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Ultrasound-assisted synthesis, biological activities and molecular docking study of 1,2,3-triazole incorporated 2-amino-3-cyanopyridines

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Abstract: An efficient, ultrasound assisted synthesis of 1,2,3-triazole incorporated 2-amino-3-cyanopyridine derivatives *via* multicomponent reaction approach were achieved in good yields. The antitubercular and antifungal activity for all the synthesized derivatives were evaluated. Some of the derivatives were displayed good antitubercular activity against Mtb H_{37}Rv strain and antifungal activity against the four tested fungal strains. Furthermore, *in silico* ADME properties for all the compounds were also studied and the results exhibited that the newly synthesized compounds shows potential to be developed as an oral drug candidates.

Keywords: 1,2,3-Triazoles, Multicomponent reactions, Ultrasound irradiation, Pyridines, Antifungal activity, Molecular docking.

1. INTRODUCTION

The development of new synthetic strategies for diversely functionalized structures with active pharmacophores in a single molecular motif have attracted great attention of chemist. Heterocyclic skeleton plays an important role among the medicinally active synthetic and natural compounds. Therefore, the development of simple and efficient route for bioactive compounds with heterocyclic skeleton has given new dimensions in the drug discovery and development.

Pyridine is one of the biologically important heterocycle, which plays vital role in biological, agrochemical and chemical coordination. Pyridine skeleton is present in many naturally occurring compounds such as Niacin (Vitamin B_3) and Pyridoxine (Vitamin B_6) [1].

The biological significance of pyridine moiety is due to its active participation in metabolism *via* oxidation or methylation pathways forming the corresponding pyridinium ions. Nicotinamide adenine dinucleotide phosphate, $NADP^+$ modulate enzymes of the living system by involving in various oxidation and reduction process in biology [2]. Many pyridine derivatives are known to possess a wide range of pharmacological activities such as antimicrobial [3], antibacterial [4], antitumor [5], antitubercular [6], antioxidant [7], antiviral [8], anticonvulsant [9], anticancer [10], anxietic [11], anti-inflammatory [12], and antimitotic activities [13]. Pyridine based compounds acts as inhibitors and receptors in biological systems such as Xia inhibitor [14], histone deacetylase inhibitor [15], TAK1 inhibitor [16], Glue R5 receptor [17], progesterone receptor agonist [18], A_3 adenosine receptor [19], cannabinoid receptor [20] and NK1 receptor antagonist [21]. Several marketed drugs containing pyridine ring includes Isoniazid, Ethionamide, Rabeprazole, Etoricoxib, Clarinex, Rosiglitazone and agrochemicals like Pyridinenitrile, Nitrapyrin, Picloram as shown in **Fig. (1)**.

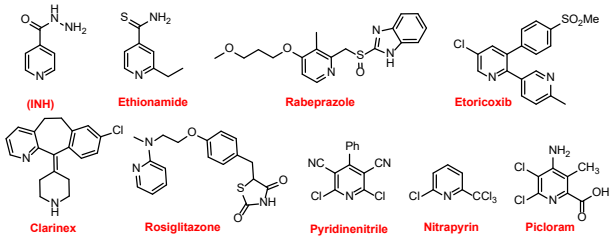


Fig. (1). Pyridine based marketed drugs and agrochemicals.

Multicomponent reactions (MCRs) are one of the advantageous tools for the generation of diversified heterocyclic scaffolds in a single transformation [22-24]. Furthermore, ultrasonic irradiation (USI) is a green energy source, which offers high yields in shorter reaction time [25-26]. Literature survey reveals that, there are several reports on the synthesis of 2-amino-3-cyanopyridine derivatives *via* the four-component multicomponent reaction between aromatic aldehyde, malononitrile, ketone and ammonium acetate in the presence of various catalyst under different reaction conditions [27-33]. Kalaria *et al.* reported [34] synthesis of 2-amino-3-cyanopyridine derivatives linked with 5-imidazopyrazole nucleus *via* one-pot four-component cyclocondensation reaction of substituted 5-(1*H*-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde, malononitrile, ammonium acetate and aromatic/heterocyclic methyl ketones under ultrasonic irradiation as well as under reflux condition. Zhang and coworkers reported [35] synthesis and *in vitro* antitumor activity of novel 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives. Konakanchi and his team reported [36] synthesis of fully substituted pyridines *via* one-pot four-component reaction between aromatic aldehyde, malononitrile, ketone and ammonium acetate in presence of zinc complex. Pagadala *et al.* developed [37] efficient synthesis of multisubstituted pyridine by using Au/MgO as catalyst for multicomponent coupling reaction in ethanol. Hauochine and coworkers [38] have been synthesized imidazopyridines linked aminopyridinyl hybrid under solvent-free condition.

1,2,3-Triazole nucleus was reported as an important pharmacophoric scaffold and possess diverse biological activities [39] such as antifungal [40], anticancer [41], antitubercular [42],

antiproliferative [43], antimicrobial [44], antibacterial [45], anti-coronavirus [46], and neuroprotective agents [47]. Some of the triazole-based marketed drug

s are shown in **Fig. (2)**, which contains Tazobactam, Cefatrizine, *tert*-butyldim ethylsilylspiroaminooxathioledioxide (TSAO) and CAI (Carboxyamidotriazole) [48-51].

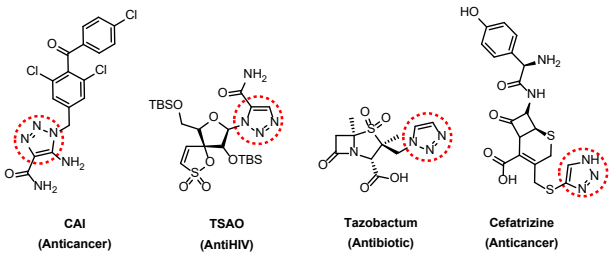


Fig. (2). 1,2,3-Triazole-based marketed drugs

The literature survey reveals that there are some reports available on the synthesis and biological evaluation of 1,2,3-triazole incorporated pyridine scaffolds. El-sayed and coworkers reported [52] pyridine substituted 1,2,3-triazole derivatives as antiviral agent. Pastor *et al.* reported [53] synthesis of triazolo pyridine as PIM kinase inhibitor. The antitubercular activity of pyridine-triazolo hybrids have been reported [54] by Sajja and coworkers. The cyctotoxic study [55], antiproliferative [56], antibiofilm [57], and antiepileptic activity [58] of triazole based pyridine derivatives are well reported.

Literature findings demonstrate that inadequate work has been reported in the recent years to make a hybrid of pyridine and triazole into a single molecular framework with potential antifungal activity. Our rationale behind choosing pyridine for hybridization is its large recurrence in bioactive natural and synthetic drugs. Therefore, considering the biological importance of pyridine and 1,2,3-triazole, we have designed and synthesized target molecule that is a hybrid of pyridine and 1,2,3-triazole heterocyclic scaffolds and further evaluation of their antitubercular, antifungal and antioxidant activities. The designing of the target molecule is on the basis of molecular hybridization approach [59-60] **Fig. (3)**.

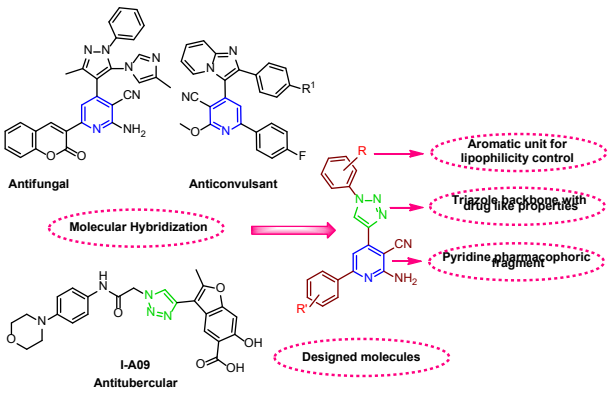


Fig. (3). Molecular hybridization strategy of 1,2,3-triazole-pyridine conjugates.

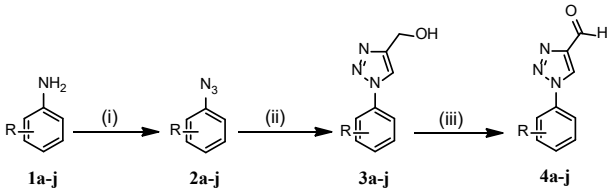
2. RESULTS AND DISCUSSION

2.1. Chemistry

The 1-aryl-1*H*-[1,2,3]triazole-4-carboxaldehydes (**4a-j**) **(Scheme 1)** were prepared according to the literature method [61]. The commercially available anilines (**1a-j**) were diazotized by using sodium nitrite to form diazonium salt, followed by substitution with sodium azide, afforded the corresponding aryl azides. These aryl azides (**2a-j**) on 1,3-dipolar cycloaddition reaction *via*

click chemistry approach with propargyl alcohol in the presence of copper sulfate ($CuSO_4$) and sodium ascorbate to the corresponding 1-aryl-1,2,3-triazolyl methanols (**3a-j**) in excellent yields. The 1-aryl-1,2,3-triazolyl methanols (**3a-j**) on oxidation by using Collins reagent to form 1-aryl-1*H*-[1,2,3]triazole-4-carboxaldehydes (**4a-j**) in good yields.

Scheme 1. Synthesis of 1-aryl-1,2,3-triazole carboxaldehydes (**4a-j**)

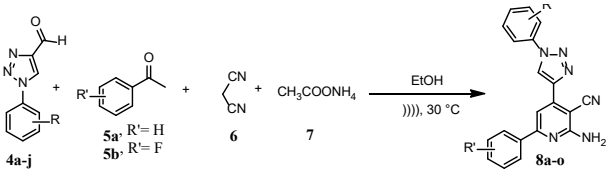


Reagents and conditions: (i) $NaNO_2$, HCl (10%); NaN_3, 1-2 h, 0 °C; (ii) Propargyl alcohol, $CuSO_4.5H_2O$, Sodium ascorbate, *tert*-BuOH-H_2O (1:1), 24-48 h, rt; (iii) Collins reagent ($CrO_3.2Py$, CH_2Cl_2), 3-6 h, rt.**)**

The aryl-1,2,3-triazolyl carbaldehydes (**4a-j)** have many applications in synthetic organic chemistry for the construction of diversely functionalized bioactive molecules like triazolyl nitrone analogues [62], bezoxazole-triazole conjugates [63], triazole based trifluoromethyl derivatives [64], triazole-biscoumarines [65], pyridine-triazole conjugates [66], triazole linked bis-pyrazoles [67], triazole appended α-aminophosphonates [68], triazolyl monocaronyl curcumin analogues [69-70], triazolyl-pyranopyrazoles [71], triazole-chromene conjugates [72], and triazole linked tetrahydrobenzopyran derivatives [73].

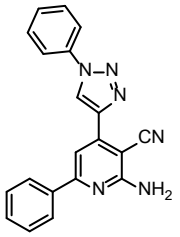
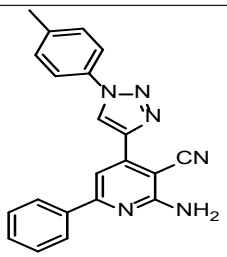
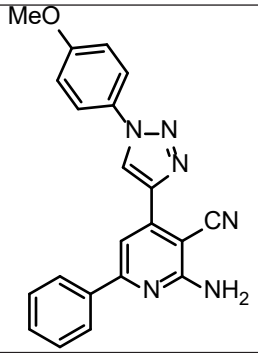
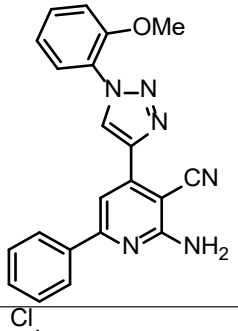
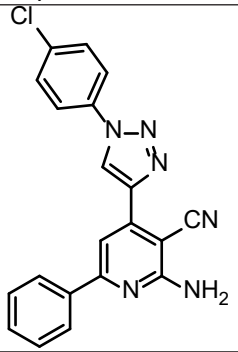
The synthesis of various highly substituted 1,2,3-triazole incorporated pyridine derivatives (**8a-o**) have been achieved *via* one-pot multicomponent reaction between aryl-1,2,3-triazolyl aldehydes (**4a-j)**, acetophenones **5**, malononitrile **6** and ammonium acetate **7**, under ultrasonic irradiation method [8] in good yields (**Scheme 2**).

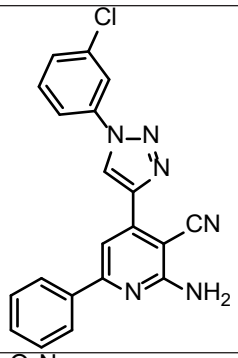
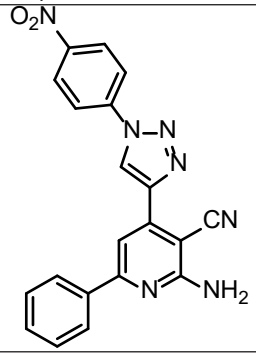
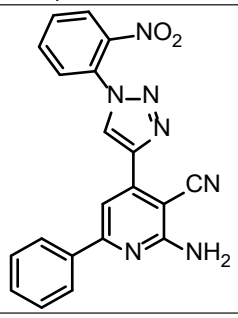
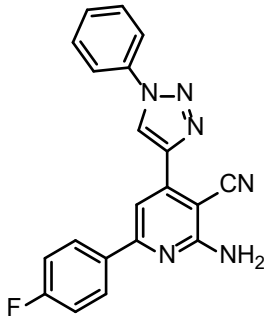
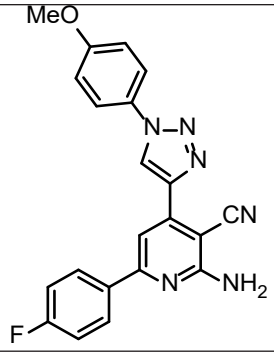
Scheme 2. Synthesis of 1,2,3-triazole incorporated 2-amino-3-cyano-pyridines.

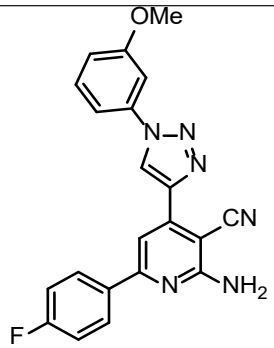
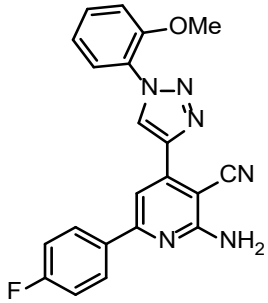
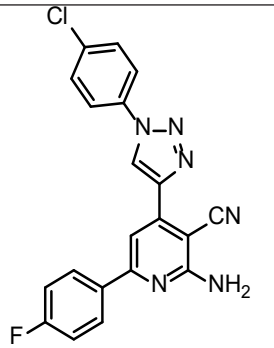
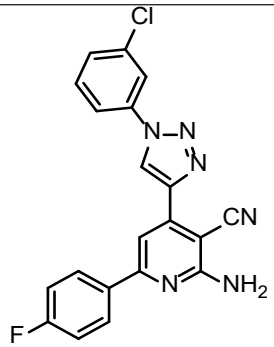
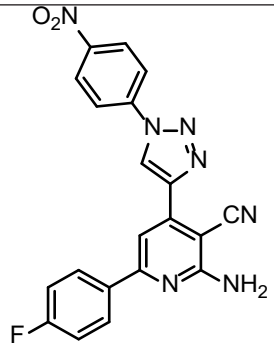


However, we have also performed all these reactions at room temperature, resulted into the corresponding products with less yield in more time as compared to ultrasound irradiation method. All the newly synthesized compounds were purified by crystallization method. The time required for the formation of products, yields and physical constants are given in **table 1**.

Table 1. Structures, yield, melting point and reaction time for the 1,2,3-triazole incorporated pyridine derivatives

Compound No.	Structure	Time (Minutes)	Yield (%)	Melting point (°C)
8a		70	90	202-204
8b		50	89	259-261
8c		60	90	248-250
8d		100	85	165-167
8e		90	85	205-207

8f		80	90	142-144
8g		100	87	210-212
8h		120	83	185-187
8i		110	85	158-160
8j		120	86	151-153

8k		130	87	160-162
8l		110	85	182-184
8m		110	89	168-170
8n		130	90	171-173
8o		100	85	155-157

The formation of the 1,2,3-triazole linked pyridine derivatives can be explained on the basis of mechanism. The plausible mechanism for the synthesis of triazole linked pyridine derivatives is as shown in **Fig. (4)**.

In the first step, reaction between triazolyl aldehyde and malononitrile gives Knoevenagel product **I**. The imine **II** formed by the reaction between ketone and ammonium acetate reacts with Knoevenagel product **I** to give intermediate **III**. The intermediate **III** on cyclization followed by isomerization and oxidation gives target molecule, 1,2,3-triazole linked pyridine derivatives **IV**.

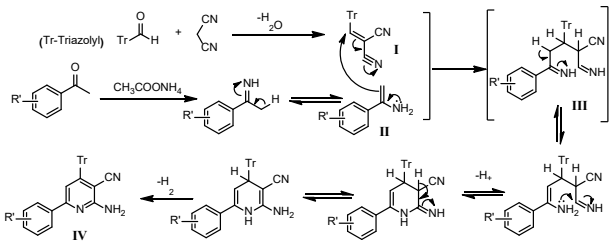


Fig. (4). Plausible mechanism for the synthesis of 1,2,3-triazole incorporated 2-amino-3-cyanopyridines (8a-o)

2.2. Biological Evaluation

2.2.1. Antifungal activity

All the newly synthesized 1,2,3-triazole incorporated 2-amino-3-cyano-pyridine derivatives **(8a-o)** were evaluated for their *in vitro* antifungal activity against four different fungal strains including *Candida albicans* (NCIM 3471), *Fusarium oxysporum* (NCIM 1332), *Aspergillus flavus* (NCIM 539) and *Aspergillus niger* (NCIM 1196). Minimum inhibitory concentration (MIC, µg/mL) values of all compounds were calculated using standard agar dilution method as per CLSI guidelines. All the experiments performed in triplicates and mean reading was taken as a final reading. 5% DMSO was used as a negative control along with Miconazole as the standard antifungal drug and the results were depicted in **Table 2**.

Among the newly synthesized highly functionalized 2-amino-3-cyanopyridine analogues, most of the compounds exhibited moderate to excellent antifungal activity. It is promising to note that some of the analogues were found to be more potent than the standard drug Miconazole. Among the series, compounds **8l**, **8m** and **8n** showed excellent antifungal activity against *C. albicans* with MIC value 17.3, 19.6 and 20.1 µg/mL, respectively. Compounds **8a**, **8c**, **8k** and **8l** were also found to be more potent than Miconazole against *F. oxysporum* with MIC value 18.7, 20.4, 20.5 and 18.9 µg/mL, respectively. Compounds **8d** and **8k** showed moderate activity against the fungal strain *A. Flavus* with MIC value 24.3 and 22.1 µg/mL, respectively. Compounds **8a**, **8d**, **8e**, **8j** and **8k** were exhibited excellent activity than Miconazole against *A. niger* with lower MIC values 22.7, 17.9, 22.9, 21.5 and 18.6 µg/mL, respectively. Remaining compounds were found to be moderately active against all the tested fungal strains.

Table 2. *In vitro* antitubercular and antifungal activity of compounds **(8a-o)**

Entry	Antifungal activity MIC (µg/mL)				Antitubercular activity Mtb $H_{37}Rv$ MIC (µg/mL)	Clog P[a]
	CA	*FO*	*AF*	*AN*		
8a	29.6	**18.7**	28.4	**22.7**	>25	3.93
8b	41.1	29.4	34.5	28.3	>25	4.43
8c	29.2	**20.4**	45.4	38.9	>25	4.06
8d	53.7	27.8	24.3	**17.9**	>25	4.06
8e	46.5	47.0	37.7	**22.9**	>25	4.72
8f	84.0	71.9	91.4	102.5	>25	4.72
8g	39.4	40.4	39.3	26.9	>25	3.94
8h	36.7	38.5	45.1	38.6	**25**	3.94

8i	65.2	68.4	62.1	59.1	**25**	4.08
8j	34.9	33.7	31.4	**21.5**	>25	4.20
8k	29.7	**20.5**	22.1	**18.6**	>25	4.20
8l	**17.3**	**18.9**	26.4	38.7	>25	4.20
8m	**19.6**	28.0	27.8	31.9	>25	4.86
8n	**20.1**	29.1	35.7	49.5	>25	4.86
8o	55.4	69.2	66.7	74.3	>25	4.09
MIZ	**25**	**25**	**12.5**	**25**	**NT**	**NT**
INH	**NT**	**NT**	**NT**	**NT**	**0.1**	**NT**
RIF	**NT**	**NT**	**NT**	**NT**	**0.2**	**NT**

[a]Calculated by using ChemBioDraw Ultra 12.0, INH: Isoniazid; RIF: Rifampicin, MIZ: Miconazole, CA: *Candida albicans*, FO: *Fusarium oxysporum*, AF: *Aspergillus flavus,* AN: *Aspergillus niger* NT: Not tested

2.2.2. **Antitubercular activity**

Antitubercular evaluation of all the newly synthesized triazole incorporated 2-amino-3-cyano-pyridines **(8a-o)** were performed by measuring the growth of inhibition against Mtb $H_{37}Rv$ strain using MABA assay method and the results are presented in **Table 2**. Among the series **(8a-o)**, none of the 2-amino-3-cyano-pyridine derivatives exhibited promising antitubercular activity. All the compounds from the series showed antitubercular activity with MIC values ≥ 25 μg/mL.

Lipophilicity parameter, Clog P value for all the synthesized compounds was also calculated and the results shows that all the compounds displayed better lipophilicity with Clog P value in the range 3.93 to 4.86 (**Table 2**)

2.3. **Computational Study**

2.3.1. **Molecular Docking Study**

In order to rationalize the promising levels of antifungal activity demonstrated by the synthesized 1,2,3-triazole incorporated 2-amino-3-cyano-pyridine derivatives **(8a-o)** and thus to understand the mechanistic basis of inhibition, molecular docking was performed against a crucial fungal target-sterol 14α-demethylase (CYP51). CYP51 is found to be crucial for the conversion of lanosterol to ergosterol and thus its inhibition could lead to accumulation of 14α-methyl sterols in the cell leading to impaired cell growth in fungi.

The in silico binding affinity analysis could lead to well clustered solutions for all the compounds (**8a-o**) into the active site of CYP51 exhibiting similar binding orientation. The binding scores were found to be in harmony with the observed antifungal data with the most active compounds showing higher affinity relative to those with moderate activities (binding energies -59.578 kcal/mol to -45.152 kcal/mol) while the average docking score was seen to be -9.365kcal/mol. An in-depth analysis of the per-residue interaction with these compounds has been carried out to identify the most significantly interacting residues and the nature of thermodynamic interactions (bonded and non-bonded interactions) governing the binding affinities. This analysis is elaborated in the next section for one of the most active analog **8l** and the results are summarized in **Table 3** for other molecules in the series.

Table 3. *In silico* molecular docking study of compounds **(8a-o)**

Entry	Glide Score	Glide Energy (Kcal/mol)	H-bond (Å)	Pi-stacking (Å)
8a	-9.865	-57.988	Tyr103(2.747)	His294(5.262), Phe290(4.204)
8b	-9.136	-50.343	Tyr103(2.478), Tyr103(2.715), Tyr116(2.452)	His294(5.251)
8c	-9.675	-54.298	Tyr116(2.111)	His294(5.413)
8d	-9.964	-58.996	Tyr103(2.652)	Tyr103(5.419)

8e	-9.302	-52.862	Tyr103(2.460), Tyr103(2.663), Tyr116(2.480)	His294(5.415)
8f	-8.441	-45.152	Tyr103(2.668), Tyr116(2.556)	His294(4.965), Phe290(4.200)
8g	-9.264	-51.355	Tyr116(2.415), Salt-bridge Glu205(4.937)	His294(5.474)
8h	-8.896	-49.466	Tyr116(2.481)	His294(5.282), Phe290(4.172), Phe110(5.312)
8i	-8.515	-47.207	Tyr103(2.664)	Tyr103(5.489)
8j	-9.524	-53.24	Tyr103(2.664)	Tyr103(5.400), Phe110(5.96)
8k	-9.916	-58.522	Tyr103(2.626), Tyr116(2.517)	His294(5.312), Phe290(4.223
8l	-9.978	-59.578	Tyr103(2.436), Tyr103(2.223), Tyr116(2.706)	Tyr116(4.232), His294(5.312), Phe290(4.396)
8m	-9.716	-56.921	Tyr103(2.573), Tyr116(2.794)	Phe110(5.419)
8n	-9.709	-55.998	Tyr103(2.724)	Phe110(5.476)
8o	-8.583	-48.361	Tyr116(2.097), Salt-bridge Glu205(4.352)	His294(5.195), Phe110(5.444)

The lowest energy docked conformation of **8l** was found to be snugly fitting into the active site of CYP51 with a significantly higher binding affinity (docking score of -9.978 and Glide binding energy of -59.578kcal/mol) engaging in a series of bonded and non-bonded interactions as shown in **Fig. (5)**.

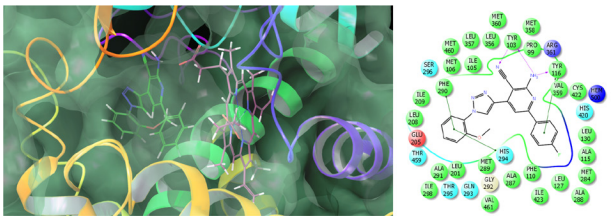


Fig. (5). Binding mode of 8l into the active site of sterol 14α-demethylase (CYP51)

It was found to be stabilized in the active site of CYP51 through a series of favorable van der Waals interactions with Val461 (-2.901Kcal/mol), Met460 (-1.932Kcal/mol), Thr459 (-1.124Kcal/mol), Thr295 (-1.178Kcal/mol), His294 (-2.287Kcal/mol), Ala291 (-2.143Kcal/mol), Phe290 (-4.002Kcal/mol), Ala287 (-1.129Kcal/mol), Leu208 (-1.713Kcal/mol), Glu205 (-1.582Kcal/mol), Met106 (-3.981Kcal/mol) and Ile105 (-1.942Kcal/mol) residues through the 2-methoxyphenyl-1*H*-1,2,3-triazole portion of the molecule while 2-Amino-3-cyano-6-(4-methyl)phenyl pyridine section exhibited similar type of interactions with Hem500 (-5.884Kcal/mol), Val359 (-1.2Kcal/mol), Leu356 (-2.148Kcal/mol), Leu127 (-1.379Kcal/mol), Tyr116 (-4.488Kcal/mol), Phe110 (-1.537Kcal/mol), Tyr103 (-3.627Kcal/mol) residues. While these van der Waals interactions were found to be the major driving force in the binding affinity, the enhanced binding shown by the molecule is also attributed to significant electrostatic interactions observed with Hem500 (-3.774Kcal/mol), Met460 (-2.147Kcal/mol), Arg361 (-1.115Kcal/mol), Phe290 (-1.34Kcal/mol), Ala287 (-1.381Kcal/mol), Glu205 (-2.707Kcal/mol), Tyr116 (-1.062Kcal/mol), Tyr103 (-2.744Kcal/mol) residues of the active site.

Further the compound was found to be engaged in close hydrogen bonding interactions with Tyr103 (2.436Å) through the cyano (-CN) function, while the amino group showed two hydrogen bonding interactions firstly with Tyr103 (2.223Å) and secondly with Tyr116 (2.706Å). The compound also exhibited a very prominent pi-pi (π-π) stacking interactions with His 294 (5.312Å and Phe290 (4.396Å) residues through the phenyl ring while the 4-methyl substituted phenyl showed similar interaction with Tyr116 (4.232Å). Such hydrogen bonding and π-π stacking interactions serve as an *anchor* to guide the orientation of the compound into the 3D space of active site and further facilitate the non-bonded (steric and electrostatic) interactions.
Similarly other compounds (**Fig. 6-17**)

in the series also exhibited a series of favorable bond and non-bonded interactions suggesting that 1,2,3-triazole incorporated 2-amino-3-cyanopyridines could serve as pertinent starting point for structure-based lead optimization to obtain potent and selective antifungal agents. All the molecular docking figures on right side shows green lines, which signify π-π stacking interactions while the pink lines represents the hydrogen bonding interactions.

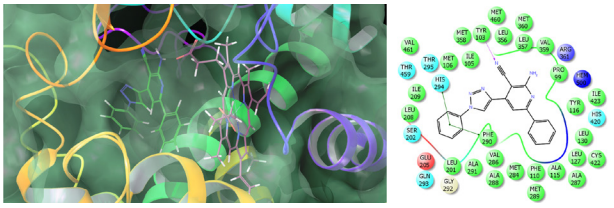


Fig. (6). Binding mode of 8a into the active site of sterol 14α-demethylase (CYP51)

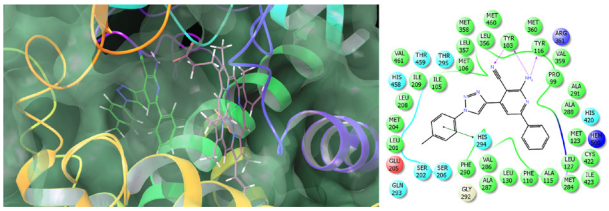


Fig. (7). Binding mode of 8b into the active site of sterol 14α-demethylase (CYP51)

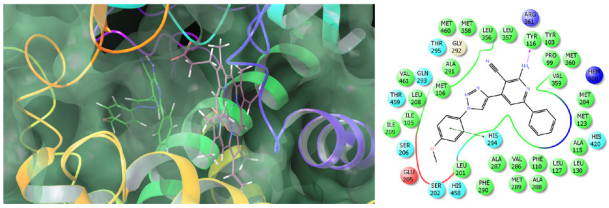


Fig. (8). Binding mode of 8c into the active site of sterol 14α-demethylase (CYP51)

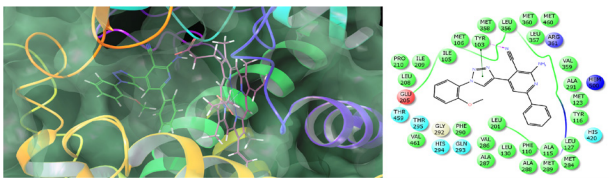


Fig. (9). Binding mode of 8d into the active site of sterol 14α-demethylase (CYP51)

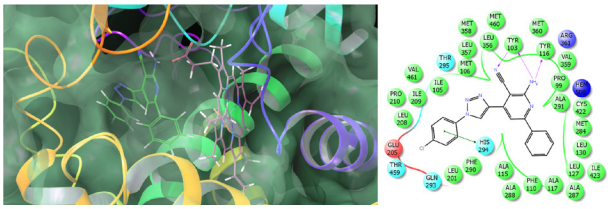


Fig. (10). Binding mode of 8e into the active site of sterol 14α-demethylase (CYP51)

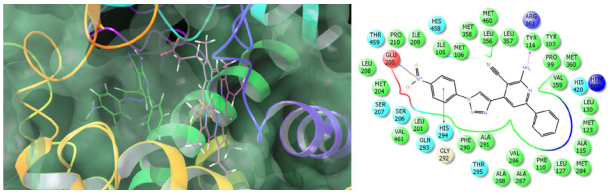


Fig. (11). Binding mode of 8g into the active site of sterol 14α-demethylase (CYP51)

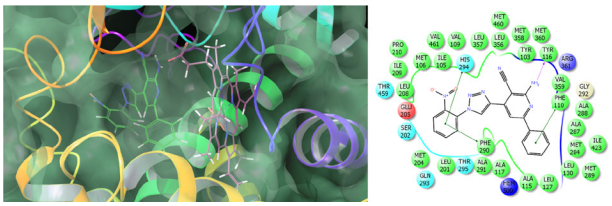


Fig. (12). Binding mode of 8h into the active site of sterol 14α-demethylase (CYP51)

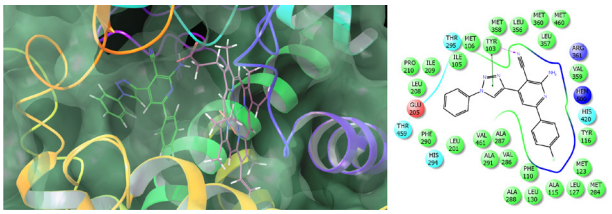


Fig. (13). Binding mode of 8i into the active site of sterol 14α-demethylase (CYP51)

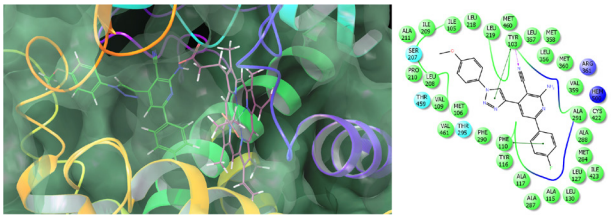


Fig. (14). Binding mode of 8j into the active site of sterol 14α-demethylase (CYP51)

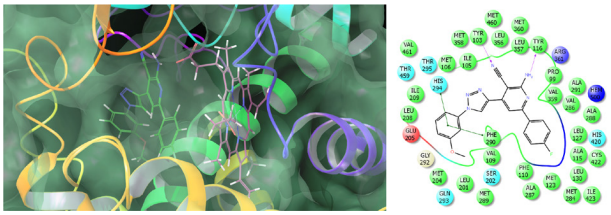


Fig. (15). Binding mode of 8k into the active site of sterol 14α-demethylase (CYP51)

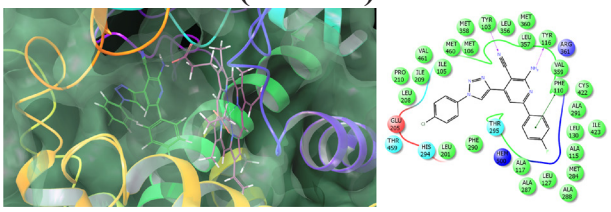


Fig. (16). Binding mode of 8m into the active site of sterol 14α-demethylase (CYP51)

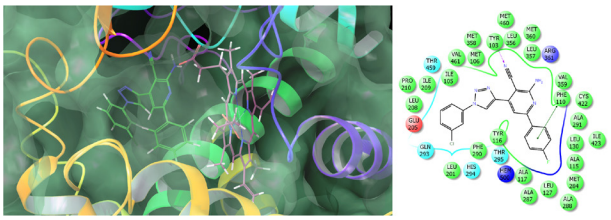


Fig. (17). Binding mode of 8n into the active site of sterol 14α-demethylase (CYP51)

2.3.2. *In silico* ADME prediction

In silico ADME properties prediction is essential for understanding success of a drug. Therefore, all the synthesized compounds were tested for various drug-like parameters and the results were mentioned in **Table 4**. From **Table 4**, it was observed that compounds possess a good % ABS (% absorption) ranging from 60.95 to 76.77 %. All the compounds were also follows Lipinski's rule of five (miLog P ≤ 5). Moreover, drug-likeness model score was calculated to study drug-like properties. From the results, it is clear that all the synthesized compounds are found to be suitable for the development of oral bioactive agents.

Table 4. Pharmacokinetic parameters for good oral bioavailability.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog P	n-ON	n-OHNH	Lipinskis violations	Drug-likeness model score
Rule	-	-	-	-	< 500	≤ 5	< 10	< 5	≤ 1	-
8a	76.77	93.42	3	299.31	338.37	2.91	6	2	0	-0.60
8b	76.77	93.42	3	315.88	352.40	3.36	6	2	0	-0.68
8c	73.58	102.66	4	324.86	368.40	2.97	7	2	0	-0.50
8d	73.58	102.66	4	324.86	368.40	3.13	7	2	0	-0.49
8e	76.77	93.42	3	312.85	372.82	3.59	6	2	0	-0.36
8f	76.77	93.42	3	312.85	372.82	3.77	6	2	0	-0.56
8g	60.95	139.25	4	322.65	383.37	2.87	9	2	0	-0.70
8h	60.95	139.25	4	322.65	383.37	3.03	9	2	0	-0.71
8i	76.77	93.42	3	304.25	356.36	3.07	6	2	0	-0.15
8j	73.58	102.66	4	329.79	386.39	3.13	7	2	0	-0.25
8k	73.58	102.66	4	329.79	386.39	3.32	7	2	0	-0.00
8l	73.58	102.66	4	329.79	386.39	3.29	7	2	0	-0.06
8m	76.77	93.42	3	317.78	390.81	3.75	6	2	0	-0.48
8n	76.77	93.42	3	317.78	390.81	3.94	6	2	0	-0.31
8o	60.95	139.25	4	327.58	401.36	3.03	9	2	0	-0.53

% ABS: Percentage absorption, TPSA: Topological polar surface area, MV: Molecular volume, MW: Molecular weight, milog p: Logarithm of partition coefficient of compound between n-octanol and water, n-ON: Number of hydrogen bond acceptors, n-OHNH: Number of hydrogen bonds donors, n-ROTB: Number of rotatable bonds

3. EXPERIMENTAL SECTION

3.1. General Methods

All the solvents and reagents were purchased from commercial suppliers Spectrochem Pvt. Ltd., Sigma Aldrich, and Rankem India Ltd. and are used without further purification. The reactions were performed using citizen (CUB 2.5) ultrasonic cleaner bath working at 40 kHz (constant frequency, 50W).

The progress of each reaction was monitored by ascending thin layer chromatography (TLC) using TLC aluminum sheets, silica gel 60 F_{254} precoated, Merck, Germany and locating the spots using UV light as the visualizing agent or iodine vapors. Melting points were determined in open capillary method and are uncorrected. Infrared (IR) spectra were recorded on a Bruker FT-IR spectrometer.^{1}H NMR spectra were recorded DMSO-d_6) on Bruker Avance 400 MHz NMR spectrometer. ^{13}C NMR spectra were recorded (DMSO-d_6) on Bruker Avance 100 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

3.2. General experimental procedure for the synthesis of 1,2,3-triazole incorporated 2-amino-3-cyano-pyridines (8a-o)

A mixture of appropriate aryl-1,2,3-triazolyl aldehyde (**4a-j**) (1 mmol), appropriate substituted acetophenone (**5a-b)** (1 mmol), malononitrile **6** (1 mmol) and ammonium acetate **7** (8 mmol) was dissolved in ethanol and the mixture was irradiated under ultrasonic irradiation with operating frequency 40 kHz at 30 °C. The progress of the reaction was monitored by TLC (*n*-Hexane/ EtOAc 9:1). After the completion of the reaction indicated by TLC, the reaction mixture was poured on crushed ice. The product was collected by filtration, dried and recrystallized from hot ethanol to give the pure desired products **(8a-o)** in good yields.

3.3. Experimental protocol for *in vitro* antifungal activity

Antifungal activity was determined by standard agar dilution method as per CLSI (formerly, NCCLS) guidelines [74]. The synthesized compounds and standard miconazole were dissolved in DMSO solvent. The medium yeast nitrogen base was dissolved in phosphate bufer of pH 7, and it was autoclaved at 110 °C for 10 min. With each set, a growth control without the antifungal agent and solvent control DMSO were included. The fungal strains were freshly subcultured onto Sabouraud dextrose agar (SDA) and incubated at 25 °C for 72 h. The fungal cells were suspended in sterile distilled water and diluted to get 105 cells/mL. Ten microliters of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25 °C for 48 h. The readings were taken at the end of 48 and 72 h. The minimum

inhibitory concentration (MIC) was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug-containing plates when there was visible growth on the drug-free control plates.

3.4. Experimental protocol for *in vitro* antitubercular activity

The antitubercular activity of the newly synthesized compounds **(8a-o)** have been screened for their in vitro effects against Mtb H37Rv (ATCC 27294) by using microplate Alamar Blue assay [75] for the determination of MIC in triplicates. The MIC (in μg/mL) was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of Mtb cultures. The MIC values of the compounds **(8a-o)** have been compared with standard drugs (rifampicin and isoniazid). The experimental method for antitubercular activity is briefly described as follows.

Initially, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1 and diluted 1:20; 100 μL was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at fourfold the final highest concentration tested. Serial twofold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100-μL 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags, and incubated at 37°C in normal atmosphere. After 7-day incubation, 30 μL of Alamar Blue solution was added to each well, and the plate was re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color.

3.5. Experimental protocol for molecular docking study

Molecular docking study was carried out using the standard protocol integrated in the GLIDE (Grid-based LIgand Docking with Energetics) program incorporated in the Schrodinger Molecular modeling package (Schrodinger, LLC, New York, NY, 2015) [76] With is objective the crystal structure of sterol 14α-demethylase (CYP51) enzyme complexed with its inhibitor-fluconazole (PDB code: 3KHM) was obtained from the RCSB Protein Data Bank (PDB) [77] and refined using the protein preparation wizard in Maestro which involves; adding the missing hydrogens/side chain atoms corresponding to pH 7.0, deleting the crystallographically observed water molecules, assignment of appropriate charge and protonation state to the obtained structure and finally energy minimization of the obtained structure using OPLS2005 force field until the average r.m.s.d. of heavy atoms reached 0.3Å.

Next the 3D structures of the compounds (**8a-8o**) to be docked were sketched in the *build* panel of Maestro and optimized through the *Ligand Preparation* wizard and then subjected to energy minimization until their average r.m.s.d. reached 0.001 Å. The shape and properties of the active site were defined using the *receptor grid*

generation panel for which a grid box of 10X10X10Å dimensions centered on the centroid of the co-crystallized ligand was generated. Using this setup, molecular docking was performed to gauze the binding affinities of the title compounds against the CYP51 using the extra precision (XP) Glide scoring function. The output files generated in the form of the docking poses were visualized and analyzed for the most significant interactions with the residues lining the active site using the Maestro's Pose Viewer utility.

3.6. Selected Spectral data

2-Amino-4-(1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-6 phenylnicotinonitrile (8d):

IR ν_{max}(cm^{-1}): 3478, 3304, 2200 and 1594. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.20 (s, 1H), 8.13-8.16 (m, 2H), 7.76-7.73 (m, 2H), 7.63-7.50 (m, 4H), 7.35-7.49 (m, 1H), 7.23-7.17 (m, 1H), 7.05 (s, 2H) and 3.9 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.1, 158.9, 151.58, 142.7, 142.3, 137.4, 131.2, 129.5, 128.7, 127.1, 126.6, 125.7, 125.2, 120.1, 117.0, 116.42, 116.0, 113.1, 106.7, 84.2 and 56.2. LC-MS: *m/z* calcd for $C_{21}H_{16}N_6O$ $[M+H]^+$: 369.1464, found: 369.

4. CONCLUSION

In summary, a series of triazole substituted 2-amino-3-cyano-pyridine derivatives were synthesized and evaluated for their biological activity. The synthesized compounds were screened for their *in vitro* antitubercular activity against Mtb H_{37}Rv strain. Among the series, compounds **8h** and **8i** were displayed moderate antitubercular activity (MIC = 25 µg/mL). All the synthesized compounds were screened for their *in vitro* antifungal activity and compounds **8a**, **8c**, **8d**, **8e**, **8j**, **8k**, **8l**, **8m** and **8n** were exhibited excellent antifungal activity against four different tested fungal strains, which were found to be more potent than Miconazole. The mechanistic basis of inhibition was investigated through molecular docking study against CYP51 which gave promising results with detailed insights into the major thermodynamic interactions contributing to the enhanced binding affinity of these molecules. Furthermore, ADME properties prediction for all the compounds were showed good drug-like properties and can be developed as an oral drug like molecule.

5. ACKNOWLEDGEMENTS

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