Synthesis and Antimicrobial Activities of Amides of Chiral Benzyl Ethers of *N*-Boc Protected Aminols of L-amino acids with Succinic Acid

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Summary: The research work presented in this article describes a synthesis of chiral amides. Chiral benzyl ethers of *N*-Boc protected aminols reacted with succinic acid using 2,4,6-trichloro-1,3,5-triazine (TCT) as a coupling agent producing chiral amides in high yields. The synthetic amides were investigated for their antifungal and antibacterial activities against different bacterial and fungal strains. All the compounds showed excellent zone of inhibition against the three tested bacterial strains and good to moderate activity against one fungal strain.

Keywords; chiral amides, TCT (2,4,6-trichloro-1,3,5-triazine), *N*-Boc protected aminols, L-amino acids, succinic acid.

Introduction

Keeping in view the growing resistance of microbes to antibiotics there is an urgent need to develop novel antimicrobial (antifungal and antibacterial) agents. Thus development of antimicrobial drugs represent one of the most important advances in therapeutics which has improved the quality of life and moved forward in many other areas of medicine [1-3]. Paracetamol (*N*-(4-hydroxyphenyl)acetamide) is a marketed drug and is an amide which prompted us work on the synthesis of different chiral amides in search of new therapeutic agents.

Being one of the important linkages, amide bond represent the key functional group in peptides, polymers, pharmaceuticals and many natural products [4]. Naturally synthesis of protein takes place through a sequence of coupling reaction between different peptides.

Amide derivatives display a broad spectrum of biological activities including antituberculosis [5] anticonvulsant [6], analgesic, anti-inflammatory [7], insecticidal [8], antifungal [9], and antitumor [10] properties. They are also involved as intermediate products in the synthesis of several therapeutic agents [11]. Amide derivatives also show anti-platelet activity [12]. When amides form conjugates with other aliphatic, aromatic or heterocyclic rings, it shows various type of biological activity. A number of aromatic amides of aromatic and heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity [13].

General method for synthesis of amides is coupling of carboxylic acids and amines by the use of either a coupling reagent [14] or by prior conversion of the carboxylic acid into reactive derivatives [15]. Alternative procedures include Staudinger ligation [16], amino carbonylation of aryl halides [17], and oxidative amidation of aldehydes [18]. There are a lot of reports on the synthesis of amides and their physical and chemical properties [19,20]. Synthesis of amidic bond by using solid support usually takes place by reactions of amines either with acid halides [21,22] or anhydrides [23] in the presence of base, or with acids. Solid support synthesis of amides also took place in the presence of coupling agents [24-26]. There are also several reports on the solid support synthesis of amides and their analogs such as ureas, urethanes and thioureas [27,28].

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2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride), an effective amidation promoter, on treatment with a carboxylic acid forms an intermediate which instantly reacts with an amine to afford an amide and an insoluble hydroxyl triazine as a byproduct. The cyanuric chloride promoted process is very advantageous in its minimization of reagent utilization and robust tolerance to presence of water during both the activation and amidation step [29].

1,3,5-Triazine derivatives have been known for a long period of time. They have found widespread applications in the pharmaceutical, textile, plastic, rubber industries, and also used as pesticides, dyestuffs, optical bleaches, explosives, and surface active agents. The chemistry of this group of compounds has been studied intensively and has been the subject of many reviews [30-35].

In an effort method chiral amides **16-20** were synthesized using acid using 2,4,6-trichloro-1,3,5-triazine (TCT) as a coupling agent in high yields. The synthetic amides **(16-20)** were evaluated for their antifungal and antibacterial activities against different bacterial and fungal strains. All the compounds showed excellent activities.

Experimental

All the reactions were carried out in an inert atmosphere under the static pressure of nitrogen gas using rubber septa and three way stopcock. Solvents were dried and distilled by standard methods.

Reactions were monitored by thin layer chromatography using pre-coated silica gel aluminium backed plates, Kiesalgel 60 HF_{254} from Merck (Germany). For spot detection on TLC, UV light (λ_{max} 254 and 365 nm) was used. Column chromatography was performed by using E. Merck silica gel (0.063-0.200 mm). Melting point of synthesized compounds were recorded in open capillaries using Gallenkamp melting point apparatus (MP-D) and uncorrected. The IR spectra were recorded on Schimadzu Fourier Transform Infrared Spectrophotometer model 270 using ATR. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-300 MHz spectrometer using TMS as internal reference. The optical rotations of compounds were measured on ATAGO AP-100 automatic polarimeter.

General Procedure for Compounds **1-5**

Amino acid (1 mmol) was dissolved in mixture of (2:1) 1, 4-dioxane and H_2O (30mL) then NaOH was added with stirring till pH of solution is basic, finally 1.2 equivalent $(BOC)_2O$ (1.2 mmol) was added and stirred overnight. After completion of reaction 1,4-dioxane was removed under reduced pressure and the crude was acidified with dil. cold $KHSO_4$. Aqueous layer was extracted with ethyl acetate (3x50 mL). Combined organic layers were washed with water and brine separately and were dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and product purity was checked by TLC using 10% methanol in chloroform.

2-(tert-Butoxycarbonylamino)-4-methylpentanoic acid **(1)**

Yield: 98%. m.p = 50-52 °C. $[\alpha]_D^{25}$ = -2.0° (c = 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 3330 (NH), 2954, 2868 (sp^3C-H), 3250-256 (O-H), 1712 (C=O acid), 1673 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm. 6.09 (bs, NH), 4.23 (1H, m, CH-NH), 1.30-1.25 (1H, m, CH-CH_3), 1.74-1.67 (2H, m, CH_2-CH), 1.45 (s, 9H, Boc), 1.29-1.25 (1H, m, CH-CH_3), 0.97(6H, d, *J* = 6.3 Hz, CH_3-CH). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm. 178.0 (C=O acid), 155.7 (C=O), 81.5 (C-O), 52.0 (CH-NH), 41.4 (CH), 28.3 (CH_3 of Boc), 25.0 (-CH_2-), 22.0 (CH_3).

2-(tert-Butoxycarbonylamino)-3-methylpentanoic acid **(2)**

Yield: 99%. m.p = 86-88 °C. $[\alpha]_D^{25}$ = -1.5° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3330 (NH), 2934, 2878 (sp^3C-H), 3210-2516 (O-H), 1711 (C=O acid), 1659 (C=O), cm^{-1}. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm. 5.07 (bs, NH), 4.87 (d, 1H, CH-NH), 1.89-1.84 (m, 1H, CH-CH_3), 1.47-1.43 (m, 1H, CHH-CH_3), 1.32-1.23 (m, 1H, CHH-CH_3), 1.03 (d, *J* = 6.9 3H, CH_3-CH), 0.93 (dd, 3H, *J* = 7.1, 4.6, Hz, CH_3-CHH) . **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm. 173.2 (C=O acid), 155.5 (C=O), 80.1 (C-O), 49.7 (CH-NH), 35.0 (CH), 29.2 ($CH_3$of Boc), 21.3 (-CH_2-), 15.5 (CH_3), 14.8 (CH_3).

4-(Methylthio)-2-(pivaloyloxyamino)butanoic acid **(3)**

Yield: 90%. m.p = 47-50 °C. $[\alpha]_D^{25}$ = -1.8° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3325 (NH), 2918, 2863 (sp^3C-H), 3232-2500 (O-H), 1708 (C=O acid), 1659 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm. 5.52 (bs, NH), 2.5 (t, *J* = 7.5 Hz, 1H, CH-NH), 2.12 (s, 3H, S-CH_3), 2.11-2.03 (m, 2H, CH_2-S), 1.90-1.80 (dt, *J* = 7.5, 4.5 Hz, 2H, CH_2- CHNH), 1.46 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm. 174.2 (C=O acid), 155.7 (C=O), 80.8 (C-O),

56.9 (CH-NH), 31.9 (CH_2-CH_2S), 30.0 (CH_2-CHNH), 28.3 (CH_3 of Boc), 15.5 (CH_3-S).

2-Amino-3-(1-(pivaloyloxy)-1H-indol-3-yl)propanoic acid **(4)**

Yield: 75%. m.p = 140-143 °C. $[\alpha]_D^{25}$ = -2.5° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3343 (NH), 2977, 2860 (sp^3C-H), 3205-2508 (O-H), 1717 (C=O acid), 1658 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 8.14 (NH), 7.64-7.12 (4H, m, aromatic), 7.28 (1H, s, H-C=C), 5.32 (bs, NH), 5.32-4.67 (1H, dd, *J* = 7.5, 5.4 Hz, CH-NH), 3.35 (2H, dd, *J* = 9.0, 6.0 Hz, CH_2.CHNH), 1.46 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 174.0 (C=O acid), 155.5 (C=O), 137.5-129.5 (4H, aromatic), 108.7 (=C-CH_2), 121.2 (HC=C), 80.1 (C-O), 48.8 (CH-NH), 28.6 ($CH_3$of Boc), 21.3 (-CH_2-).

1-(Pivaloyloxy)pyrrolidine-2-carboxylic acid **(5)**

Yield: 98%. m.p = 133-135 °C. $[\alpha]_D^{25}$ = -3.0° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 2932, 2895 (sp^3C-H), 3220-2536 (O-H), 1733 (C=O acid), 1699 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 4.35 (1H, dd, *J* = 8.7, 7.5, CH-CO_2H), 3.58-3.325 (2H, m, CH_2-N), 2.32-2.08 (2H, m, CH_2-CH_2N), 2.06-1.92 (2H, m, CH_2- CH-CO_2H), 1.49 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 179.0 (C=O acid), 156.9 (C=O), 81.4 (C-O), 59.1 (CH-CO_2H), 47.0 (CH-NH), 28.31 (CH_3 of Boc), 24.8 (-CH_2-CHNH), 22.0 (CH_2- CH-CO_2H).

General Procedure for Synthesis of Aminols of N-Boc Protected Amino Acids

N-Boc protected amino acid (1 mmol) was dissolved in dry, distilled THF in anhydrous conditions and reacted with *N*-methyl morpholine (1.5eq) at 0 °C, after stirring for 30 min, methyl chloroformate (2 eq) was added, the reaction mixture got milky. After 1 h stirring the reaction mixture was filtered through Celite bed and $NaBH_4$ (5 eq.) was added in distilled water to the filtrate at 0 °C. The reaction mixture was stirred for four h and solvent was evaporated under reduced pressure. Aqueous layer was diluted with saturated solution of ammonium chloride and extracted with ethyl acetate (3x50 mL), the combined organic layer was washed with water, brine and dried over anhydrous $MgSO_4$. The product was purified by column chromatography using 2% methanol in chloroform as eluent.

tert-Butyl-1-hydroxy-4-methylpentan-2-ylcarbamate **(6)**

Yield: 91%.Gel like. $[\alpha]_D^{25}$ = -4.12° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 3420 (NH), 2933, 2870 (sp^3C-H), 3336 (O-H, alcohol), 1682 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 4.85 (bs, NH), 4.34 (1H, m, CH-NH), 3.48 (1H, OH), 3.22-3.18 (2H, m, CH_2-OH), 1.32-1.23 (1H, m, CH-CH_3), 1.70-1.63 (2H, m, CH_2-CH), 1.46 (s, 9H, Boc), 1.28-1.25 (1H, m, CH-CH_3), 0.95(6H, d, *J* = 6.3 Hz, CH_3-CH). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 155.5 (C=O), 80.1 (C-O), 64.1(CH_2-OH), 51.9 (CH-NH), 42.3 (CH), 29.2 (CH_3 of Boc), 24.0 (-CH_2-), 22.2 (CH_3).

tert-Butyl-1-hydroxy-3-methylpentan-2-ylcarbamate **(7)**

Yield: 90%.Gel like. $[\alpha]_D^{25}$ = -3.87° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3380 (NH), 2931, 2877 (sp^3C-H), 3338 (O-H, alcohol), 1682 (C=O), cm^{-1}. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 4.71 (bs, NH), 3.7 (1H, m, CH-NH), 3.64-3.58 (2H, dd, *J* = 6, 6.6 CH_2OH), 3.51 (1H, OH), 1.62 (1H, m, CH-CH_3), 1.46 (9H, s, Boc), 1.25-1.1 (m, 1H, CHH-CH_3), 0.90 (3H, d, *J* = 5.4, CH_3-CHH), 0.93 (dd, 3H, *J* = 5.1, 4.5, Hz, CH_3-CHH) . **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 156.9 (C=O), 79.6 (C-O), 64.0 (CH_2OH), 57.1 (CH-NH), 36.2 (CH), 28.4 ($CH_3$of Boc), 25.5 (-CH_2-), 15.5 (CH_3), 11.5 (CH_3).

tert-Butyl-1-hydroxy-4-(methylthio)butan-2-ylcarbamate **(8)**

Yield: 80%.Gel like. $[\alpha]_D^{25}$ = -4.75° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3367 (NH), 2931, 2874 (sp^3C-H), 3411 (O-H, alcohol), 1678 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 4.85 (bs, NH), 3.76-3.57 (2H,m, CH_2-S), 3.68(1H, m, CH-NH), 2.64-2.5(CH_2-OH), 2.56 (1H, OH), 2.12 (s, 3H, S-CH_3), 1.455 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 156.4 (C=O), 79.81(C-O), 65.5 (CH_2-OH), 52.0 (CH-NH), 30.9 (CH_2-S), 30.7 (CH_2-CH_2S), 28.9 (CH_3 of Boc), 15.6 (CH_3-S).

tert-Butyl-3-(2-amino-3-hydroxypropyl)-1H-indole-1-carboxylate **(9)**

Yield: 97%. m.p = 107-110 °C. $[\alpha]_D^{25}$ = -5.20° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3300 (NH), 2917, 2866 (sp^3C-H), 3338 (O-H, alcohol), 1681 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 8.10 (NH), 7.66-7.0 (4H, m, aromatic), 6.98 (1H, s, H-C=C), 4.98 (bs, NH), 5.11-4.62 (1H, dd, *J* = 6.5,

4.4 Hz, CH-NH), 3.25 (2H, m, CH_2-OH) 3.35 (2H, m, CH_2-CHNH), 1.45 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 156.6 (C=O), 136.0-129.32 (4H, aromatic), 109.4 (=C- CH_2), 118.2 (HC=C), 79.5 (C-O), 48.7 (CH-NH), 28.4 ($CH_3$of Boc), 26.9 (CH_2-OH) 21.3 (CH_2-).

tert-Butyl-2-(hydroxymethyl)pyrrolidine-1-carboxylate **(10)**

Yield: 95%. m.p = 65-67 °C. $[\alpha]_D^{25}$ = -4.23° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 2932, 2895 (sp^3C-H), 3220-2536 (O-H), 1733 (C=Os acid), 1699 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 3.95 (2H, CH_2OH), 3.72 (1H, OH), 3.69-3.355 (1H, m, CH-N), 3.46 (2H, m, CH_2-N), 2.06-1.95 (2H, m, CH_2-CH_2N), 1.89-1.75 (2H, m, CH_2- CH-CH_2OH), 1.47 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 156.7 (C=O), 80.2 (C-O), 67.1 (CH_2-OH), 59.91 (CH_2-OH), 47.4 (CH-NH), 28.7 (CH_3 of Boc), 23.9 (-CH_2-CHNH).

General Procedure for Synthesis of Benzyl Ethers of *N*-Boc Protected Aminols

N-Boc protected aminols were converted to benzyl ethers by reacting *N*-Boc protected aminol (1 mmol) with NaH (1.2 eq) in THF (20mL) at 0 °C for 30 min. After 30 min, benzyl bromide (1.2 eq) was added at 0 °C with stirring and reaction mixture was stirred at room temperature for more 20 h. After the completion of reaction dry methanol was added. The solvent was removed under reduced pressure. Crude mixture was diluted with chloroform (3x50mL), the combined organic layer was washed with water and brine, dried over anhydrous magnesium sulphate and was evaporated under reduced pressure. Product was purified by column chromatography using 2% ethyl acetate in *n*-hexane.

tert-Butyl-1-(benzyloxy)-4-methylpentan-2-ylcarbamate **(11)**

Yield: 80%.Colorless oil. $[\alpha]_D^{25}$ = -7.40° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 3300 (NH), 2955, 2867 (sp^3C-H), 1685 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.28-7.29 (5H, m, aromatic), 6.1 (bs, NH), 4.25 (1H, m, CH-NH), 4.12 (2H, dd, J = 3.5, 6.5 CH_2-OPh), 1.8 (2H, s), 1.67(1H, m, CH-CH_3), 0.97 (6H, d, CH_3-CH), 1.45 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 155.9 (C=O), 138.0-127.9 (aromatic), 79.0 (C-O), 73.0 (CH_2-Ph), 70.0 (CH_2-OPh), 51.5 (CH-NH), 41.4 (CH_2-CHNH), 28.4 (CH_3 of Boc), 24.8 (-CH_2- CH_3), 21.8 (CH_3).

tert-Butyl-1-(benzyloxy)-3-methylpentan-2-ylcarbamate **(12)**

Yield: 78%. Colorless oil. $[\alpha]_D^{25}$ = -6.73° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3250 (NH), 2924, 2854 (sp^3C-H), 1694 (C=O). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.38-7.28 (5H, aromatic), 4.8 (bs, NH), 4.51 (2H, dd, J = 3.8, 6.7 CH_2-O), 3.57 (1H, m, CH-NH), 3.50 (1H, m, CH-CH_3), 1.28 (2H, m, CHH-CH_3), 0.94 (3H, d, J = 5.7, CH_3-CH), 0.92 (dd, 3H, J = 7.2, 6.0, Hz, CH_3-CHH) . **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 155.5 (C=O), 138.2-128.4 (aromatic), 79.1 (C-O), 73.5 (CH_2Ph), 70.1 (CH_2-Ph), 54.4 (CH-NH), 36.5 (CH), 29.4 ($CH_3$of Boc), 25.4 (-CH_2-), 15.6 (CH_3), 14.1 (CH_3).

tert-Butyl-1-(benzyloxy)-4-(methylthio)butan-2-ylcarbamate **(13)**

Yield: 75%. Colorless oil. $[\alpha]_D^{25}$ = -5.90° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3320 (NH), 2917, 2866 (sp^3C-H), 1697 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.30-7.28 (5H, aromatic), 4.82 (bs, NH), 3.80 (1H, m, CH-NH), 3.50 (2H, m, CH_2-S), 2.68 (2H, s, CH_2-Ph), 2.55(2H, m, CH_2-O), 2.11 (3H, s, S-CH_3), 1.458 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 155.6 (C=O), 138.1-127.6 (aromatic), 79.4 (C-O), 73.2 (CH_2PH), 71.8 (CH_2-O), 49.7 (CH-NH), 32.0 (CH_2-S), 30.8 (CH_2-CH_2S), 28.4 (CH_3 of Boc), 15.6 (CH_3-S).

tert-Butyl-1-(benzyloxy)-3-(1H-indol-3-yl)propan-2-ylcarbamate **(14)**

Yield: 70%. Colorless oil. $[\alpha]_D^{25}$ = -7.0° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3267 (NH), 3021 (aromatic), 2917, 2866 (sp^3C-H), 1681 (C=O urethane). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.76 (NH), 7.38-7.10 (9H, m, aromatic), 6.88 (1H, s, H-C=C), 5.23 (bs, NH), 5.3 (1H, s, CH_2Ph), 4.56-4.45 (1H, dd, J = 7.5, 4.0 Hz, CH-NH), 3.46 (2H, m, CH_2-O), 3.1 (2H, m, CH_2-CHNH), 1.48 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 155.6 (C=O), 138.3-126.3 (9H, aromatic), 109.5 (=C- CH_2), 119.2 (HC=C), 79.8 (C-O), 73.2 (CH_2Ph), 70.3, (CH_2O), 49.9 (CH-NH), 28.5 ($CH_3$of Boc), 27.2 (-CH_2-).

tert-Butyl-2-(benzyloxymethyl)pyrrolidine-1-carboxylate **(15)**

Yield: 77%. Colorless oil. $[\alpha]_D^{25}$ = -8.45° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 2930, 2875 (sp^3C-H), 1690 (C=O urethane). **^{1}H-NMR** (300 MHz,

$CDCl_3$): δ ppm 7.33-7.27 (5H, aromatic), 4.54 (2H, s, CH_2Ph), 3.62 (1H, m, CH-N), 3.45 (2H, m, CH_2-O), 3.42(2H, m, CH_2-N), 1.97 (2H, m, CH_2- CH), 1.84 (2H, m, CH_2-CH_2N), 1.46 (s, 9H, Boc). **^{13}C-NMR** (75 MHz, $CDCl_3$): 154.6 (C=O), 138.5-126.9 (aromatic), 79.2 (C-O), 73.2 (CH_2Ph), 70.9 (CH_2-OH), 56.5 (CH-NH), 46.6 (CH_2-N), 28.5 (CH_3 of Boc), 27.1 (-CH_2-CHNH), 23.3 (CH_2- CH-CH_2O).

Deprotection of Boc-group

The *N*-Boc-protected amino acid (5 mmol) was dissolved in CH_2C1_2 (12 mL) and cooled to 0 °C under nitrogen atmosphere. An equal volume of trifluoroacetic acid (TFA) (8 eq.) was added and the mixture was allowed to slowly warm to room temperature, and then stirred for further 6 h. Removal of solvent under reduced pressure yielded the crude TFA salt, which was used without further purification.

General Procedure for Synthesis of Amides

Triethyl amine (0.02 mol) was added to the solution of succinic acid (0.01 mol) and TCT (0.01 mol) in acetone at 0 °C. After stirring for 3 h when all the TCT was consumed in reaction, salt of deprotected benzyl ether of *N*-Boc protected aminol (0.02 mol) was added and stirred for 2 h. After the completion of reaction, the reaction mixture was filtered to remove triazine derivative and excess acetone was removed from filtrate under reduced pressure. Reaction mixture was diluted with ethyl acetate. Organic layer was washed with water, brine and dried over anhydrous magnesium sulphate. Solvent was evaporated under reduced pressure and product was purified by column chromatography using 2% methanol in chloroform as eluent.

4-(1-(Benzyloxy)-4-methylpentan-2-ylamino)-4-oxobutanoic acid **(16)**

Yield: 95%. Colorless oil. $[\alpha]_D^{25}$ = -5.32° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 3268 (NH), 2956, 2868 (sp^3C-H), 1728 (C=O acid), 1638 (C=O amide). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 10.9 (1H, OH), 7.30-7.27 (5H, m, aromatic), 6.23 (bs, NH), 4.2 (1H, dd, *J* = 4.0, 6.0 CH-NH), 4.11 (2H, dd, *J* = 3.3, 7.5 CH_2-OCH_2Ph), 2.48 (2H, m, CH_2-COOH), 2.36 (CH_2-CONH), 2.2 (2H, s, O-CH_2), 1.70 (2H, m, CH_2-CHNH), 1.30 (1H, m, CH-CH_3), 0.97 (6H, d, CH_3-CH), 1.455. **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 175.8 (C=O acid), 170.9 (C=O amide), 138.2-129.0 (aromatic), 73.0 (CH_2-Ph), 70.5 (CH_2-OPh), 51.3 (CH-NH), 41.6 (CH_2-CHNH), 30.2 (CH_2-COOH), 29.8 (CH_2-CONH), 24.0 (-CH_2- CH_3), 21.7 (CH_3).

4-(1-(Benzyloxy)-3-methylpentan-2-ylamino)-4-oxobutanoic acid **(17)**

Yield: 93%. Colorless oil. $[\alpha]_D^{25}$ = -4.72° (c = 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3031 (NH), 2930, 2874 (sp^3C-H), 1716 (C=O acid), 1653 (C=O amide). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 10.8 (1H, bs, OH), 7.47-7.28 (5H, aromatic), 5.3 (bs, NH), 4.4 (2H, m, CH_2-O), 3.68 (1H, m, CH-NH), 3.50 (1H, m, CH-CH_3), 2.5 (2H, m, CH_2CON), 2.64 (2H, m, CH_2COOH), 1.28 (2H, m, CHH-CH_3), 0.96 (3H, dd, *J* = 6.9, 4.8 CH_3-CH), 0.90 (dd, 3H, *J* = 6.9Hz, CH_3-CHH) . **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 174.8 (C=O acid) 172.2 (C=O amide), 138.3-128.4 (aromatic), 80.2 (C-O), 73.0 (CH_2Ph), 70.5 (CH_2-Ph), 54.6 (CH-NH), 36.5 (CH), 30.2 (CH_2COOH), 29.8 (CH_2CON), 29.4 ($CH_3$of Boc), 25.4 (-CH_2-), 15.6 (CH_3), 15.2 (CH_3).

4-(1-(Benzyloxy)-4-(methylthio)butan-2-ylamino)-4-oxobutanoic acid **(18)**

Yield: 90%. Colorless oil. $[\alpha]_D^{25}$ = -5.0° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3260 (NH), 2920, 2862 (sp^3C-H), 1725 (C=O acid), 1660 (C=O amide). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.38-7.28 (5H, aromatic), 4.57 (2H, m, CH_2-S), 4.50 (1H, m, CH-NH), 3.65(2H, m, CH_2-O), 2.66 (2H, s, CH_2-Ph), 2.40 (2H, m, CH_2COOH), 2.30 (2H, m, CH_2CON), 2.08 (3H, s, S-CH_3). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm 177.7 (C=O acid), 176.5(C=O amide), 137.9-127.7 (aromatic), 72.8 (O- CH_2), 68.6 (CH_2PH), 51.1 (CH-NH), 31.1 (CH_2-S), 28.0 (CH_2COOH), 27.1 (CH_2CON), 15.6 (CH_3-S).

4-(1-(Benzyloxy)-3-(1H-indol-3-yl)propan-2-ylamino)-4-oxobutanoic acid **(19)**

Yield: 92%. Colorless oil. $[\alpha]_D^{25}$ = -5.90° (c= 5mg/15mL Chloroform). **IR** (υ_{max}, cm^{-1}). 3291 (NH), 3029 (aromatic), 2917, 2860 (sp^3C-H), 3205-2510 (O-H, acid), 1724 (C=O acid), 1647 (C=O amide). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 10.6 (1H, OH), 7.77 (NH), 7.38-7.08 (9H, m, aromatic), 6.86 (1H, s, H-C=C), 6.47 (bs, NH), 4.46 (1H, m, CHNH), 3.45 (2H, m, CH_2-O), 2.60 (2H, dt, *J* = 9.6, 4.2 Hz, CH_2-CHNH, 2.40 (2H ,m, CH_2COOH), 2.20 (2H ,m, CH_2CON). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ ppm. 176.6 (C=O acid), 172.6 (C=O amide), 138.1-111.1 (9H, aromatic), 121.8 (=C- CH_2), 109.7 (HC=C), 73.2 (CH_2-Ph), 60.7

(CH_2-O), 49.9 (CH-NH), 30.0 (CH_2-COOH), 29.2 (CH_2-CON), 26.8 (-CH_2-).

4-(2-(Benzyloxymethyl)pyrrolidin-1-yl)-4-oxobutanoic acid **(20)**

Yield: 97%. Colorless oil. $[\alpha]_D^{25}$ = -6.7° (c= 5mg/15mL $CHCl_3$). **IR** (υ_{max}, cm^{-1}). 3000 (aromatic), 2916, 2871 (sp^3C-H), 1716 (C=O acid), 1637 (C=O amide). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm 7.35-7.29 (5H, aromatic), 4.51 (2H, s, CH_2Ph), 4.49 (1H, m, CH-N), 3.62 (2H, m, CH_2-O), 3.42(2H, m, CH_2-N), 2.66 (2H, m, COOH), 2.58 (2H, m, CON), 2.05 (2H, m, CH_2- CH), 1.92 (2H, m, CH_2-CH_2N). **^{13}C-NMR** (75 MHz, $CDCl_3$): 176.4 (C=O acid), 171.7 (C=O amide), 138.5-127.4 (aromatic), 73.14 (CH_2-Ph), 71.2(CH_2-O), 69.8 (CH-NH), 47.4(CH_2-N), 29.8 (CH_2COOH), 27.6 (-CH_2-CON), 25.4 (CH_2- CH-CH_2O).

Material and Method

Assay for Antifungal Activity

The agar tube dilution method is used for determination of antifungal activity.[14] Fungal strain *Fusarium moniliforme* was used in this study. Fungal strain was maintained on Sabouraud's dextrose agar (Oxoid) medium at 4 °C.

The samples for antifungal assay were prepared from initial stock solution of 0.12 g of compound in 1 mL of dimethyl sulfoxide (DMSO). Culture media was prepared by dissolving 6.5 g of Sabouraud dextrose agar per 100 mL of distilled water pH was adjusted at 5.6. Test tubes were marked to the 10 mL mark. The Sabouraud's dextrose agar (Oxoid) dispensed as 10 mL volume into screw capped tubes or cotton plugged test tubes and were autoclaved at 121 °C for 21 min. Tubes were allowed to cool to 50 °C and Sabouraud's dextrose agar (Oxoid) was loaded with 67 μL of compound pipette from the stock solution. This would give the final concentration of 200 µg/mL of the pure compound in media. Tubes were then allowed to solidify in slanting position at room temperature. Three slants of the compound sample were prepared for fungus species. The tubes containing solidified media and sample compound were inoculated with 4 mm diameter piece of inoculum, taken from a seven days old culture of fungus. One sample of each compound was prepared, which was used for positive control. Slants without compound were used for negative control.

The test tubes were incubated at 28 °C for 7 days. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length of fungus in slant by measuring growth (mm) and growth inhibition was calculated with reference to negative control and all tests were carried out in triplicate.

Assay for Antibacterial Activity

Antibacterial activity of the methanolic solution of selected compounds was determined by agar well diffusion method.[15] Nutrient broth medium was prepared by dissolving 0.4 g of nutrient broth in 50 mL of distilled water. pH was adjusted at 7.0 and was sterilized by autoclaving. Nutrient agar medium was prepared by dissolving 2.3 g agar in 100 mL of distilled water; pH was adjusted to 7.0 and was autoclaved at 121 °C. Three strains of bacteria *Klebsiella pneumoniae*, *E. coli* and *Pseudomonas aeruginosa* were used in the study. The organisms were maintained on nutrient agar medium at 4 °C. Bacterial pallets obtained after centrifugation of 24 h old culture in nutrient broth of selected bacterial strains were mixed with physiological normal saline solution until a McFarland turbidity standard [106 colony forming unit (CFU) mL-1] was obtained. Then this inoculum was used for seeding the nutrient agar.

Nutrient agar medium was prepared by adding nutrient agar 2.3 g in 100 mL of distilled water; pH was adjusted at 7.0, and was autoclaved. It was allowed to cool to 45 °C. Petri plates were prepared by pouring 75 mL of seeded nutrient agar and allowed to solidify. Four wells per plate were made with sterile cork borer (5 mm).

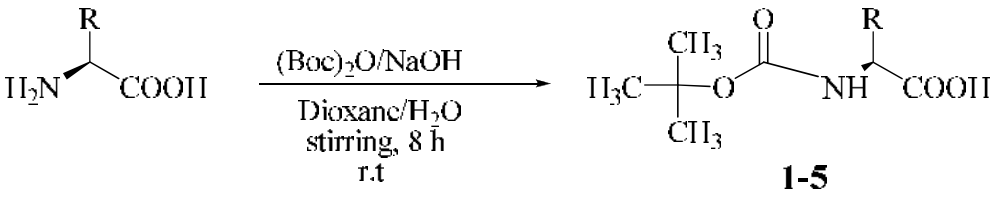
Using micropipette, 100 μL of test solutions was poured in respective wells. These plates were incubated at 37 °C. After 24 h of incubation the diameter of the clear zones of inhibitions was measured by a ruler. Antibacterial activity of three dilutions of each compound was determined against three bacterial strains. All tests were carried out in triplicate.

Results and Discussion

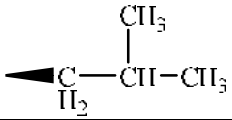
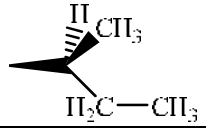
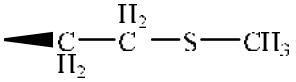
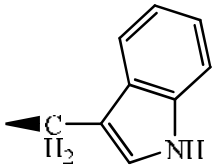
Chemistry

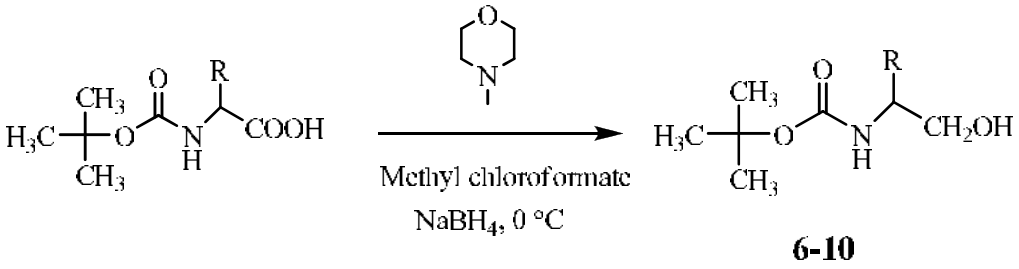
Keeping in view the importance of amides in organic synthesis and their biological importance we planned to synthesize some new chiral amides of naturally occurring L-amino acids [1,36]. During the course of research work, inexpensive and

commercially available starting materials *i.e.* L-amino acids and succinic acid were selected. Amides were synthesized by the route shown in **Scheme-1-4** in four steps. In first step NH_2 group of amino acids was protected using Boc anhydride **Scheme-1**. In subsequent step carboxylic group of *N*-Boc protected amino acid was converted to alcoholic functionality S**cheme-2**, which was protected as benzyl ether **Scheme-3.** Finally after deprotection of Boc group amides were prepared by reacting deprotected benzyl ethers of amino acids with succinic acid. Synthetic amides (**16-20**) were purified by column chromatography and their structures were characterized by spectroscopic techniques such as IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

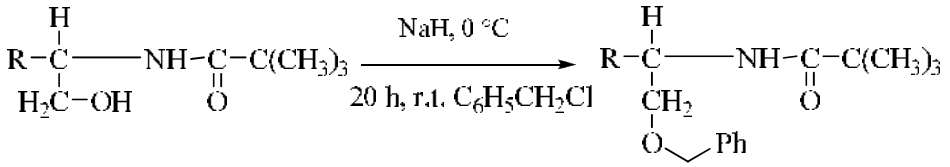


Scheme 1: Boc protection of amino acid.

Compound No.	R
1	
2	
3	
4	
5	

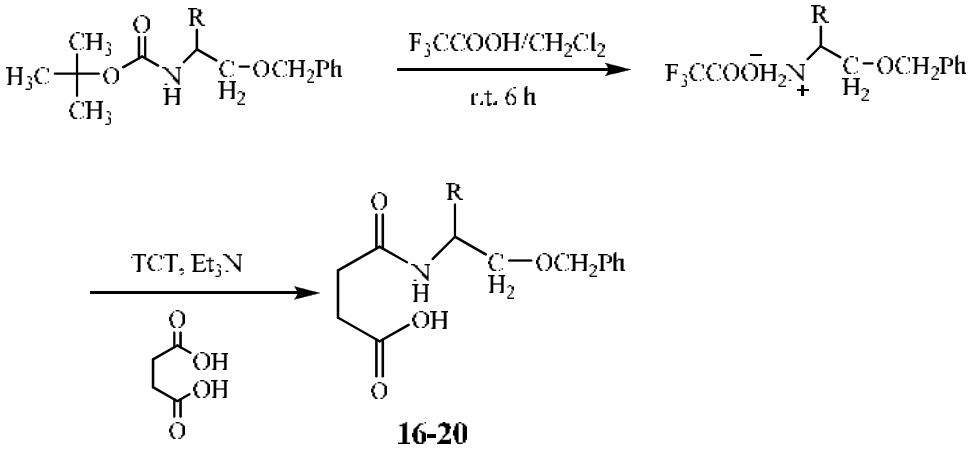


Scheme 2: Reduction of acid group to alcohol functionality.



11-15

Scheme 3: Protection of alcohol as benzyl ether.



16-20

Scheme 4: Synthesis of amides **16-20**.

The infrared absorption spectrum of the benzyl ethers of *N*-Boc protected aminols showed characteristic bands at 3250, 3000 and 2930 cm^{-1}. These absorptions were attributed to NH, Ar-*H* and C-H respectively.

In ^{1}H-NMR spectrum a singlet at 7.28 ppm with integration of five protons was attributed to aromatic protons, a singlet at 1.46 ppm with integration of nine protons was attributed to two CH_3 groups of Boc. The infrared absorption spectrum of amides showed the characteristic bands at 1640, 1728 and 2956 cm^{-1}. These absorptions were assigned to CO of (amide), (acid), and C-H stretching, respectively. In ^{1}H-NMR spectrum, singlet at 2.20 ppm with the integration of six protons was assigned to two CH_3 groups of esters. Another singlet appeared at 5.91 ppm with the integration of two protons which was attributed to two CH groups. A triplet appeared at 4.92 ppm with the integration of one proton was assigned to the CH group adjacent to nitrogen atom with the coupling constant of 7.1 Hz. Similarly a multiplet was observed in the range of 3.33-3.31 ppm with the integration of two protons. In ^{13}C-NMR peaks at 169.2, 170.2 and 172.0 ppm indicated the presence of three carbonyls of imide, ester, and acid, respectively. Peaks at 72.7, and 18.8 ppm indicated the presence of methyl and methine carbons, respectively. IR described in the experimental protocol further supported the structures of all the synthetic compounds **16-20**.

Bioassay

All the synthetic compounds **16-20** were screened for their *in vitro* antibacterial and antifungal activities using selected three strains of bacteria and one strain of fungi. DMSO was used as control solvent, streptomycin, and chloramphenicol were used as standard drugs for antifungal and antibacterial activities respectively.

Antifungal Activity

All five amides **16-20** showed varying degree of growth inhibition against tested fungal strain and results are tabulated in Table-1.

Compounds **18** displayed comparatively good activity against the tested fungi. Compounds **18** demonstrated (60%) inhibition against *Fusarium moniliforme*. Compounds **16**, **19** and **20** showed (20%), (40%), and (20%) inhibition, respectively, however, compound **18** was inactive.

Antibacterial Activity

Synthetic chiral amides **16-20** showed excellent percentage inhibition against all three

selected bacterial strains *i.e. Klebsiella pneumoniae*, *E. coli* and *Pseudomonas aeruginosa* and results are summarized in Table 2. Compound **16** displayed excellent growth inhibition potential against *klebsiella pneumoniae* (26.7%), *E. coli* (14.7%), and *Pseudomonas aeruginos* (10.33%), respectively, as compared to standard streptomycin and chloramphenicol. Compound **16** exhibited good percentage inhibition against *Klebsiella pneumoniae* (13%), *E. coli* (11%) and *Pseudomonas aeruginosa* (10%), respectively. Compound **19** also exhibited percentage inhibition against *Klebsiella pneumonia* (16%), *E. coli* (15%) and *Pseudomonas aeruginosa* (15%) respectively. Compound **20** exhibited percentage inhibition against *Klebsiella pneumonia* (17%), *E. coli* (17%) and *Pseudomonas aeruginosa* (14%), respectively.

Table-1: Antifungal activities of the synthesized amides **16-20**.

Compounds Concentration(1 mg/mL)	Mycelial growth (cm)	Growth inhibition %
16	8 cm	20
17	10 cm	0.0
18	4 cm	60
19	6 cm	40
20	8 cm	20
Streptomycin (Std.)	2 cm	98

Table 2: Antibacterial activities of the synthesized amides **16-20**.

Compounds (1 mg/mL)	*Klebsiella pneumoniae*	*E. coli*	*Pseudomonas aeruginosa*
	% inhibition	*% inhibition*	*% inhibition*
16	26	15	10
17	13	11	10
18	Inactive	Inactive	Inactive
19	16	15	15
20	17	17	14
Streptomycene (Std.)	13	13	0
Chloramphenicol (Std.)	17	17	0

Compound **17** was found to be inactive against all the three tested strains of bacteria.

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