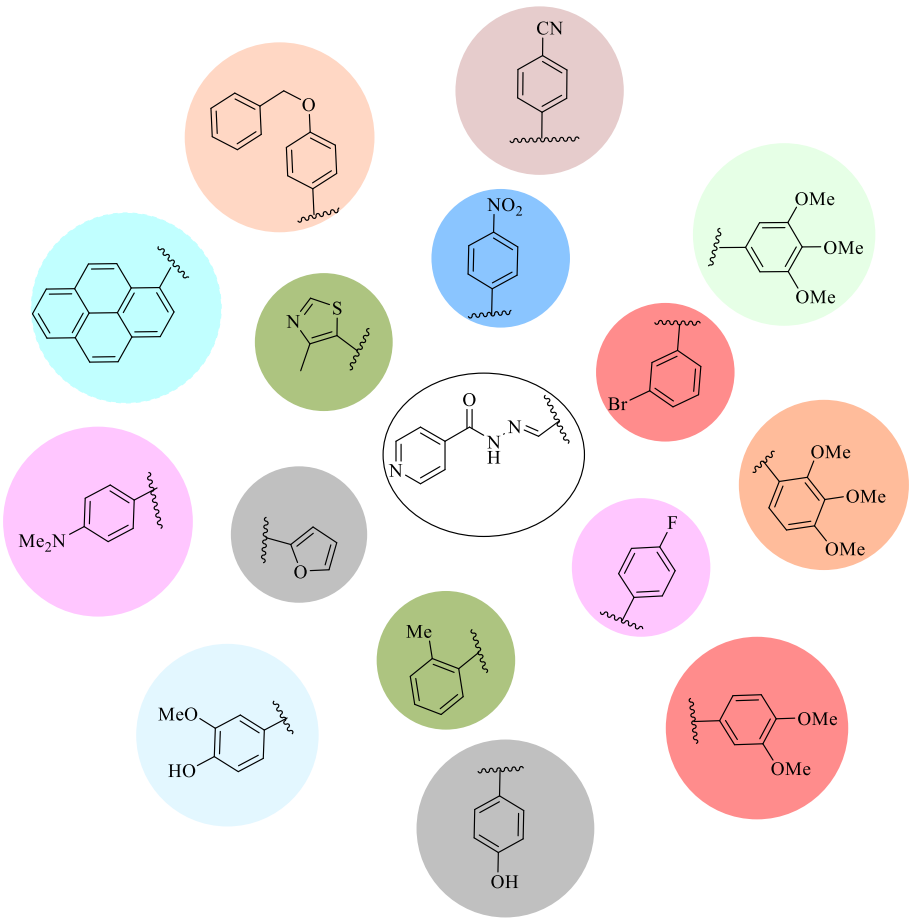


Fig. 1. Library of synthesized isonicotinohydrazide derivatives.

runs, there is a decrease in the yield of product as observed with increased time (Fig. 2). The XRD of recovered catalyst after fifth run is illustrated in Fig. 3.

2.2. Antimicrobial activity

The synthesized isonicotinohydrazide derivatives were screened for antimicrobial activity using a broad panel of gram-positive bacteria *S. aureus* (ATCC No. 25923), *B. subtilis* (ATCC No. 29212), gram-negative bacteria *E. coli* (ATCC No. 25922), *B. shigella* (ATCC No. 27853) and fungi *P. chrysogenum* (ATCC No. 25922), *M. furfur* (ATCC No. 25922), *A. niger* (ATCC No. 25922), *P. notatum* (ATCC No. 25922). It was observed that most of the compounds exhibited excellent activity and few of them exhibited moderate/poor activity. The results of antimicrobial activity, their zone of inhibition and MIC values are summarized in Tables 5 and 6.

From the MIC results, it was found that compound 3h was active against *S. aureus* and others showed low to moderate activity. The synthesized derivatives (3a-o) showed poor activity against *B. subtilis*. Compounds 3a, 3b, 3e, 3f, 3l, 3 m, 3n, and 3o were more potent than the reference drug ciprofloxacin against *E. coli* whereas 3c, 3d, 3g, and 3j have comparable activity to reference drug against *E. coli*. Compounds 3a, 3e, 3g, and 3n were found active against *B. shigella* and compound 3f was more active than the reference drug against *B. shigella*. In general, the synthesized derivatives showed significant activity against Gram negative bacteria in comparison to Gram positive bacteria. For anti-fungal activity, it was observed that compounds 3a and 3l were active against *P. chrysogenum* and compounds 3a, 3b, and 3e have comparable activity and 3g, 3 m, 3n, and 3o have higher activity against *M. furfur* as compared to fluconazole. Compounds 3b, 3f, 3g, and 3h were found active against *A. niger*. Compound 3a was more active whereas compound 3c was similar active as reference drug against *P. notatum*. It was found that compounds were more active against fungal strains as compared to bacterial strains. As per SAR, the compounds having electron donating group (OMe, NMe_2, OH, Me) were more active than the compounds having electron withdrawing group. The functional groups like OMe, OH, NMe_2, Me had crucial interactions and can have H-bonding due to this they showed greater activity.

2.3. Anti-tubercular activity

The newly synthesized compounds 3a-o were screened for their *in-vitro* anti-tubercular activity at 100 μg/mL against *Mycobacterium tuberculosis* $H_{37}RV$ strain in BACTEC 12B medium *via* microplatealamar blue assay [42]. The anti-tubercular studies results for the isonicotinohydrazide derivatives exhibiting more than 85% inhibition at 100 μg/mL were re-screened at lower concentration (serial dilution up to 6.25 μg/mL) (Table 7). Compounds 3a, 3b, and 3 m demonstrated more than 85% inhibition against Mtb, making them acceptable for further testing at lower concentration. The derivative 3 m exhibited 88% inhibition against Mtb on single dilution compared to the initial concentration *i.e.* 50 μg/mL indicating it to be the most active compound among the series of derivatives. Based on the structure activity relationship (SAR) study, it was observed that the substitution in the derivatives has highly influenced the *in-vitro* results against the *Mycobacterium tuberculosis* $H_{37}RV$ strain. Compound 3 m possessing electron-donating groups at position 3 and 4 on the phenyl ring *i.e.* 3-methoxy and 4-hydroxy demonstrated highest anti-tubercular inhibition among the synthesized derivatives. The schiff base skeleton present in all the derivatives also exhibited an impact on their anti-tubercular potential.

2.4. Molecular docking studies

Furthermore, docking studies were also performed to find the potent drug candidates among the synthesized derivatives. The selection of proteins is done on the basis of literature study and PASS prediction. Receptor 1KZN for *Escherichia coli* [50], 1IYL for *Candida albicans* [45], and 2NSD for *Mycobacterium tuberculosis* $H_{37}Rv$ strain [51] were selected from the RCSB database [43]. The synthesized compounds (3a-o) were docked against the respective enzyme using the default docking parameters.

2.4.1. Protein 1KZN

On conducting docking studies on *E. coli* (1KZN), it was observed that all the synthesized compounds were able to dock within the

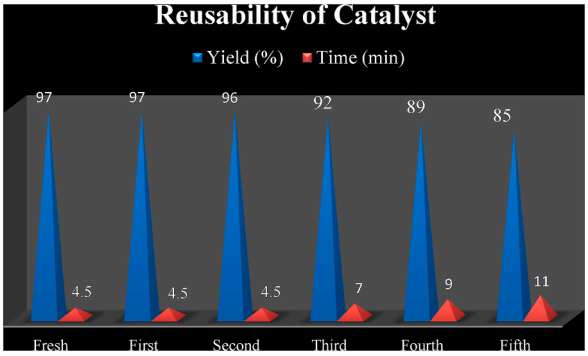


Fig. 2. Reusability of glycerol based sulfonic acid catalyst for the synthesis of N'-(4-cyanobenzylidene)isonicotinohydrazide.

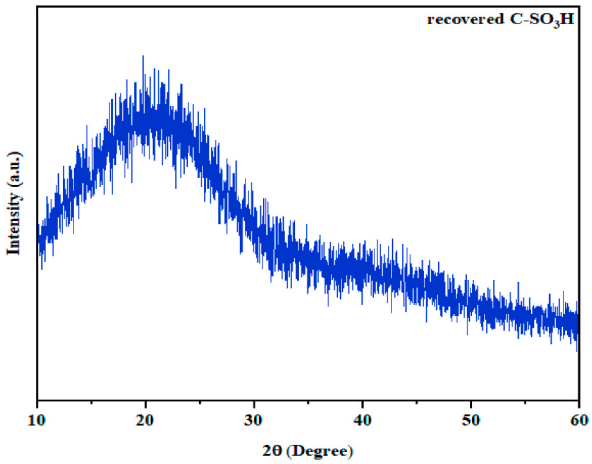


Fig. 3. XRD spectrum of recovered catalyst.

binding site of the protein. From the (Supplementary Table 3) it can be observed that among the fifteen compounds, 3c displayed the highest docking score of −22.89 kcal/mol followed by 3j, 3a, 3b, 3 m, 3h, 3k, 3i, 3f, 3o, 3d, 3e, 3g, 3l, and 3n (Fig. 4). All the compounds bind within the same binding domain as represented in the 3D coordinates of the protein-ligand complex (Supplementary Fig. 3). From the 2D interaction plot, it was observed that all the crucial interactions, *i.e.* interactions with Asn46, Asp73, and Arg136, were seen in the form of hydrogen bonding or hydrophobic interactions (Supplementary Figs 3 and 4) [44]. The same interactions have been reported in (Table 8).

2.4.2. Protein 1IYL

Docking studies conducted on 1IYL showed that among the synthesized derivatives, 3h (−33.83 kcal/mol) displayed the highest docking score followed by 3b, 3 m, 3j, 3c, 3o, 3l, 3a, 3d, 3g, 3k, 3n, 3e, 3i, and 3f as shown in the (Fig. 5 and Supplementary Table 3)). All the fifteen synthesized compounds showed binding within the same binding cavity of the protein (Supplementary Fig. 6). Moreover, all the crucial interactions of the compounds with His227, and Lau451 were seen in the 2D-interaction plot in the form of hydrogen bonding and hydrophobic interactions (Table 8) [45].

2.4.3. Protein 2NSD

While performing docking studies on 2NSD, it was observed that 3i (−23.53 kcal/mol) showed the highest score followed by 3g, 3l, 3c, 3j, 3a, 3 m, 3k, 3n, 3f, 3e, 3d, 3b, 3o as shown in the (Fig. 6 and Supplementary Table 3). Apart from 3h, all compounds were able to bind within the same active site of the protein (Supplementary Fig. 7). Also, all the docked complexes displayed the crucial interaction with Tyr158 as hydrogen bonding and hydrophobic bonding (Supplementary Fig. 7) [46].

Molecular docking studies revealed the importance of the substitution at R position on isonicotinohydrazide scaffold. The nitrogen atoms of hydrazide moiety were involved in forming hydrogen bonding with the active site amino acids, and the substitution at the R position occupy the hydrophobic position in the binding pocket. Also, the functional groups present on the substituted R position were involved in forming hydrogen bonding with the crucial active site residues.

3. Experimental

The drug isoniazid was procured from Sigma-Aldrich. The aldehydes were bought from Alfa-Aesar, Sigma-Aldrich, Avra, and Spectrochem. The melting points (m.p.) of synthesized isonicotinohydrazide analogs were determined using an open-tube capillary procedure. The ^{1}H or ^{13}C NMR spectrum were analyzed on a Bruker Avance NEO 500 MHz and 125 MHz spectrometer using TMS as an internal standard in DMSO-d_6 as a solvent. The progress of reaction was investigated by thin layer chromatography, eluting with hexane: ethyl acetate (4:6).

3.1. General method for synthesis of isonicotinohydrazide analogs

For the synthesis of isonicotinohydrazide analogs (3a-o), equal mmol of isoniazid and diversely substituted aldehydes (2a-o) in ethanol were taken. Glycerol-based carbon sulfonic acid catalyst (35 mg) was taken and refluxed for an adequate time. The completion of reaction was monitored using TLC. After the completion of reaction, methanol was added to it and filtered to recover the catalyst. The methanol was evaporated using rotary evaporator. The crude was washed with ethanol and desired product was obtained in excellent yields (91–98%) in short reaction time 4–10 min.

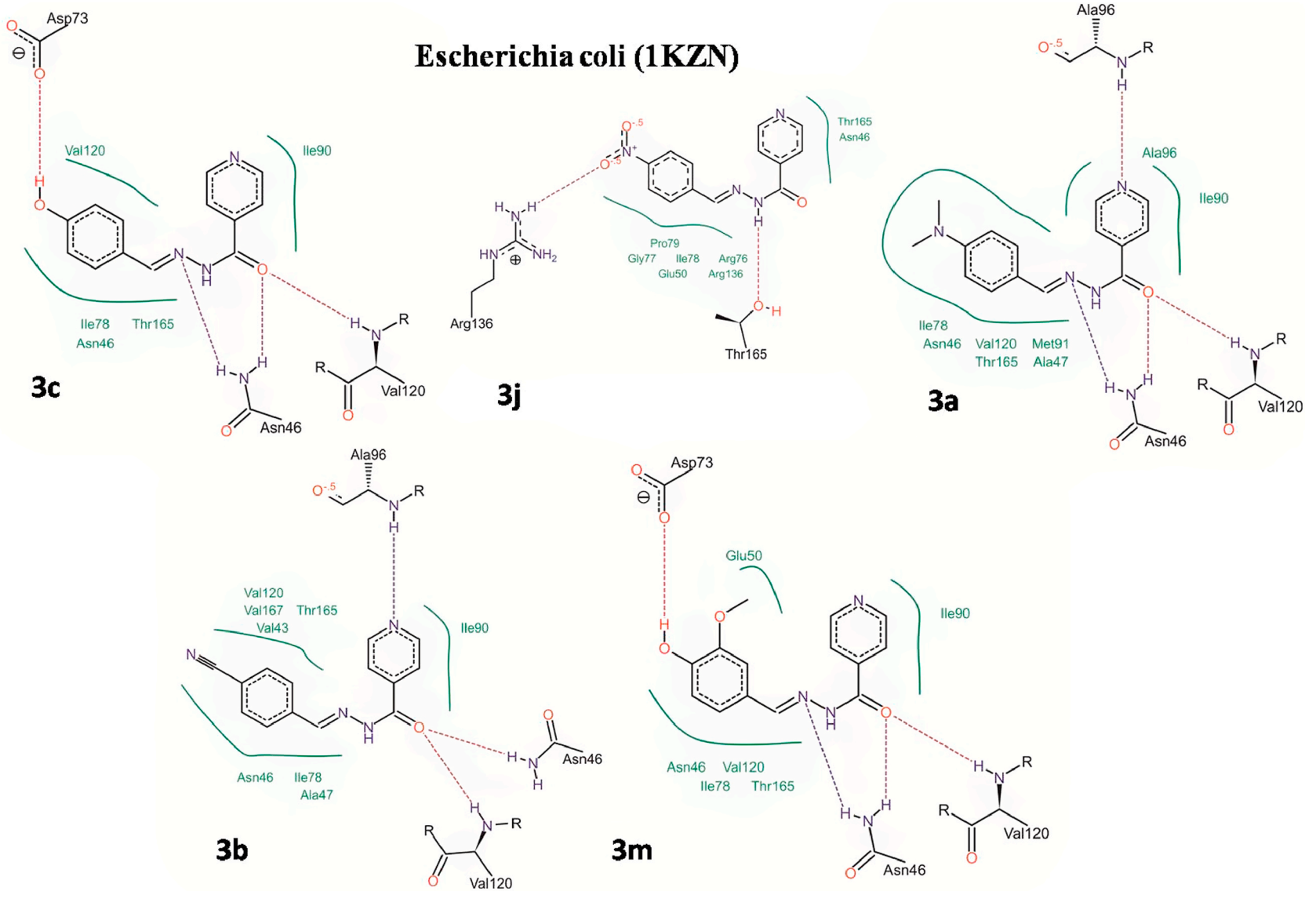


Fig. 4. 2D coordinates of top five docked protein-ligand complexes of 1KZN.

Table 5
Zone of inhibition of synthesized compounds against different bacterial and fungal strains.

Zone of inhibition (1000 μg/mL)								
S. No.	Bacterial strains				Fungal strains			
	S. aureus ATCC 25923	*B. subtilis* ATCC 6051	*E. coli* ATCC 25922	*B. shigella* ATCC 23354	*P. chrysogenum* ATCC 10106	*M. furfur* ATCC 14521	*A. niger* ATCC 1015	*P. notatum (wide strain)*
3a	15	10	18	5	**25**	20	20	**25**
3b	8	10	19	7	15	15	**22**	12
3c	10	8	10	7	15	7	16	19
3d	8	11	10	11	12	15	14	15
3e	10	12	12	7	14	12	15	21
3f	9	8	10	8	11	11	18	7
3g	10	12	17	6	12	17	19	13
3h	8	10	12	5	11	14	20	16
3i	12	10	15	4	20	17	16	16
3j	10	11	15	14	16	13	16	13
3k	13	10	10	4	17	15	6	20
3l	15	11	13	10	15	13	17	12
3 m	14	11	14	10	13	11	16	17
3n	10	11	20	10	11	12	17	14
3o	12	12	12	15	12	13	19	15
Ciprofloxacin	24	25	25	23	–	–	–	–
Fluconazole					25	25	23	25

Table 6
Minimum inhibitory concentration of synthesized compounds against different bacterial and fungal strains.

Minimum Inhibitory Concentration (μg/mL)								
S. No.	Bacterial strains				Fungal strains			
	S. aureus ATCC 25923	*B. subtilis* ATCC 6051	*E. coli* ATCC 25922	*B. shigella* ATCC 23354	*P. chrysogenum* ATCC 10106	*M. furfur* ATCC 14521	*B. niger* ATCC 1015	*P. notatum (wide strain)*
3a	250	500	62.5	125	62.5	125	125	31.25
3b	500	250	62.5	250	125	125	62.5	250
3c	500	1000	125	250	250	500	500	62.5
3d	1000	250	125	500	125	1000	500	250
3e	1000	125	31.25	125	125	125	1000	125
3f	125	62.5	62.5	62.5	500	500	62.5	1000
3g	125	1000	125	125	500	62.5	62.5	1000
3h	62.5	500	500	250	1000	500	62.5	500
3i	125	500	500	500	1000	1000	500	500
3j	1000	1000	125	1000	500	1000	125	500
3k	500	250	250	1000	125	1000	125	500
3l	250	125	62.5	1225	62.5	500	500	500
3 m	250	125	31.25	250	500	62.5	250	1000
3n	125	62.5	31.25	125	1000	62.5	250	250
3o	500	500	62.5	250	1000	62.5	500	125
Ciprofloxacin	62.5	31.25	125	125	–	–	–	–
Fluconazole					62.5	125	62.5	62.5

3.2. Biological screening

3.2.1. Antimicrobial activity

To determine the antimicrobial activity of library of synthesized compounds (3a-o) microbroth dilution procedure was used [47]. The synthesized isonicotinohydrazide derivatives were screened for antimicrobial activity using different gram-positive bacteria, *S. aureus* (ATCC No. 25923), *B. subtilis* (ATCC No. 29212), gram-negative bacteria *E. coli* (ATCC No. 25922), *B. shigella* (ATCC No. 27853) and fungi *P. chrysogenum* (ATCC No. 25922), *M. furfur* (ATCC No. 25922), *A. niger* (ATCC No. 25922), *P. notatum* (ATCC No. 25922). The antimicrobial evaluation was done by micro dilution/broth titer method. The stock solution (1000 μg/mL) for was prepared for each compound and screened for antimicrobial activity by diluting the stock solution from 1000, 500, 250, 125, 62.5, 31.25, 15.62 up to 7.8 μg/mL. The tubes along with the control were then kept for incubation at 37 °C for 24 h. The suspensions were further inoculated on an appropriate media and the growth was noted after 48 h. The results of the antibacterial and antifungal activities in MIC (μg/mL) have been reported in Table 6. 1000 μg/mL concentration was used to estimate the zone of inhibition.

Table 7
Anti-tubercular activity of synthesized compounds (3a-o).

S. No	code	R	% Inhibition at 100 μg/mL	% Inhibition at 50 μg/mL	% Inhibition at 25 μg/mL	% Inhibition at 12.5 μg/mL	% Inhibition at 6.25 μg/mL
1	3a	4-$NMe_2C_6H_4$	87	72	61	44	26
2	3b	4-CN C_6H_4	89	77	54	41	17
3	3c	4-OH C_6H_4	65	34	NI	NI	NI
4	3d	3-Br C_6H_4	65	12	NI	NI	NI
5	3e	2,3,4-$(OMe)_3$ C_6H_2	71	44	NI	NI	NI
6	3f	4-Methylthiazole	62	32	NI	NI	NI
7	3g	2-Benzyloxy	74	68	44	12	NI
8	3h	1-Pyrene	71	61	21	8	NI
9	3i	Furfural	75	42	12	NI	NI
10	3j	4-NO_2 C_6H_4	75	37	NI	NI	NI
11	3k	4-F C_6H_4	71	54	29	10	NI
12	3l	3,4-$(OMe)_2$ C_6H_3	73	68	34	14	NI
13	3 m	4-OH-3-OMe C_6H_3	92	88	65	36	22
14	3n	3,4,5-$(OMe)_3$ C_6H_2	61	26	NI	NI	NI
15	3o	2-Me C_6H_4	60	31	NI	NI	NI
Standard Pyrazinamide			93	89	86	82	79

*NI=No inhibition.

3.2.2. Anti-tubercular activity

The synthesized compounds (3a-o) were screened for their *in-vitro* anti-tubercular activity at 100 μg/mL concentration against *Mycobacterium tuberculosis* H_{37}RV strain in BACTEC 12B medium *via* microplate alamar blue assay [42]. Pyrazinamide was used as a reference drug.

3.2.3. Docking assessment

All the compounds were docked by using the *FlexX* module [48,49] of the *LeadIT* 2.1.8 suit [52]. This model uses an incremental-based construction algorithm to dock the molecule in the protein binding site. To conduct the docking studies, receptor 1KZN for *Escherichia coli*, 1IYL for *Candida albicans*, and 2NSD for *Mycobacterium tuberculosis H37Rv* strain were selected from the RCSB database [43]. The synthesized compounds (3a-o) were docked against the respective enzyme using default docking parameters. The co-crystallized ligands CBN (1KZN), R64 (1IYL), and 4PI (2NSD) were selected as the center with a radius of 6.5 Å to act as the binding domain for the synthesized ligands. During docking calculations, the top 50 poses were requested from each analysis. These poses differ based on their docking score calculated by employing the modified Böhm's scoring function [53]. The docking results are represented in the form of 2D and 3D interaction plots, which were generated by using the *PoseView* module [54] of the *LeadIT*2.1.8 suit(LeadIt).

3.3. Spectral characterization

3.3.1. (E)-N'-(4-(dimethylamino)benzylidene)isonicotinohydrazide

Mustard color, 95% yield, m.p. 193–195 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.82 (s, 6H, $N(CH_3)_2$), 7.73 (d, J = 5.5 Hz, 1H, Ar–H), 7.84 (d, J = 4.9 Hz, 1H, Ar–H), 8.01 (d, J = 8.5 Hz, 2H, Ar–H), 8.30 (d, J = 8.4 Hz, 2H, Ar–H), 8.57 (s, 1H, N═CH), 8.70–8.81 (m, 2H, Ar–H), 9.95 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.3, 40.9, 111.9, 112.1, 121.0, 121.4, 128.2, 128.8, 129.1, 129.9, 131.7, 141.3, 142.9, 146.7, 149.7, 149.9, 164.9; ESI-MS: *m/z* 268.32 [M+].

3.3.2. E)-N'-(4-cyanobenzylidene)isonicotinohydrazide

Off white, 97% yield, m.p. 270 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.83 (d, J = 5.8 Hz, 2H, Ar–H), 7.91 (d, J = 7.95 Hz, 4H, Ar–H), 8.52 (s, 1H, N═CH), 8.80 (d, J = 3.7 Hz, 2H, Ar–H), 12.30 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.8, 118.4, 121.2, 121.9, 128.7, 131.9, 132.5, 133.8, 139.8, 140.8, 149.8, 165.6; ESI-MS: *m/z* 250.26 [M+].

3.3.3. E)-N'-(4-hydroxybenzylidene)isonicotinohydrazide

Pale yellow, 94% yield, m.p. 250–252 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.35–7.48 (m, 1H, Ar–H), 7.58 (d, J = 8.6 Hz, 1H, Ar–H), 7.65–7.71 (m, 2H, Ar–H), 7.80–7.82 (m, 2H, Ar–H), 8.42 (s, 1H, N═CH), 8.73–8.78 (m, 2H, Ar–H), 9.97 (s, 1H, OH), 11.94 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 115.1, 115.6, 121.3, 121.7, 124.8, 129.9, 130.1, 140.5, 146.8, 149.5, 149.7, 161.8; ESI-MS: *m/z* 241.25 [M+].

3.3.4. (E)-N'-(3-bromobenzylidene)isonicotinohydrazide

Light yellow, 92% yield, m.p. 245–247 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42 (t, J = 7.92 Hz, 1H, Ar–H), 7.64 (dd, J = 7.9 Hz, 0.9 Hz, 1H, Ar–H), 7.75 (d, J = 7.8 Hz, 1H, Ar–H), 7.82 (dd, J = 4.5 Hz, 1.5 Hz, 2H, Ar–H), 7.95 (s 1H, Ar–H), 8.44 (s, 1H, N═CH), 8.79

Table 8
List of the interactions (hydrogen bonding and hydrophobic interactions) reported in the docking of the fifteen synthesized molecules (3a-o) with *Escherichia coli,* fungal (*Candida albicans*), and Mycobacterium tuberculosis H37Rv strain.

PDB ID	Ligands	Interactions
Escherichia coli		
1KZN	3c	Asn46, Asp73, Val120(Val 120, Ile78, Thr165, Asn46, Ile90)
	3j	**Arg136, Thr165** (Thr165, Asn46, Arg76, Arg136, Ile78, Pro79, Gly77, Glu50)
	3a	**Ala96, Val120, Asn46** (Ile78, Asn46, Val120, Thr165, Met91, Ala47, Ile90, Ala96)
	3b	**Ala96, Val120, Asn46** (Ile90, Val120, Val167, Val43, Thr165, Asn46, Ile78, Ala47)
	3m	**Asp73, Asn46, Val120** (Ile90, Glu50, Asn46, Val120, Ile78, Thr165)
	3h	**ASp73** (Pro79, Ile78, Arg76, Gly77, Glu50, Gln72, Asp73, Val71, Thr165, Ala47)
	3k	**Ala96, Asn46, Val120** (Ala95, Ile90, Asn46, Val120, Glu50, Ile78)
	3i	**Asn46, Val120, Ala96** (Asn46, Ile90)
	3f	**Ala96, Asn46, Val120** (Ile90, Ala96, Asp45, Asn46)
	3o	**Thr165** (Ala47, Thr165, Asn46, Gly77, Ile78, Glu50, Pro79, Arg76)
	3d	**Thr165** (Asn46, Thr165, Ala47, Pro79, Ile78, Glu50, Gly77, Arg76)
	3e	**Asn46, Val120** (Asn46, Thr165, Val120, Ala47, Glu50, Ile90, Ile78)
	3g	**Thr165** (Ala47, Thr165, Asn46, Pro79, Glu50, Ile78, Gly77, Arg76)
	3l	**Asn46, Val120** (Ile90, Ala96, Ile78, Met91, Asn46, Thr165, Val120, Val167)
	3n	**Ala96, Asn46, Val120** (Ile90, Ala96, Thr165, Val120, Ile78, Asn46, Glu50)
***Mycobacterium tuberculosis* H37Rv**		
2NSD	**3i**	**Tyr158** (Met199, Glu219, Pro193, Phe149, Tyr158, Nad300)
	3g	Met199, Tyr158, Val203, Phe149, Ile202, Nad300, Phe97, Gly96, Ala198
	3l	**Tyr158** (Met199, Ile202, Met161, Nad300, Tyr158, Phe149, Pro193, Leu218, Glu219)
	3c	Met103, Met161, Nad300, Ile202, Tyr158, Val203
	3j	**Tyr158** (Pro193, Met190, Gly96, Nad300, Met161, Met103, Ile202, Tyr158, Phe149)
	3a	**Tyr158** (Phe149, Pro193, Tyr158, Ile202, Met199, Nad300, Phe97, Gly96, Met103, Met161)
	3m	Tyr158, Phe149, Met155, Nad300, Ile202, Met161
	3k	**Tyr158**(Nad300, Met161, Gly96, Phe97, Met103, Phe149, Pro193, Met199, Tyr158, Ile202)
	3n	**Tyr158** (Gly96, Met161, Ile202, Met199, Pro193, Val203, Tyr158, Phe149, Nad300)
	3f	**Tyr158** (Met103, Met161, Ile202, Gly96, Nad300, Met199, Phe149, Tyr158)
	3e	**Tyr158,Met103**(Met199, Leu218, Tyr158, Phe149, Nad300, Ile202, Met161, Met103, Gly96)
	3d	**Tyr158**(Met161, Gly96, Nad300, Ile202, Tyr158, Pro193, Val203, Phe149, Met199)
	3b	**Tyr158, Met103**(Phe149, Ile202, Nad300, Met161, Met103, Leu218, Tyr158)
	3o	**Tyr158**(Met161, Ile202, Phe149, Met155, Tyr158, Pro193, Nad300, Met199)
Candida albicans		
11 YL	**3h**	**Tyr107, Leu451**(Tyr225, Tyr354, Phe117, Phe339, His227, leu415, Asn392, Asn175, Thr211, Leu451)
	3b	**Tyr335, Cys393, Gln226, Tyr119**(Phe117, Tyr119, Leu451, Leu337, Cys393, Leu394, Tyr354, Asn392, Tyr225)
	3m	**Tyr335, Cys393, Gln226, Tyr119**(Cys393, Leu394, Phe117, Leu451, Leu337, Tyr225, Tyr354, Asn392)
	3j	**Tyr335, Tyr119, Gln226, Cys393**(Leu394, Tyr354, Phe117, Asn392, Tyr225, Leu337)
	3c	**Tyr335, Tyr119, Cys393, Gln226**(Phe117, Leu451, Leu337, Leu394, Tyr225, Tyr354, Asn392)
	3o	**Tyr335, Tyr119**(Leu451, Tyr225, Tyr354, Leu394, Asn392, Phe117, Leu337)
	3l	**Tyr107, Gln226, Cys393**(Phe117, Tyr225, Asn392, Cys393, Tyr354, Tyr107, Leu337)
	3a	**Tyr107, Phe176, Leu451**(Asn175, Tyr107, Phe176, Tyr225, Tyr354, Leu394, Leu451, Asn392)
	3d	**Asn392, His227**(Ile352, Phe339, Phe240, Tyr225, Leu394, Asn392, Tyr354, Cys393, His227)
	3g	**Tyr107, Leu451, Phe176**(Leu451, Tyr107, Asn175, Tyr225, Phe240, Tyr354, His227, Phe339, Phe117)
	3k	**Tyr335, Tyr119**(Leu451, Phe117, Leu337, Asn392, Tyr354, Tyr225, Leu394, Cys393)
	3n	**Tyr335, Tyr119, His227**(Leu451, Leu337, Tyr119, Asn392, Cys393, Phe117, Leu394, Tyr354, Tyr225)
	3e	**Tyr335, Tyr354, Tyr225, Ile111**(Asp110, Phe117, Tyr354, Tyr225, Phe339, Glu109)
	3i	**Tyr107, Thr211, Leu451**(Asn175, Thr211, Leu451)
	3f	**Tyr119, Tyr335**(Leu451, Tyr335, Tyr119, Leu337, Tyr354, Leu394)

(d, $J = 5.85$ Hz, 2H, Ar–H), 12.21 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 121.4, 122.0, 122.9, 126.2, 129.2, 130.9, 132.7, 136.3, 140.1, 147.0, 149.4, 150.2, 161.6; ESI-MS: *m/z* 304.15 [M+].

3.3.5. (E)-N'-(2,3,4-trimethoxybenzylidene)isonicotinohydrazide

White color solid, 91% yield, m.p. 178–180 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.78 (s, 3H, OCH_3), 3.86 (d, $J = 2.1$ Hz, 6H, OCH_3), 6.94 (d, $J = 8.95$ Hz, 1H, Ar–H), 7.63 (d, $J = 8.85$ Hz, 1H, Ar–H), 7.83 (dd, $J = 4.5$ Hz, 1.45 Hz, 2H, Ar–H), 8.66 (s, 1H, N═CH), 8.78 (d, $J = 5.75$ Hz, 2H, Ar–H), 11.98 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.9, 60.3, 61.7, 108.6, 119.9, 120.5, 121.3, 123.0, 140.4, 141.4, 144.4, 149.3, 150.1, 152.6, 155.3, 161.6; ESI-MS: *m/z* 315.33 [M+].

3.3.6. E)-N'-((4-methylthiazol-5-yl)methylene)isonicotinohydrazide

White color solid, 95% yield, m.p. 254–256 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.35 (s, 3H, OCH_3), 7.80 (d, $J = 5.85$ Hz, 2H, Ar–H), 8.77 (s, 1H, N═CH), 8.79 (d, $J = 5.7$ Hz, 2H, Ar–H), 9.10 (s, 1H, Ar–H), 12.08 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.2, 121.2, 123.0, 127.2, 140.1, 141.9, 149.3. 150.2, 154.6, 155.2, 161.1; ESI-MS: *m/z* 246.29 [M+].

3.3.7. (E)-N'-(4-(benzyloxy)benzylidene)isonicotinohydrazide

White color solid, 92% yield, m.p. 169–170 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.18 (s, 2H, OCH_2), 7.11 (d, $J = 8.75$ Hz, 2H, Ar–H),

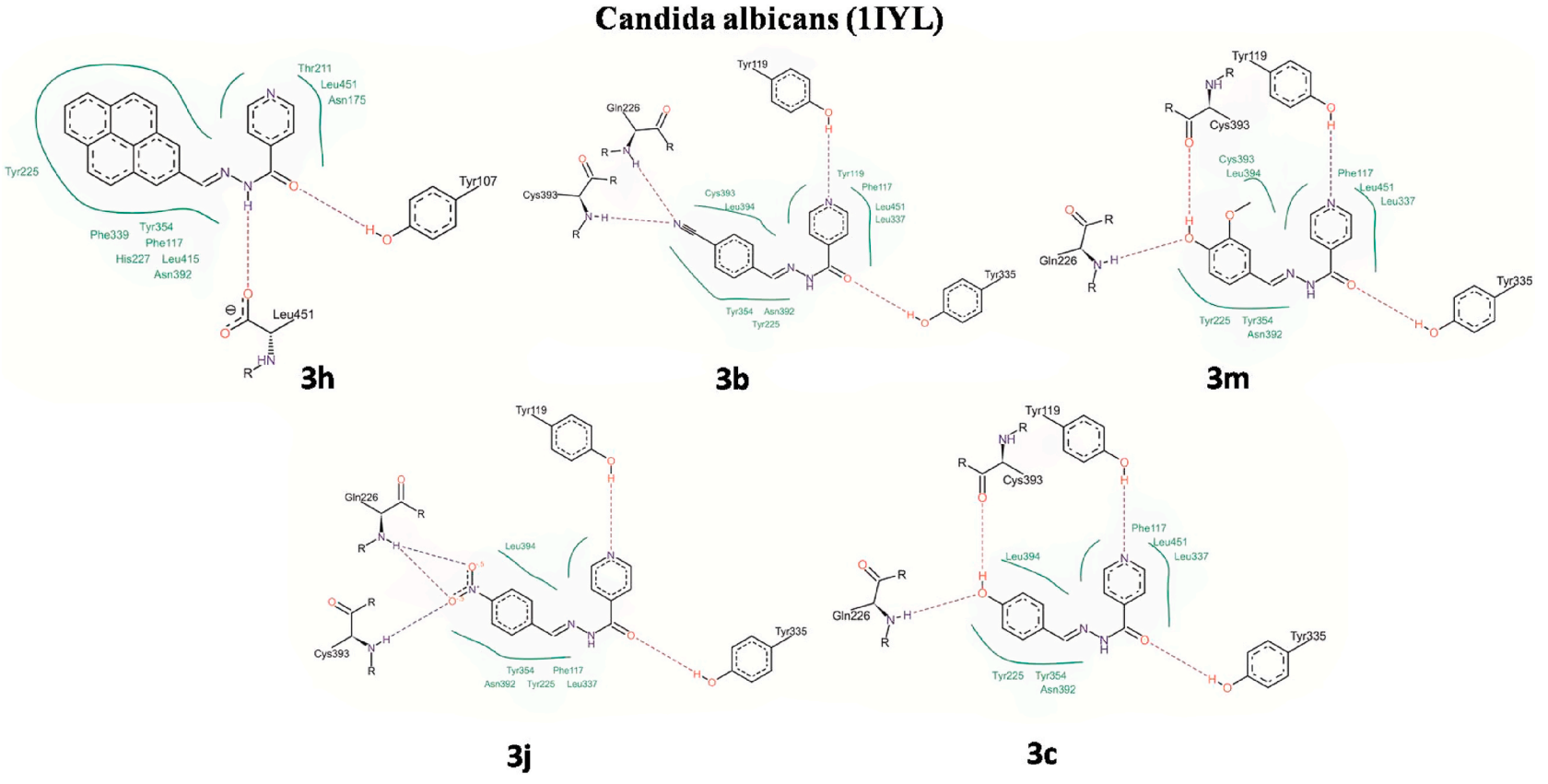


Fig. 5. 2D coordinates of top five docked protein-ligand complexes of 1IYL.

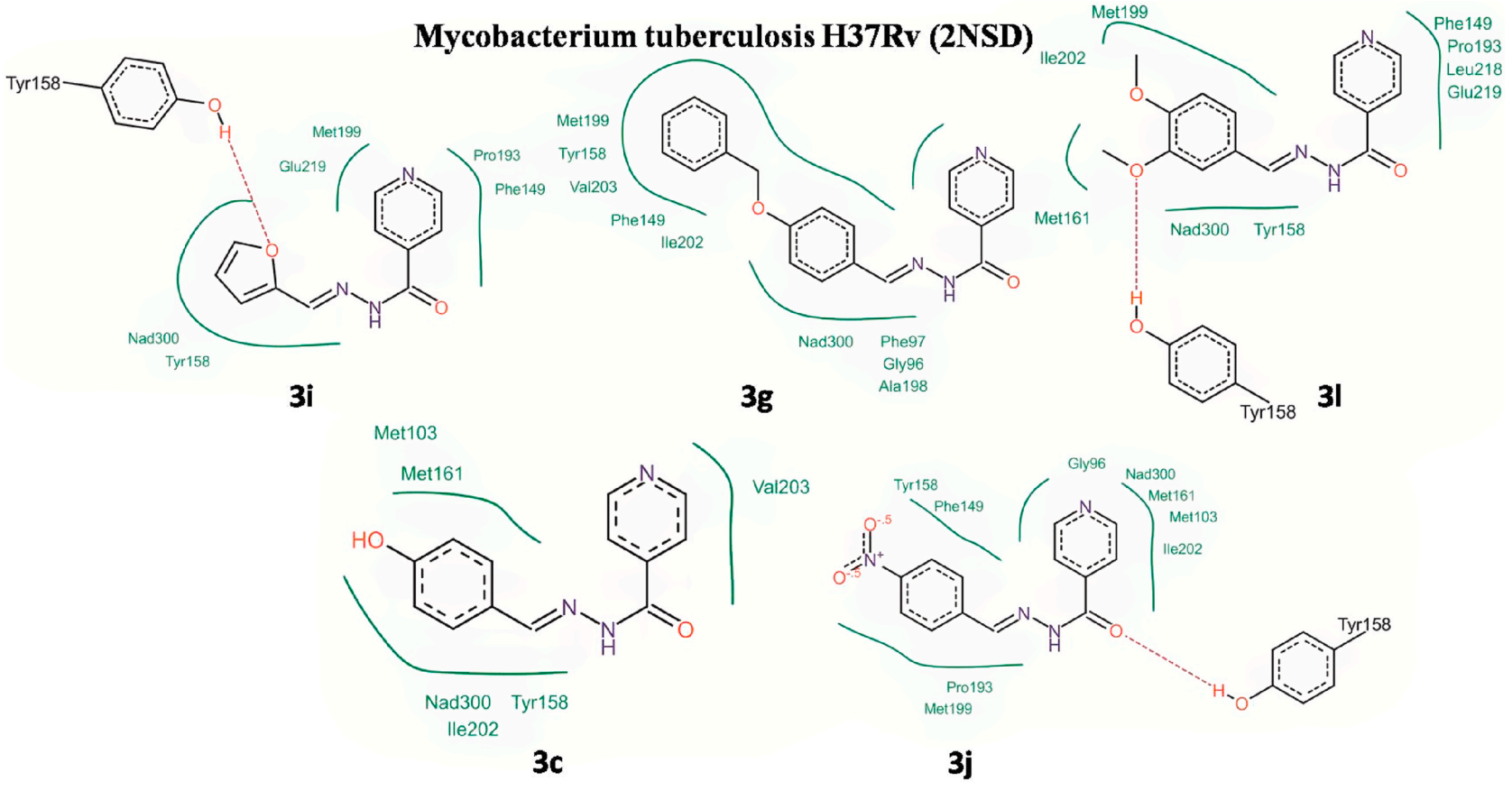


Fig. 6. 2D coordinates of top five docked protein-ligand complexes of 2NSD.

7.35 (d, J = 7.25 Hz, 1H, Ar–H), 7.40 (m, 2H, Ar–H), 7.47 (d, J = 7.15 Hz, 2H, Ar–H), 7.69 (d, J = 8.75 Hz, 2H, Ar–H), 7.81 (dd, J = 4.45 Hz, 1.6 Hz, 2H, Ar–H), 8.41 (s, 1H, N═CH), 8.78 (dd, J = 4.45 Hz, 1.55 Hz, 2H, Ar–H), 11.94 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 69.2, 114.9, 115.1, 121.3, 121.5, 126.6, 127.1, 127.6, 127.8, 128.3, 128.7, 130.2, 130.5, 136.6, 140.5, 146.7, 149.9, 150.1, 161.2, 163.2; ESI-MS: *m/z* 331.38 [M+].

3.3.8. (E)-N'-(pyren-2-ylmethylene)isonicotinohydrazide

Pale yellow color solid, 93% yield, m.p. 200–202 °C, ^{1}H NMR 500 MHz, DMSO-d_6) δ 7.92 (dd, J = 4.5 Hz, 1.45 Hz, 2H, Ar–H), 8.11 (t, J = 7.6 Hz, 1H, Ar–H), 8.21–8.28 (m, 2H, Ar–H), 8.32–8.38 (m, 4H, Ar–H), 8.58 (d, J = 8.1 Hz, 1H, Ar–H), 8.81 (d, J = 9.35 Hz, 1H, Ar–H), 8.85 (d, J = 4.55 Hz, 2H, Ar–H), 9.53 (s, 1H, N═CH), 12.26 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 121.4, 122.2, 122.9, 123.6, 124.0, 125.1, 125.1, 125.7, 126.0, 126.4, 126.5, 127.2, 128.4, 128.6, 128.7, 129.9, 130.7, 132.0, 140.3, 147.7, 149.5, 150.3, 161.4; ESI-MS: *m/z* 349.39 [M+].

3.3. 9(E)-N'-(furan-2-ylmethylene)isonicotinohydrazide

White color solid, 94% yield, m.p. 188–190 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.65 (dd, J = 3.3 Hz, 1.7 Hz, 1H, Ar–H), 6.99 (d, J = 3.35 Hz, 1H, Ar–H), 7.80 (dd, J = 4.5 Hz, 1.4 Hz, 2H, Ar–H), 7.88 (d, J = 1.1 Hz, 1H, Ar–H), 8.36 (s, 1H, N═CH), 8.78 (d, J = 5.9 Hz, 2H, Ar–H), 12.00 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.1, 114.1, 121.3, 122.9, 138.5, 140.2, 145.4, 149.0, 149.4, 150.2, 161.3; ESI-MS: *m/z* 215.21 [M+].

3.3.10. (E)-N'-(4-nitrobenzylidene)isonicotinohydrazide

White color solid, 96% yield, m.p. 191–193 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (d, J = 4.9 Hz, 2H, Ar–H), 8.01 (d, J = 8.45 Hz, 2H, Ar–H), 8.30 (d, J = 8.4 Hz, 2H, Ar–H), 8.57 (s, 1H, N═CH), 8.80 (d, J = 4.5 Hz, 2H, Ar–H), 12.37 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.5, 117.9, 121.4, 121.7, 129.4, 129.8, 133.8, 139.5, 140.1, 146.4, 150.0, 150.2, 163.7; ESI-MS: *m/z* 270.25 [M+].

3.3.11. (E)-N'-(4-fluorobenzylidene)isonicotinohydrazide

White color solid, 95% yield, m.p. 257–259 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.30 (t, J = 8.85 Hz, 2H, Ar–H), 7.81–7.83 (m, 4H, Ar–H), 8.46 (d, J = 4.25 Hz, 1H, N═CH), 8.78 (d, J = 4.5 Hz, 2H, Ar–H), 12.08 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 115.4, 116.8, 121.0, 121.6, 130.1, 130.9, 133.8, 139.5, 140.3, 149.8, 149.9, 162.5, 164.7; ESI-MS: *m/z* 243.24 [M+].

3.3.12. (E)-N'-(3,4-dimethoxybenzylidene)isonicotinohydrazide

Light yellow color solid, 90% yield, m.p. 168–170 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 7.03 (d, J = 8.3 Hz, 1H, Ar–H), 7.23 (dd, J = 8.25 Hz, 1.6 Hz, 1H, Ar–H), 7.36 (d, J = 1.5 Hz, 1H, Ar–H), 7.81 (d, J = 5.9 Hz, 2H, Ar–H), 8.40 (s, 1H, N═CH), 8.78 (d, J = 5.6 Hz, 2H, Ar–H), 11.95 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.3, 55.4, 108.2, 111.4, 121.3, 122.0, 123.1, 126.6, 140.5, 148.9, 149.1, 149.3, 150.1, 150.9, 161.3; ESI-MS: *m/z* 285.30 [M+].

3.3. 13(E)-N'-(4-hydroxy-3-methoxybenzylidene)isonicotinohydrazide

Lemon yellow color solid, 96% yield, m.p. 210–212 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 6.86 (d, J = 8.1 Hz, 1H, Ar–H), 7.11 (dd, J = 8.15 Hz, 1.8 Hz, 1H, Ar–H), 7.33 (d, J = 1.75 Hz, 1H, Ar–H), 7.81 (dd, J = 4.5 Hz, 1.6 Hz, 2H, Ar–H), 8.36 (s, 1H, N═CH), 8.77 (d, J = 5.95 Hz, 2H, Ar–H), 9.61 (s, 1H, OH), 11.90 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.4, 109.0, 115.3, 121.3, 122.3, 125.3, 140.6, 147.9, 149.2, 149.5, 150.1, 150.4, 161.2; ESI-MS: *m/z* 271.28 [M+].

3.3.12. E)-N'-(3,4,5-trimethoxybenzylidene)isonicotinohydrazide

White color solid, 94% yield, m.p. 198–201 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.72 (s, 3H, OCH_3), 3.85 (s, 6H, OCH_3), 7.26 (s, 2H, Ar–H), 7.81 (dd, J = 4.5 Hz, 1.55 Hz, 2H, Ar–H), 8.40 (s, 1H, N═CH), 8.73–8.80 (m, 2H, Ar–H), 12.05 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 54.9, 59.6, 61.3, 109.6, 118.9, 121.5, 121.9, 122.5, 139.9, 140.4, 143.4, 148.8, 149.1, 151.6, 156.3, 162.6; ESI-MS: *m/z* 315.33 [M+].

3.3.15. (E)-N'-(2-methylbenzylidene)isonicotinohydrazide

White color solid, 91% yield, m.p. 178–180 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.01 (s, 3H, CH_3), 6.84 (d, J = 8.65 Hz, 1H, Ar–H), 7.11 (dd, J = 8.8 Hz, 1H, Ar–H), 7.35–7.48 (m, 2H, Ar–H), 7.69 (d, J = 8.8 Hz, 2H, Ar–H),7.80–7.82 (m, 2H, Ar–H), 8.36 (s, 1H, N═CH), 11.94 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.9, 121.5, 121.9, 125.8, 126.5, 129.1, 129.9, 135.5, 140.2, 144.5, 149.7, 149.8, 164.6; ESI-MS: *m/z* 239.28 [M+].

4. Conclusion

In summary, a simple, efficient, and facile approach for the synthesis of isonicotinohydrazide derivatives has been developed. The designed pathway was robust and gave excellent yield of products in a very short reaction time. Moreover, the results of antimicrobial activity revealed that the synthesized compounds were more active against tested Gram negative bacterial strains and some of them also possessed significant results against tested fungal strains. As per the results of anti-tubercular activity, compounds 3a, 3b, and 3 m were found active against *M. tuberculosis* H_{37}RV strain having 87%, 89% and 92% inhibition at 100 μg/mL concentrations respectively. These compounds possessed inhibition at a low concentration of 6.25 μg/mL. The SAR study revealed that the presence of electron donating group (OH, OMe, NMe_2) has great impact on the biological activity of compounds. From the docking outcome, we can

conclude that all the complexes apart from 3h (in *M. tuberculosis H37Rv* strain), all were able to bind well in the active site of the respective proteins. Moreover, all the essential interactions were witnessed in the 2D interactions plots of the respective protein-ligand complex, which enhance their chances to act as antibacterial, antifungal, and anti-tubercular agents as reported in the in vitro studies too.

Author contribution statement

Ayushi Sethiya: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Deepkumar Joshi: Performed the experiments.

Anu Manhas: Performed the experiments; Wrote the paper.

Nusrat Sahiba: Conceived and designed the experiments.

Dinesh K. Agarwal: Analyzed and interpreted the data.

Prakash C. Jha: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Shikha Agarwal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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