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Synthesis and biological evaluation of some new Imidazole - Amino acids Schiff's bases

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ABSTRACT

A series of imidazole-amino acid Schiff's bases were synthesized by the reaction of 3-methyl-1-benzyl-4-(2-hydroxypropan-2-yl)-2-propyl-1H-imidazole-5-carboxylic acid with amino acids and further reacting with hydrazine hydrate and substituted benzaldehydes. The structures of the compounds were confirmed by physical and spectroscopic techniques followed by the antimicrobial evaluation by agar well diffusion methods. Results of the activities reveal that some compounds exhibited moderate to good antimicrobial activity.

Keywords: Antimicrobial, Schiff's bases.

1. INTRODUCTION

The growing interest in heterocyclic compounds is basically because of their raised biological activity and also they make possible development of novel materials with unique properties. One very interesting and promising class of heterocycle is the series of imidazole and its derivatives. Imidazoles have been widely used in biological screening resulting in numerous applications and constitute an attractive pharmacological scaffold present in several drugs[1]. Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. Heterocyclic chemistry is a vast expending area of chemistry because of the obvious application of compound derived from heterocyclic rings in pharmacy medicine, agriculture, photography, veterinary products and also other field. About half of the known compounds have structures that incorporate at least one heterocyclic compound [1,2].

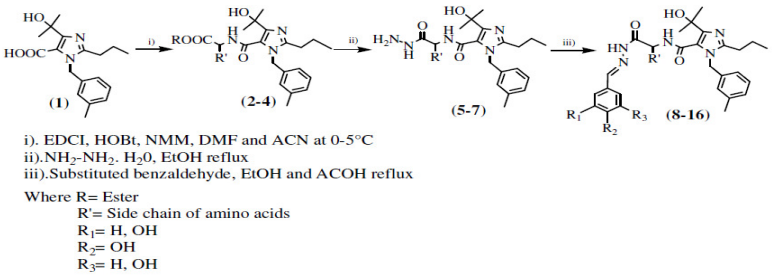
Extensive work has been reported on the conjugation of different amino acids to various biologically active moieties[3-4] which reveals that conjugation plays a paramount role in exerting the activity. Also involving amino acids in drugs makes the low toxic, ample bioavailability and permeability, modest potency and good metabolic and pharmacokinetic properties [3-5].

2. MATERIALS AND METHODS

All the amino acids used except glycine were of *L*-configuration unless otherwise mentioned. Hydrazine hydrate, EDCI, HOBt and NMM were purchased from Padmashri Scintific. All reagents and solvents used for the synthesis were of analytical grade. All the reagents used for antimicrobial studies were of bacteriological grade unless otherwise indicated. Silica gel (60-120 mesh) for column chromatography was purchased from Sisco Research Laboratories Pvt. Ltd., (Bombay, India). The pathogens used for the microbial studies were obtained from a local hospital. The progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/methanol/acetic acid in the ratio 95:5:3 throughout the study and the compounds on TLC plates were detected by iodine vapours. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on VARIAN 400MHz instrument using $CDCl_3$ and the chemical shifts are reported as parts per million (δ ppm) using TMS as an internal standard. Mass spectra were obtained on LCMSD-Trap-XCT instrument.

2.1. General Procedure for the Coupling of Imidazole Derivative with Amino acids

To 3-methyl-1-benzyl-4-(2-hydroxypropan-2-yl)-2-propyl-1H-imidazole-5-carboxylic acid (0.005 mol) and HOBt (0.765g, 0.005 mol) dissolved in DMF (10ml/mg) and cooled to 0°C was added NMM (0.55 mL, 0.005 mol). EDCI (0.956g, 0.005 mol) was added under stirring while maintaining the temperature at 0°C.



i). EDCI, HOBt, NMM, DMF and ACN at 0-5°C
ii).NH_2-NH_2. H_20, EtOH reflux
iii).Substituted benzaldehyde, EtOH and ACOH reflux

Where R= Ester
R'= Side chain of amino acids
R_1= H, OH
R_2= OH
R_3= H, OH

Scheme - 1: Schematic representation of synthesis imidazole-amino acids schiff's bases

The reaction mixture was stirred for an additional 10 min and pre-cooled solution of ROOC-Xaa-NH_2.HCl (1.31g, 005 mol) and NMM (0.55 ml, 0.005 mol) in DMF (13 mL) was added slowly. After 20 min, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. DMF was removed under reduced pressure and the residue was poures into about 200 ml ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated product was taken into $CHCl_3$ and washed with 5% $NaHCO_3$ solution (2 x 20 ml), 0.1N cold HCl solution (2 x 20ml) and finally brine solution (2 x 20ml). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The products so obtained were recrystallized from ether/petroleum ether to get white to yellow coloured desired conjugates (2-4).

2.2. General Procedure for the Synthesis of Hydrazides

To a solution of the conjugates 2, 3 and 4 (0.001 mol) in ethanol (10 ml/g of compound) were added hydrazine hydrate (0.001 mol) and mixture was stirred at reflux for 8h. After completion of the reaction monitored by TLC. The solvent was evaporated under reduced pressure and quenched residue to ice cold water, precipitate was filtered and washed with ice cold water to obtain hydrazides 5, 6 and 7.

2.3. General Procedure for the Condensation of Hydrazides with Substituted Benzaldehyde

Each hydrazides 5, 6 and 7 was conjugated with substituted benzaldehydes using ethanol as solvent and acetic acid as catalyst at reflux for 10h. The completion of reaction was monitored by TLC, solvent was removed under reduced pressure, quenched to ice cold water to get desired product (8-16).

2.4. Antibacterial Activity

In vitro antibacterial activity was evaluated against human pathogens of both gram positive organisms namely *Coagulase positive staphylococcus* and *Klebsiella pneumonia* and gram negative organisms namely *Xanthomonasoryzae* and *Escherichia coli* by agar well diffusion method.

2.5. Agar well diffusion method

Microorganisms were inoculated into the sterilized nutrient broth and maintained at 37 °C for 24 h. On the day of testing, bacteria were subcultured separately into 25 mL of sterilized nutrient broth. Inoculated subcultured broths were kept at room temperature for the growth of inoculums. Each test compound and standard drug (streptomycin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 50 µg/mL. About 15–20 mL of molten nutrient agar was poured into each of the sterile plates. With the help of cork borer of 6 mm diameter, the cups were punched and scooped out of the set agar and the plates were inoculated with the suspension of particular organism by spread plate technique. The cups of inoculated plates were then filled with 0.1 mL of the test solution, streptomycin solution and DMSO (negative control). The plates were allowed to stay for 24h at 37°C and zone of inhibition (mm) was then measured.

2.6. Antifungal Activity

In vitro antifungal activity was evaluated against three fungal species namely *Aspergillusniger, A. flavus and Fusariumoxysporum* by agar well diffusion method.

2.7. Agar well diffusion method

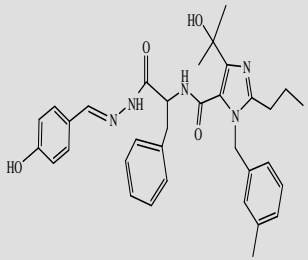
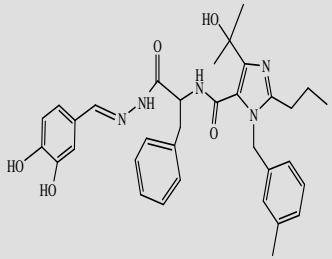
The fungal strains were subcultured separately into 25 mL of sterilized nutrient broth and incubated for 1 day to obtain the inoculums. Each test compound and standard drug (Bavistin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 50 µg/mL. Molten media of Sabouraud agar of 10–15 mL was poured into the petriplates and allowed to solidify. Fungal subculture was inoculated on the solidified media. With the help of 6 mm cork borer, the cups were punched and scooped out of the set agar. The cups of inoculated plates were then filled with 0.1 mL of the test solution, bavistin solution and DMSO (negative control). The plates were allowed to stay for 3 days at room temperature and zone of inhibition (mm) was then measured.

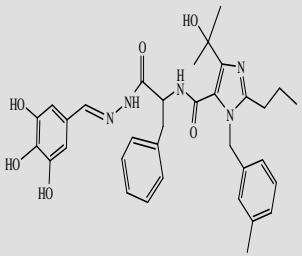
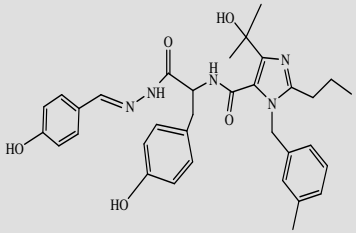
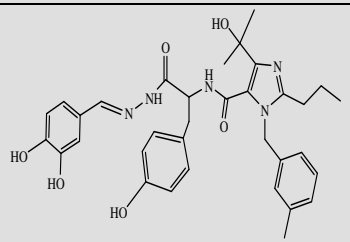
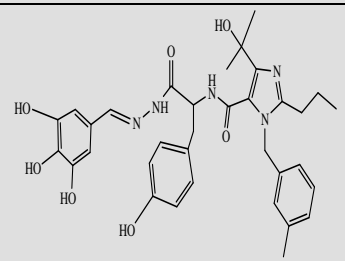
3. RESULTS AND DISCUSSION

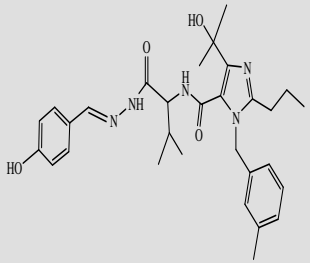
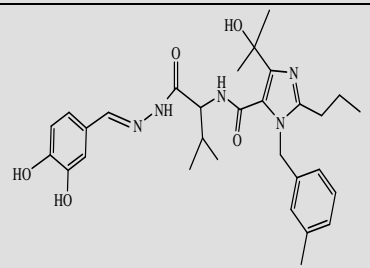
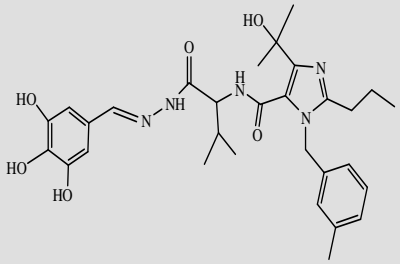
3.1. Chemistry

Starting material 1 was conjugated with different amino acids using EDCI/HOBt as coupling agent and NMM as base. Synthesis of compound (2-4) was confirmed by absence of –COOH peak and appearance of an ester group peak, compounds (2-4) were separately made to react with hydrazine hydrate to get hydrazides (5-7) which were ascertainted by presence of –NH proton peak. Compounds (5-7) separately reacted with different substituted aldehydes to obtain final imines (8-16). Synthesis of final imines was confirmed by the presence of all aquisite peaks and absence of extraneous peaks in ^{1}H NMR and also mass values were in accordance with the mol. wt calculated. The analytical and spectroscopic data of the synthesized compounds are given in table 1.

Table - 1: Physical and spectroscopic studies of synthesized compounds

Entry	Structures	R^f	Yield (%)	MP (°C)	Formula HRMS [M^++Na]	IR,^{1}H NMR and ^{13}C NMR
8		0.45	96.0%	188°C	$C_{34}H_{39}N_5O_4$ 582.34	IR υmax (nujol, cm-1): 1678 (C=O); 3320 (NH) ^{1}H NMR: 0.94 (3H, t, -CH_3 Propyl), 1.12 (6H, d, CH_3), 1.60 (2H, m, Propyl), 2.32 (3H, s, Ar-CH_3), 2.49 (2H, t, CH_2 of Propyl), 2.72 (1H, m, β-CH), 4.53 (1H, d, α-CH), 5.25 (2H, s, CH_2 benzyl), 6.22 (1H, s, OH), 7.88-8.30 (2H, s, NH), 6.80-7.15 (13H, m, Ar-H), 8.09 (1H, s, -CH). ^{13}H NMR: 13.91, 21.06, 23.12, 31.18, 39.72, 46.91, 52.69, 70.51, 115.83, 117.35, 117.89, 120.78, 128.55, 128.77, 130.90, 131.22, 133.89, 138.19, 147.66, 147.65, 148.44, 150.1, 155.16, 156.78, 161.43, 173.23.
9		0.44	92.0%	192°C	$C_{34}H_{39}N_5O_5$ 598.30	IR υmax (nujol, cm-1): 1683 (C=O); 3324 (NH) ^{1}H NMR: 0.91 (3H, t, -CH_3 Propyl), 1.15 (6H, d, CH_3), 1.58 (2H, m, Propyl), 2.29 (3H, s, Ar-CH_3), 2.52 (2H, t, CH_2 of Propyl), 2.70 (1H, m, β-CH), 4.55 (1H, d, α-CH), 5.20 (2H, s, CH_2 benzyl), 6.19 (2H, s, OH), 7.86-8.29 (2H, s, NH), 6.82-7.18 (12H, m, Ar-H), 8.10 (1H, s, -CH). ^{13}H NMR: 13.89, 21.00, 23.11, 31.22, 39.44, 46.56, 53.21, 69.33, 115.66, 117.23, 118.11, 121.80, 128.88, 128.90, 130.76, 131.12, 134.08, 139.16, 147.43, 147.88, 149.11, 150.15, 155.54, 156.80, 161.56, 173.66.

10		0.42	95.0%	195°C	$C_{34}H_{39}N_5O_6$ 639.29 M^++Na	IR υmax (nujol, cm-1): 1686 (C=O); 3328 (NH) ^{1}H NMR: 0.89 (3H, t, -CH_3 Propyl), 1.19 (6H, d, CH_3), 1.60 (2H, m, Propyl), 2.30 (3H, s, Ar-CH_3), 2.49 (2H, t, CH_2 of Propyl), 2.69 (1H, m, β-CH), 4.52 (1H, d, α-CH), 5.25 (2H, s, CH_2 benzyl), 6.23-6.80 (3H, s, OH), 7.84-8.30 (2H, s, NH), 6.82-7.25 (11H, m, Ar-H), 8.12 (1H, s, -CH). ^{13}H NMR: 14.21, 26.22, 27.65, 33.31, 38.40, 41.12, 55.84, 66.60, 116.40, 117.55, 118.66, 119.20, 121.61, 128.59, 129.67, 130.54, 131.36, 131.90, 133.19, 138.40, 140.89, 145.99, 149.55, 151.40, 154.10, 156.09, 161.14, 175.81.
11		0.48	89.0%	180°C	$C_{34}H_{39}N_5O_5$ 598.32	IR υmax (nujol, cm-1): 1666 (C=O); 3314 (NH) ^{1}H NMR: 0.95 (3H, t, -CH_3 Propyl), 1.12 (6H, d, CH_3), 1.56 (6H, s, CH_3), 1.60 (2H, m, Propyl), 2.27 (3H, s, Ar-CH3), 2.49 (2H, t, CH_2 of Propyl), 2.67 (1H, m, β-CH), 4.53 (1H, d, α-CH), 5.18 (2H, s, CH_2 benzyl), 6.00 (1H, s, OH), 7.89-8.19 (2H, s, NH), 6.82-7.10 (12H, m, Ar-H), 8.10 (1H, s, -CH). ^{13}H NMR: 15.12, 29.02, 29.78, 31.74, 39.29, 39.71, 40.13, 51.43, 113.14, 116.01, 116.40, 117.45, 123.21, 128.80, 29.19, 130.56, 131.87, 132.11, 134.44, 138.66, 143.0, 147.45, 149.61, 150.76, 155.78, 171.47, 191.49.
12		0.44	90.0%	182°C	$C_{34}H_{39}N_5O_6$ 614.31	IR $υ_{max}$ (nujol, cm^{-1}): 1694 (C=O); 3320 (NH) ^{1}H NMR: 0.90 (3H, t, -CH_3 Propyl), 1.15 (6H, d, CH_3), 1.55 (6H, s, CH_3), 1.63 (2H, m, Propyl), 2.34 (3H, s, Ar-CH_3), 2.52 (2H, t, CH_2 of Propyl), 2.66 (1H, m, β-CH), 4.51 (1H, d, α-CH), 5.22 (2H, s, CH_2 benzyl), 6.02-6.21 (2H, s, OH), 7.91-8.01 (2H, s, NH), 6.86-7.04 (11H, m, Ar-H), 8.14 (1H, s, -CH). ^{13}H NMR: 13.91, 24.78, 28.67, 32.0, 37.72, 40.33, 54.95, 69.80, 115.44, 115.50, 117.28, 122.81, 128.60, 129.11, 130.65, 131.43, 131.90, 134.34, 138.55, 139.89, 147.22, 149.56, 150.67, 155.78, 156.08, 160.65, 177.27.
13		0.41	91.0%	170°C	$C_{34}H_{39}N_5O_6$ 655.29 M^++Na	IR υmax (nujol, cm-1): 1693 (C=O); 3325 (NH) ^{1}H NMR: 0.89 (3H, t, -CH_3 Propyl), 1.14 (6H, d, CH_3), 1.54 (6H, s, CH_3), 1.60 (2H, m, Propyl), 2.38 (3H, s, Ar-CH_3), 2.51 (2H, t, CH_2 of Propyl), 2.63 (1H, m, β-CH), 4.50 (1H, d, α-CH), 5.22 (2H, s, CH_2 benzyl), 6.10-6.44 (3H, s, OH), 7.94-8.05 (2H, s, NH), 6.90-7.02 (10H, m, Ar-H), 8.15 (1H, s, -CH). ^{13}H NMR: 13.51, 24.33, 28.56, 32.33, 37.86, 40.11, 55.40, 56.23, 64.80, 106.52, 115.34, 128.65, 129.32, 130.34, 131.53, 132.1, 134.40, 138.66, 141.51, 143.0, 150.71, 150.90, 155.77, 156.42, 177.55.

14		0.52	88.0%	148°C	$C_{30}H_{39}N_5O_4$ 535.34	IR υmax (nujol, cm-1): 1670 (C=O); 3323 (NH) 1H NMR: 0.98 (3H, t, -CH_3 Propyl), 1.15 (6H, d, CH_3), 1.58 (6H, s, CH_3), 1.62 (2H, m, Propyl), 2.31 (3H, s, Ar-CH3), 2.52 (2H, t, CH_2 of Propyl), 2.70 (1H, m, β-CH), 4.50 (1H, d, α-CH), 5.22 (2H, s, CH_2 benzyl), 6.03 (1H, s, OH), 7.91-8.2 (2H, s, NH), 6.84-7.12 (8H, m, Ar-H), 8.12 (1H, s, -CH). ^{13}H NMR: 14.01, 18.89, 21.80, 24.07, 30.26, 30.94, 47.58, 61.49, 75.42, 124.61, 126.0, 128.49, 130.98, 136.11, 136.44, 138.0, 140.23, 150.69, 156.11, 156.84, 170.74, 174.75.
15		0.50	85.0%	139°C	C30H39N5O5 551.34	IR υmax (nujol, cm-1): 1695 (C=O); 3319 (NH) 1H NMR: 0.96 (3H, t, -CH3 Propyl), 1.10 (6H, d, CH3), 1.54 (6H, s, CH3), 1.66 (2H, m, Propyl), 2.35 (3H, s, Ar-CH3), 2.55 (2H, t, CH2 of Propyl), 2.68 (1H, m, β-CH), 4.52 (1H, d, α-CH), 5.20 (2H, s, CH2 benzyl), 6.0-6.21 (2H, s, OH), 7.90-8.0 (2H, s, NH), 6.87-7.02 (7H, m, Ar-H), 8.10 (1H, s, -CH). 13H NMR: 14.13, 18.75, 21.82, 24.22, 30.33, 30.986, 47.52, 61.50, 75.56, 124.60, 126.03, 128.77, 131.03, 136.14, 136.43, 138.03, 140.43, 150.44, 156.13, 156.86, 170.43, 174.88.
16		0.47	90.0%	Gummy	C30H39N5O6 551.34	IR υmax (nujol, cm-1): 1697 (C=O); 3321 (NH) 1H NMR: 0.92 (3H, t, -CH3 Propyl), 1.09 (6H, d, CH3), 1.50 (6H, s, CH3), 1.62 (2H, m, Propyl), 2.38 (3H, s, Ar-CH3), 2.51 (2H, t, CH2 of Propyl), 2.63 (1H, m, β-CH), 4.50 (1H, d, α-CH), 5.22 (2H, s, CH2 benzyl), 6.02-6.50 (3H, s, OH), 7.94-8.05 (2H, s, NH), 6.89-7.05 (6H, m, Ar-H), 8.16 (1H, s, -CH). 13H NMR: 13.90, 19.00, 21.77, 24.65, 30.33, 31.02, 47.60, 61.54, 76.00, 124.58, 126.09, 128.50, 130.92, 136.15, 136.48, 138.11, 140.27, 150.70, 156.14, 156.80, 170.66, 175.63.

3.2. Biological evaluation

The efficacy of the synthesized compounds as antimicrobials were evaluated for their antibacterial studies against different strains of human pathogens of both gram positive organisms namely *Coagulase positive staphylococcus* and *Klebsiella pneumonia* and gram negative organisms namely *Xanthomonasoryzae* and *Escherichia coli* and antifungal studies against *Aspergillusniger, A. flavus* and *Fusariumoxysporum.* The results obtained as zone of inhibition (mm) are presented in Table 2 respectively. Streptomycin and bavastin were used as standard drugs for antibacterial and antifungal assays respectively.

Our earlier investigation (Ullas et al. 2012) revealed promising activity by the conjugation of imidazole derivatives with amino acids. Encouraged by this, in the present study it was felt worthy to link imidazole moiety to these amino acids. It is evident from the results that all the heterocycle conjugated amini acids exhibited enhanced activity compared to heterocycle or free amino acids tested alone which are inactive or weakly active (>50µg/ml).

Earlier studies report the significance of activity revealed by aromaticity and hydrophobicity (Ullas et al. 2012) and hence initially more hydrophobic and aromatic amino acids such as Phe, Tyr and Trp were selected for conjugation [6]. Intially, only the heterocycle 1 was tested which revealed that they have very less/negligible activity (data not shown). But when they

were conjugated to amino acids, the resulting conjugates exhibited enhanced activity.

From the activity profile, it may be observed that most of the compounds presented in this study have shown better antimicrobial activity. Analogues containing simple amino acids like Val 14, 15 and 16 are less active than compounds containing Phe/Tyr 8, 9, 10, 11, 12 and 13 also in this compounds which hold two and three hydroxyl group 9, 10, 12 and 13 has shown enhanced activity. The excellent percentage of inhibition which could be due to the presence of aromatic side chain in amino acids like Phe/Tyr along with the presence of electron withdrawing groups like hydroxyl on the other end of the molecules.

Table - 2: Inhibitory zone (diameter) mm of the synthesized derivatives against tested bacterial and fungal strains by agar well diffusion method

Entry	Antibacterial activity				Antifungal activity		
	Zone of Inhibition (mm) ±SD (n=3)						
	C. staphylococcus	*K. pneumonia*	*X. oryzae*	*E. coli*	*A. niger*	*A. flavus*	*F. oxysporum*
8	06±0.52	08±0.33	09±0.12	05±0.11	19±0.66	15±0.12	16±0.17
9	**14±0.44**	**12±0.52**	**15±0.16**	**20±0.12**	**21±0.52**	**18±0.67**	**16±0.33**
10	**16±0.52**	**13±0.23**	**15±0.24**	**22±0.34**	**24±0.45**	**19±0.45**	**16±0.09**
11	09±0.45	08±0.41	11±0.61	6±0.10	18±0.56	16±0.32	14±0.88
12	**15±0.34**	**12±0.35**	**16±0.10**	**20±0.44**	16±0.51	13±0.42	16±0.34
13	**18±0.16**	**14±0.32**	**16±0.13**	**22±0.12**	18±0.76	18±0.50	18±0.54
14	12±0.11	08±0.56	13±0.10	15±0.44	12±0.52	10±0.45	14±0.32
15	14±0.50	09±0.42	12±0.34	15±0.52	17±0.88	14±0.30	14±0.77
16	**15±0.52**	**12±0.37**	**16±0.55**	**20±0.45**	**20±0.66**	**16±0.54**	**18±0.49**
Streptomycin	**14**	**10**	**14**	**19**	**-**	**-**	**-**
Bavastin	**-**	**-**	**-**	**-**	**20**	**15**	**15**

4. CONCLUSION

A novel series of Schiff's base analogues of amino acids-imidazole conjugates were evaluated for antibacterial and antifungal activities by agar well diffusion method. Activity profile revealed that compounds containing aromatic amino acids have shown profound results along with the beneficiary electron loving hydroxyl groups. Compounds like 14, 15 and 16 showed least activity which could be due to alky group in amino acids attached.

Acknowledgements

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