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SYNTHESIS OF NOVEL IMIDAZO[4,5-b] PYRIDINE DERIVATIVES AS NEW ANTIMICROBIAL AGENTS

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ABSTRACT

The targeted compounds 3-(1H-imidazo [4,5-b] pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI) derivatives were evaluated for antimicrobial activity by using cup plate method. The synthesized compounds were characterized and evaluated for antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Staphylococcus typhi and antifungal activity against Aspergillus niger and Candida albicans by using Ampicillin sodium and Clotrimoxazole as standards respectively. Among all the compounds, Compound VId was more effective against all the testing bacteria. Among this series Compound VIb and Compound VIf were exhibited more effective against Gram Negative bacteria and Compound VId was also more effective against fungal.

KEY WORDS

imidazo[4,5-b] pyridine, IR Spectrum, NMR Spectrum, Mass Spectrum, Antibacterial, Antifungal

INTRODUCTION:

The main problem of existing treatment of infectious diseases is demanding due to resistance to antimicrobial agents and their drawbakcs. In order to beat this state, it is necessary to persist the search for new antibacterial agents. In recent situation heterocycles plays a main role in drug synthesis. In that respect imidazopyridines plays a noteworthy role among other heterocycles. From the literature survey imidazopyridine was found to be having varied activity like antimicrobial (Bukowski L, et al, 1991), anti-cancer activity on human colon carcinoma (Pawel.Lukasiket al, 2012), anti-convulsant (Adam J. Rosenberg et al, 2012), anti-tuberculosis (Dathu ReddY et al, 2014), anti-inflammatory (Gaozhi Chenet al, 2013), Cardiac agent (Chakravatre et al, 1994) anti-viral (Li, X et al, 2016) etc. So, it was planned to synthesize a novel series of imidazo pyridine derivatives and to check their activity as antibacterial and antifungal agents.

MATERIALS AND METHODS

All melting points were taken in open capillaries on a veego VMP-1 apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer FT IR 240-c spectrometer. The ^{1}H NMR spectra were recorded on Varian-Gemini 200 MHz spectrometer in DMSO-d6 using TMS as an internal standard and mass spectra were recorded on Schimadzu QP 5050A spectrometer.

i) Synthesis of Ethyl 3-(1H-imidazo[4,5-b] pyridin-2-ylamino) propanoate (III).

An equimolar ratio of 1H-imidazo[4,5-b] pyridin-2-amine (0.01mol) and ethylacrolate (0.01mol) was refluxed in 20ml of glacial acetic acid for around 15hr. After completion of the reaction (monitored by TLC), the reaction mixture was cooled, and poured in to a mixture of crushed ice. The resultant precipitate was filtered and recrystallized from alcohol to give ethyl 3-(1H-imidazo[4,5-b] pyridin-2-ylamino) propanoate (III).

ii) Synthesis of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino) propanehydrazide (IV)

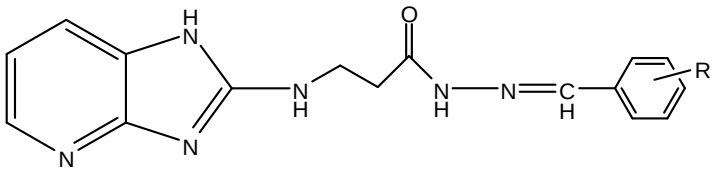
A mixture of ethyl 3-(1H-imidazo[4,5-b] pyridin-2-ylamino) propanoate (III, 0.01mol) and hydrazine hydrate (99%) (0.01mol) were taken in 50ml of alcohol, heated under reflux on a water bath for 5hrs. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and washed with small portions of cold alcohol first, and then with cold water repeatedly and dried. The product was purified by recrystallization from ethyl alcohol.

iii) Synthesis of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(3-arylidene) propanehydrazones (Va-i)

3-(1H-imidazo[4,5-b] pyridin-2-ylamino) propane hydrazide (IV, 0.01mol) and benzaldehyde (0.01mol) in absolute alcohol containing the drops of acetic acid was refluxed for 8hrs. the product thus separated was filtered, and dried and purified by recrystallization from ethyl alcohol.

Other compounds in this series (Va-i) were prepared similarly and their characterization data recorded in **Table-1.**

Table 1: Physical data of 3-(1H-imidazo [4, 5-b] pyridin-2-ylamino)-N'-arylidene propanehydrazide(V)



S.No	Compound	Ar	Chemical formula	M.P (^{O}C)	Yield (%)	Elemental Analysis (Found (calc (%)		
						C	H	N
1	**Va**	Phenyl	$C_{16}H_{16}N_6O$	212-214	75	62.30 (62.32)	5.20 (5.23)	27.24 (27.26)
2	**Vb**	2-hydroxy phenyl	$C_{16}H_{16}N_6O_2$	281-283	70	59.30 (59.25)	4.95 (4.97)	25.89 (25.91)
3	**Vc**	4-Chlorophenyl	$C_{16}H_{15}N_6OCl$	222-224	71	56.10 (56.06)	4.37 (4.41)	24.55 (24.52)
4	**Vd**	4-hydroxyphenyl	$C_{16}H_{16}N_6O_2$	249-251	75	59.30 (59.25)	4.98 (4.97)	25.89 (25.91)
5	**Ve**	3,4,5-trimethylphenyl	$C_{19}H_{22}N_6O$	295-297	78	65.14 (65.12)	6.30 (6.33)	23.94 (23.98)
6	**Vf**	4-dimethylamino	$C_{18}H_{21}N_7O$	267-269	79	61.51 (61.52)	6.06 (6.02)	27.86 (27.90)
7	**Vg**	Cinnamyl	$C_{18}H_{18}N_6O$	210-212	75	64.68 (64.66)	5.40 (5.43)	25.12 (25.13)
8	**Vh**	4-methoxyphenyl	$C_{17}H_{18}N_6O_2$	289-291	73	60.35 (60.34)	5.34 (5.36)	24.90 (24.84)
9	**Vi**	3,4-dimethoxyphenyl	$C_{18}H_{20}N_6O_3$	302-303	75	58.68 (58.69)	5.44 (5.47)	22.80 (22.81)

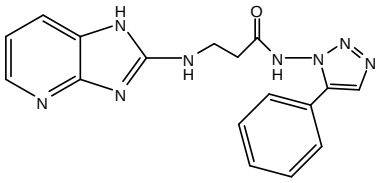
iv) Synthesis of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(5-phenyl-1H-1,2,3-triazol-1-yl) propanamides (VIa)

To an ice-cold benzene solution (10 ml) of diazomethane (15 mL, 0.4 mmol) was added 3-(1*H*-imidazo[4,5-*b*] pyridin-2-ylamino)-*N*'-(3-benzylidene) propane hydrazone (V, 0.1 mmol) in benzene (10 ml) and reaction mixture was kept at -15^{O}C for 44-48 hrs. Reaction progress was monitored by TLC. After completion of formation of triazoline (as indicated by TLC), aq. $KMnO_4$ solution and tetrabutyl ammonium chloride (TBA) was added, and the contents are heated under reflux with stirring for 6 hr. Then the reaction mixture was treated with Na_2SO_3 and filtered. The filtrate was treated with ice water, and then extracted with ethyl acetate. The solvent was removed on a rotary

evaporator, and the resultant solid was recrystallized from ethanol.

Other compounds in this series (VI a-i) were prepared similarly and their characterization data recorded in **Table-2.**

Table 2: Physical data of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI)



S.No	Compound	Ar	Chemical formula	M.P(^{O}C)	Yield (%)	Elemental Analysis (Found (calc (%)		
						C	H	N
1	**VIa**	phenyl	$C_{17}H_{16}N_8O$	228-230	78	58.57 (58.61)	4.59 (4.63)	32.15 (32.17)
2	**VIb**	2-hydroxy phenyl	$C_{17}H_{16}N_8O_2$	298-300	75	56.02 (56.04)	4.38 (4.43)	30.74 (30.75)
3	**VIc**	4-Chlorophenyl	$C_{17}H_{15}ClN_8O$	184-186	66	53.29 (53.34)	3.89 (3.95)	29.28 (29.27)
4	**VId**	4-hydroxyphenyl	$C_{17}H_{16}N_8O_2$	202-204	65	56.02 (56.04)	4.38 (4.43)	30.78 (30.75)
5	**VIe**	3,4,5-trimethylphenyl	$C_{20}H_{22}N_8O$	254-256	63	61.50 (61.52)	5.66 (5.68)	28.71 (28.70)
6	**VIf**	4-dimethylamino	$C_{19}H_{21}N_9O$	233-235	68	58.31 (58.30)	5.39 (5.41)	32.22 (32.21)
7	**VIg**	Cinnamyl	$C_{19}H_{18}N_8O$	285-287	65	60.98 (60.95)	4.88 (4.85)	29.90 (29.93)
8	**VIh**	4-methoxyphenyl	$C_{18}H_{18}N_8O_2$	265-267	56	57.19 (57.14)	4.74 (4.79)	29.60 (29.61)
9	**VIi**	3,4-dimethoxyphenyl	$C_{19}H_{20}N_8O_3$	284-286	78	55.83 (55.88)	4.99 (4.94)	27.30 (27.44)

IR Spectrum data of compound III:

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3345 (N-H), 2945 (C-H, Aromatic), 1745 (C=O), 1506 (C=N), 1215 (C-O-C).

^{1}H NMR Spectrum data of compound III:

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at:12.9(s,1H,NH), 8.2(m, 1H,ArH), 7.8(dd,1H,ArH), 7.4(s,1H,NH), 7.0(m, 1H,ArH) 4.1(q, 2H, CH_2), 3.4 (t, 2H, CH_2), 2.6(t, 2H, CH_2), 1.3(t,3H,CH_3).

IR Spectrum data of compound IV:

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3456, 3450(NH_2), 3314 (NH), 3012 (N-H), 2936 (C-H, Aromatic), 1645 (C=O),1610(C=C), 1545 (C=N).

^{1}H NMR Spectrum data of compound IV:

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at:13.0(s,1H,NH, imidazole ring), 8.8 (1s, -CONH,-NH.NH_2), 8.4(d,1H,ArH), 7.8(d,1H,ArH), 7.3(s,1H,NH),7.0 (m, 1H, ArH), 4.2(s, 2H, NH_2), 3.4 (t, 2H, CH_2), 2.6(t, 2H, CH_2).

IR Spectrum data of compound Va:

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3328 (N-H), 3021 (NH), 2965 (C-H, CH_2), 1645 (C=O), 1624 (C=N), 1554 (C=C, Ar).

^{1}H NMR Spectrum data of compound Va:

^{1}H NMR Spectrum (DMSO-d_6) has been found to exhibit characteristic proton signals (δ, ppm) at: 13.4 (s, 1H, NH (Imidazo[4,5-b]pyridine ring)), 11.1(s, 1H, CONH), 8.6 (s, 1H,-N=CH-), 8.2 (s,1H, ArH), 7.8 (m, 2H, Ar-H), 7.6(d, 2H,Ar-H),7.4 (d, 2H, ArH), 7.3 (s, 1H, NH), 7.0(m, 1H,ArH), 3.4 (t, 2H,CH_2), 2.6(t,2H,CH_2).

IR Spectrum data of compound VIa:

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3452 (N-H), 3022 (-N-H, Ar), 2248 (CH-Ar), 1668 (C=O), 1588 (C=C, Ar), 1445(N=N).

^{1}H NMR Spectrum data of compound VIa:

^{1}H NMR Spectrum (DMSO-d_6) has been found to exhibit characteristic proton signals (δ, ppm) at: 13.0 (s, 1H, NH, Imidazole ring), 8.6 (s, 1H, -CONH,), 8.2 (s,1H, ArH), 8.0 (m, 2H, ArH), 7.8 (d, 1H, Ar-H), 7.2(d,1H,Ar-H),7.4 (s, 1H, NH), 7.1 (m, 4H, Ar-H), 3.1 (t, 2H,CH_2), 2.7(t,2H,CH_2).

Mass Spectrum data of compound VIa:

ESI-MS:m/z 349$[M+H]^+$

SCREENING FOR ANTI-MICROBIAL PROPERTIES

1. **Antibacterial activity by cup plate method (**Indian Pharmacopoeia, 1996)

The antibacterial activity of synthesized compounds was tested against two Gram-positive bacteia viz., *Bacillus subtilis* and *Staphylococcus aureus*and two Gram-negative bacteia viz., Escherichia *coli* and *Salmonella typhi* by using cup plate method. Ampicillin sodium was employed as standard to compare the results.

The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37°C ± 1°C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacteria inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in conical flasks (250 ml). The flasks were incubated at 37°C ± 1°C for 48 hours before the experimentation.Solution of the test compounds were prepared by dissolving 10 mg each in dimethylformamide (10 ml, AnalaR grade). A reference standard for both gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately. The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 min. The petriplates, tube and flasks plugged with cotton were sterilized in hot-air oven at 160°, for an hour. Into each sterilized petriplate (10 cm diameter), about 27 ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred asceptically. The plates were left at room temperature to allow the solidification. In each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 ml of the test solution was added to the respective cups asceptically and labeled, accordingly. The plates were kept undisturbed for atleast 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. After incubation of the plates at 37°± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethyl formamide to observe the solvent effects. The results are presented in Table 3.

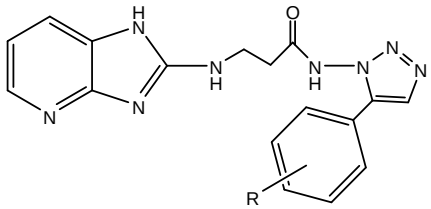
2) Antifungal activity [British Pharmacopoeia, 1953**]:**

All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were: *Candida albicans*and *Aspergillus niger.*

The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 hours, they were stored at 4°C in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml conical flasks. The flasks were incubated at 25°C for 24 hours before use. The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. A reference standard (1 mg/ml conc.) was prepared by dissolving 10 mg of Clotrimazole in 10 ml of dimethylformamide (AnalaR grade). Further, the dilution was made with dimethylformamide itself to obtain a solution of 100 µg/ml concentration. The potato-dextrose-agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes. The petriplates, tubes and flasks with cotton plugs were sterilized in hot-air oven at 150°, for an hour. In each sterilized petriplate, about 27 ml of molten potato-dextrose-agar medium inoculated with respective fungus (6 ml of inoculum in 300 ml of potato-dextrose medium) was added, aseptically. After solidification of the medium at room temperature three discs of 6 mm

diameter were made in each plate with a sterile borer. Accurately 0.1 ml (100 µg/disc) of test solution was transferred to the discs aseptically and labelled, accordingly. The reference standard 0.1 ml (10 mg/cup) was also added to the discs in each plate. The plates were kept undisturbed at room temperature for 2 hours, at least to allow the solution to diffuse properly into the potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hours. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors. The results are presented in Table 4.

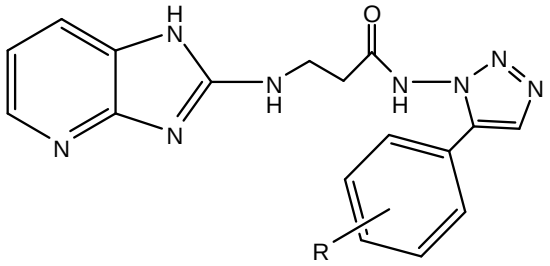
TABLE-3: Antibacterial activity of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl) propanamides (V)



S.No	Compound.	Zone of inhibition (mm) Gram positive bacteria		Gram negative bacteria	
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***S.typhi***
1	**VIa**	10	10	22	27
2	**VIb**	16	11	23	27
3	**VIc**	19	18	21	28
4	**VId**	24	28	28	34
5	**VIe**	14	17	21	22
6	**VIf**	12	11	24	25
7	**VIg**	18	16	20	24
8	**VIh**	12	14	18	22
9	**VIi**	19	21	18	24
Standard	**Ampicillin(10µg/ml)**	**22**	**20**	**18**	**22**

Solvent: Dimethylformamide; **Concentration**: 0.1 mg/ml

Table 4: Antifungal activity of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl) propanamides (VI)



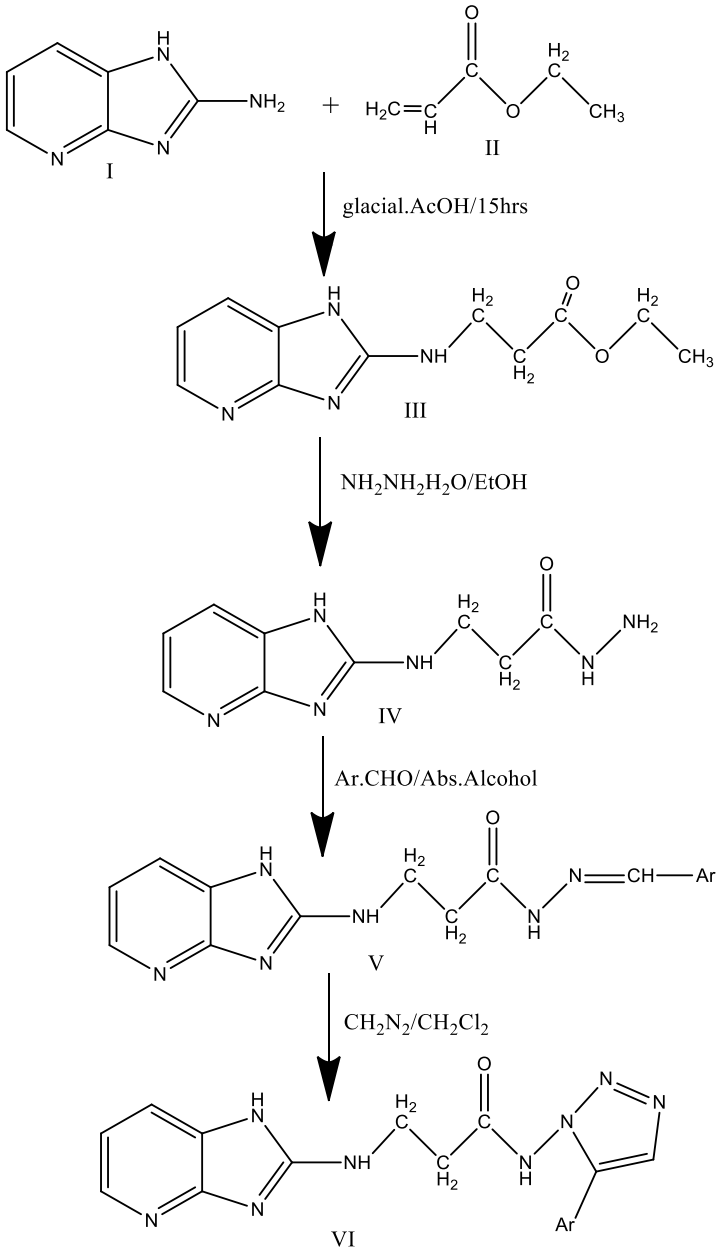
S.No	Compound	Zone of inhibition (in mm)	
		A.niger	*C.albicans*
1	VIa	15	16
2	VIb	19	15
3	VIc	--	12
4	VId	25	20
5	VIe	14	15
6	VIf	16	13
7	VIg	22	17
8	VIh	21	15
9	VIi	21	21
10	Clotrimazole (10mg/cup)	19	22

Concentration of the test compound: 100 mg/cup

RESULTS AND DISCUSSION

The reaction of 1H-imidazo[4,5-b] pyridin-2-amine (I) with ethylacrylate (II) in glacial acetic acid produced the intermediate ethyl-3-(1H-inidazo[4,5-b] pyridin-2-ylamino) propanoate (III). Compound III upon condensation with hydrazine hydrate (99%, 0.01mol) in alcohol afforded 3-(1H-imidazo[4,5-b]pyridin-2ylamino)propanehydrazide (IV).Compound 3-(1H-imidazo[4,5-b]pyridin-2ylamino)propanehydrazide (IV) on reflux with various aromatic aldehydes in 20ml of absolute alcohol containg few drops of acitic acid, gave the corresponding 3-(1H-imidazo[4,5-b]pyridin-2ylamino)-N′-(3-arylidene)propanehydrazones (V) in good yields. Finally 3-(1H-imidazo[4,5-b]pyridin-2ylamino)-N′-(3-arylidene)propanehydrazones (V) on treatment with diazomethane in benzene, followed by treatment with aq. $KMnO_4$ in presence of tetrabutyl ammonium chloride *in situ* produced targetted compound 3-(1H-imidazo [4,5-b]pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI) in excellent yields. (Scheme-1).

A. Synthesis of 3-(1H-imidazo[4,5-b] pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI)



Ar=

a	Phenyl	**d**	4-hydroxyphenyl	**g**	Cinnamayl
b	2-hydroxyphenyl	**e**	3,4,5-trimehylphenyl	**h**	4-methoxyphenyl
c	4-chlorophenyl	**f**	4-methylamino	**i**	3,4-dimethoxyphenyl

Scheme-1

The structures of compounds (III-VI) have been established on the basis of analytical and spectral data. All the synthesized compounds were characterized and evaluated for antibacterial activity against *Bacillus subtilis Staphylococcus aureus*, *Escherichia coli and Salmonella typhi* and antifungal activity against *Aspergillu sniger*, and *Candida albicans*by using Ampicillin sodium and Clotrimoxazole as a standards.

The antibacterial activity results of 3-(1H-imidazo[4,5-b]pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI) showed in Table3, only the compound VId (Ar=4-hydroxyphenyl) active against

both Gram Positive i.e *B.subtilis and S.aureaus* Gram negative bacteria *E.coli* and *S.typhi* with zone of inhibition of 24mm, 28mm, 28mm and 34 mm respectively. The three compounds i.e Compound VIb (Ar=2-hydroxy pheyl), VIf(Ar=4-dimethylaminophenyl) and compound VIa (Ar=Phenyl) were active only against Gram negative bacteria *E.coli* and *S.typhi* with zone of inhibition of 23mm& 27mm, 24mm & 25mm and 22mm & 27mm respectively. Rest of the compounds showed mild to moderate activity against both Gram positive and Gram-negative bacteria. Remaining compounds showed mild to moderate activity against test organisms. Surprisingly some of the compounds were inactive against test organism.

The antifungal activity results revealed that 3-(1H-imidazo[4,5-b]pyridin-2-ylamino)-N'-(5-aryl-1H-1,2,3-triazol-1-yl)propanamides (VI, Table 4) compound VId (Ar= 4-hydroxyphenyl) and compound VIi (Ar=3,4-dimethoxyphenyl) are active compared to the standard drug with zone of inhibition of 25mm, 20mm and 21mm, 21mm respectively. Compound VIc only inactive against *A.niger* and remaining all the compounds is showed mild to moderate activity against tested organisms.

CONCLUSION

This study reports the successful synthesis of the imidazo pyridine derivatives in good yields and moderate to potent anti-microbial activity when compared with standard drug.

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