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Synthesis, Characterization and Biological Evaluation of Ammonium carboxylate salts of 1-[2-(2-tert-Butylcarbamoyl-Benzoylamino)-*alkyl* acyl]-Piperidine-4- Carboxylic Acid

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ABSTRACT

A series of novel "Ammonium salt of piperidine-4-carboxylic acid coupled with several N-phthaloyl amino acids derivatives were synthesized, characterized, and evaluated for their antimicrobial as well as antifungal properties. These compounds were synthesized using DCC / HOBt coupling of piperidine-4-carboxylic acid methyl ester with N-phthaloyl amino acids followed by ring opening reaction using *tert*-butyl amine, the resulting ester derivatives were hydrolyzed using Sodium hydroxide, desired "Ammonium 1-[2-(2-*tert*-butylcarbamoyl-benzoylamino)-*alkyl*-acyl]- piperidine -4-carboxylate" derivatives were afforded by treatment of ammonia solution in methanol. These compounds synthesized were characterized using IR, NMR and Mass spectroscopy and screened for in vitro activity against *S. aureus, E. coli, P. aeruginosa, S. typhimurium, F. oxysporum* and *A. alternata*. Some of these compounds exhibited moderate to good activity, whereas some were found inactive, against pathogens being evaluated.

Keywords : Piperidine, Ammonium salts, Antibecterial, Antifungal.

I. INTRODUCTION

Drugs marketed today, if assessed reveals that more than half of the drugs are administered in the form of salts, also termed as pharmaceutical salts. These are ionizable drugs those have been combined with counter ions to form pharmaceutical salts. Salt formation is achieved by neutralization of parent drug molecule with an acid or base.

Today's need to develop drugs with higher potency and lower toxicity or side effect as well as development of drugs for un-curable ailments pushed researcher to explore more complex scaffold or scaffolds which are relatively un-explored till now for potential drug candidates. These attempts has resulted in the drug candidates with limited physicochemical property favorable for a potential drug[1], solubility of the drug being one of the prominent property of them[1]. Researcher has applied several strategies to overcome physicochemical limitation of drug candidates. One of the commonly applied one is derivatization of the scaffold, apart from this the other common strategy applied to overcome physicochemical constraints of drug candidate is salt formation[2].

As researcher tries to discover newer drugs for non-curable ailments as well as drugs with higher activity and lower toxicity, novel moieties are being

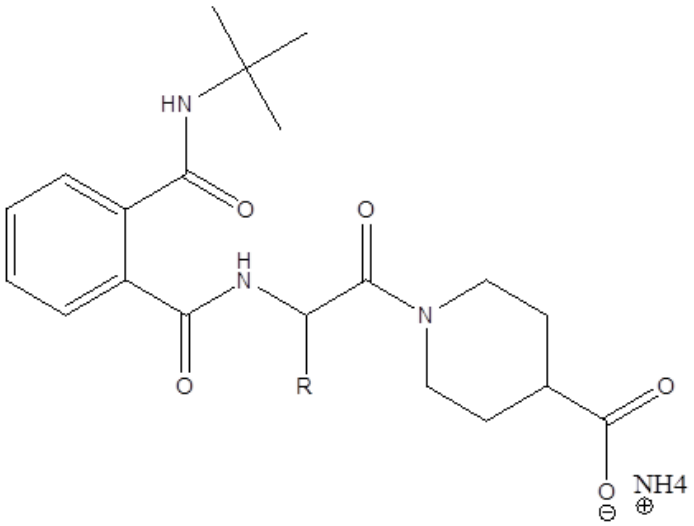
discovered and tried for their biological activity. In these attempts researcher developed more complex moieties or moieties which have limited favorable physicochemical properties. Solubility of drug in water is one of the key physicochemical properties of drug and this is evident from the fact that almost half the drugs being administered today is in their salt forms, as solubility of salts are better compared to their free amines or carboxylic acids. Solubility of drug has direct impact on drugs bioavailability[3] and hence salt formation has become one of the significant aspects in drug development [4]. Drug candidate having some undesired features are also overcome by applying this simple technique of salt formation[4].

Literature reveals that amine drugs are mostly applied as hydrochloride salt, apart from that other salts applied are Sulphate, Oxalate, Acetate etc. Whereas carboxylic acid drugs are applied as sodium, potassium , calcium and ammonium salt of various organic bases like Ammonia, Morpholine, Lysine , Monoethanolamine, Miglumine, Benzyl amine, Diethyl amine etc.

Some of the drugs marketed in their salt forms are Atorvastatin calcium a lipid lowering agent, Clorazepate dipotessium a tranquilizer, Montelukast sodium a leukotriene receptor antagonist(LTRA). Diclofenac sodium a nonsteroidal anti-inflammatory drug (NSAID). Drugs being administered as their ammonium salt are Ammonium salt of Acetoxolone an antiulcerative and Flugenamic acid an anti-inflammatory drug, Morpholine salt of Acediasulfone an antibacterial drug, Lysine salt of Clonixin an analgesic, Diethylamine salt of Diclofenac an anti-inflammatory drug and Miglumine salt of Flunixin an inti-inflammatory drug.

In this study we have investigated biological application of ammonium salt of "1-[2-(2-*tert*-butylcarbamoyl-Benzoylamino)-*Alkyl*-Acyl]-Piperidine-4-Carboxylic Acid derivatives". The parent methyl ester derivatives 1-[2-(2-*tert*-butylcarbamoyl-Benzoylamino)-*Alkyl*-Acyl]-Piperidine-4-CarboxylicAcid have already demonstrated its biological potential as antibacterial and anti-fungal agents[5] .

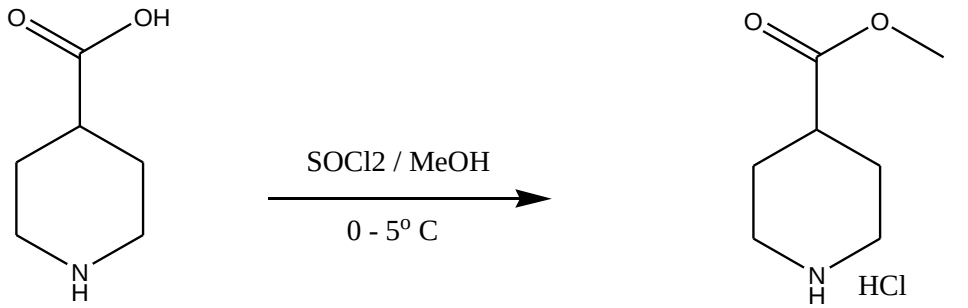
Preparation of these Piperidine 4- carboxylic acid sodium salt derivatives were achieved by methyl ester formation of isonipecotic acid using conventionally known method in the literature i.e. using thionyl chloride and methanol at lower temperature (Scheme 1). N-phthaloyl derivative **2a-2i** were synthesized using triethyl amine (TEA) and toluene according to known methods [6,7,8] (Scheme 2). Coupling of isonipecotic acid methyl ester and amino acid derivatives of N-phthaloyl were carried at lower temperature using coupling reagent N,N'-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) in tetrahydrofuran (THF) as solvent and triethylamine (TEA) as base. Ring opening reaction were carried out using *tert*-butyl amine in dichloromethane (DCM) and methanol (MeOH) mixture as solvent at room temperature to get compounds **4a – 4i** (Scheme 3) in reasonable yield[5] . Finally hydrolysis of compounds **4a – 4i** was performed using well known method in literature i.e. Sodium hydroxide in methanol followed by acidification of reaction mass to get respective acid which was converted in to ammonium salt by applying solution of ammonia in methanol to yield target compounds **5a – 5i**. (Scheme 4).



1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-*alkyl* acetyl]-piperidine-4-carboxylic acid ammonium salt

REACTION SCHEME

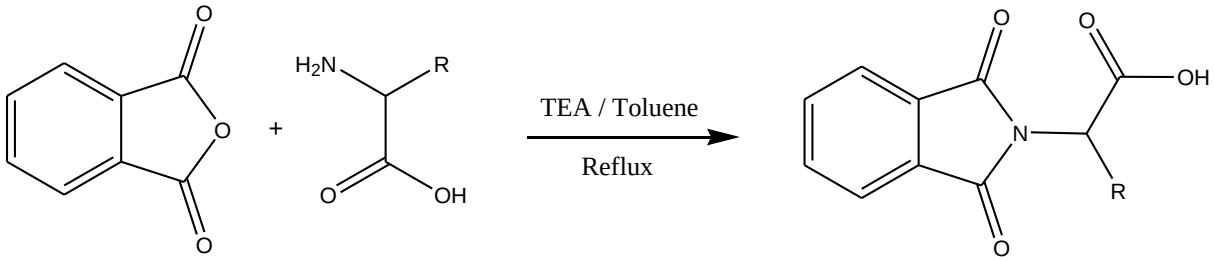
Scheme 1 : Synthesis of methyl ester of isonipecotic acid



Isonipecotic acid
[Piperidine-4-carboxylic acid]

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

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



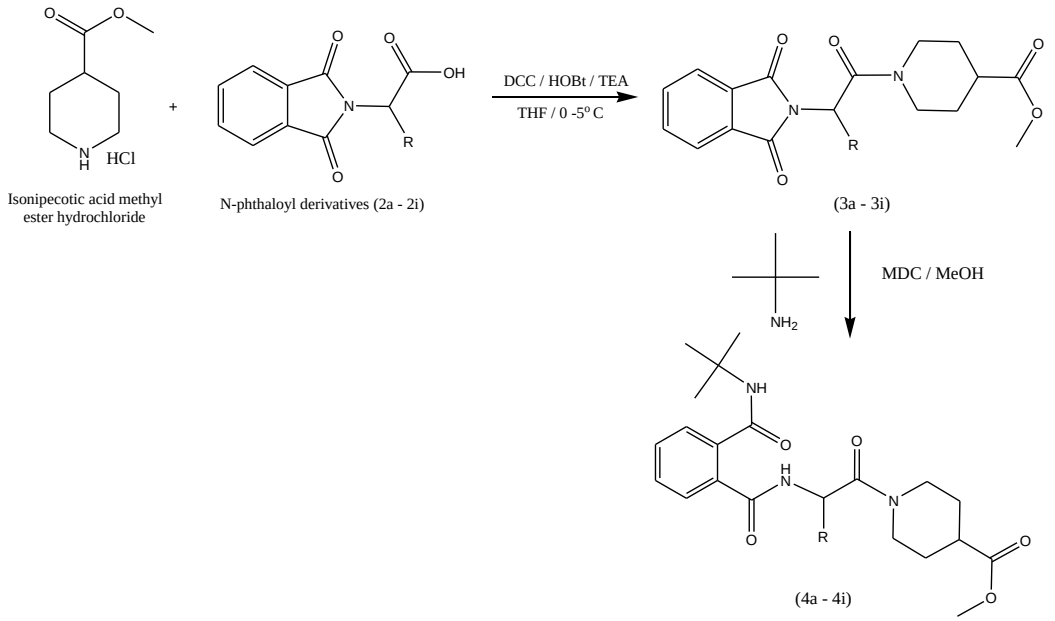
Phthalic anhydride

Amino acid (1a - 1i)

N-phthaloyl derivatives (2a - 2i)

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

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



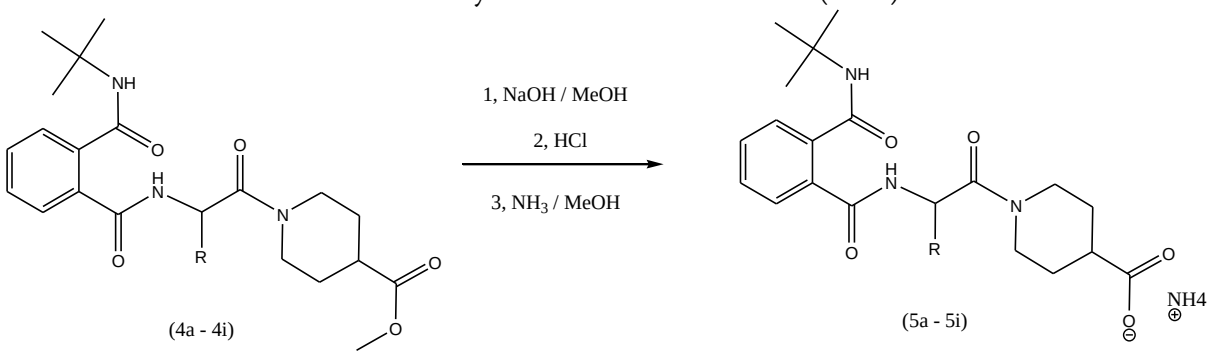
Isonipecotic acid methyl ester hydrochloride

N-phthaloyl derivatives (2a - 2i)

(3a - 3i)

(4a - 4i)

Scheme 4 : Synthesis of Ammonium salt (5a-5i)



(4a - 4i)

(5a - 5i)

Various natural as well as un-natural amino acid used in the synthesis to get different derivatives of base compounds, the details of amino acid used and respective amino acid, their structure and respective structural residue resulted in final compound is listed in below table 01 and 02.

TABLE 01 : List of natural amino acid

Sr. no	Code	Name of Amino acid used	Amino acid structure	Structure of substituent "-R"	Formula of Substituent "-R"
1	a	Glycine	H_2N COOH	- H	- H
2	b	Alanine	CH_3 H_2N COOH	- CH_3	- CH_3

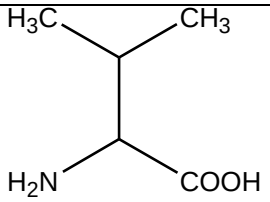
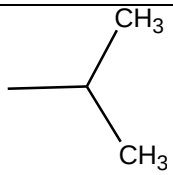
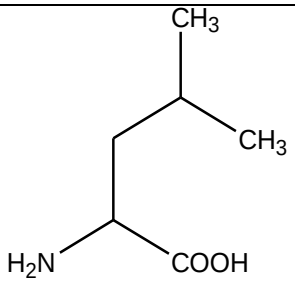
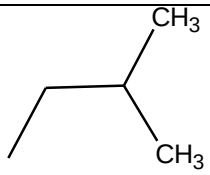
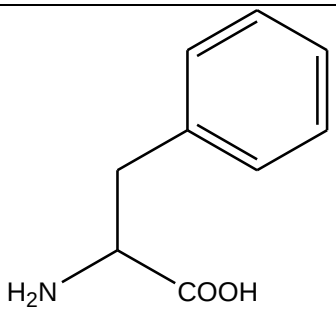
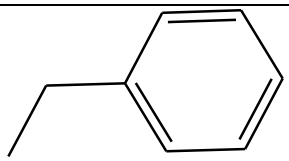
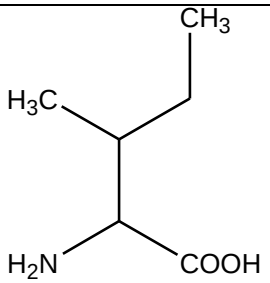
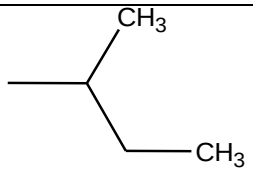
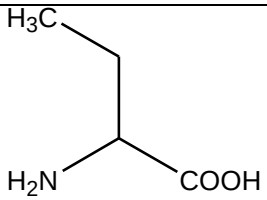
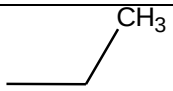
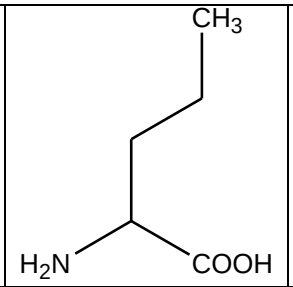
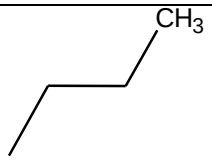
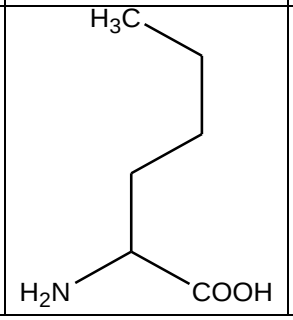
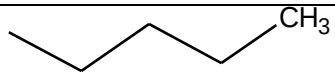
3	c	Valine			- $CH(CH_3)_2$
4	d	Leucine			- $CH_2CH(CH_3)_2$
5	g	Phenyl alanine			- CH_2-Ph
6	i	Isoleucine			- $CH(CH_3)CH_2$-CH_3

TABLE 02 : List of un-natural amino acid

Sr. no	Code	Name of amino acid used	Amino acid structure	Structure of substituent "-R"	Formula of Substituent "-R"
1	e	2-Aminobutyric acid			- CH_2CH_3

2	f	Norvaline			- $CH_2CH_2CH_3$
3	h	Norleucine			- $CH_2CH_2CH_2CH_3$

II. METHODS AND MATERIAL

All the chemicals used 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 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) using DCM-MeOH system as mobile phase and the spots were visualized under UV illumination (254nm) or using staining reagent 1% $KMnO_4$ solution. Mass spectra was done by direct mass analysis, melting points are not corrected and IR peaks were recorded in nm using KBr pallet.

Synthesis of methyl ester of isonipecotic acid (Scheme-1): Isonipecotic acid (10 mmol) was suspended in methanol (10 volume) and the mixture was cooled to 0 - 5°C. Thionyl chloride (15 mmol) was added slowly to this mixture maintaining temperature below 5° C. Reaction mass was stirred till completion of reaction. Reaction was monitored with TLC (DCM : MeOH :: 9:1) for disappearance of starting material, Ninhydrin (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 (5 vol) 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-2i) (General Method) (Scheme 2): Phthalic anhydride

(1.48 g, 10 mmol) and appropriate amino acids **(1a – 1i)** (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 hours. The reaction mass was concentrated under reduced pressure to get residue as sticky oily mas. 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 – 2i)**.

Synthesis of Substituted 1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-*alkyl* acyl]-piperidine-4-carboxylic acid methyl ester(3a – 3i) (General Method)(Scheme 3): N-phthaloyl derivatives (2a – 2f) (10 mmol) and Isonipecotic acid methyl ester hydrochloride (12 mmol) was dissolved in THF (10 volume) 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 volume) 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, Ninhydrin (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 Di-isopropyl 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 – 4i) (General Method) (Scheme 3): 1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-*alkyl*-acyl]-piperidine-4-carboxylic acid methyl ester **(3a–3i)** (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-4i**).

Synthesis of ammonium salt of 1-[2-(2-*tert*-butylcarbamoyl-benzoylamino)-*alkyl*-acyl]-piperidine-4-carboxylic acid (5a – 5i) (General Method) (Scheme 4): 1-[2-(2-*tert*-butylcarbamoyl-benzoylamino)-*alkyl* acyl]-piperidine-4-carboxylic acid methyl ester **(4a – 4i)** (10 mmol) was dissolved Methanol (10 volume). Added to that Sodium hydroxide pallets (25 mmol) and stirred the heterogeneous mixture at 40-45^{o}C for 2 to 3 days. Reaction mass was acidified to pH 1-2 using 10% Methanolic HCl solution. Concentrated the reaction mass to dryness and dissolved the material in Ethyl acetate. Insoluble mass was removed by filtration. Added to the filtrate 2M Ammonia in Methanol (100 mmol) and stirred the reaction mass overnight. Reaction mass was concentrated to dryness and solid was obtained by tituration with Di-isopropyl ether to get solid, which on drying gave desired compound of sufficient purity.

1-[2-(2-*tert*-butylcarbamoyl-benzoylamino)-acetyl]-piperidine-4-carboxylic acid ammonium salt (5a): Yield, (56.1%); m.p., 242-245°C; $C_{20}H_{30}N_4O_5$; Mol.Wt : 406.48; IR (KBr,cm^{-1}): 3248 (NH), & 3229 (NH) 1734 (C=O) 1592 (NH), 1618(NH), 1620(C=O), 1639(C=O) 1660 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.10 (s, 2H, -CH_2), 8.55- 7.77 (m, 4H,C_6H_4), 1.48, (s, 9H, -$C(CH_3)_3$, 3.14-3.00 (m, 2H, -C**Ha**Hb-N), 2.77-2.58

(m, 2H, -CHa**Hb**-N), 2.36-2.24 (m, 1H, -CH-C=O), 1.98-1.81 (m, 2H, -C**Ha**Hb-CH), 1.72-1.54 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 390.2 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-propionyl]-piperidine-4-carboxylic acid ammonium salt (5b): Yield, (61.3%); m.p., 221-226°C; $C_{21}H_{32}N_4O_5$; Mol.Wt: 420.5 ; IR (KBr, cm^{-1}) : 3240 (NH), & 3230 (NH) 1729 (C=O) 1600 (NH), 1624(NH), 1627(C=O), 1641(C=O) 1665 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.25-4.11 (m, 1H, -C**H**-CH_3), 1.40-1.38 (d,3H, -CH-C**H**$_3$), 8.56 – 7.69 (m, 4H,C_6H_4), 1.48, (s, 9H, -$C(CH_3)_3$, 3.08-3.04 (m, 2H, -C**Ha**Hb-N), 2.78-2.60 (m, 2H, -CHa**Hb**-N), 2.41-2.30 (m, 1H, -CH-C=O), 1.98-1.81 (m, 2H, -C**Ha**Hb-CH), 1.71-1.54 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 404.2(M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-3-methyl-butyryl]-piperidine-4-carboxylic acid ammonium salt (5c): Yield, (66.13%); m.p., 206-211°C; $C_{23}H_{36}N_4O_5$; Mol.Wt: 448.56; IR (KBr,cm^{-1}): 3240 (NH), & 3232 (NH) 1726 (C=O) 1592 (NH), 1611(NH), 1630(C=O), 1649(C=O) 1659 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.28-4.17 (m, 1H, -C**H**-CH-), 2.46-2.33 (m, 2H, -C**H**-$(CH_3)_2$ & -CH-C=O) 1.44 (d,6H, -CH-(CH_3)$_2$, 8.47 – 7.71 (m, 4H,C_6H_4), 1.50, (s, 9H, -$C(CH_3)_3$, 3.22-3.05 (m, 2H, -C**Ha**Hb-N), 2.78-2.64 (m, 2H, -CHa**Hb**-N), 2.02-1.84 (m, 2H, -C**Ha**Hb-CH), 1.73-1.57 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 432.2 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-4-methyl-pentanoyl]-piperidine-4-carboxylic acid ammonium salt (5d): Yield, (49.5%); m.p., 188-291°C; $C_{24}H_{38}N_4O_5$; Mol.Wt: 462.58; IR (KBr,cm^{-1}): 3257 (NH), & 3225 (NH) 1726 (C=O) 1599 (NH), 1620(NH), 1629(C=O), 1633(C=O) 1659 (C=O); 1H NMR spectrum in DMSO-d6 (δ ppm): 4.30-4.13 (m, 1H, -C**H**-CH_2-), 1.33-1.20 (m, 2H, -CH-C**H**$_2$-CH-), 1.52-1.34 (m, 10H, -CH_2-C**H**-& -$C(CH_3)_3$), 1.01-0.89 (d, 6H, -CH-(CH_3)$_2$, 8.31 – 7.71 (m, 4H,C_6H_4), 3.21-3.06 (m, 2H, -C**Ha**Hb-N), 2.74-2.60 (m, 2H, -CHa**Hb**-N), 2.37-2.23 (m, 1H, -CH-C=O), 1.93-1.81 (m, 2H, -C**Ha**Hb-CH), 1.74-1.54 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 446.3 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-butyryl]-piperidine-4-carboxylic acid ammonium salt (5e): Yield, (61.0%); m.p., 199-203°C; $C_{22}H_{34}N_4O_5$; Mol.Wt: 434.53; IR (KBr,cm^{-1}): 3250 (NH), & 3220 (NH) 1711 (C=O) 1613 (NH), 1631(NH), 1637(C=O), 1641(C=O) 1670 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.27-4.09 (m, 1H, -C**H**-CH_2-), 1.08-1.00 (m,3H, -CH_2-C**H**$_3$), 8.53 – 7.71 (m, 4H,C_6H_4), 1.48, (s, 9H, -$C(CH_3)_3$, 3.16 -3.00 (m, 2H, -C**Ha**Hb-N), 2.72-2.54 (m, 2H, -CHa**Hb**-N), 2.38-2.21 (m, 1H, -CH-C=O), 1.98-1.81 (m, 2H, -C**Ha**Hb-CH), 1.76-1.56 (m, 4H, -CHa**Hb**-CH & -C**H**$_2$-CH_3).; MS (m/z) :418.2 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-pentanoyl]-piperidine-4-carboxylic acid ammonium salt (5f) : Yield, (59.20%); m.p., 203-205°C; $C_{24}H_{35}N_3O_5$; Mol.Wt: 448.56; IR (KBr,cm^{-1}): 3251 (NH), & 3241 (NH) 1740 (C=O) 1594 (NH), 1617(NH), 1622(C=O), 1637(C=O) 1660 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm) : 4.26-4.10 (m, 1H, -C**H**-CH_2-), 1.48-1.33 (m, 2H, -CH-C**H**$_2$-CH_2-), 1.31-1.18 (m, 2 H, -CH_2-C**H**$_2$-CH_3), 1.03-0.91 (t, 3H, -CH_2-C**H**$_3$), 8.37 – 7.66 (m, 4H, C_6H_4), 1.50, (s, 9H, -$C(CH_3)_3$, 3.21-3.08 (m, 2H, -C**Ha**Hb-N), 2.81-2.66 (m, 2H, -CHa**Hb**-N), 2.33-2.20 (m, 1H, -CH-C=O), 2.00-1.83 (m, 2H, -C**Ha**Hb-CH), 1.74-1.58 (m, 2H, -CHa**Hb**-CH); MS (m/z) : 432.2 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-3-phenyl-propionyl]-piperidine-4-carboxylic acid ammonium salt (5g): Yield (51.4%); m.p 237 to 239 °C; $C_{27}H_{36}N_4O_5$. Mol. Wt : 496.6 ; IR (KBr,cm^{-1}): 3258 (NH), & 3249 (NH) 1741 (C=O) 1600 (NH), 1622 (NH), 1630(C=O), 1650(C=O) 1666 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.36-4.20 (m, 1H, -C**H**-CH_2-), 7.58-7.32 (m, 5H, C_6H_5), 8.57 – 7.91 (m, 4H, C_6H_4), 1.50, (s, 9H, -$C(CH_3)_3$, 3.19- 2.99 (m, 4H, -C**Ha**Hb-N & -CH-C**H**$_2$-),), 2.74-2.58 (m, 2H, -CHa**Hb**-N), 2.48-2.32 (m, 1H, -CH-C=O), 1.94-1.81 (m, 2H, -

C**Ha**Hb-CH), 1.72-1.56 (m, 2H, -CHa**Hb**-CH).; MS (m/z) : 480.2 ($M^+ + 1$).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-hexanoyl]-piperidine-4-carboxylic acid ammonium salt (5h): Yield : 47.0%; m.p 259-264 °C; $C_{24}H_{38}N_4O_5$; Mol. Wt. : 462.58; IR (KBr,cm^{-1}): 3244 (NH), & 3233 (NH) 1733 (C=O) 1599 (NH), 1619(NH), 1631 (C=O), 1636(C=O) 1658 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.24-4.13 (m, 1H, -C**H**-CH_2), 1.52-1.26 (m, 13H, -CH_2-, -CH_2- & -$C(CH_3)_3$), 1.03-0.90 (t, 3H, -CH_3), 8.39 – 7.84 (m, 4H,C_6H_4), 3.18-3.03 (m, 2H, -C**Ha**Hb-N), 2.79-2.60 (m, 2H, -CHa**Hb**-N), 2.35-2.21 (m, 1H, -CH-C=O), 2.02 - 1.81 (m, 4H, -C**Ha**Hb-CH & -CH-C**H**$_2$-CH_2), 1.74-1.57 (m, 2H, -CHa**Hb**-CH) ; MS (m/z) : 446.3 (M+1).

1-[2-(2-*tert*-Butylcarbamoyl-benzoylamino)-3-methyl-pentanoyl]-piperidine-4-carboxylic acid ammonium salt (5i): Yield : 28%; m.p 194 – 196°C; $C_{25}H_{40}N_4O_5$; Mol. Wt : 476.6; IR (KBr,cm^{-1}): 3249 (NH), & 3231 (NH) 1738 (C=O) 1594 (NH), 1613(NH), 1621(C=O), 1633(C=O) 1662 (C=O); 1H NMR spectrum in DMSO-d6(δ ppm): 4.26-4.11 (m, 1H, -CH-), 1.01-0.89 (m, 6H, -CH3 & -CH3), 1.40-1.21 (m, 2H, CH2), 1.99-1.81 (m, 3H, -C**H**-CH_2- & -C**Ha**Hb-CH), 8.50 – 7.93 (m, 4H,C_6H_4), 1.49, (s, 9H, -$C(CH_3)_3$, 3.23-3.08 (m, 2H, -C**Ha**Hb-N), 2.75-2.59 (m, 2H, -CHa**Hb**-N), 2.47-2.36 (m, 1H, -CH-C=O), 1.72-1.54 (m, 2H, -CHa**Hb**-CH) ; MS (m/z) : 460.3 (M+1).

Biological screening : Preliminary examination of the biological activity of these newly synthesized compounds was performed by the disc diffusion method[9] 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.

III. RESULTS AND DISCUSSION

All the synthesized compounds were characterized using various spectroscopic techniques. IR spectra showed characteristic bands of amide N-H stretch (3220 – 3258 cm^{-1}), amide N – H bend (1592 - 1631 cm^{-1}), carbonyl C=O stretch (1620 – 1741 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 desired m/z M + 1.

Biological assay : 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% Ampicillin and Chloramphenicol as antibacterial agent while Nystatin was used as reference drugs as antifungal agent. Results were summarized in Table 03.

Table 03 : In *vitro* antimicrobial activities for ammonium salt of carboxylic acid.

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*
5a	7	6	5	13	19	19
5b	**13**	**9**	**11**	**12**	**43**	**38**
5c	**11**	**6**	**9**	**10**	**33**	**29**
5d	**8**	**7**	**8**	**10**	**48**	**22**
5e	3	2	0	0	11	7
5f	6	3	4	5	0	13
5g	**14**	**16**	**10**	**13**	**61**	**49**
5h	5	0	3	6	22	21
5i	2	4	2	1	9	19
Ampicillin	22	10	-	-	-	-
Chloramphenicol	18	20	12	14	-	-
Nystatin	-	-	-	-	69	53

IV. CONCLUSION

Biological activity evaluation showed that compound with methyl substituent 5b [R = - CH_3], isopropyl substituent 5c [R= -$CH(CH_3)_2$], sec-Butyl substituent 5d [R= -$CH_2CH(CH_3)_2$] and benzyl substituent 5g [R= -CH_2-Ph] substituent exhibited higher activity against all the pathogen being evaluated when compared to reference standard drugs. While the compound with no substituent 5a [R= - H], ethyl substituent 5e [R= -CH_2CH_3], propyl substituent 5f [R= -$CH_2CH_2CH_3$] , butyl substituent 5h [R= -$CH_2CH_2CH_2CH_3$] and isobutyl substituent 5i [R= -$CH(CH_3)CH_2CH_3$] exhibit very poor activity against the pathogen being evaluated in comparison to reference drugs. Overall the evaluation indicates that mostly ammonium salts derivatives prepared from natural amino shows relatively higher over-all activity compared to those prepared of non-natural amino acids, except ammonium salt prepared from glycine 5a and isoleucine 5i, which even after being prepared from natural amino acid (i.e. contains natural amino acid residue) showed lower activity in comparison to other compounds being evaluated.

V. CONCLUSION

We have disclosed rational design for synthesis of a series of novel and potent ammonium salts of Piperidine 4-caroboxylinc acid derivatives (**5a-5i)** using different natural and non-natural amino acids and evaluated their biological activity for antibacterial and antifungal activity. Some of the compounds show excellent activity, some showed good activity while some were not significantly activity.

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VII. REFERENCES

[1]. Prentis R. A., Lis Y. and Walker S. R., (1988), Pharmaceutical innovation by the seven UK-owned pharmaceutical companies. British Journal of Clinical Pharmacology, 25, 387-396.

[2]. Tessella Scientific Software Solutions, Automated Salts and Polymorph Screening. www.tessella.com/Services/CaseStudies/pdfs/e_GSK_ASAP.pdf. Accessed Dec. 15, 2006.

[3]. Prakash K., Jieun R., Hyeongmin K., Iksoo K., Jeong T. K.,Hyunil K., Jae M. C., Gyiae Y. and Jaehwi L., (2014), Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability, Asian journal of pharmaceutical sciences, 9, 304 -316.

[4]. Patrick M., (2014), Principles of salt formation, UK Journal of Pharmaceutical and Biosciences, 2(4), 01-04.

[5]. Kharatkar R. and Joshi K A., (2015), Synthesis, characterization and biological evaluation of novel 1-[2-(2-tert-butylcarbamoyl-benzoylamino)-alkyl acyl]-piperidine-4-carboxylic acid methyl ester, J Pharm Sci Bioscientific Res. 2015 5(6) 599-604.

[6]. Abdel-Aziz A. A.,(2007), Novel and versatile methodology for synthesis of cyclic imides and evaluation of their cytotoxic, DNA binding, apoptotic inducing activities and molecular modeling study, European Journal of Medicinal Chemistry, 42(5), 614–626.

[7]. Collin X, Robert J, Wielgosz G, et al•, New anti-inflammatory N-pyridinyl(alkyl)phthalimides acting as tumour necrosis factor-alpha production inhibitors, European Journal of Medicinal Chemistry, 2001; 36(7–8): 639–649.

[8]. Zavyalov S. I, Dorofeeva OV, Rumyantseva EE, et al•, (2002). Synthesis of 2-aminothiazole derivatives, Pharmaceutical Chemistry Journal,2002; 36(8): 440–442.

[9]. Cruickshank R, Duguid JP, Marmion BP and Swain RH, Medicinal Microbiology, Churchill Livingstone, London, Twelth Edition , 1975, 196-202.

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