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Synthesis, Characterization and Biological Evaluation of Novel 1-[2-(2-Tert-Butylcarbamoyl-Benzoylamino)-Alkyl Acyl]-Piperidine-4-Carboxylic Acid Methyl Ester

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ABSTRACT:

A series of novel piperidine-4-carboxylic acid methyl ester coupled with several N-phthaloyl amino acids derivatives were synthesized, characterized and their antimicrobial as well as antifungal properties were evaluated. These compounds were synthesized by reaction of DCC/HOBt coupling of piperidine-4-carboxylic acid methyl ester and N-phthaloyl amino acids followed by ring opening reaction using tert-butyl amine and characterized using IR, 1H NMR 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.

KEY WORDS: Carboxamide, N-phthaloyl amino acids, tert-butyl amine, antimicrobial activity, antifungal activity, DCC.

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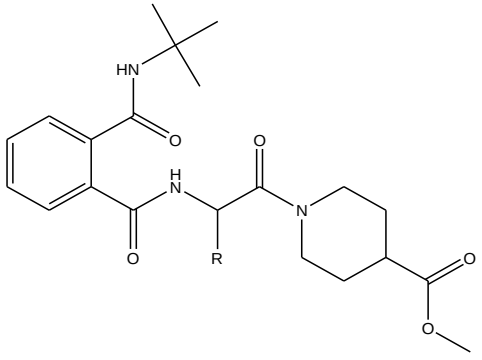
1. INTRODUCTION

Carboxamide derivatives are very useful as preventives and therapeutic agents. It has been found that alkyl as well as aryl substituted carboxamide derivatives show very good antibacterial[1], Antifungal[1], antiviral[2,3], anti-inflammatory-analgesic[4], anti-tuberculosis[5], anticancer[6], and respiratory analeptic[7] activities and offer a potential emerging target for the treatment of pain[8]. Amino acids play very important role in almost all metabolic activities and are important constituents of nutrients and building block of proteins and enzymes. Both these class of compounds have remained important target in designing of new antimetabolites for various metabolic disorders. Currently there is a growing tendency to use amino acid and peptidyl residues during the pro-drug design process. Literature reports that bioactive compounds shows enhanced activity and reduced side effect when linked to amino acids[9]. Uses of unusual (non-proteinogenic) amino acids have further stimulated interest in new synthetic methodology and strategies to obtain a target structure for desired medicinal activity[10]. The goal of the present work was to synthesize 1,2-dicarboxamide derivatives bearing various proteinogenic as well as non-proteinogenic amino acids reside at position 2 of carboxamide and an amine at position 1 of target structure A.

Preparation of these Dicarboxamide compounds were achieved by methyl ester formation of isonepecotic acid using conventionally known method in

the literature i.e. using thionyl chloride and methanol at lower temperature (Scheme 1). N-phthaloyl derivative 2a-2j were synthesized using triethyl amine (TEA) and toluene according to known methods[11-13] (Scheme 2). Coupling of isonipecotic acid ester and amino acid derivatives of N-phthanolyl were carried at lower temperature using coupling reagent N,N'-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) in tetrahydrofuran (THF) as solvent and triethylaminde (TEA) as base. Final ring opening reaction were carried out using tert-butyl amine in dichloromethane (DCM) and methanol (MeOH) mixture as solvent at room temperature to get target molecules 4a – 4j in reasonable yield[14,15].



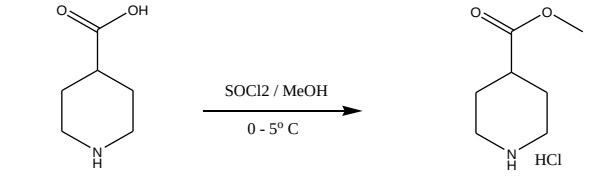
Structure A : 1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-*alkyl* acetyl]-piperidine-4-carboxylic acid methyl ester

2. MATERIAL AND METHODS

All chemicals were purchased from commercial 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 either $CDCl_3$ or DMSO-d6 as solvent and 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 DCM-MeOH (9:1) solvent system and the spots were visualized under UV illumination(254nm) or using staining reagent Ninhydrine (1% solution in Ethanol).

General Procedure: Synthesis of methyl ester of isonipecotic acid (Scheme-1): Isonipecotic acid (10 mmol) was suspended in methanol (10V) and the mixture was cooled to 0 - 5°C. Thionyl chloride (15 mmol) was added

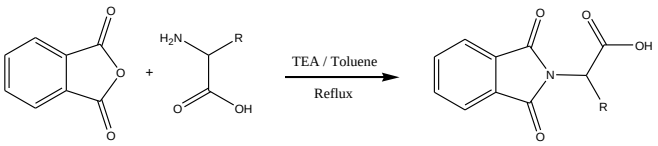
Scheme 1: Synthesis of methyl ester of isonipecotic acid



Isonipecotic acid [Piperidine-4-carboxylic acid]

Isonipecotic acid methyl ester hydrochloride [Methyl piperidine-4-carboxylate hydrochloride]

Scheme 2: Synthesis of N-phthaloyl derivative (2a-2j)



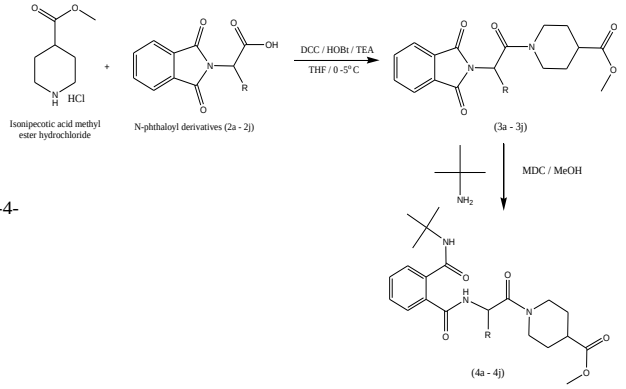
Phthalic anhydride

Amino acid (1a - 1j)

N-phthaloyl derivatives (2a - 2j)

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

Scheme 3: Synthesis of Carboxamide derivatives (4a-4j)



Isonipecotic acid methyl ester hydrochloride

N-phthaloyl derivatives (2a - 2j)

(3a - 3j)

(4a - 4j)

slowly to this mixture maintaining temperature below 5o C. Reaction was stirred till completion of reaction. Reaction was monitored with TLC (DCM : MeOH :: 9:1) for disappearance of starting material, Ninhydrine (1% in Ethanol) was used as TLC visualization reagent. After completion of reaction, methanol was distilled off under reduced pressure and resulting mass was repeatedly dissolved in methanol and distilled off to remove traces of thionyl chloride. Acetone (5V) was added and distilled under reduced pressure to bring methanol and thionyl chloride content to a minimum level. The product so obtained Isonipecotic acid methyl ester was sufficiently pure for characterization as well as to use in next stage (Amide coupling).

Yield: 95.0%; m.p.190-192 °C; $C_7H_{17}ClNO_2$; Mol. Wt : 179.64; IR (KBr,cm^{-1}): 3466 (NH), 1739 (C=O); 1H NMR spectrum in DMSO-d6 (*δ* ppm): 3.61, (s, 3H, -OCH_3), 3.13-3.06 (m, 2H, -C**Ha**Hb-N), 2.70-2.60 (m, 2H, -CHa**Hb**-

N), 2.45-2.35 (m, 1H, -CH-C=O), 1.95-1.81 (m, 2H, -C**Ha**Hb-CH), 1.75-1.54 (m, 2H, -CHa**Hb**-CH).; MS (*m/z*): 144.0 (M+1).

Synthesis of N-Phthaloyl Amino Acids (2a-2j) (General Method) (Scheme 2): Pthalic anhydride (1.48 g, 10 mmol) and appropriate amino acids **(1a – 1j)** (10 mmol) were mixed in round bottom flask fitted with Dean-stark apparatus and reflux condenser, the mixture was refluxed in toluene in the presence of 0.1 ml triethylamine for 3 h. The reaction mass was concentrate under reduced pressure to get residue as sticky oily mass. Water was added to this oily mass and the mixture was acidified with hydrochloric acid, and stirred for 30 min to get solid. This solid product was filtered off, washed with water, and dried to get a target compound **(2a – 2j)**.

Synthesis of Substituted 1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-*alkyl* acyl]-piperidine-4-carboxylic acid methyl ester(3a – 3j) (General Method)(Scheme 3):N-phthaloyl derivatives (2a – 2f) (10 mmol) and Isonipecotic acid methyl ester hydrochloride (12 mmol) was dissolved in THF (10V) and triethyl amine (36 mmol). Added to this hydroxybenzotriazole (12 mmol) and the resulting mixture was cooled to 0 – 5 ^{o}C. Solution of DCC (11 mmol) in THF (2.5 V) was added to the above mixture maintaining temperature below 5 ^{o}C. The mixture was stirred for 30 minutes at low temperature and then was allowed to stir overnight at ambient temperature. Reaction pH was adjusted to alkaline using triethylamine, if required. Completion of reaction was monitored using TLC (DCM:MeOH:AcOH :: 85:10:5) for disappearance of isonipecotic acid methyl ester, Ninhydrine (1% in Ethanol) was used as TLC visualization reagent. After completion of reaction, dicyclohexylurea was filtered, washed with THF and filtrate was concentrated under reduced pressure, resulting mass was dissolved in Ethyl acetate and washed with saturated $NaHCO_3$, 0.1 N HCl solution and then with brine solution. Resulting organic layer was dried using anhydrous sodium sulphate and concentrated to get solid or syrup, If syrup was obtained then solidified by stirring with Hexane or Diisopropyl ether and filtered. The product so obtained was dried under vacuum.

Synthesis of 1-[2-(2-tert-butylcarbamoyl-benzoylamino)-*alkyl* acyl]-piperidine-4-carboxylic acid methyl ester (4a – 4j) (General Method) (Scheme 3): 1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-*alkyl*-acyl]-piperidine-4-carboxylic acid methyl ester **(3a–3f)**(10 mmol) were dissolved in MeOH:MDC (1:2, 12V) mixture and *tert*-Butylamine (20 mmol) was added. Reaction mixture was stirred at ambient temperature for 10 – 12 h. The reaction mass was concentrated under reduced pressure and the resulting oily residue was repeatedly triturated with hexane and then stirred in ethyl acetate – hexane mixture, filtered and dried to get respective carboxamide derivatives (**4a-4j).**

1-[2-(2-tert-butylcarbamoyl-benzoylamino)-acetyl]-piperidine-4-carboxylic acid methyl ester (4a): Yield, (73.2%); m.p., 215°C; $C_{21}H_{29}N_3O_5$; Mol.Wt : 403.47; IR (KBr,cm^{-1}): 3233 (NH), & 3249 (NH) 1727 (C=O) 1590 (NH), 1621(NH), 1619(C=O), 1641(C=O) 1655 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.09 (s, 2H, -CH_2), 8.53- 7.75 (m, 4H,C_6H_4),1.49, (s, 9H, -$C(CH_3)_3$, 3.62, (s, 3H, -OCH_3), 3.1-3.03 (m, 2H, -C**Ha**Hb-N), 2.69-2.61 (m, 2H, -CHa**Hb**-N), 2.44-2.36 (m, 1H, -CH-C=O), 1.96-1.80 (m, 2H, -C**Ha**Hb-CH), 1.71-1.54 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 404.1 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-propionyl]-piperidine-4-carboxylic acid

methyl ester (4b): Yield, (75.02%); m.p., 196°C; $C_{22}H_{31}N_3O_5$; Mol.Wt: 417.5 ; IR (KBr, cm^{-1}) : 3237 (NH), & 3241 (NH) 1723 (C=O) 1596 (NH), 1627(NH), 1620(C=O), 1635(C=O) 1661 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.19-4.13 (m, 1H, -C**H**-CH_3), 1.37-1.31 (d,3H, -CH-C**H**$_3$).8.54 – 7.68 (m, 4H,C_6H_4),1.48, (s, 9H, -$C(CH_3)_3$, 3.64, (s, 3H, -OCH_3), 3.11-3.04 (m, 2H, -C**Ha**Hb-N), 2.68-2.59 (m, 2H, -CHa**Hb**-N), 2.42-2.32 (m, 1H, -CH-C=O), 1.95-1.80 (m, 2H, -C**Ha**Hb-CH), 1.69-1.50 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 418.2 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-3-methyl-butyryl]-piperidine-4-carboxylic acid methyl ester (4c): Yield, (70.02%); m.p., 187°C; $C_{24}H_{35}N_3O_5$; Mol.Wt: 445.55; IR (KBr,cm^{-1}): 3229 (NH), & 3233 (NH) 1720 (C=O) 1601 (NH), 1620(NH), 1622(C=O), 1639(C=O) 1655 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm):4.24-4.16 (m, 1H, -C**H**-CH-), 2.46-2.39 (m, 2H, -C**H**-$(CH_3)_2$ & -CH-C=O) 1.45 (d,6H, -CH-(CH_3)$_2$, 8.45 – 7.70 (m, 4H,C_6H_4), 1.50, (s, 9H, -$C(CH_3)_3$, 3.60, (s, 3H, -OCH_3), 3.15-3.07 (m, 2H, -C**Ha**Hb-N), 2.73-2.65 (m, 2H, -CHa**Hb**-N), 1.99-1.83 (m, 2H, -C**Ha**Hb-CH), 1.71-1.53 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 446.2 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-4-methyl-pentanoyl]-piperidine-4-carboxylic acid methyl ester (4d): Yield, (66.1%); m.p., 159°C; $C_{25}H_{37}N_3O_5$; Mol.Wt: 459.58; IR (KBr,cm^{-1}): 3231 (NH), & 3251 (NH) 1719 (C=O) 1596 (NH), 1622(NH), 1631(C=O), 1635(C=O) 1660 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.22-4.15 (m, 1H, -C**H**-CH_2-), 1.41-1.33 (m, 2H, -CH-C**H**$_2$-CH-), 1.53-1.45 (m, 10H, -CH_2-C**H**- & -$C(CH_3)_3$), 0.98-0.89 (d, 6H, -CH-(CH_3)$_2$, 8.39 – 7.69 (m, 4H,C_6H_4), 3.59, (s, 3H, -OCH_3), 3.11-3.09 (m, 2H, -C**Ha**Hb-N), 2.71-2.59 (m, 2H, -CHa**Hb**-N), 2.44-2.36 (m, 1H, -CH-C=O), 1.93-1.80 (m, 2H, -C**Ha**Hb-CH), 1.76-1.56 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 460.3 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-butyryl]-piperidine-4-carboxylic acid methyl ester (4e): Yield, (75.21%); m.p., 167°C; $C_{23}H_{33}N_3O_5$; Mol.Wt: 431.53; IR (KBr,cm^{-1}): 3220 (NH), & 3251 (NH) 1705 (C=O) 1611 (NH), 1625(NH), 1630(C=O), 1635(C=O) 1669 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.22-4.11 (m, 1H, -C**H**-CH_2-), 1.06-0.99 (m,3H, -CH_2-C**H**$_3$), 8.58 – 7.71 (m, 4H,C_6H_4), 1.49, (s, 9H, -$C(CH_3)_3$, 3.61, (s, 3H, -OCH_3), 3.09 - 3.01 (m, 2H, -C**Ha**Hb-N), 2.67-2.56 (m, 2H, -CHa**Hb**-N), 2.47-2.36 (m, 1H, -CH-C=O), 1.91-1.79 (m, 2H, -C**Ha**Hb-CH), 1.77-1.55 (m, 4H, -CHa**Hb**-CH & -C**H**$_2$-CH_3).; MS (m/z) :432.2 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-pentanoyl]-piperidine-4-carboxylic acid methyl ester (4f) : Yield, (71.21%); m.p., 181°C; $C_{24}H_{35}N_3O_5$; Mol.Wt: 445.55; IR (KBr,cm^{-1}): 3240 (NH), & 3245 (NH) 1731 (C=O) 1590 (NH), 1630(NH), 1621(C=O), 1632(C=O) 1659 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm):) : 4.18-4.10 (m, 1H, -C**H**-CH_2-), 1.45-1.34 (m, 2H, -CH-C**H**$_2$-CH_2-), 1.28-1.19 (m, 2 H, -CH_2-C**H**$_2$-CH_3), 0.98-0.91 (t,3H, -CH_2-C**H**$_3$), 8.42 – 7.65 (m, 4H,C_6H_4), 1.49, (s, 9H, -$C(CH_3)_3$, 3.60, (s, 3H, -OCH_3), 3.15-3.09 (m, 2H, -C**Ha**Hb-N), 2.75-2.65 (m, 2H, -CHa**Hb**-N), 2.44-2.33 (m, 1H, -CH-C=O), 1.97-1.83 (m, 2H, -C**Ha**Hb-CH), 1.72-1.55 (m, 2H, -CHa**Hb**-CH); MS (m/z) : 446.3 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-3-phenyl-propionyl]-piperidine-4-carboxylic acid methyl ester (4g): Yield (68.5%); m.p 212 to 215 °C; $C_{28}H_{35}N_3O_5$. Mol. Wt : 493.59 ; IR (KBr,cm^{-1}): 3244 (NH), & 3249 (NH) 1733 (C=O) 1599 (NH), 1624 (NH), 1621(C=O), 1644(C=O) 1658 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.32-4.21 (m, 1H, -C**H**-CH_2-), 7.48-7.31 (m, 5H, C_6H_5), 8.54 – 7.90 (m, 4H, C_6H_4), 1.50, (s, 9H, -$C(CH_3)_3$, 3.65, (s, 3H, -OCH_3), 3.13-2.98 (m, 4H, -C**Ha**Hb-N & -CH-C**H**$_2$-),), 2.70-2.59 (m, 2H, -CHa**Hb**-N), 2.47-2.35 (m, 1H, -CH-C=O), 1.91-1.81 (m, 2H, -C**Ha**Hb-CH), 1.71-1.55 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 516.5 (M^+ + Na).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-hexanoyl]-piperidine-4-carboxylic acid methyl ester (4h): Yield : 91.0%; m.p 229 °C; $C_{25}H_{37}N_3O_5$; Mol. Wt. : 459.58; IR (KBr,cm^{-1}): 3237 (NH), & 3240 (NH) 1720 (C=O) 1595 (NH), 1626(NH), 1619(C=O), 1633(C=O) 1660 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.20-4.11 (m, 1H, -C**H**-CH_2), 1.53-1.28 (m, 13H, -CH_2-,-CH_2- & -$C(CH_3)_3$), 0.97-0.91 (t, 3H, -CH_3), 8.33 – 7.85 (m, 4H,C_6H_4), 3.60, (s, 3H, -OCH_3), 3.15-3.06 (m, 2H, -C**Ha**Hb-N), 2.73-2.60 (m, 2H, -CHa**Hb**-N), 2.45-2.37 (m, 1H, -CH-C=O), 2.06 - 1.81 (m, 4H, -C**Ha**Hb-CH & -CH-C**H**$_2$-CH_2), 1.71-1.57 (m, 2H, -CHa**Hb**-CH) ; MS (m/z) : 460.2 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-3-methyl-pentanoyl]-piperidine-4-carboxylic acid methyl ester (4i): Yield : 46%; m.p 179 – 180°C; $C_{25}H_{37}N_3O_5$; Mol. Wt : 459.58; IR (KBr,cm^{-1}): 3230 (NH), & 3240 (NH) 1726 (C=O) 1591 (NH), 1622(NH), 1620(C=O), 1636(C=O) 1657 (C=O);

1H NMR spectrum in $CDCl_3$ (δ ppm): 4.21-4.09 (m, 1H, -CH-), 0.97-0.87 (m, 6H, -CH3 & -CH3), 1.41-1.20 (m, 2H, CH2), 1.96-1.80 (m, 3H, -C**H**-CH_2- & -C**Ha**Hb-CH), 8.47 – 7.93 (m, 4H,C_6H_4), 1.48, (s, 9H, -$C(CH_3)_3$, 3.67, (s, 3H, -OCH_3), 3.15-3.09 (m, 2H, -C**Ha**Hb-N), 2.73-2.61 (m, 2H, -CHa**Hb**-N), 2.46-2.38 (m, 1H, -CH-C=O), 1.72-1.53 (m, 2H, -CHa**Hb**-CH) ; MS (m/z) : 460.2 (M+1).

1-[2-(2-tert-Butylcarbamoyl-benzoylamino)-3-methyl-but-3-enoyl]-piperidine-4-carboxylic acid methyl ester (4j) : Yield : 31%; m.p 139 – 146°C; $C_{24}H_{33}N_3O_5$; Mol. Wt.: 443.54; IR (KBr,cm^{-1}): 3242 (NH), 3251 (NH) 1729 (C=O) 1607 (NH), 1633(NH), 1626(C=O), 1641(C=O) 1671 (C=O); 1622 (C=C);

1H NMR spectrum in $CDCl_3$ (δ ppm): 5.17-4,98 (m, 1H, -C**H**-$C(CH_3)$, 2.06-1.95 (m, 1H, -C**H**$_3$-$C(CH)_3$, 4.82-4,73 (m, 2H, -C=C**H**$_2$**),** 8.33 – 7.9 (m, 4H,C_6H_4), 1.44 (s, 9H, -$C(CH_3)_3$, 3.55, (s, 3H, -OCH_3), 3.10-3.02 (m, 2H, -C**Ha**Hb-N), 2.70-2.61 (m, 2H, -CHa**Hb**-N), 2.45-2.37 (m, 1H, -CH-C=O), 1.95-1.83 (m, 2H, -C**Ha**Hb-CH), 1.71-1.55 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 466.2 (M + Na).

Biological Screening: Preliminary testing of the antibacterial activity of the newly synthesized compounds was performed by the disc diffusion method[16] 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 sets of 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 spared 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 labelled 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, Maharastra, India.

3. RESULT AND DISCUSSION

All the synthesized compounds were characterized using various spectroscopic techniques. IR spectra showed characteristic bands of amide N-H stretch (3220 – 3251 cm^{-1}), amide N – H bend (1590 - 1633 cm^{-1}), carbonyl C=O stretch (1619 – 1733 cm^{-1}). 1H spectrum was recorded at 500 MHz and showed characteristics pattern of peaks supporting formation of the desired compound. Electron ionization mass spectrometric analysis confirms the molecular weight of compounds giving m/z either M + 1 or M + Na.

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. alternate* 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**.

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	16	8	7	9	15	38
4b	17	10	10	11	38	18
4c	19	7	8	9	22	26
4d	12	6	7	8	49	24
4e	20	11	12	11	53	33
4f	19	10	11	11	38	32
4g	20	4	8	10	26	37
4h	9	10	7	10	37	22
4i	11	6	9	12	33	39
4j	19	9	12	7	55	41
Amplicillin e	20	11	-	-	-	-
Chloramp henicol	17	20	12	12	-	-
Nystatin	-	-	-	-	70	50

4. CONCLUSION

In summary, we have disclosed the rational design for synthesis of a series of novel and potent dicarboxamide derivatives (**4a-4j)** using different proteogenic and non-proteogenic amino acids as well as ester of isonipecotic acid. The biological data indicate that carboxamide derivatives having non-proteogenic amino acid residue

shows trend of relatively higher activity compared to that of proteogenic amino acid.

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