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Synthesis, Characterization and Pharmacological Evaluation of Novel Derivatives of 5-(2-Acetylamino-2-Alkyl-Acetylamino)-1-Methyl-1H-Imidazole-4-Carboxylic Acid Amide

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ABSTRACT:

A series of 1-methyl imidazole carboxamide coupled with N-protected amino acid derivatives were synthesized, characterized and their antimicrobial as well as antifungal properties were evaluated. These compounds were synthesized by coupling reaction in presence of POCl3 and Pyridine and characterized using IR, 1H and mass spectroscopy. The synthesized compounds were screened for their in vitro antimicrobial activity against S. aureus, E. coli, P. aeruginosa, S. typhimurium, F. oxysporum and A. alternata. Some of these compounds exhibited moderate to good activity, where as some were found inactive, in comparison to pathogens being evaluated.

KEYWORDS: Imidazole, Amino acid, Carboxamide, Antibecterial, Antifungal & 5-Amino-1-methyl-1H-imidazole-4-carboxylic acid amide

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INTRODUCTION:

Carboxamide is a common feature in small or complex synthetic or natural pharacologically active compounds. It is ubiquitous in metabolic system. Nearly all known enzymes are proteins, which are carboxamides and play a crucial role in virtually all biological processes such as enzymatic catalysis, transport/storage (haemoglobin), immune protection (antibodies) and mechanical support (collagen). Carboxamide of amino acid are of particular interest to researchers as it shows wide range of pharmacological activities like antibacterial[1], anticancer [2], anti-inflammatory[3], anti HIV[4] etc. An in-depth analysis of the Comprehensive Medicinal Chemistry database revealed that the carboxamide group appears in more than 25% of known drugs [5] .

Another biologically important back bone of drug design is Imidazole. Imidazole and its derivatives are of great significance due to their important roles in biological system, particularly in enzymes, as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes[6]. Imidazole derivatives possess a broad spectrum of pharmacological activities such as anticonvulsant[6,7], anti-Parkinson[7,8] and mono-aminooxidase (MAO) inhibitory[6,9] activity. Imidazole is back bone of many existing generic drugs like Temosolomide[2,10] and Dacarbazine[2]. Derivatives of imidazole have exhibited various pharmacological activities against various pathogens[11], for example antibacterial[12], antifungal[12], antiprotozoal[13] and anthelmintic[14,15].

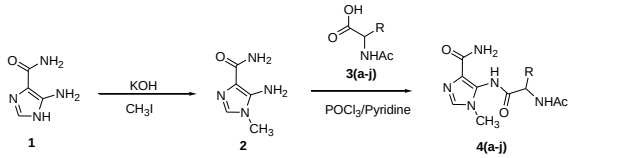
In continuation with our previous work of napthaloyl moiety attached to amino acids we wish to explore the effect of amino acids attached to biologically important imidazole moiety through carboxamide linkage.

Synthesis of N-alkyl imidazole was performed according to reported method in literature[16]. Coupling of N-protected amino acids was carried out by known method[17] using $POCl_3$ and Pyridine.

1. MATERIALS AND METHODS

All chemicals were purchased from laboratory chemical suppliers and used without further purification. Melting points (m.p.) were determined using a Veego VMP-PM melting point apparatus and are uncorrected. Mass spectra (MS) were recorded on a Waters Q-TOF instrument in only positive ion detection mode. The 1H NMR spectra were recorded on a Bruker Avance II 500 (500 MHz) instrument using DMSO-d6 as solvent and Tetramethyl sillane (TMS) as internal reference. Chemical shifts are expressed as δ values (ppm). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR spectrophotometer. The course of reactions was monitored and the purity of synthesized compounds was checked by TLC using silica gel 60 F 254 Al-plates (Merck, Germany) in Dichloromethane : Methanol : Acetic acid (80;:15:5) and Butanol : Acetic acid : water (4:1:1) as mobile phase and the spots were visualized under 254nm UV illumination and 1 % $KMnO_4$ solution.

General Reaction Scheme:



a = R : - H d = R : - $CH_2CH(CH_3)_2$ g = R : - CH2-Ph j = R : - $C(=CH_2)CH_3$
b = R : - CH_3 e = R : - CH_2CH_3 h = R : - $CH_2CH_2CH_2CH_3$
c = R : - $CH(CH_3)_2$ f = R : - $CH_2CH_2CH_3$ i = R : - $CH(CH_3)CH_2$-CH_3

Synthesis of 5-Amino-1-methyl-1H-imidazole-4-carboxylic acid amide. (2)

Potassium hydroxide (3.45 g, 61.5 mmol) was added to an ice cooled solution of 4-aminoimidazole-5-carboxamide hydrochloride (5.0 g, 30.8 mmol) in N,N-dimethyl-formamide (50 mL) and stirred for 3 hours at 0°C. Methyl iodide (1.91 mL, 30.8 mmol) was added and the mixture was stirred over night at 0°C. Filtration followed by wash with methanol and evaporation of the organic phase gave 5-amino-1-methyl-1H-imidazole-4-carboxylic acid amide (2.2 g, 51%) as a brown solid, The product obtained is sufficiently pure for analysis and further use.

Coupling of 5-Amino-1-methyl-1H-imidazole-4-carboxylic acid amide with n-protected amino acids. (4a-j) : 5-amino-1-methyl-1H-imidazole-4-carboxylic acid amide (2) (10 mmol) and n-protected amino acids **(3a-j)** (25 mmol) were dissolved in anhydrous pyridine. The solution was cooled to 10−15 °C and $POCl_3$ (12 mmol) was added drop wise under vigorous stirring. The reaction mixture then was stirred at 10−15 °C for 30 minutes. The solution was allowed to warm to room temperature and then stirred for 48 to 72 h at same temperature. The reaction was quenched by pouring reaction mass on crushed ice-water. The desired compound was extracted repeatedly using Dichloromethane. The combined organic layers were washing with water, treated with activated charcoal and dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get brown to dark brown semi-solid or oil. The crude material was further purified by flash column chromatography using 230-400 mesh silica, Mixture of dichloromethane and methanol were used as mobile phase, fractions were checked using TLC plates, the fractions with single spot were combined and concentrated under reduced pressure to yield pure product.

5-(2-Acetylamino-acetylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide ; (4a); Yield : 36%; (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 134°C (dec); $C_9H_{13}N_5O_3$; M. wt. : 239.23.; MS (*m/z*): 262.1 (M + Na); IR (KBr,cm^{-1}): 3229 (NH), 3250 (NH), 1580 (NH), 1635(NH), 1619(C=O), 1641(C=O) 1660 (C=O); 1H NMR spectrum in DMSO-d6 (*δ* ppm) ; 7.9 (s, 1H, Ar-H), 3.6 (s, 3H, N-CH_3), 1.98 (s, 3H, -CO-CH_3), 4.1 (s, 1H, -CH_2-).

5-(2-Acetylamino-propionylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide; (4b); Yield : 24% ;(from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 142°C (dec); $C_{10}H_{15}N_5O_3$; M. wt. : 253.26.; MS (*m/z*): 276.1 (M + Na); IR (KBr,cm^{-1}): 3225 (NH), 3249 (NH), 1581 (NH), 1627(NH), 1614(C=O), 1639(C=O) 1651 (C=O); 1H NMR spectrum in DMSO-d6 (*δ* ppm) ; 7.88 (s, 1H, Ar-H), 3.59 (s, 3H, N-CH_3), 1.98 (s, 3H, -CO-CH_3), 4.15-4.13 (m, 1H, -C**H**-CH_3), 1.36-1.33 (d,3H, -CH-C**H**$_3$).

5-(2-Acetylamino-3-methyl-butyrylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4c) ; Yield : 13 %;

(from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 153°C ; $C_{12}H_{19}N_5O_3$; M. wt. : 281.31.; MS (*m/z*): 282.21 (M + 1); IR (KBr,cm^{-1}): 3234 (NH), 3257 (NH), 1591 (NH), 1633(NH), 1619(C=O), 1641(C=O) 1665 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.88 (s, 1H, Ar-H), 3.6 (s, 3H, N-CH_3), 1.99 (s, 3H, -CO-CH_3), 4.2-4.16 (m, 1H, -C**H**-CH-), 2.41-2.39 (m, 1H, -C**H**-$(CH_3)_2$ 1.39 (d,6H, -CH-$(CH_3)_2$.

5-(2-Acetylamino-4-methyl-pentanoylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4d); Yield : 35 %; (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 144°C (dec); $C_{13}H_{21}N_5O_3$; M. wt : 295.34.; MS (*m/z*): 318.1 (M + Na); IR (KBr,cm^{-1}): 3233 (NH), 3251 (NH), 1589 (NH), 1631(NH), 1620(C=O), 1643(C=O) 1667 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.9 (s, 1H, Ar-H), 3.61 (s, 3H, N-CH_3), 2.01 (s, 3H, -CO-CH_3), 4.15-4.13 (m, 1H, -C**H**-CH_2-), 1.45-1.43 (m, 2H, -CH-C**H**$_2$-CH-), 1.53-1.5 (m, 1H, -CH_2-C**H**-), 1.01-0.97 (d, 6H, -CH-(C**H**$_3$)$_2$).

5-(2-Acetylamino-butyrylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4e) ; Yield : 11 % (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 121°C (dec); $C_{11}H_{17}N_5O_3$; M. wt : 267.28.; MS (*m/z*): 290.1 (M + Na); IR (KBr,cm^{-1}): 3229 (NH), 3247 (NH), 1591 (NH), 1629(NH), 1621(C=O), 1644(C=O) 1666 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.91 (s, 1H, Ar-H), 3.58 (s, 3H, N-CH_3), 2.0 (s, 3H, -CO-CH_3), 4.2-4.16 (m, 1H, -C**H**-CH_2-), 1.74-1.69 (m, 2H, -C**H**$_2$-CH_3), 1.01-0.99 (m,3H, -CH_2-C**H**$_3$);

5-(2-Acetylamino-pentanoylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4f); Yield : 9 % (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 128°C (dec); $C_{12}H_{19}N_5O_3$; M. wt : 281.31.; MS (*m/z*): 304.1 (M + Na); IR (KBr,cm^{-1}): 3228 (NH), 3251 (NH), 1588 (NH), 1630(NH), 1624(C=O), 1652(C=O) 1676 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.87 (s, 1H, Ar-H), 3.58 (s, 3H, N-CH_3), 1.99 (s, 3H, -CO-CH_3), 4.13-4.1 (m, 1H, -C**H**-CH_2-), 1.44-1.38 (m, 2H, -CH-C**H**$_2$-CH_2-), 1.3-1.21 (m, 2 H, -CH_2-C**H**$_2$-CH_3), 1.0-0.92 (t,3H, -CH_2-C**H**$_3$).

5-(2-Acetylamino-3-phenyl-propionylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4g); Yield : 15 % (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 165°C (dec); $C_{16}H_{19}N_5O_3$; M. wt : 329.35.; MS (*m/z*): 352.1 (M + Na); IR (KBr,cm^{-1}): 3230 (NH), 3249 (NH), 1589 (NH), 1635(NH), 1625(C=O), 1650(C=O) 1675 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.88 (s, 1H, Ar-H), 3.57 (s, 3H, N-CH_3), 1.97 (s, 3H, -CO-CH_3), 6.01 – 6.27 (b, 2H, -CO-NH_2), 4.27-4.22 (m, 1H, -C**H**-CH_2-), 7.45-7.33 (m, 5H, C_6H_5), 3.10- 3.0 (m, 2H, -CH-C**H**$_2$-) ;

5-(2-Acetylamino-hexanoylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4h); Yield : 22 % (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 117°C (dec); $C_{13}H_{21}N_5O_3$; M. wt : 295.34.; MS (*m/z*): 318.2 (M + Na); IR (KBr,cm^{-1}): 3244 (NH), 3261 (NH), 3230 (NH), 1599 (NH), 1630(NH), 1635(C=O), 1651(C=O), 1683 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.91 (s, 1H, Ar-H), 3.55 (s, 3H, N-CH_3); 4.11-4.07 (m, 1H, -C**H**-CH_2), 2.04 - 1.89 (m, 5H, -CH-C**H**$_2$-CH_2 & -CO-CH_3), 1.53-1.28 (m, 4H, -CH_2- & -CH_2-), 0.97-0.91 (t, 3H, -CH_3).

5-(2-Acetylamino-3-methyl-pentanoylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4i); Yield : 17 % (from 4-aminoimidazole-5-carboxamide hydrochloride);M. P. : 159°C (dec); $C_{13}H_{21}N_5O_3$; M. wt : 295.34.; MS (*m/z*): 318.2 (M + Na); IR (KBr,cm^{-1}): 3230 (NH), 3245 (NH), 1565 (NH), 1630(NH), 1618(C=O), 1644(C=O) 1666 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.88 (s, 1H, Ar-H), 3.6 (s, 3H, N-CH_3), 4.21-4.1 (m, 1H, -CH-), 1.0-0.96 (m, 6H, -CH3 & -CH3), 1.38-1.31 (m, 2H, CH2), 1.97-1.92 (m, 4H, -C**H**-CH_2- & -CO-CH_3),

5-(2-Acetylamino-3-methyl-but-3-enoylamino)-1-methyl-1H-imidazole-4-carboxylic acid amide (4j); Yield : 31 % (from 4-aminoimidazole-5-carboxamide hydrochloride); M. P. : 188 – 191°C, $C_{12}H_{17}N_5O_3$; M. wt : 289.3.;MS (*m/z*): 302.1 (M + Na);IR (KBr,cm^{-1}): 3241 (NH), 3247 (NH), 1566 (NH), 1631(NH), 1627(C=O), 1643(C=O) 1676 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm) ; 7.9 (s, 1H, Ar-H), 3.61 (s, 3H, N-CH_3), 5.34-5.24 (m, 1H, -C**H**-C(CH_3), 2.01-1.96 (m, 1H, -C**H**$_3$-C(CH)$_3$ & -CO-CH_3), 4.75-4,63 (m, 2H, -C=CH_2**).**

Biological Screening: Preliminary examination of the biological activity of these newly synthesized compounds was performed by the disc diffusion method[18] using Muller Hinton Agar (MHA) medium. In hard glass screw cap test tube, sterile slants of MHA were prepared. Stored pure cultures were transferred to the freshly prepared MHA slants separately for each organism using sterilized inoculating loop. In this way, four test tubes were freshly prepared for each bacterial pathogen. Freshly prepared pure culture tubes slants were used for inoculation of nutrient broths. These tubes were incubated at (35 - 2°C) for 24 hours to get bacterial suspensions used to study antibacterial activity. The

microorganisms were spread on the surface of MHA plate. Five wells of equal size were created using gel puncher (4 mm) in each plate. These wells were then filled with 10 µL of each sample) and labeled accordingly. DMSO was used as a solvent. The micro-organisms of *Staphylococcus aureus* NCIM 2127 (*S. aureus*), *Escherichia coli* NCIM 2065 (*E. coli*), *Pseudomonas aeruginosa* NCIM-2036 (*P. aeruginosa)* and *Salmonella typhimurium* NCIM 2501 (*S. typhimurium*) were purchased from the National Chemical Laboratory (NCL), Pune, India.

2. RESULT AND DISCUSSION

All the synthesized compounds were characterized using various spectroscopic techniques. IR spectra showed characteristic bands of amide at 3244-3225 cm^{-1}, 3261-3245 cm^{-1}, 1599-1565 cm^{-1} & 1635-1627 cm^{-1}; and carbonyl at 1635-1614 $cm^{-1,}$ 1652-1639 cm-1, 1683-1651 cm^{-1} . The 1H spectrum was carried out at 500 MHz and showed all characteristics pattern of peaks in their respective range. Electron ionization mass spectrometric fragmentation pattern further confirmed the existence of desired compound.

Biological Assays : All the synthesized compounds were evaluated *in vitro* for their antibacterial activities against *S. aureus as* examples of Gram positive bacteria and *E. coli, P. aeruginosa and S. typhimurium as* examples of Gram negative bacteria. They were also evaluated *in vitro* for their antifungal activities against the *F. oxysporum* and *A. alternata* fungal strains. The results were compared with the standard 0.3% Amplicilline and Chloramphenicol as antibacterial agent while Nystatin was used as reference drugs as antifungal agent. Results were summarized in Table 1.

3. CONCLUSION

In summary, we have disclosed the rational design for synthesis of series of potent and novel protected amino acid carboxamides of Imidazole **(4a-4f)**. The biological evaluation of these carboxamide derivatives indicate that carboxamides of imidazole having *alkyl* substituent as methyl, ethyl, propyl and isopropyl group are significantly active than the carboxamides of imidazole having isobutyl, benzyl, butyl, isobutyl and 1-Methyl ethylene groups when compared to Amplicilline, Chloramphenicol and Nystatin as standards.

Table I. In *vitro* antimicrobial activities of all synthesized compounds

Compound code	Zone of inhibition (in mm)					
	Bacteria				Fungi	
	Gram +ve	Gram -ve				
	S. aureus	*E. coli*	*P. aeruginosa*	*S. typhimurium*	*F. oxysporum*	*A. alternata*
4a	11	2	5	3	16	8
4b	24	15	8	16	48	33
4c	22	12	8	8	51	35
4d	9	10	0	4	21	3
4e	25	11	4	9	45	37
4f	15	19	7	12	55	34
4g	4	0	3	14	17	7
4h	8	4	6	01	12	22
4i	13	7	7	3	54	11
4j	12	6	2	11	33	18
Amplicillin e	26	11	-	-	-	-
Chloramph enicol	20	20	10	15	-	-
Nystatin	-	-	-	-	57	38

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