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RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Activity of Methyl 1-(-2-amine-alkylcarbonyl) piperidine-4-carboxylate

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ABSTRACT

A series of Methyl 1-(-2-amine-alkylcarbonyl) piperidine-4-carboxylate derivatives of amino acids (3a-e) was synthesized. These new derivative was achieved by treating Isonipecotic acid methyl ester (1) with Boc-protected amino acids (2a-e) using CDI as coupling reagent in MDC at room temperature. Further deprotection with HCl/Dioxane gives the desired product. Structures of the synthesized compounds were established on the basis of spectral and elemental analysis. The synthesized compounds were screened for antimicrobial activity.

KEYWORDS

Protected amino acid, Isonipecotic acid, CDI, antimicrobial activity.

INTRODUCTION

Nitrogen-containing heterocyclic compounds are well-known pharmacophores in drug discovery. Isonipecotic acid is a heterocyclic compound consists of a piperidine ring with a carboxylic acid moiety in the *iso* position. Isonipecotic acid mainly acts as a $GABA_A$ receptor partial agonist.[1-3] The derivative Isonipecotic acid also show potent anticonvulsive activity.[4] The esters of isonepecitc acids also have many therapeutic applications. Literature reveals that tert-butylphenyl ester of Isonipecotic acid act as a trypsin inhibitor can specifically inhibit the activity of proteinase which plays a not only key role in DNA synthesis initiation but is also necessary for survival of certain cell lines[5] Pethidine or meperidine hydrochloride is a isonepecotic acid methyl ester derivative , use as a fast-acting opioid analgesic drug.

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Mostly it is prescribed for acute pain and chronic severe pain. Anileridine[6] is a synthetic opioid analgesic. Lofentanil[7,8] is one of the most potent opioid analgesics known and is an analogue. Cyclic amino acid esters of propofol were synthesized in an attempt to develop new water-soluble anesthetic agents [9]

The presence of an unusual amino acid has stimulated interest in new synthetic methodology and strategies to obtain a target structure. Also in addition to that it is well known fact that compounds containing free carboxylic acids ester having analgesic and anti-inflammatory. In this connection, we have synthesized novel derivatives of Methyl 1-(-2-amine-alkylcarbonyl) piperidine-4-carboxylate of amino acids and subjected to microbial screening.

MATERIAL AND METHOD

All chemicals were purchased from commercial suppliers and used without further purification. Melting points were determined using a Veego

microprocessor based melting point and are uncorrected. MS and MS/MS spectra samples were recorded on Waters LCMS-Q-TOF instrument in only positive ion detection mode. 1H and 13C NMR-spectra were recorded either in $CDCl_3$ on a Bruker Avance II 500 (500MHz) spectrometer and chemical shifts are reported in ppm. IR spectra recorded on Perkin Elmer spectrometer as a KBr pellet.

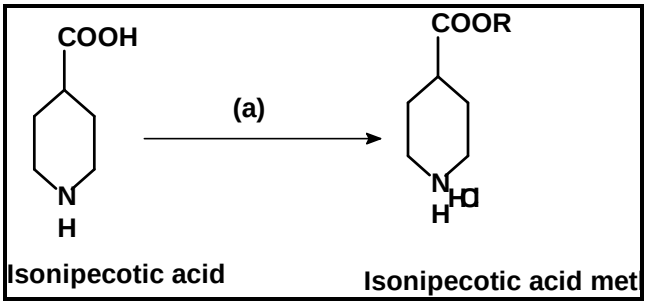
Antibacterial Assay

Muller Hinton Agar (MHA) medium was used for growing bacterial strains and studying their antimicrobial activity. 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 such a 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 ± 2^0C) for 24 hours to get bacterial suspension then used to study antimicrobial activity. The microorganisms were spared on the surface of MHA plate. Five wells of equal size were created using gel puncher (4mm) in each plate. These wells were then filled with the 10µl of each sample was prepared in DMSO (0.05g in 5ml DMSO) and labeled accordingly.

After sampling, plates were incubated and after 24 hours these plates were studied for zone of inhibition.

Synthesis of Isonipecotic acid Methyl ester[1]

A mixture of Isonipecotic acid (1.0 equiv) in Methanol (10 vol) was cooled to 5-10°C. Thionyl chloride (1.5 equiv) was added drop wise by maintaining temperature between 5-10°C. After addition the reaction mixture was stirred at 50-60°C till complition of reaction.Reaction progress was monitored over TLC using 80:20 Chloroform: Methanol as solvent system and TLC was visualized using 1% Ninhydrine solution in Ethanol. After completion solvent was completely stripped off to get white to off white solid. Filtered the solid using Acetone to get pure Isonipecotic acid methyl ester.



Scheme 1: Synthesis of Isonipecotic acid methyl ester 1

***Methyl piperidine-4-carboxylate hydrochloride* (1a):** Yield: 95.0%; m.p.190-192 °C; (KBr, nmax, cm-1): 3615 (NH), 3412 (OH), 1720 (COOH), 1610 (C=O), 1516 (C-N), 770 (1, 2-disubstitution); ^{1}H-NMR (500 MHz, CDCl3, δ / ppm): δ = 9.2 (s,1H, HCl), 9.0(s,1H,NH), 3.62 (s, 3H, -$COOCH_3$), 3.2-3.18(m, 2H, >N-CH_2-), 2.92-2.86 (m, 2H, >N-CH_2-), 2.7(m, 1H),1.98(m, 2H), 1.8-1.72(m,2H) MS (*m/z*, (relative abundance, %)): 206.22 (M+1, 98%) 228.21(M + Na, 100%) ;

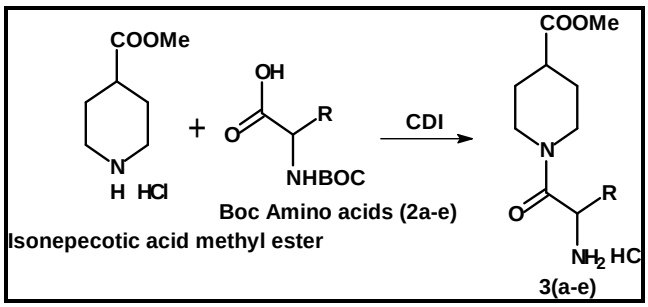
Synthesis of (3a-e)

Boc protected amino acids (2a-e) (1 equiv) and CDI (1.5 equiv) were dissolved in MDC (20 ml).The content was stirred for 1 hrs at room temperature. Added Isonipecotic acid ester HCl (1 equiv) and stirred the mixture overnight. After conletion of reaction acidified the reaction mixture using 20ml 1N HCl. Organic layer was washed using saturated bicarbonate solution followed by water. Organic layer was dried over anhy. Sodium sulfate and evaporated completely, which was further dissolve in HCl / Dioxane and stirred till complete deprotection of BOC. Solvent was completely evaporated to get crude product which was further crystallize using ethanol/Ether to give 3a-e

Compound 3a- Glycine

Yield: 60%, Appearance: off white solid, molecular formula: $C_9H_{16}N_2O_3.HCl$, molecular weight: 236.69 mass spectrum (FAB+) m/e 236.12 (M+1); IR (KBr, nmax, cm-1): 3615 (NH), 3412 (OH), 1720 (COOH), 1610 (C=O), 1516 (C-N), 770 (1, 2-disubstitution).

^{1}H NMR ($CDCl_3$, 500MHz): δ = 3.67 (s, -CH_3), 2.33 (m,1H), 1.85 (m, 4H, -CH_2), 3.34 (m, 4H, >N-CH_2), 3.54 (s, 2H, -CH_2): ^{13}C NMR ($CDCl_3$, 500MHz): δ = 176.00 (-$COOCH_3$), 172.7 (-$CONH_2$), 50.7 (-OCH_3), 44.1 (-CH_2), 43.1 (d, >N-CH_2), 37.8 (-CH), 24.9 (d, -CH_2).



Scheme 2: Synthesis of Isonipecotic acid methyl ester with Boc-protected amino acids

Compound 3b- Alanine

Yield: 55%, Appearance: off white solid, molecular formula: $C_{10}H_{18}N_2O_3$.HCl, molecular weight: 250.72 mass spectrum (FAB+) m/e 251.61 (M+1);

^{1}H NMR ($CDCl_3$, 500MHz): δ = 3.67 (s, -CH_3), 2.33 (m,1H), 1.85 (m, 4H, -CH_2), 3.34 (m, 4H, >N-CH_2), 3.74 (q, 2H, -CH_2), 1.28(d, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 500MHz): δ = 176.00 (-$COOCH_3$), 177.2 (-$CONH_2$), 50.7 (-OCH_3), 53.2 (-CH), 43.41 (d, >N-CH_2), 37.8 (-CH), 24.9 (d, -CH_2),20.2(-CH3)

Compound 3c- Valine

Yield: 51%, Appearance: off white solid m.p.:215-217^{o}C, molecular formula: $C_{12}H_{22}N_2O_3$,HCl molecular weight: 278.77 mass spectrum (FAB+) m/e 279.52 (M+1);

^{1}H NMR ($CDCl_3$, 500MHz): δ = 3.67 (s, -CH_3), 2.33 (m,1H), 1.85 (m, 4H, -CH_2), 3.34 (m, 4H, >N-CH_2), 3.5 (d, 2H, -CH_2), 2.29(m, 1H, -CH), 1.01(d, 6H,-CH3); ^{13}C NMR ($CDCl_3$, 500MHz): δ = 50.7 (-OCH_3), 176.00 (-$COOCH_3$), 37.8 (-CH), 24.9 (d, -CH_2), 43.41 (d, >N-CH_2), 176.5 (-$CONH_2$), 63.5 (-CH), 31.0(-CH), 16.2 (d, -CH3)

Compound 3d -Leucine

Yield: 71%, Appearance: off white solid m.p.:140-142^o C, molecular formula: $C_{13}H_{24}N_2O_3$,HCl; molecular weight: 292.80; mass spectrum (FAB+) m/e 293.21 (M+1), (2M+Na); ^{1}H NMR ($CDCl_3$, 500MHz): δ = 3.67 (s, -CH_3), 2.33 (m,1H), 1.85 (m, 4H, -CH_2), 3.34 (m, 4H, >N-CH_2), 3.56 (d, 1H, -CH), 1.75 (m, 2H, -CH2), 1.83(m, 1H, -CH), 1.01(d, 6H,-CH3); ^{13}C NMR ($CDCl_3$, 500MHz): δ =50.7 (-OCH_3), 176.00 (-$COOCH_3$), 37.8 (-CH), 24.9 (d, -CH_2), 43.41 (d, >N-CH_2), 176.5 (-$CONH_2$), 53.9 (-CH), 44.2 (-CH2), 22.4 (-CH), 21.6 (d, -CH3)

Compound 3e -Phenyl alanine

Yield: 47%, Appearance: White solid m.p.:149-151^o C, molecular formula: $C_{16}H_{22}N_2O_3$.HCl, molecular weight: 326.81 mass spectrum (FAB+) m/e ; 327.23 (M+ 1); IR (KBr, nmax, cm-1): 3615 (NH), 3412 (OH), 1720 (COOH), 1610 (C=O), 1516 (C-N), 770 (1, 2-disubstitution).; ^{1}H NMR ($CDCl_3$, 500MHz): δ =3.67 (s, -CH_3), 2.33 (m, 1H), 1.85 (m, 4H, -CH_2), 3.34 (m, 4H, >N-CH_2), 3.95 (t, 1H, -CH), 3.05 (d, 2H, -CH2), 7.08-7.21(m, 5H, Ar-H).; ^{13}C NMR ($CDCl_3$, 500MHz): δ = 50.7 (-OCH_3), 176.00 (-$COOCH_3$), 37.8 (-CH), 24.9 (d, -CH_2), 43.4 (d, >N-CH_2), 177.2 (-$CONH_2$), 58.8 (-CH), 40.0 (-CH2), 140.0 (-C, Ar), 127.9 (d, -CH, Ar), 128.4 (d, -CH, Ar), 125.7 (-CH, Ar)

RESULTS AND DISCUSSION

All the synthesized compounds were characterized using different spectroscopic techniques. IR spectrum showed characteristic band of carbonyl group at 1781 and C=N at 1652 cm-1. 1H-NMR spectrum was carried out at 500 MHz and showed some characteristics pattern of peaks. Whereas aromatic protons appeared at 6.89-8.12 ppm. Electron ionization mass spectrometric fragmentation pattern of all the compounds were same.

Antibacterial Activity

All the compounds were tested for their antibacterial activity against the microorganism *Staphylococcus aurous, Escherichia coli Pseudomonas aeruginosa* and *Salmonella typhi*. The results were compared with the standard 0.3% Amplicilline and Chloramphenicol in which compound no 3a and 3c shows good

Table 1: Antibacterial activity (Concentration used 1% of each compound), Zone of inhibition was measured in (mm)

Sr. No	Product code	Gram +ve	Gram -ve		
		S. aurous	E. coli	Pseudomonas SPP	Salmonella SPP
1	**3a**	8	9	9	11
2	**3b**	7	3	3	3
3	**3c**	7	11	3	3
4	**3d**	7	8	-	-
5	**3e**	8	9	7	-
6	**Amplicilline**	20	11	-	-
7	**Chloramphenicol**	17	20	12	12

**Effectively was classified in to four zones on the bases of the diameter of zone of inhibition*

antibacterial activity against *Salmonella typhi* and *Escherichia coli.*

CONCLUSION

A series of novel Methyl 1-(-2-amine-alkylcarbonyl) piperidine-4-carboxylate derivatives of amino acids were designed and synthesized, and their structures were characterized by ^{1}H NMR, high-resolution mass spectroscopy and elemental analysis. The antimicrobial studies of the new compounds were evaluated. The results of preliminary bioassays indicate that a number of these molecules exhibit antibacterial activities against Gram-positive bacteria that are comparable to commercially available drugs. The modification of the heterocyclic ring of the parent compound offers a promising prospect and more active analogues are expected to be found.

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