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A FACILE, EFFICIENT AND CATALYST FREE SYNTHESIS OF IMIDAZOLE, TETRAZOLE AND PYRIMIDINE COMBINED MOIETY AS POTENTIAL ANTIMICROBIAL AND ANTITUBERCULAR AGENTS

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Abstract – The tetrazole fused pyrimidine system possesses a broad spectrum of biological activities. So, we have synthesized 5-(substituted-phenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine derivatives **(5a-n)** by reaction of chalcones with 5-aminotetrazole without catalyst in appropriate solvent. The structural elucidation of these compounds is based on MS, IR, ^{1}H-NMR and ^{13}C-NMR spectral data. The in vitro antimicrobial activity was investigated against Gram-positive and Gram-negative bacterial and fungal strains. It was found that the compounds **5a**, **5b**, **5c**, **5d**, **5e** showed significant activities against tested organisms as compared to standard drugs (Ampicillin and Griseofulvin) while compounds **5a**, **5c**, **5i**, and **5k** showed good percentage of average inhibition in the dormant and active stage of tuberculosis.

INTRODUCTION

The synthesis of novel tetrazole derivatives and the investigation of their chemical and biological behaviour have gained more importance in the recent decades for biological and pharmaceutical reasons.[1] On the other hand, pyrimidine scaffold was the base of many bioactive molecules.[2] In view of the above-mentioned facts, if two active pharmacophores, linked together, would generate novel molecular structures which are likely to exhibit interesting biological properties. Tetrazolo-pyrimidine condensed derivatives as the pharmacophore exhibit broad spectrum of biological activities encompassing antimicrobial,[3-8] antifungal,[9-11] anticancer,[12-18] antimalarial,[19] antitubercular,[19] anti-inflammatory,[20] analgesics,[21,22] antiviral,[23] anti-oxidant,[24] antiproliferative,[25,26] and antibacterial agents.[27] Hence the preparation of tetrazolo[1,5-*a*]pyrimidine core unit has gained much importance. Various methods for the

preparation of tetrazolo[1,5-*a*]pyrimidine derivatives have been cited in literature.[28-40] However, many of these methods suffered from toxic reagents, harsh reaction conditions, strongly acidic or basic conditions, poor yields, and prolonged reaction-times. We have developed a catalyst free method for the synthesis of 5-(substituted-phenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidines **(5a-n)**. The isolated products were screened for the antimicrobial and antitubercular activities.

RESULTS AND DISCUSSION

In the first step, chalcones **(3a-n)** were prepared by using PEG-400 as a reaction solvent, in the second step tetrazolo[1,5*a*]pyrimidines **(5a-n)** were prepared by reaction between chalcones **(3a-n)** and 5-aminotetrazole **(4)** at different reaction conditions. The optimization in reaction conditions, in terms of time, solvent, catalyst and yields are reported in **Table 1**.

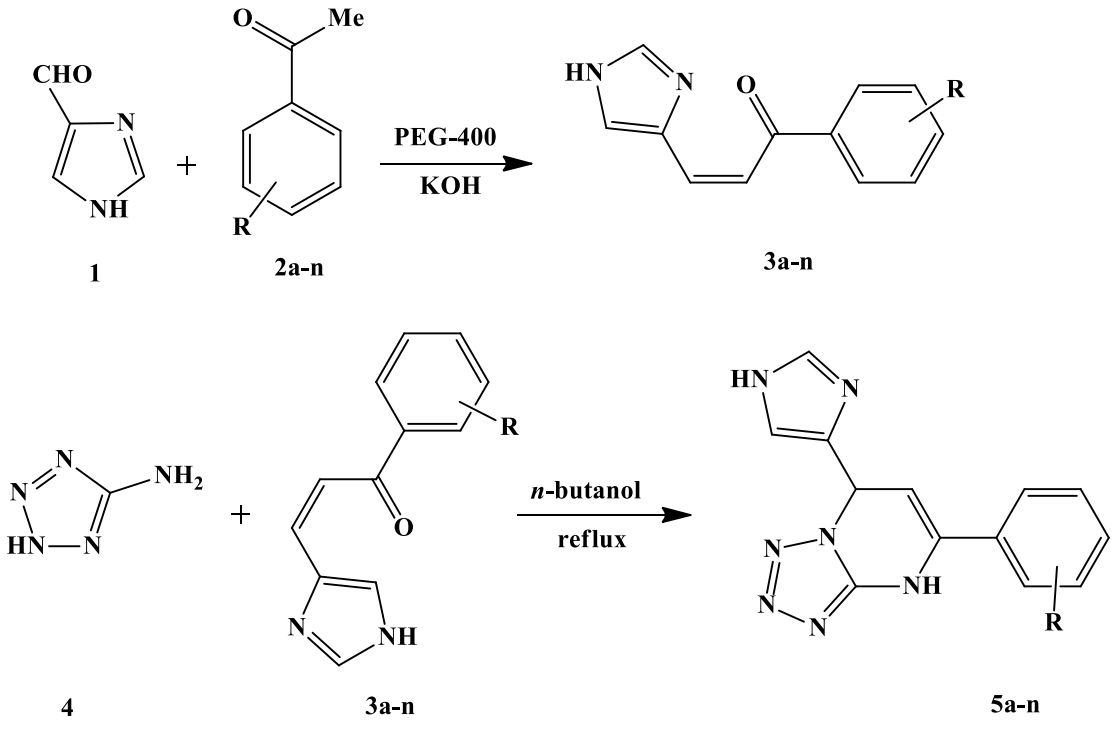
Table 1. Various conditions for the synthesis of tetrazolo[1,5-*a*]pyrimidines

Entry	Catalyst	Solvent	Time (h)	Temperature	Yield (%)
1	HCl (2 drops)	ethanol 95%	15	reflux	60
2	piperidine (2 drops)	ethanol 95%	12	reflux	54
3	-	-	1	fusion	30
4	-	methanol	12	reflux	40
5	-	ethanol 95%	12	reflux	52
6	-	*n*-propanol	10	reflux	55
7	**-**	***n*-butanol**	**10**	**reflux**	**87**
8	-	DMF	15	reflux	42
9	-	isopropanol	12	reflux	45
10	-	1,4-dioxane	15	reflux	56

We carried out the synthesis of tetrazolo[1,5-*a*]pyrimidines in the acidic and basic catalytic amount at reflux temperature, but there was not much satisfied with the yield and reaction time of obtained products. Again the same reaction was carried out without any solvent or catalyst in fused condition but yield was very low. So from the above conclusion, we have chosen, an alcoholic solvents such as methanol, *n*-propanol, isopropanol, *n*-butanol and polar solvent like dimethylformamide (DMF) and 1,4-dioxane. After doing various optimizations in the experimental conditions, it was concluded that, when the reaction

was carried out in *n*-butanol solvent showed better yield (87%) in less reaction time.

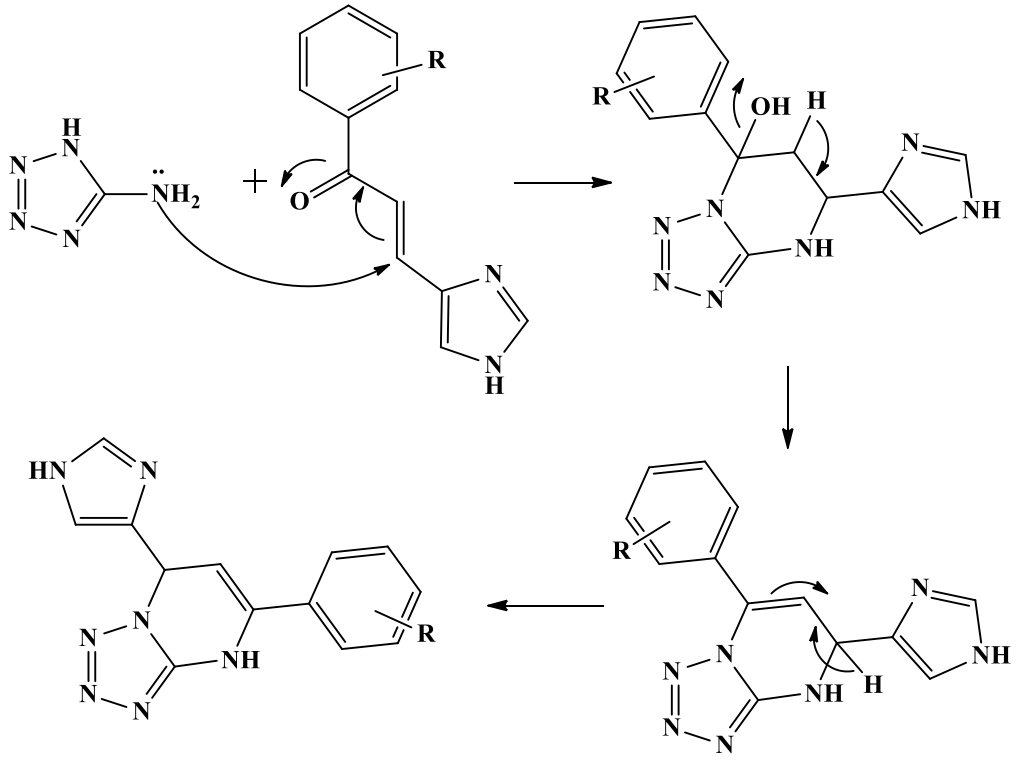
All the newly synthesized compounds were characterized using IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. The IR spectrum of synthesized compounds exhibited characteristic absorption band at 3075 cm^{-1} for aromatic C=C stretching vibration band. Characteristic absorption band observed at 1315 cm^{-1} for C-N stretching in imidazole ring and at 1605 for C=N of the pyrimidine ring system and all other general frequency band are in good agreement with the structure. ^{1}H-NMR spectra of the compound **5b** showed characteristic singlet signals at 12.16 ppm due to the proton of imidazole-NH and at 10.44 ppm due to the proton of fused pyrimidine-NH. Chiral carbon proton was observed doublet at 5.28 ppm with 2.80 Hz coupling constant value. The ^{1}H-NMR spectrum of compound **5c** exhibited two doublets, arising from the two equivalent phenyl ring protons at 7.54 and 7.24 ppm. The ^{13}C-NMR spectrum of compound **5c** showed signal at 53.23 ppm for the chiral carbon while at 96.00 ppm for the pyrimidine ring C=C carbon and at 21.12 ppm for the methyl carbon attached to phenyl ring. Molecular ion peak was observed in agreement with molecular weight of respective compound i.e. the molecular formula of compounds **5c** and **5m** by displaying molecular ion peaks at *m/z* 279 (M^{+}) and 325 (M^{+}) respectively.



R= 4-Cl (a); 4-Br (b); 4-Me (c); 4-OH (d); 4-MeO (e); 3-MeO (f); 2-MeO (g); 2-OH (h); 4-F (i); 3-Cl (j); 3-NO_2 (k); -H (l); 2,4-$(MeO)_2$ (m); 4-NO_2 (n)

Scheme 1

PLAUSIBLE REACTION MECHANISM:



Scheme 2

The examination of the data of the **Table 2** revealed that many of the compounds shown good activity against bacterial strain when compared with the standard drugs like Ampicillin, Chloramphenicol, and Ciprofloxacin. From the results of antimicrobial activity we can say that compound **5e** has shown excellently active with MIC of 62.5 μg/mL against *P. aeruginosa* with respect to the standard drug Ampicillin. Compounds **5a**, **5b** and **5c** were moderately active against *S. aureus* with respect to the standard drug Ampicillin. Compound **5j** has exhibited comparatively activity with MIC of 100 μg/mL against *S. pyogenus* with respect to the standard drug Ampicillin. Moderate activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) with respect to standard drug Ampicillin was shown by compounds **5b**, **5c** and **5h** (100 μg/mL).

Looking at the antifungal results compounds **5c** and **5j** (200 μg/mL) were more active with respect to standard antifungal drug Griseofulvin against *C.albicans*while. Compounds **5d**, **5h** and **5k** were comparative active with the MIC of 500 μg/mL with respect to standard Griseofulvin against *C. albicans*. Compound **5d** was comparative active with the MIC of 100 μg/mL with respect to standards Nystatin and Griseofulvin against *A. niger* while compound **5j** was equally active with the MIC of 100 μg/mL with respect to standards Nystatin and Griseofulvin against *A. clavatus.*

Table 2. Antibacterial and antifungal screening of compounds **5a-n** as a MIC

Antimicrobial screening of the compounds								
Sr. No.	-R	Antibacterial activity (MIC) in µg mL^{-1}				Antifungal activity (MIC) in µg mL^{-1}		
		Gram-positive		Gram-negative		Fungi		
		S. aureus	*S. pyogenus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*
5a	4-Cl	**100**	250	200	200	1000	500	1000
5b	4-Br	**100**	200	250	**100**	>1000	500	500
5c	4-Me	**100**	250	**100**	250	**200**	>1000	>1000
5d	4-OH	500	500	200	500	500	**100**	500
5e	4-OMe	500	200	125	**62.5**	1000	>1000	>1000
5f	3-OMe	250	250	250	200	>1000	1000	500
5g	2-OMe	500	250	200	200	1000	>1000	500
5h	2-OH	250	250	250	**100**	500	500	1000
5i	4-F	250	125	500	250	1000	500	500
5j	3-Cl	500	**100**	250	500	**200**	250	**100**
5k	3-NO_2	250	250	200	250	500	500	500
5l	-H	500	500	200	500	1000	1000	1000
5m	2,4-$(OMe)_2$	200	200	250	200	>1000	>1000	>1000
5n	4-NO_2	250	200	125	250	1000	500	500
Ampicillin		250	100	100	100	-	-	-
Chloramphenicol		50	50	50	50	-	-	-
Ciprofloxacin		50	50	25	25	-	-	-
Nystatin		-	-	-	-	100	100	100
Griseofulvin		-	-	-	-	500	100	100

Staphylococcus aureus MTCC 96; *Streptococcus pyogenes* MTCC 442; *Escherichia colli* MTCC 443; *pseudomonas aeruginosa* MTCC 1688; *Aspergillus niger* MTCC 282; *Candida albicans* MTCC 227; *Aspergillus clavatus* MTCC 1323.

From the results of antitubercular activity data **Table 3** reveals that, all the synthesized compounds have shown moderate activity against dormant and active stage of *Mycobacterium tuberculosis* $H_{37}Rv$ at µg/mL concentration level. **5a**, **5c**, **5i**, and **5k** are showing good percentage of average inhibition in the dormant

and active stage of tuberculosis. Compound **5c**, is most active and showing 90.54% and 92.58% inhibition at 100 μg/mL concentration at dormant and active stage, respectively. Compound **5c** showing 85.69% and 85.45% inhibition at 50 μg/mL concentrations in dormant and active stage, respectively. Compound **5i** is active and showing 80.21% and 80.54% inhibition at 100 μg/mL concentrations at dormant and active stage respectively. At all concentration compounds **5d**, **5f**, **5h** and **5l** are not active in active stage. Compounds **5d**, **5e** and **5h** are not showing activity in the dormant stage at 25 μg/mL and 12.5 μg/mL concentrations.

Table 3. Antitubercular activity of compounds **5a-n**

		Antitubercular activityof the compounds							
Sr. No.	-R	% average inhibition dormant stage μg/mL concentration				% average inhibition active stage μg/mL concentration			
		100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL	100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL
5a	4-Cl	**80.30**	77.25	60.87	20.54	**80.45**	78.47	60.21	40.25
5b	4-Br	70.25	60.14	52.36	21.58	77.36	68.25	54.77	42.25
5c	4-Me	**90.54**	**85.69**	**80.12**	62.54	**92.58**	**85.45**	58.56	48.75
5d	4-OH	12.36	10.53	NA	NA	NA	NA	NA	NA
5e	4-OMe	50.25	39.87	25.41	18.35	25.36	12.54	10.65	5.84
5f	3-OMe	25.32	10.54	NA	NA	NA	NA	NA	NA
5g	2-OMe	33.21	21.57	10.23	8.79	24.23	15.21	11.47	6.23
5h	2-OH	9.78	5.48	NA	NA	NA	NA	NA	NA
5i	4-F	**80.21**	74.25	62.23	55.14	**80.54**	70.54	62.74	54.21
5j	3-Cl	74.12	58.23	47.14	20.56	78.52	63.49	51.98	47.25
5k	3-NO_2	**85.63**	**81.24**	75.14	56.58	78.21	62.45	52.21	42.06
5l	-H	25.41	14.28	10.25	5.12	NA	NA	NA	NA
5m	2,4-$(OMe)_2$	50.21	32.62	21.47	12.54	10.23	6.54	NA	NA
5n	4-NO_2	**80.21**	78.56	65.25	50.21	70.45	57.23	45.02	36.32

NA= Not Active

STRUCTURE-ACTIVITY RELATIONSHIP

On considering the relationships between the structure of the heterocyclic scaffolds **5a-n** and the antimicrobial property, the uniqueness of individual substitutions proved to be a vital parameter for

influencing the activity of the synthesized compound. From the data obtained it is clear that compound **5e** (4-OMe) was the most active compound at MIC 62.5 mg/mL towards Gram-negative bacteria while compounds **5f** (2-OMe) and **5g** (3-OMe) were not much active though all posses methoxy group. The position of the methoxy group was an important part of the activity.

CONCLUSION

We carried out catalyst free synthesis of tetrazolo[1,5-*a*]pyrimidines using *n*-butanol as a solvent at reflux temperature. We have confirmed the structure based on spectroscopic technique. The protons and carbons belonging to the tetrazolo-pyrimidine ring, imidazole ring and phenyl substituents were observed at expected chemical shift and integral values. Considering the results obtained from antibacterial and antifungal tests together, it can be interpreted that compounds with electron withdrawing groups like chloro, bromo, nitro etc. substitution showed good activity compared to those possessing electron donating group substitution. From the results of antitubercular activity, we concluded, compounds with aromatic ring substituted with 4-methyl and 3-nitro have shown good activity against dormant and active stage of *Mycobacterium tuberculosis H*$_{37}$*Rv* at μg/mL concentration level.

EXPERIMENTAL

All chemicals were purchased and used without any further purification. Most of the reactions were carried out by standard techniques for the exclusion of moisture. Reactions were monitored by thin-layer chromatography (TLC) on silica gel-G plates of 0.5 mm thickness (Merck $^{60}F_{254}$), visualizing with ultraviolet light and appropriate solvents were used as mobile phase. Melting points were recorded in open capillary tubes and were uncorrected. IR spectra were recorded on a Shimadzu FT-IR-8400 instrument using DRS (diffusive reflectance system) method and are expressed in cm^{-1} (KBr). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance II spectrometer at 400 and 101.1 MHz, respectively, using DMSO-d_6 as a solvent; the chemical shifts are referenced to tetramethylsilane (TMS). Mass spectra were recorded on the Shimadzu GC-MS-QP-2010 model using a direct inlet probe technique.

General procedure for the synthesis of 1-(substituted-phenyl)-3-(1*H*-imidazol-4-yl)prop-2-en-1-ones (2a-n). An equimolar mixture of 4-formylimidazole (1 mmol), substituted acetophenones (1 mmol) and KOH (2 mmol) was stirred in PEG-400 (12 mL) at 50 ^{o}C for 2 h. On completion of the reaction monitored by TLC, the crude mixture was worked up in ice-cold water (100 mL). The separated product was filtered. The filtrate was evaporated to remove water leaving PEG-400 behind. The same PEG-400 was utilized to synthesize further chalcones. Purification of the product was carried out using Et_2O to afford analytically pure products.

General procedure for the synthesis of 5-(substituted-phenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydro-tetrazolo[1,5-*a*]pyrimidines (5a-n). To a stirred solution of substituted chalcone (10 mmol) in *n*-butanol, 5-aminotetrazole (10 mmol) was added. The mixture was stirred to reflux for 10 h. On completion of the reaction monitored by TLC, the separated solid was filtered and washed with Et_2O to obtain the solid crude product. Crystallization of the synthesized products was carried out in appropriate solvent.

5-(4-Chlorophenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5a): Light yellow solid; yield 83%; mp 210-212 °C; IR (KBr), (ν max, cm^{-1}): 3075 (C-H_{phenyl}), 1675 (C=$N_{pyrimidine}$), 1550, 1290 (C-N), 1105 (C-H), 817(*p*-disubstituted aromatic ring), 723 (C-Cl); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.26-5.27 (d, 1H, *J* = 2.80 Hz, -CH_{chiral}), 6.56 (s, 1H, ArH), 7.26 (s, 1H, ArH), 7.63-7.65 (d, 2H, *J* = 8.40 Hz, Ph-H), 7.70-7.72 (d, 2H, *J* = 8.00 Hz, Ph-H), 7.77 (s, 1H, ArH), 10.40 (s, 1H, -$NH_{pyrimidine}$), 12.19 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 52.4, 58.4, 95.7, 110.1, 118.7, 125.1, 128.5, 131.5, 133.2, 148.1; MS: *m/z* 299 (M^+).

5-(4-Bromophenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5b): Yellow solid; yield 86%; mp 150-151 °C; IR (KBr), (ν max, cm^{-1}): 3074(C-H_{phenyl}), 1660(C=$N_{pyrimidine}$), 1548, 1313(C-N), 1095 (C-H), 819 (*p*-disubstituted aromatic ring), 680 (C-Br); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.27-5.28 (d, 1H, *J* = 2.80 Hz, -CH_{chiral}), 6.54 (s, 1H, ArH),7.24 (s, 1H, ArH), 7.58-7.60 (d, 2H, *J* = 8.40 Hz, Ph-H), 7.64-7.66 (d, 2H, *J* = 8.00 Hz, Ph-H), 7.78 (s, 1H, ArH), 10.44 (s, 1H, -$NH_{pyrimidine}$), 12.16 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 53.3, 60.3, 97.6, 114.2, 122.2, 128.0, 131.9, 133.8, 135.9, 150.5; MS: *m/z* 343 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(*p*-tolyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5c): white solid; yield 82%; mp 121-123 °C; IR (KBr), (ν max, cm^{-1}): 3010 (C-H_{phenyl}), 1655 (C=$N_{pyrimidine}$), 1580, 1220 (C-N), 1110 (C-H), 865 (*p*-disubstituted aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.17 (s, 3H, -CH_3), 5.18 (s, 1H, -CH_{chiral}), 6.54 (s, 1H, ArH), 7.12 (s, 1H, ArH), 7.22-7.24 (d, 2H, *J* = 7.20 Hz, Ph-H), 7.52-7.54 (d, 2H, *J* = 7.16 Hz, Ph-H), 7.63 (s, 1H, ArH), 10.38 (s, 1H, -$NH_{pyrimidine}$), 12.22 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 21.1, 53.2, 60.3, 96.0, 114.1, 125.7, 128.3, 129.3, 130.9, 134.6, 136.1, 139.7, 143.6, 150.6, 155.1, 175.1; MS: *m/z* 279 (M^+).

4-(7-(1*H*-Imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidin-5-yl)phenol (5d): Light green solid; yield 67%; mp 212-215 °C; IR (KBr), (ν max, cm^{-1}): 3525 (O-H_{phenol}), 3012 (C-H_{phenyl}), 1630 (C=$N_{pyrimidine}$), 1565, 1341 (O-H_{phenol}), 1230 (C-N), 1121 (C-H), 857 (*p*-disubstituted aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.26 (s, 1H, -CH_{chiral}), 5.38 (s, 1H, -OH), 6.36 (s, 1H, ArH), 7.40 (s, 1H, ArH), 7.56-7.57 (d, 2H, *J* = 7.82 Hz, Ph-H), 7.62-7.64 (d, 2H, *J* = 7.32 Hz, Ph-H), 7.72 (s, 1H, ArH), 10.10 (s, 1H, -$NH_{pyrimidine}$), 12.04 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 51.3, 56.2, 98.1, 110.2, 116.4, 119.8, 123.5, 133.6, 137.2, 144.8, 158.3, 167.2; MS: *m/z* 281 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(4-methoxyphenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5e): Brown solid;

yield 87%; mp 145-146 °C; IR (KBr), (ν max, cm^{-1}): 2978 (C-H_{phenyl}), 1643 (C=$N_{pyrimidine}$), 1512, 1257(C-N), 1180 (C-H), 1033 (C-O_{ether}), 875 (*p*-disubstituted aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 3H, -OCH_3), 5.25 (s, 1H, -CH_{chiral}), 6.42 (s, 1H, ArH), 7.42 (s, 1H, ArH), 7.53-7.55 (d, 2H, *J* = 7.80 Hz, Ph-H), 7.63-7.65 (d, 2H, *J* = 7.31 Hz, Ph-H), 7.67 (s, 1H, ArH), 10.03 (s, 1H, -$NH_{pyrimidine}$), 12.10 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 54.2,55.1, 57.4, 95.2, 111.3, 114.5, 121.5, 125.2, 129.2, 132.6, 136.4, 142.2, 155.6, 161.2; MS: *m/z* 295 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(3-methoxyphenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5f): Brown solid; yield 74%; mp 205-207 °C; IR (KBr), (ν max, cm^{-1}): 3055 (C-H_{phenyl}), 1654 (C=$N_{pyrimidine}$), 1575, 1246 (C-N), 1132 (C-H), 1014 (C-O_{ether}); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H, -OCH_3), 5.27 (s, 1H, -CH_{chiral}), 6.51 (s, 1H, ArH), 7.32 (s, 1H, ArH), 7.48-7.61 (m, 4H, Ph-H), 7.81 (s, 1H, ArH), 10.07 (s, 1H, -$NH_{pyrimidine}$), 12.21 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 56.2, 57.1, 93.2, 110.8, 115.1, 120.7, 126.1, 133.6, 140.1, 145.4, 156.2, 162.2; MS: *m/z* 295 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(2-methoxyphenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5g): Brown solid; yield 78%; mp 154-155 °C; IR (KBr), (ν max, cm^{-1}): 3080 (C-H_{phenyl}), 1614 (C=$N_{pyrimidine}$), 1533, 1240 (C-N), 1150 (C-H), 1033 (C-O_{ether});^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 3H, -OCH_3), 5.35 (s, 1H, -CH_{chiral}), 6.21 (s, 1H, ArH), 7.35 (s, 1H, ArH), 7.45-7.58 (m, 4H, Ph-H), 7.78 (s, 1H, ArH), 10.10 (s, 1H, -$NH_{pyrimidine}$), 12.08 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 53.2, 57.1, 96.7, 113.2, 116.2, 121.6, 127.2, 134.5, 144.2, 155.1, 166.4; MS: *m/z* 295 (M^+).

2-(7-(1*H*-Imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidin-5-yl)phenol (5h): white solid; yield 75%; mp 178-180 °C; IR (KBr), (ν max, cm^{-1}): 3554 (-OH_{phenol}), 3020 (C-H_{phenyl}), 1662 (C=$N_{pyrimidine}$), 1563, 1340 (-OH_{phenol}), 1245 (C-N), 1138 (C-H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.19 (s, 1H, -CH_{chiral}), 5.39 (s, 1H, -OH), 6.20-6.22 (d, 1H, *J* = 3.14 Hz, ArH), 7.32 (s, 1H, ArH), 7.55-7.69 (m, 4H,Ph-H), 7.78 (s, 1H, ArH), 10.12 (s, 1H, -$NH_{pyrimidine}$), 12.13 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 53.2, 55.3, 97.4, 112.7, 118.7, 122.6, 125.7, 135.6, 138.1, 147.4, 159.2, 169.0; MS: *m/z* 281 (M^+).

5-(4-Fluorophenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5i): Reddish brown solid; yield 80%; mp 174-175 °C; IR (KBr), (ν max, cm^{-1}): 3066 (C-H_{phenyl}), 1658 (C=$N_{pyrimidine}$), 1574, 1254 (C-N), 1255 (C-F), 1147 (C-H), 825 (*p*-disubstituted aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.30-5.32 (d, 1H, *J* = 2.78 Hz, -CH_{chiral}), 6.57 (s, 1H, ArH), 7.27 (s, 1H, ArH), 7.60-7.62 (d, 2H, *J* = 8.42 Hz, Ph-H), 7.66-7.68 (d, 2H, *J* = 8.12 Hz, Ph-H), 7.76 (s, 1H, ArH), 10.38 (s, 1H, -$NH_{pyrimidine}$), 12.21 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 54.2, 61.3, 98.5, 112.3, 125.5, 130.1, 132.2, 136.7, 152.7; MS: *m/z* 283 (M^+).

5-(3-Chlorophenyl)-7-(1*H*-imidazol-4-yl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5j): Light yellow solid; yield 62%; mp 214-216 °C; IR (KBr), (ν max, cm^{-1}): 3060 (C-H_{phenyl}), 1658 (C=$N_{pyrimidine}$), 1560, 1268 (C-N), 1156 (C-H), 730 (C-Cl); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.30-5.32 (d, 1H, *J* = 2.82 Hz,

-CH_{chiral}), 6.58 (s, 1H, ArH), 7.25 (s, 1H, ArH), 7.65-7.76 (m, 4H,Ph-H), 7.80 (s, 1H, ArH), 10.22 (s, 1H, -$NH_{pyrimidine}$), 12.28 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 56.5, 60.1, 97.4, 108.2, 115.3, 123.3, 130.7, 134.1, 152.5; MS: *m/z* 299 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(3-nitrophenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5k): Dark yellow solid; yield 72%; mp 187-189 ºC; IR (KBr), (ν max, cm^{-1}): 3056 (C-H_{phenyl}), 1645 (C=$N_{pyrimidine}$), 1554, 1354 (C-NO_2), 1254 (C-N), 1141 (C-H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.32-5.34 (d, 1H, *J* = 2.74 Hz, -CH_{chiral}), 6.61 (s, 1H, ArH), 7.32 (s, 1H, ArH), 7.64-7.75 (m, 4H, Ph-H),7.78 (s, 1H, ArH), 10.03 (s, 1H, -$NH_{pyrimidine}$), 12.24 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 52.1, 59.2, 97.6, 105.4, 123.3, 130.3, 135.1, 141.1, 158.0; MS: *m/z* 310 (M^+).

7-(1*H*-Imidazol-4-yl)-5-phenyl-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5l): White solid; yield 74%; mp 241-243 ºC; IR (KBr), (ν max, cm^{-1}): 3065 (C-H_{phenyl}), 1650 (C=$N_{pyrimidine}$), 1570, 1236 (C-N), 1156 (C-H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.20 (s, 1H, -CH_{chiral}), 6.52 (s, 1H, ArH), 7.10 (s, 1H, ArH), 7.20-7.33 (m, Ph-H),7.60 (s, 1H, ArH), 10.08 (s, 1H, -$NH_{pyrimidine}$), 12.15 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 54.1, 65.7, 99.5, 115.4, 127.1, 129.8, 137.4, 139.1, 145.7, 154.2, 174.5; MS: *m/z* 265 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(2,4-dimethoxyphenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5m): Brown solid; yield 80%; mp 164-166 ºC; IR (KBr), (ν max, cm^{-1}): 3073 (C-H_{phenyl}), 1685 (C=$N_{pyrimidine}$) 1540, 1277 (C-N), 1155 (C-H), 1058 (C-O_{ether}); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 6H, -OCH_3), 5.28 (s, 1H, -CH_{chiral}), 6.21 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.50-7.63 (m, 3H, Ph-H), 7.75 (s, 1H, ArH), 10.12 (s, 1H, -$NH_{pyrimidine}$), 12.10 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 57.1, 59.5, 95.4, 112.4, 117.0, 125.4, 137.7, 142.4, 147.1, 158.1, 164.0; MS: *m/z* 325 (M^+).

7-(1*H*-Imidazol-4-yl)-5-(4-nitrophenyl)-4,7-dihydrotetrazolo[1,5-*a*]pyrimidine (5n): Dark yellow solid; yield 68%; mp 154-155 ºC; IR (KBr), (ν max, cm^{-1}): 3093 (C-H_{phenyl}), 1662 (C=$N_{pyrimidine}$), 1552, 1315 (C-NO_2), 1209 (C-N), 1093 (C-H), 821 (*p*-disubstituted aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.33-5.35 (d, 1H, *J* = 2.76 Hz, -CH_{chiral}), 6.60 (s, 1H, ArH), 7.31 (s, 1H, ArH), 7.63-7.65 (d, 2H, *J* = 8.28 Hz, Ph-H), 7.70-7.72 (d, 2H, *J* = 8.30 Hz, Ph-H), 7.77 (s, 1H, ArH), 10.06 (s, 1H, -$NH_{pyrimidine}$), 12.31 (s, 1H, -$NH_{imidazole}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 55.3, 62.5, 99.1, 110.4, 127.1, 133.7, 136.4, 139.4, 155.3; MS: *m/z* 310 (M^+).

ANTIMICROBIAL SCREENING PROTOCOL

For evaluation of antibacterial activity of newly synthesized entities **(5a-n)**, we used *Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442 from Gram-positive group of bacterial strain and *Escherichia colli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688 from Gram-negative group of bacterial strain. For evaluation of antifungal activity of newly synthesized entities **(5a-n)**, we used

Aspergillus niger MTCC 282, *Candida albicans* MTCC 227and *Aspergillus clavatus* MTCC 1323 as a fungal strains. The strains were procured from the Institute of Microbial Technology, Chandigarh. The samples for antibacterial and antifungal evaluation were tested by the conventional broth dilution method. The antibacterial testing standards were Ampicillin, Chloramphenicol and Ciprofloxacin while Nystatin and Griseofulvin for antifungal activity at different concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62 up to 7.8 μg mL^{-1} as shown in **Table 2**. Many of them had proven their antimicrobial potency varied from moderate to excellent. The MIC value of ciprofloxacin was noted to be 50 μg/mL against Gram-positive bacterial strain *S. aureus*, whereas the same standard responded at 25 μg/mL; when used against Gram-negative bacterial strain *E. coli*, and *P. aeruginosa*. Nystatin, when used as a standard drug against fungal strains *C. albicans*, *A. niger*, and *A. clavatus* MIC value was observed at 100 μg/mL.

ANTITUBERCULAR SCREENING PROTOCOL

All the synthesized compounds **5a-n** were evaluated for anti-tubercular activity against *M. tuberculosis H37Rv* in dormant and active stage at μg/mL concentration using XTT Reduction Menadione (XRMA) method.[41] Isoniazide is taken as standard drug for the comparison of activity. All experiments were performed in triplicates and the quantitative value was expressed as the percentage average of inhibition showed in **Table 3**.

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