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Synthesis of imidazolo-amino acids conjugates as biologically active agents

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ABSTRACT

In the present work, some imidazole derivatives have been covalently conjugated to different amino acids. Structures of the resultant compounds were characterized by physical and spectroscopical techniques. All the compounds were screened for their antibacterial, antifungal and antioxidant activities. The antibacterial activity revealed that compounds containing tyrosine exhibited better potency against tested *coagulate positive Staphylococcus* and *E. coli* and also the same set of compounds were found to have good antifungal activity than rest of the compounds. Further, these have exhibited very good antioxidant property. This study leads us to conclude that the conjugates may be regarded as better therapeutic agents to treat infection.

Keywords: Imidazoles, Amino acids, Antimicrobial, Antioxidant

1. INTRODUCTION

The increasing incidence of infection caused by the rapid development of bacterial resistance to most of the known antibiotics is a serious health problem. [1-4] Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, it is known that antifungal drugs do not have selective activity because of the biochemical similarity between human cell and fungi forms.[5-7] Therefore, the development of drug resistance as well as the appearance of undesirable effects of certain antibiotics has stimulated the research of new antimicrobial agents.

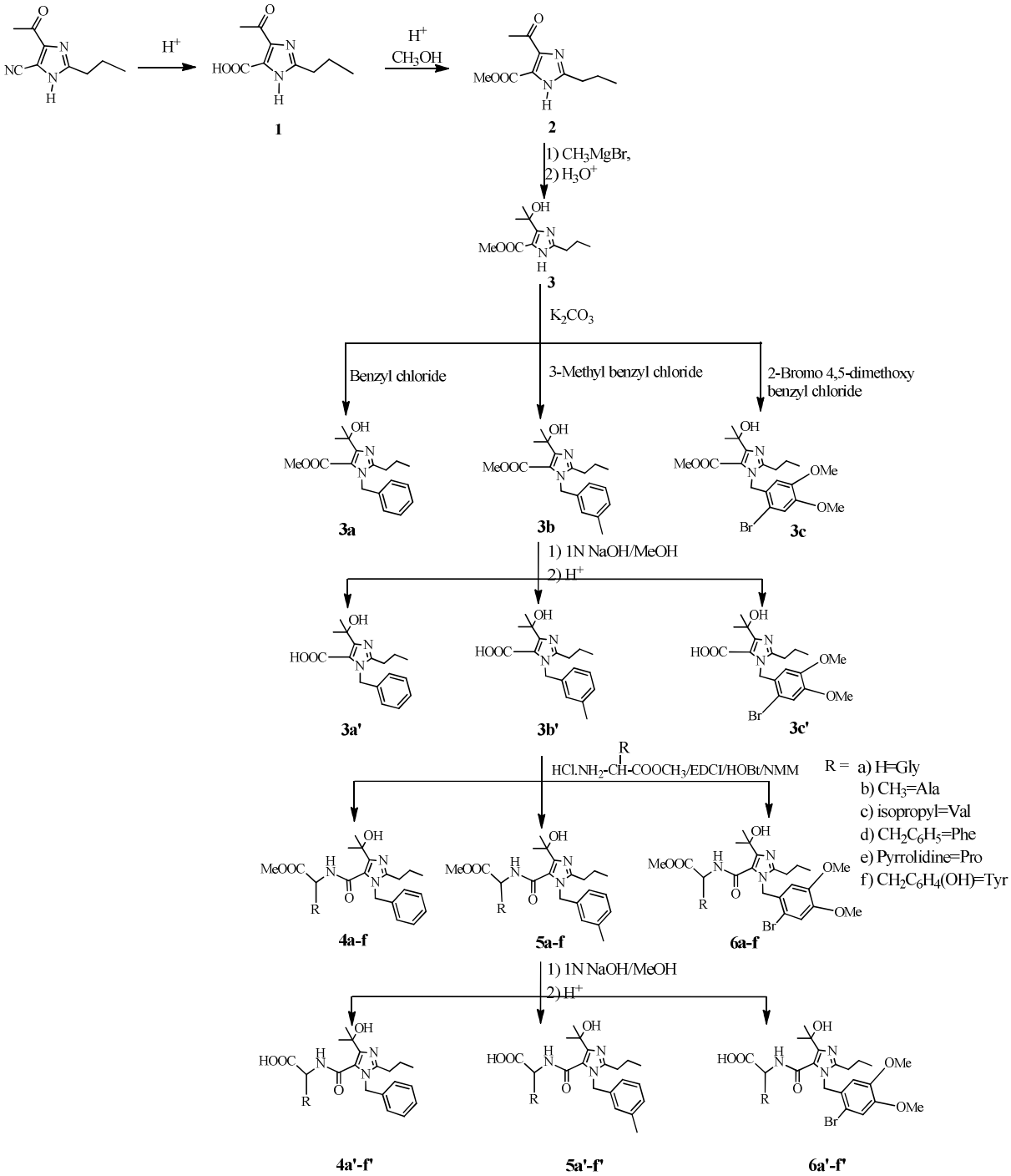
Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially. *N*–Heterocycles were involved at the very beginning of life in the genesis of DNA and play an essential role in many living systems. Compounds containing imidazole ring have been described as antiprotozoal, [8-10] anti-inflammatory, antifungal, antibacterial, antiviral agents [11] etc. Various 1-substituted imidazoles have anticonvulsant properties. [12]

Earlier reports have shown that conjugation of different amino acids/peptides to various biologically active moieties [13-17] has shown remarkable results which reveal that conjugation of these entities play a paramount role in exerting the activity. Also, involving amino acids/peptides in drugs makes them low toxic, enhances bioavailability and permeability, modest potency and good metabolic and pharmacokinetic properties [18]. In view of the above, the present investigation was aimed at the synthesis and biological evaluation of imidazoles conjugated to amino acids.

2. MATERIAL AND METHODS

2.1. General

Starting material, 4-acetyl-2-propyl-1*H*-imidazole-5-carbinitrile was purchased from Sigma Aldrich. All amino acid methyl esters, 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide.HCl (EDCI), 1-Hydroxybenzotriazole (HOBt) and *N*-methyl morpholine (NMM) were purchased from Sigma Chemical Co (St. Louis, MO). Chemicals and reagents used for antimicrobial studies were of bacteriological grade. Silica gel (60-120 mesh) for column chromatography was purchased from Sisco Research Laboratories Pvt. Ltd. (Bombay, India). Progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvents comprising chloroform/methanol/acetic acid in the ratio 95:5:3 and the compounds on TLC plates were detected by iodine vapors. Melting point was determined on Buchi Labortechnik AG:CH-9230 flawil and is uncorrected. ^{1}H NMR spectra were obtained on Varian 400 MHz



Scheme - 1: Schematic representation of the synthesis of title compounds.

instrument using $CDCl_3$ and the chemical shifts are reported as parts per million (δ ppm) using TMS as an internal standard. ^{13}C NMR spectra were recorded in DMSO-d_6 at 100 MHz. Mass spectra were obtained on API 2000 Applied Biosystems instrument. Elemental analysis was obtained on Elementar Vario EL-III analyzer.

2.2. Chemistry

Compound **1** was synthesized by acid hydrolysis of 4-acetyl-2-propyl-1*H*-imidazole-5-carbonitrile using dilute HCl and on esterification using methanol and conc. H_2SO_4 as catalyst to obtain compound **2**, the completion of reaction was monitored by TLC. Compound **2** was made to react with CH_3MgBr in THF at reflux for 3 h, after completion reaction mass was quenched with water, filtered inorganic solid extracted to Methylenedichloride (MDC), solvent was evaporated to obtain compound **3** (Scheme 1).

2.2.1. General procedure for *N*-alkylation

Compound **3** was dissolved in acetone and added slowly benzyl chloride, 3-methyl benzyl chloride and 2-bromo-4,5-dimethoxy benzyl chloride separately followed by K_2CO_3 (2 mol) and the reaction was maintained for 10h at reflux. Completion of the reaction was monitored by TLC, K_2CO_3 was filtered, solvent was evaporated, cooled and recrystallized using ethanol. The *N*-alkylated products **3a, 3b** and **3c** were characterized by physical and spectroscopic techniques.

2.2.2. General procedure for hydrolysis

Compounds **3a, 3b** and **3c** (0.01 mol) were hydrolysed separately in methanol (10 mL/g of compound) separately using cold solution of 1N NaOH (30 mL) for 4h. Completion of the reaction was monitored by TLC, solvent was evaporated, cooled and neutralized with cold 1N HCl, extracted with $CHCl_3$, washed with 1N HCl followed by water and dried over anhydrous Na_2SO_4. Solvent was removed under pressure and triturated with ether, filtered, washed with ether to obtain **3a', 3b'** and **3c'**.

2.2.3. General procedure for the conjugation of 3a'-c' with different amino acids (HCl. NH_2-Xaa-COOMe) where Xaa = Side chain of Gly, Ala, Val, Phe, Pro and Tyr

To **3a', 3b'** and **3c'** (0.005 mol) and HOBt (0.305g, 0.002mol) dissolved in DMF (10 mL/g of compound) and cooled to 0 °C was added NMM (0.55mL, 0.002 mol). EDCI (0.383g, 0.005 mol) was added under stirring while maintaining the temperature at 0 °C. The reaction mixture was stirred for an additional 10min and a pre-cooled solution of HCl.NH_2-Xaa-COOMe (0.631g, 0.002 mol) and NMM (0.55 mL, 0.002 mol) in DMF (10 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 over night at room temperature. DMF was removed under reduced pressure and the residue was poured 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 × 20 mL), water (1 x 20 mL), 0.1N cold HCl (2 x 20 mL) followed by brine (1 x 20 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The products so obtained were recrystalized from ether/petroleum ether to get desired conjugates (**4a-f**), (**5a-f**) & (**6a-f**). [Hydrolysis of conjugates (4a-f), (5a-f) & (6a-f): Procedure followed for the hydrolysis of these conjugates is same as above which resulted in (**4a'-f'**), (**5a'-f'**) and (**6a'-f'**)].

2.2.4. Biology

2.2.4.1. Antibacterial activity

In vitro antibacterial activity was evaluated against pathogens of both gram positive organisms namely *C. positive staphylococcus* and *K. pneumoniae* and gram negative organisms namely *X. oryzae* and *E. coli* by agar well diffusion method.

The microorganisms were inoculated in to 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 compounds (4a'- 6f') and standard drug (amoxicillin) of 10mg was dissolved in 10 mL of DMSO to get a concentration of 1 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 6mm 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, amoxicillin solution and DMSO (negative control). The plates were allowed to stay for 24 h at 37 °C and zone of inhibition (mm) was then measured.

2.2.4.2. Antifungal activity

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

The fungal strains were subcultured separately into 25 mL of sterilized nutrient broth and compounds and standard drug (bavistin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL. Molten media of sabouraud agar of 10-15 mL was poured into the petri plates 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.

2.2.4.3. Antioxidant activity

2.2.4.3.1. DPPH radical scavenging assay

Antioxidant activity of compounds was determined using DPPH as described by Blios.[16] All the synthetic compounds were taken at a concentration of 1 mg/mL and mixed with 5 mL of 0.1 mM methanolic solution of DPPH and incubated at 20 °C for 20 min in darkness. The control was prepared as above without a compound, and methanol was used for the base line correction. Changes in the absorbance of the

samples were measured at 517 nm using UV–visible spectrophotometer (Shimadzu 160A). All the tests were performed in triplicates. RSA was expressed as percentage activity using the formula

$$RSA\ (\%) = [(A_0 - A_1 / A_0 \times 100)]$$

Where A_0 is the measurement of DPPH solution without compound and A_1 the measurement of DPPH solution with compound. The RSA of ascorbic acid was also measured and compared with all **s**ynthesized compounds.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Starting material was subjected to acid hydrolysis to obtain **1** which was confirmed by the appearance of a peak at δ 11.88 in the ^{1}H NMR spectrum. Synthesis of compound **2** was confirmed by the PMR spectrum which showed absence of –COOH peak and appearance of a peak at δ 3.28, which on Grignard reagent treatment gave **3** which exhibited the $-Me_2$ and OH peaks at δ 1.56 and 5.02 respectively. Compound **3** was divided into three parts and subjected to *N*-alkylation using different benzyl chlorides, syntheses of which was ascertained by the absence of N-H proton peak. Compounds **3a-c** were hydrolysed to get free acid containing derivatives which were used for conjugation to different amino acids using EDCI/HOBt as coupling agent and NMM as base. The PMR spectrum showed peaks for NH at δ ~9.00. All the other peaks were exactly matching the structures. Further, the syntheses were confirmed by ^{13}C and mass spectrometry data also, which were found to be in good agreement with the structures assigned. The physical and analytical data of the synthesized compounds are provided in table 1.

Table - 1: Physical and analytical data of the conjugated compounds (4a-f), (5a-f) and (6a-f)

Entry	R_f Value	Yield (%)	M.P. (°C)	Theoretical Mol. wt.	Actual MS	IR (ν cm^{-1}), ^{1}H and ^{13}C NMR ($CDCl_3$, δ ppm)
1	0.56	88	98	196.20	197.23 [M$^+$+1]	PMR: 0.96 (3H, t, -CH_3 of Propyl), 1.65 (2H, m, -CH_2 of Propyl), 2.54 (2H, t, -CH_2 of Propyl), 2.60 (3H, s, -CH_3), 10.10 (1H, s,-NH), 11.0 (1H, s,- COOH) CMR: 13.6, 23.9, 26.3, 34.6, 136.7, 142.8, 160.7, 167.5, 196.0
2	0.60	76	100	210.23	210.68	PMR: 0.94 (3H, t, -CH_3 of Propyl), 1.66 (2H, m, -CH_2 of Propyl), 2.53 (2H, t, -CH_2 of Propyl), 2.59 (3H, s, -CH_3), 3.89 (3H, s, -OCH_3), 9.97 (1H, s, -NH) CMR:14.0, 24.0, 26.4, 35.4, 51.5, 135.7, 143.0, 160.3, 167.9, 196.2
3	0.80	90	132	226.27	226.52	PMR: 0.94 (3H, t, -CH_3 of Propyl),1.55 (6H, s, -CH_3) 1.70 (2H, m, -CH_2 of Propyl), 2.52 (2H, t, -CH_2 of Propyl), 3.89 (3H, s, -OCH_3), 10.12 (1H, s, -NH) CMR:14.0, 23.0,31.4, 34.9, 51.50, 69.9, 124.6, 143.8, 152.7, 167.9
3a'	0.54	86	152	316	339.12 [M$^+$+Na]	PMR: 0.92 (3H, t, -CH_3 of Propyl),1.57 (6H, s, -CH_3) 1.69 (2H, m, -CH_2 of Propyl), 2.50 (2H, t, -CH_2 of Propyl), 4.82 (2H, s, - CH_2), 6.9-7.20 (5H, m, Ar-H) CMR : 13.7, 24.2, 28.7, 31.4, 40.5, 70.2, 125.8, 128.2, 128.7, 129.1, 136.3, 144.2, 152.5, 167.9
3b'	0.61	80	144	316.2	317.20 [M$^+$+1]	PMR: 0.98 (3H, t, -CH_3 of Propyl),1.58 (6H, s, -CH_3) 1.70 (2H, m, -CH_2 of Propyl), 2.36 (3H, s, -CH_3), 2.55 (2H, t, -CH_2 of Propyl), 4.82(2H, s, -CH_2), 6.88-7.20(4H, m, Ar-H) CMR: 13.3, 24.6,24.7, 28.5, 32.0, 40.8, 70.5, 125.4, 128.1, 128.2, 129.0, 136.0, 141.9, 151.9, 166.8
3c'	0.70	82	153	441.2	441.2	PMR: 0.99 (3H, t, -CH_3 of Propyl),1.60 (6H, s, -CH_3) 1.67 (2H, m, -CH_2 of Propyl), 2.50 (2H, t, -CH_2 of Propyl), 3.73 (6H, s, -OCH_3), 4.82 (2H, s, -CH_2), 6.70-6.80 (2H, s, Ar-H) CMR: 13.5, 24.2, 28.7, 31.4, 33.0, 56.2, 70.2, 116.3, 117.3, 118.5, 128.2, 134.3, 144.2, 148.7, 149.0, 152.5, 167.9
4a	0.61	89	76-78	373.20	373.45	IR: 3250 (NH), 1690 (CO) PMR: 0.90 (3H, t, -CH_3 of propyl), 1.57 (6H, s, -CH_3), 1.68 (2H, m, -CH_2 of propyl), 2.45 (2H, t, -CH_2 of propyl) 3.51 (3H, s, -OMe), 3.92 (2H, d, $^{\alpha}CH_2$), 5.25 (2H, s, -CH_2) 6.8-7.02 (5H, m, Ar-H), 9.30 (1H, t, -NH). CMR: 13.7, 24.2, 28.3, 31.0, 40.1, 40.2, 51.6, 69.8, 125.8, 128.7, 129.1, 136.3, 138.6, 150.7, 156.4, 161.1, 169.6

4b	0.59	91	101	343.23	343.10	IR: 3260 (NH), 1695 (CO) PMR: 0.92 (3H, t, -CH_3 of propyl), 1.48 (3H, d, $^{\beta}CH_3$) 1.58 (6H, s, -CH_3), 1.70 (2H, m, -CH_2 of propyl) 2.48 (2H, t, -CH_2 of propyl), 3.50 (3H, s, -OMe) 4.60 (1H, m, $^{\alpha}CH_2$), 5.30 (2H, s,- CH_2) 6.8-7.02 (5H, m, Ar-H), 9.34 (1H, d, -NH) CMR: 13.9, 24.8, 28.7, 32.0, 43.1, 47.9, 51.9, 70.0, 128.0, 129.6, 129.5, 136.8, 140.0, 151.7, 155.0, 160.8, 171.0
4c	0.62	92	113-114	415.25	415.50	IR: 3258 (NH), 1680 (CO) PMR: 1.0 (3H, t, -CH_3 of propyl), 1.10 (6H, d, $^{\gamma}CH_3$) 1.60 (6H, s, -CH_3), 1.69 (2H, m, -CH_2 of propyl) 2.01 (1H, t, $^{\alpha}CH$), 2.42 (2H, t, -CH_2 of propyl) 3.09 (1H, m, $^{\beta}CH$), 3.53 (3H, s, -OMe) 5.21 (2H, s, -CH_2), 6.9-7.1 (5H, m, Ar-H) 9.40 (1H, d, NH) CMR: 13.2, 17.5, 26.2, 27.2, 30.5, 33.0, 42.1, 51.0, 55.3, 70.8, 123.8, 125.6, 130.1, 138.3, 138.9, 152.0, 158.4, 160.0, 171.6
4d	0.55	85	120-122	463.57	463.20	IR: 3264 (NH), 1691 (CO) PMR: 0.94 (3H, t, -CH_3 of propyl), 1.05 (6H, s, -CH_3) 1.65 (2H, m, -CH_2 of propyl), 2.40 (2H, t, -CH_2 of propyl), 2.81 (2H, d, $^{\beta}CH_2$), 3.55 (3H, s, -OMe) 4.81 (1H, q, $^{\alpha}CH$), 5.30 (2H, s, -CH_2), 6.8-7.02 (10H, m, Ar-H), 9.35 (1H, d, -NH) CMR: 13.0, 25.2, 28.8, 31.8, 36.0, 40.5, 51.9, 53.3, 69.8, 125.8, 126.0, 127.8, 130.7, 133.1, 136.4, 139.6, 140.5, 153.7, 154.4, 157.8, 167.6
4e	0.58	92	Gum	489.61	490.0	IR: 3245 (NH), 1698 (CO) PMR: 0.95 (3H, t, -CH_3 of propyl), 1.50 (6H, s, -CH_3) 1.64 (2H, m, $^{\gamma}CH_2$), 1.78 (2H, m, -CH_2 of propyl) 1.95 (2H, q, $^{\beta}CH_2$), 2.45 (2H, t, -CH_2 of propyl) 3.40 (2H, t, $^{\delta}CH_2$), 5.26 (2H, s, -CH_2) 5.34 (2H, s, -CH_2), 7.0-7.62(10H, m, Ar-H) CMR: 14.1, 22.6, 26.1, 28.0, 28.9, 30.0, 38.0, 45.7, 58.3, 68.4, 69.6, 121.6, 125.6, 125.8, 128.7, 129.1, 129.2, 136.3, 136.6, 150.7, 151.4, 156.4, 165.4, 168.4
4f	0.70	88	175	479.56	479.32	IR: 3246 (NH), 1686 (CO) PMR: 0.98 (3H, t, -CH_3 of propyl), 1.40 (6H, s, -CH_3) 1.69 (2H, m, -CH_2 of propyl), 2.39 (2H, t, -CH_2 of propyl), 3.29 (2H, d, $^{\beta}CH_2$), 3.50 (3H, s, -OMe) 4.80 (1H, q, $^{\alpha}CH$),5.20 (2H, s, -CH_2), 6.8-7.02 (9H, m, Ar-H), 9.20 (1H, d, -NH) CMR: 14.0, 26.2, 28.3, 31.8, 37.0, 40.1, 51.9, 52.8, 70.0, 115.8,125.8, 128.7, 129.1, 129.2, 132.1,136.3, 138.6, 150.7, 155.7, 156.4, 160.8, 171.6
5a	0.66	96	Gum	387.47	388.10	IR: 3266 (NH), 1670 (CO) PMR: 0.82 (3H, t, -CH_3 of propyl), 1.37 (6H, s, -CH_3) 1.68 (2H, m, -CH_2 of propyl), 2.35 (3H, s, -CH_3) 2.45 (2H, t, -CH_2 of propyl),3.51 (3H, s, -OMe) 3.92 (2H, d, $^{\alpha}CH_2$), 4.99 (2H, s, -CH_2) 6.8-7.02 (4H, m, Ar-H), 9.30 (1H, t, -NH) CMR: 12.0, 22.2, 23.6, 24.3, 33.0, 37.2, 40.4, 52.6, 70.8, 128.1, 130.0, 130.9, 137.0, 140.2, 140.6, 143.1, 146.2, 161.1, 169.6
5b	0.57	86	89-90	401.23	401.50	IR: 3261 (NH), 1675 (CO) PMR: 0.88 (3H, t, -CH_3 of propyl),1.49 (3H, d, $^{\beta}CH_3$) 1.51 (6H, s, -CH_3), 1.60 (2H, m, -CH_2 of propyl) 2.21 (3H, s, -CH_3), 2.36 (2H, t, -CH_2 of propyl) 3.79 (3H, s, -OMe), 4.63 (1H, m, $^{\alpha}CH_2$) 5.34 (2H, s, -CH_2), 6.8-7.02 (4H, m, Ar-H) 9.12 (1H, d, -NH) CMR: 11.7, 17.2, 24.7, 24.9, 28.3, 31.3, 40.4, 47.9, 51.1, 71.2, 119.1, 128.7, 133.9, 135.3, 139.3, 139.6, 149.6, 153.4, 157.2, 174.4

5c	0.60	90	96	429.55	453.1 [M⁺+Na]	IR: 3258 (NH),1660 (CO) PMR: 0.82 (3H, t, -CH_3 of propyl), 1.20 (6H, d, $^{\gamma}CH_3$) 1.42 (6H, s, -CH_3),1.55 (2H, m, -CH_2 of propyl) 2.33 (3H, s, -CH_3), 2.51 (2H, t, -CH_2 of propyl) 3.11 (1H, m, $^{\beta}CH$), 3.42 (3H, s, -OMe) 4.38 (1H, t, $^{\alpha}CH$), 5.33 (2H, s, -CH_2) 6.8-7.02 (4H, m, Ar-H), 9.36 (1H, d, -NH) CMR: 11.0, 17.5, 21.2, 22.7, 25.3, 29.5, 30.3, 39.4, 51.0, 55.3, 59.2, 124.0, 126.6, 131.1, 134.3, 135.3, 136.0, 148.0, 152.4, 157.8, 174.0
5d	0.55	92	102	477.6	477.4	IR: 3244 (NH), 1685(CO) PMR: 0.86 (3H, t, -CH_3 of propyl), 1.65 (6H, s, -CH_3) 1.72 (2H, m, -CH_2 of propyl), 2.35 (3H, s, -CH_3) 2.56 (2H, t, -CH_2 of propyl), 3.29 (2H, d, $^{\beta}CH_2$) 3.66 (3H, s, -OMe), 4.81 (1H, q, $^{\alpha}CH$), 5.41 (2H, s, -CH_2), 6.8-7.02 (4H, m, Ar-H), 9.80 (1H, d, -NH) CMR: 12.7, 24.1, 26.0, 31.0, 32.1, 37.0, 44.1, 49.9, 52.8, 70.8, 123.0, 127.1, 127.8, 128.6, 128.2, 134.1, 138.1, 138.7, 140.5, 141.5, 150.7, 156.4, 160.8, 171.6
5e	0.60	90	109-110	503.20	503.50	IR:3256 (NH),1669 (CO) PMR: 1.12 (3H, t, -CH_3 of propyl), 1.52 (6H, s, -CH_3) 1.68 (2H, m, $^{\gamma}CH_2$), 1.70 (2H, m, -CH_2 of propyl) 1.95 (2H, q, $^{\beta}CH_2$), 2.26 (3H, s, -CH_3), 2.32 (2H, t, -CH_2 of propyl), 3.40 (2H, t, $^{\delta}CH_2$), 5.28 (2H, s, -CH_2), 5.34 (2H, s, -CH_2) , 6.8-7.02 (9H, m, Ar-H) CMR:15.1, 20.4, 24.2, 25.1, 28.3, 28.9, 31.4, 40.4, 45.7, 58.7, 68.5, 68.8, 126.5, 127.2, 127.7, 128.7, 129.0, 130.9, 136.3, 136.6, 137.6, 137.9, 141.2, 146.6, 153.3, 165.4, 168.4
5f	0.68	93	Gummy	493.59	493.20	IR: 3244 (NH), 1692 (CO) PMR: 1.09 (3H, t, -CH_3 of propyl), 1.41 (6H, s, -CH_3) 1.59 (2H, m, -CH_2 of propyl), 2.38 (3H, s, -CH_3) 2.55 (2H, t, -CH_2 of propyl), 3.29 (2H, d, $^{\beta}CH_2$) 3.39 (3H, s, -OMe), 4.80(1H, q, $^{\alpha}CH$), 5.22 (2H, s, -CH_2), 6.7-7.10 (8H, m, Ar-H), 9.37 (1H, d, -NH) CMR: 11.7, 23.1, 24.8, 26.3, 31.9, 35.0, 39.1, 51.9, 52.8, 66.6, 115.8, 126.4, 128.3, 129.4, 130.1, 132.1, 134.1, 138.3, 138.6, 140.4, 152.2, 154.3, 164.8, 172.7
6a	0.55	94	Gummy	512.39	512.52	IR: 3267 (NH),1700 (CO) PMR: 0.99 (3H, t, -CH_3 of propyl),1.39 (6H, s, -CH_3) 1.62 (2H, m, -CH_2 of propyl), 2.53 (2H, t, -CH_2 of propyl), 3.51-3.85 (9H, s, -OMe), 3.92 (2H, d, $^{\alpha}CH_2$), 5.10 (2H, s, -CH_2), 6.7-7.12 (2H, m, Ar-H), 9.19 (1H, t, NH) CMR: 14.8, 24.7, 27.3, 29.0, 32.6, 38.2, 51.6, 55.1, 70.8, 116.3, 117.3, 118.5, 138.6, 148.7, 149.0, 150.7, 156.4, 161.1, 169.6
6b	0.56	88	Gummy	526.24	526.00	IR: 3270 (NH), 1695 (CO) PMR: 1.20 (3H, t, -CH_3 of propyl),1.48 (3H, d, $^{\beta}CH_3$) 1.43 (6H, s, -CH_3), 1.59 (2H, m, -CH_2 of propyl) 2.33 (2H, t, -CH_2 of propyl), 3.53-3.90 (9H, s, -OMe) 4.60 (1H, m, $^{\alpha}CH$), 5.26 (2H, s, -CH_2), 6.9-7.02 (2H, m, Ar- H), 9.10 (1H, d, -NH) CMR: 14.5, 21.2, 29.3, 32.2, 32.6, 47.9, 51.9, 56.2, 71.2, 115.2, 118.1, 119.4, 138.2, 147.6, 150.1, 151.2, 154.1, 160.8, 171.6
6c	0.60	92	108	554.57	578.1 [M⁺+Na]	IR: 3240 (NH), 1689 (CO) PMR: 0.90 (3H, t, -CH_3 of propyl), 1.01 (6H, d, $^{\gamma}CH_3$) 1.45 (6H, s, -CH_3), 1.73 (2H, m, -CH_2 of propyl) 2.49 (2H, t, -CH_2 of propyl), 3.09 (1H, m, $^{\beta}CH_2$) 3.60-3.92 (9H, s, -OMe), 4.40 (1H, t, $^{\alpha}CH$) 5.15 (2H, s, -CH_2), 6.9-7.10 (2H, m, Ar-H), 9.22 (1H, d, -NH) CMR: 11.6, 17.2, 24.2, 26.1, 31.1, 31.4, 52.8, 55.3, 56.6, 67.2, 114.1, 116.7, 117.8, 141.0, 147.7, 146.0, 149.6, 154.0, 161.5, 169.5

6d	0.56	91	118	601.20	624.1 [M^++Na]	IR: 3243 (NH), 1695 (CO) PMR: 0.88 (3H, t, -CH_3 of propyl), 1.57 (6H, s-CH_3) 1.68 (2H, m, -CH_2 of propyl), 2.45 (2H, t, -CH_2 of propyl) 3.29 (2H, d, $^{\beta}CH_2$), 3.45-3.75 (9H, s, -OMe) 4.81 (1H, q, $^{\alpha}CH$), 5.30 (2H, s, -CH_2) 6.8-7.02 (7H, m, Ar-H), 9.34 (1H, d, -NH) CMR: 13.6, 23.1, 26.3, 32.2, 32.6, 37.0, 51.9, 52.8, 56.2, 69.8, 116.3, 117.0, 118.4, 126.0, 127.8, 128.7, 134.3, 138.6, 139.5, 148,7, 149.0, 150.7, 156.4, 162.4, 170.3
6e	0.59	86	Gummy	627.19	627.10	IR: 3260 (NH), 1698 (CO) PMR: 0.94 (3H, t, -CH_3 of propyl),1.50 (6H, s, -CH_3) 1.64 (2H, m, $^{\gamma}CH_2$), 1.69 (2H, m, -CH_2 of propyl) 1.95 (2H, q, $^{\beta}CH_2$), 2.47 (2H, t, -CH_2 of propyl) 3.40 (2H, t, $^{\delta}CH_2$), 3.73 (6H, s, -OMe), 4.18 (1H, t, $^{\alpha}CH$), 5.26 (2H, s, -CH_2), 5.34 (2H, s, -CH_2) , 6.82 (2H, m, Ar-H) CMR: 13.7, 22.6, 24.2, 28.3, 28.9, 31.0, 32.6, 45.7, 56.2, 58.7, 68.5, 69.8, 116.3, 117.3, 118.5, 127.2, 127.7, 129.0, 134.3, 138.6, 141.2, 148.7, 149.0, 150.7, 156.4, 165.4, 171.6
6f	0.60	90	148	618.48	641.51 [M^++Na]	IR: 3256 (NH), 1696 (CO) PMR: 1.0(3H, t, -CH_3 of propyl), 1.48 (6H, s, -CH_3), 1.67 (2H, m, -CH_2 of propyl), 2.43 (2H, t, -CH_2 of propyl), 3.29 (2H, d, $^{\beta}CH_2$), 3.50-3.85 (9H, s, -OMe), 4.80 (1H, q, $^{\alpha}CH$), 5.20 (2H, s, -CH_2), 6.7-7.10 (6H, m, Ar- H), 9.25 (1H, d, -NH) CMR: 12.5, 24.1, 27.3, 31.8, 33.4, 37.0, 51.9, 52.8, 54.3, 68.6, 115.8, 116.1, 118.2, 118.5, 129.2, 132.1, 135.3, 138.4, 148.7, 149.6, 151.6, 155.7, 156.2, 160.8, 171.2

Table - 2: Inhibitory zone (diameter) mm of the synthesized conjugates against tested bacterial and fungal strains by agar well diffusion method; % inhibition in the antioxidant activity

Entry	Antibacterial[a]					Antifungal[a]		Antioxidant activity[a] (% inhibition)
	Zone of inhibition (mm) ± SD (n=3)							
	EC	***XO***	***RS***	***XCC***	***CPS***	***AN***	***FO***	
4a	6±0.52	4±0.20	6±0.30	5±0.30	14±0.20	-	-	-
4b	7±0.20	7±0.52	6±0.15	-	12±0.15	-	-	-
4c	5±0.50	6±0.35	6±0.15	-	12±0.43	2±0.5	-	-
4d	6±0.50	5±0.41	6±0.32	-	10±0.10	-	-	-
4e	5±0.41	5±0.50	5±0.32	-	10±0.10	5±0.30	-	-
4f	6±0.46	20±0.15	10±0.32	7±0.15	10±0.10	5±0.41	-	87.50%
5a	4±0.45	20±0.15	20±0.10	10±0.26	16±0.05	10±0.45	-	-
5b	8±0.47	10±0.25	12±0.20	10±0.32	16±0.20	-	-	-
5c	5±0.41	10±0.26	12±0.43	4±0.10	12±0.10	-	-	-
5d	6±0.50	10±0.30	8±0.30	-	12±0.15	-	-	-
5e	10±0.20	8±0.20	6±0.25	-	9±0.20	5±0.32	-	-
5f	4±0.40	4±0.10	4±0.15	-	9±0.15	-	-	84.80%
6a	12±0.45	19±0.20	9±0.10	8±0.15	10±0.30	-	-	-
6b	5.5±0.36	6±0.10	4±0.26	6±0.45	8±0.20	-	-	-
6c	4±0.30	6±0.20	5±0.10	5±0.26	8±0.40	-	-	-
6d	4±0.35	6±0.20	5±0.15	5±0.17	8±0.37	-	-	-
6e	9±0.30	12±0.20	11±0.20	10±0.49	14±0.20	-	-	-

6f	4±0.35	7±0.26	5±0.15	6±0.25	9±0.40	-	-	-
4a'	10±0.36	9±0.05	5±0.32	-	10±0.41	6±0.30	2±0.30	-
4b'	10±0.30	12±0.35	5±0.20	-	13±0.40	7±0.43	4±0.45	-
4c'	12±0.30	10±0.23	5±0.11	-	10±0.05	7±0.30	3±0.20	-
4d'	10±0.40	10±0.20	7±0.20	-	12±0.10	6±0.30	-	-
4e'	9±0.52	9±0.34	5±0.20	-	9±0.20	5±0.40	-	-
4f'	12±0.45	10±0.23	7±0.11	-	12±0.10	6±0.46	4±0.37	-
5a'	8±0.30	6±0.23	7±0.05	-	12±0.20	-	-	87.90%
5b'	8±0.41	6±0.05	4±0.10	-	9±0.20	-	-	-
5c'	9±0.30	7±0.10	11±0.26	-	17±0.20	-	-	-
5d'	7±0.40	8±0.23	7±0.30	-	10±0.40	-	-	-
5e'	9±0.41	8±0.32	10±0.20	-	20±0.20	-	-	-
5f'	10±0.26	9±0.20	7±0.11	-	9±0.15	5±0.30	-	86.10%
6a'	9±0.40	9±0.34	9±0.15	-	13±0.36	-	-	-
6b'	9±0.30	9±0.11	6±0.15	-	12±0.30	-	-	-
6c'	14±0.45	10±0.20	9±0.20	-	12±0.40	9±0.30	8±0.11	-
6d'	12±0.30	10±0.20	6±0.20	-	15±0.26	7±0.43	7±0.30	-
6e'	10±0.45	9±0.30	9±0.20	-	16±0.25	8±0.46	6±0.20	-
6f'	14±0.41	12±0.25	9±0.15	-	15±0.20	7±0.40	8±0.20	86.10%
Std	14±0.23	10±0.15	14±0.24	8±0.30	19±0.09	20±0.14	10±0.22	93.0%

[a]Values are mean of three determinations, the ranges of which are <5% of the mean in all cases

3.2. Biology

The ability of the synthesized compounds as antimicrobials were evaluated for their antibacterial studies against different strains of both gram positive bacteria namely *C. positive staphylococcus, Ralstonia Solanacearurm* and *Xanthomonas Campestris pv. Campestris* and gram negative organisms like *E. coli* and *X. oryzae* and antifungal studies against *A. niger,* and *F. oxysporum*. The result obtained as zone of inhibition (mm) is presented in table 2.

Imidazole and its derivatives present varied biological activities. Further, conjugation of amino acids/peptides is the emerging area in the field of medicinal chemistry which has good therapeutic values. Initially, only the heterocycles **3a-b** and **3a'-b'** were 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. Compounds containing *C*-terminal methyl esters have shown less activity compared to their acid containing counterparts, which reveals that presence of polar groups may help in obtaining antimicrobial agents. Among the three *N*-substituents, presence of Br and OMe were found to be worthy. Hence, it may be suggested that presence of two or more substituents would be helpful in designing the molecules.

From the activity profile, it may be observed that most of the compounds presented in this study have shown better antibacterial activity compared to antifungal. In most cases, analogues containing simple amino acids like Gly/Ala/Val are less active than those compounds containing Pro/Phe/Tyr. This trend might be due to the presence of side chain functions like pyrolidine/phenyl/hydroxyl phenyl in the amino acids Pro/Phe/Tyr respectively.

As for as antioxidant activity is concerned, as expected, compounds with Tyr residue (**4f, 5f, 5f'** and **6f'**) have shown excellent percentage inhibition which could be due to the presence of phenolic functional groups which acts as radical scavengers. One exception to this is the Gly containing compound **5a'**.

4. CONCLUSION

In this study, novel imidazolo-amino acids conjugates have been synthesized and tested for their antibacterial, antifungal and antioxidant assays. Most of the compounds with –COOH free group showed good activity. Presence of two or more functional groups were found to be essential to obtain active compounds. Those moieties having Tyr as the conjugate exhibited superior activities compared to rest of the analogues. It was observed that most of the compounds tested were

strain specific. Though much information on SAR could not be obtained from the series, this work would be a platform for our group to develop more active therapeutic agents.

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