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SYNTHESIS AND BIOLOGICAL EVALUATION OF AZETIDINONE DERIVATIVES AS ANTIBACTERIAL AND ANTIFUNGAL AGENTS

DARPAN B. PATEL*[1] AND VIKAS A. DESAI[1]

Department of Chemistry, BKM Science College, Valsad, 396001,Veer Narmad South Gujarat University

ABSTRACT

We have developed a novel approach for the preparation of *N*-[(*Z*)-arylmethylidene] pyrrolidine-2-carboxamide using catalytic acetic acid through the reaction of different substituted benzaldehyde with *L*-Prolinamide. These molecules were further cyclized with chloroacetylchloride under nucleophilic substitution reaction condition to finally afford 3-chloro-4-ary*L*-1-[(2*S*)-pyrrolidin-2-ylcarbonyl]azetidin-2-one. These synthesized compounds were further confirmed by IR, ^{1}H NMR, and mass spectroscopy. All these final synthesized compounds have been screened for their biological activity against different strains for their antibacterial and antifungal activity.

KEYWORDS : ***L*-Prolinamide, Chloroacetylchloride, *N*-[(*Z*)-arylmethylidene] pyrrolidine-2-carboxamide, TEA.**

DARPAN B. PATEL*
Department of Chemistry, BKM Science College, Valsad, 396001,Veer Narmad South Gujarat University

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INTRODUCTION

Azetidin-2-one, a four-membered cyclic lactam (*β*-lactam) skeleton is well known for the synthesis of a large number of molecules by exploring the Baeyer's strain associated with it.[1-3] These structural motifs are associated with wide spectrum *β*-lactam antibiotics, including penicillin, cephalosporins, carbapenems and monobactams that have been widely used as pharmacological agents to treat bacterial infections and microbial diseases.[4-7] Synthesis of Azetidin-2-ones has been reported synthesized by enolate-imine condensations and cyclization reactions.[8,9] They have been used as a template to build the heterocyclic structure fused to the four-membered ring. Azetidin-2-ones and its derivatives possess various pharmacological properties such as antihypertensive, anti-inflammatory, anticancer, antihyperlipidemic, antimicrobial, antitubercular, and anticonvulsant.[10-15] Most of the researches up to early 90s and till present focused on synthesis of 2-azetidinones and studied their antibacterial property using different functional groups.[16-18] It has been reported that Azetidinone derivatives showed interesting antibacterial and antifungal properties.[19] So, we were interested to synthesize and investigate the *L*-prolinamide Azetidinone derivatives to screen for such activities.

MATERIALS AND METHODS

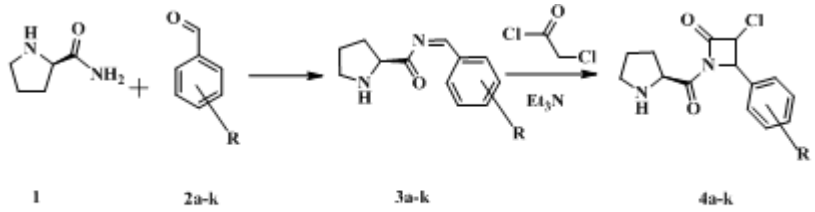
All the chemicals and reagents were used in the synthesis process of analytical grade chemicals. All commercial solvents were used after purified. The prepared compound 3-chloro-4-phenyl*L*-1-((S)-pyrrolidine-2-carbonyl) azetidin-2-one derivatives were confirmed by Mass, IR, ^{1}H NMR and CHN analysis. Instrumental analyses ^{1}H NMR were performed at Centre of Excellence, Vapi. Mass spectra analysed on Agilent 6420 LCMS at Atul Ltd. IR spectra analysed on Perkin-Elmer 1600 FTIR spectrometer. Melting points measured using a Buchi-610 apparatus. Reactions monitoring carried out using TLC (Thin Layer Chromatography) on Merck silica gel 60F_{254}, the solvent systems and compound spots were detected either in UV irradiation or by developing ninhydrin.

Antibacterial activity

The purified products were screened for their antibacterial activity. The nutrient agar broth prepared by the usual method, was inoculated aspetically with 0.5 ml of 24 hours old subcultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* in separate conical flasks at 40-50^{o}C and mixed well by gentle shaking. About 25 ml content of the flask were poured and evenly spreaded in a petri dish (13 cm in diameter) and allowed to set for 2 hrs. The cups (10 mm in diameter) were formed by the help of borer in agar medium and filled with 0.04 mL (40 mg) solution of sample in DMSO. The plates were incubated at 37^{o}C for 24 hours and the control was also maintained with 0.04 mol of DMSO in a similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and are recorded in Table 2.

Antifungal activity

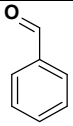
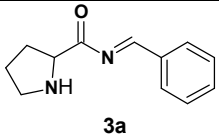
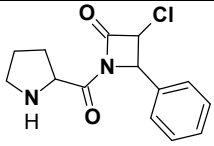
Candida albicans/ Aspergillus niger/ Aspergillus clavatus was employed for testing antifungal activity using cup-plate method. The culture was maintained on Sabouraud's agar slants. Sterilised Sabouraud's agar medium was inoculated for 72 hrs. Old 0.5 mL suspension of fungal spores in a separate flask was taken. About 25 mL of the inoculated medium was evenly spreaded in a petridish and allowed to set for 2 hrs. The cups (10 mm in diameter) were punched. The plates were incubated at 30^{o}C for 48 hrs. After the completion of incubation period, the zone of inhibition of growth in the form of diameter in mm was measured. Along the test solution in each petridish one cup was filled with solvent which act as control. The zones of inhibition are recorded in Table 3.

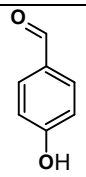
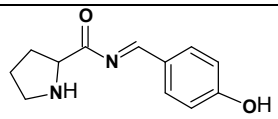
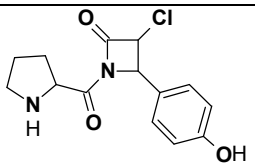
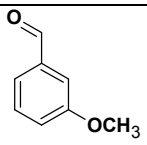
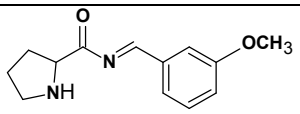
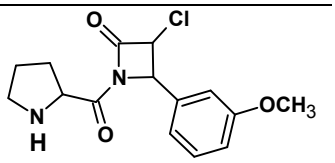
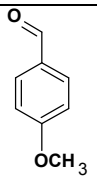
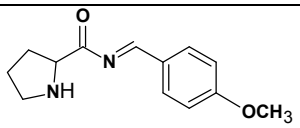
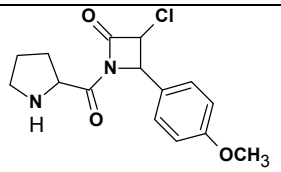
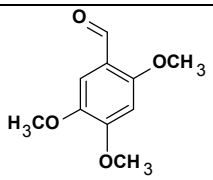
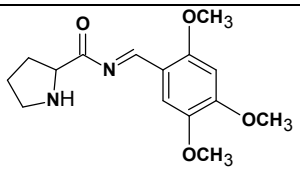
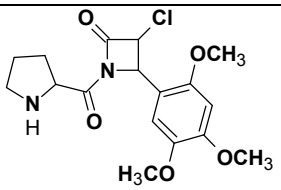
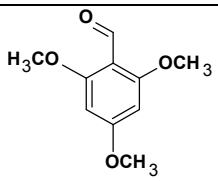
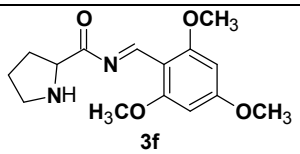
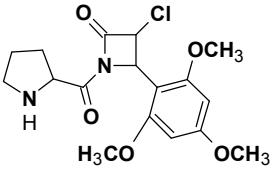
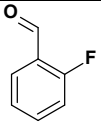
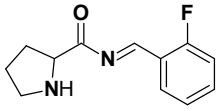
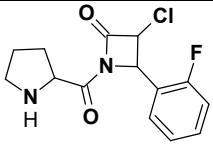
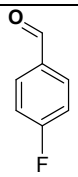
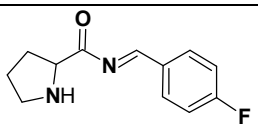
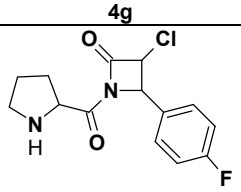
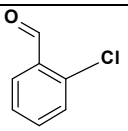
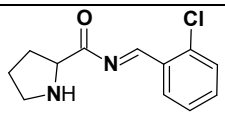
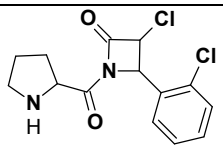
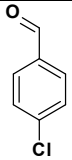
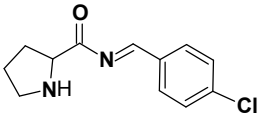
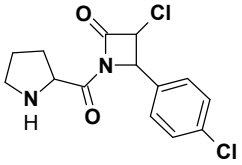
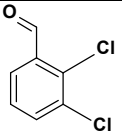
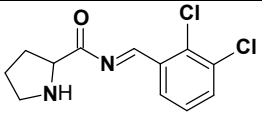
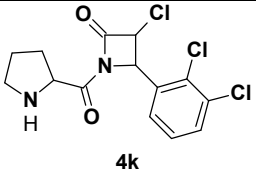


R = -H, -OH, 3-OCH_3, 4-OCH_3, 2,4,5 tri-OCH_3, 2,4,6 tri-OCH_3, 2-F, 4-F, 2-Cl, 4-Cl, 2,3-diCl

Table 1

Synthetic scheme for the preperation of 3-chloro-4-phenyL-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4a-k.

Entry	Aldehyde 2a-k	Schiff-base 3a-k	Azetidinone derivative 4a-k
1	 2a	 3a	 4a

2	 2b	 3b	 4b
3	 2c	 3c	 4c
4	 2d	 3d	 4d
5	 2e	 3e	 4e
6	 2f	 3f	 4f
7	 2g	 3g	 4g
8	 2h	 3h	 4h
9	 2i	 3i	 4i
10	 2j	 3j	 4j
11	 2k	 3k	 4k

General procedure for the synthesis of (S,Z)-N-benzylidenepyrrolidine-2-carboxamide (3a)

5.0 g (0.043 moles) of *L*-prolinamide (1) was condensed with 6.0 g (0.056 moles) benzaldehyde (2a) at room temperature under stirring in methanol for 8-10 hour. Few drops of acetic acid was used as catalyst to enhance the rate of reaction. The compound of reaction was monitored by TLC. Methanol was removed under reduce pressure to give light yellow oily residue. The residue was quenched in ice-water to give Schiff base as a free flowing yellow solid **(3a)** 7.5 g (61.42% yield).[20]

General procedure for the synthesis of 3-chloro-4-phenyL-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one (4a)

2.0 g (0.0098 moles) of the schiff base and 1.67 g (0.0147 moles) of chloroacetyl chloride were refluxed in 1,4-dioxane using TEA as a base for 10-12 hours. 1,4-Dioxane was then distilled off and the resulting viscous liquid was washed with 10% $NaHCO_3$ solution to remove any unreacted Chloroacetylchloride. The resulting product separated was washed with water, dried and recrystallized from methanol to finally yield the 3-chloro-4-phenyL-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one.[21]
The product was obtained as offwhite soild, mp: 156-158°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.34 – 7.27 (m, 1H), 7.26 – 7.18 (m, 1H), 7.20 – 7.13 (m, 2H), 6.26 (dt, *J* = 7.5, 1.0 Hz, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 3.70 (t, *J* = 8.6 Hz, 1H), 3.04 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.10 (ddt, *J* = 13.0, 8.6, 7.8 Hz, 1H), 1.99 – 1.68 (m, 2H), 1.82 (s, 1H). Anal. Calcd for: $C_{13}H_{13}ClN_2O_2$ (264.71) Calculation: (C, 58.99; H, 4.95; Cl, 13.39; N, 10.58); found (C, 58.95; H, 4.93; Cl, 13.36; N, 10.56). MS (EI) *m*/*z*: 264.71 (M^+), 265.82(M+1). IR (KBr, cm^{-1}): v= 3400 (-NH), 1678(C=O), 1360 (C-N).

3-chloro-4-(4-hydroxyphenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4b

The product was obtained as off white soild, mp: 181–184 °C^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.12 – 7.05 (m, 2H), 6.82 – 6.76 (m, 2H), 6.16 (dt, *J* = 7.5, 1.0 Hz, 1H), 5.87 (d, *J* = 7.5 Hz, 1H), 4.62 (s, 1H), 3.71 (t, *J* = 8.7 Hz, 1H), 3.03 (dt, *J* = 9.5, 6.7 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.6 Hz, 1H), 2.09 (ddt, *J* = 13.1, 8.6, 7.8 Hz, 1H), 1.92 (ddt, *J* = 13.0, 8.6, 7.7 Hz, 1H), 1.81 (s, 1H), 1.87 – 1.67 (m, 2H). Anal. Calcd for: $C_{14}H_{15}ClN_2O3$ (294.73); Calculation (C, 57.05; H, 5.13; Cl, 12.03; N, 9.50; O, 16.29%); found (C, 57.03; H, 5.10; Cl, 12.01; N, 9.48%). MS (EI) *m*/*z*: 294.73 (M^+), 295.8 (M+1). IR (KBr, cm^{-1}): v= 3410 (-NH), 1670(C=O), 1290 (C-N).

3-chloro-4-(3-methoxyphenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4c

The product was obtained as off white needles, mp: 198–201°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.29 – 7.22 (m, 1H), 6.86 (dtd, *J* = 7.5, 2.0, 0.9 Hz, 1H), 6.77 (dq, *J* = 5.7, 2.0 Hz, 2H), 6.13 (dt, *J* = 6.6, 1.0 Hz, 1H), 5.88 (d, *J* = 6.6 Hz, 1H), 3.75 (t, *J* = 7.1 Hz, 1H), 3.71 (s, 2H), 3.01 (dt, *J* = 9.5, 5.9 Hz, 1H), 2.80 (dt, *J* = 9.5, 5.9 Hz, 1H), 2.33 – 2.22 (m, 1H), 2.12 (s, 1H), 1.87 – 1.65 (m, 3H). Anal. Calcd for: $C_{15}H_{17}ClN_2O_3$ (308.76); Calculation (C, 58.35; H, 5.55; N, 9.07;%); found (C, 58.32; H, 5.52; N, 9.05%). MS (EI) *m*/*z*: 308.76 (M^+), 309.92 (M+1). IR (KBr, cm^{-1}): v= 3408 (-NH), 1666(C=O), 1356 (C-N).

3-chloro-1-((S)-pyrrolidine-2-carbonyl)-4-(2,4,5-trimethoxyphenyl)azetidin-2-one 4d

The product was obtained as yellow needles, mp: 223–226°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.20 – 7.13 (m, 2H), 6.88 – 6.81 (m, 2H), 6.23 (dt, *J* = 7.3, 0.9 Hz, 1H), 5.84 (d, *J* = 7.3 Hz, 1H), 3.80 (s, 2H), 3.70 (t, *J* = 8.7 Hz, 1H), 3.04 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.11 (ddt, *J* = 13.1, 8.7, 7.8 Hz, 1H), 1.94 (ddt, *J* = 13.0, 8.6, 7.7 Hz, 1H), 1.83 (s, 1H), 1.88 – 1.68 (m, 2H). Anal. Calcd for: $C_{15}H_{17}ClN_2O_3$ (308.76); Calculation (C, 58.35; H, 5.55; N, 9.07;%); found (C, 58.33; H, 5.53; N, 9.04%). MS (EI) *m*/*z*: 308.76 (M^+), 309.87 (M+1). IR (KBr, cm^{-1}): v= 3399 (-NH), 1660(C=O), 1348 (C-N).

3-chloro-1-((S)-pyrrolidine-2-carbonyl)-4-(2,4,5-trimethoxyphenyl)azetidin-2-one 4e

The product was obtained as off-white needles, mp: 186–188°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.86 (d, *J* = 1.0 Hz, 1H), 6.66 (s, 1H), 6.05 – 5.95 (m, 2H), 3.90 (s, 4H), 3.81 – 3.72 (m, 3H), 3.05 – 2.97 (m, 1H), 2.84 – 2.76 (m, 1H), 2.33 – 2.22 (m, 1H), 2.10 (s, 1H), 1.87 – 1.74 (m, 2H), 1.78 – 1.67 (m, 1H). Anal. Calcd for: $C_{17}H_{21}ClN_2O_5$ (368.81); Calculation (C, 55.36; H, 5.74; Cl, 9.61; N, 7.60%); found (C, 55.33; H, 5.72; Cl, 9.58; N, 7.56%). MS (EI) *m*/*z*: 368.81 (M^+), 369.86 (M+1). IR (KBr, cm^{-1}): v= 3403 (-NH), 1672(C=O), 1299 (C-N).

3-chloro-1-((S)-pyrrolidine-2-carbonyl)-4-(2,4,6-trimethoxyphenyl)azetidin-2-one 4f

The product was obtained as white needles, mp: 182–185°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.26 (s, 1H), 6.14 (d, *J* = 6.4 Hz, 1H), 5.87 (d, *J* = 6.4 Hz, 1H), 4.05 (t, *J* = 7.8 Hz, 1H), 3.82 (d, *J* = 20.0 Hz, 6H), 3.00 – 2.92 (m, 2H), 2.72 (dt, *J* = 9.3, 6.0 Hz, 1H), 2.25 – 2.14 (m, 1H), 2.03 – 1.92 (m, 1H), 1.87 – 1.66 (m, 2H). Anal. Calcd for: $C_{17}H_{21}ClN_2O_5$ (368.81); Calculation (C, 55.36; H, 5.74; Cl, 9.61; N, 7.60%); found (C, 55.32; H, 5.71; Cl, 9.57; N, 7.55%). MS (EI) *m*/*z*: 368.81 (M^+), 369.71 (M+1). IR (KBr, cm^{-1}): v= 3392 (-NH), 1658(C=O), 1308 (C-N).

3-chloro-4-(2-fluorophenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4g

The product was obtained as off white needles, mp: 162–165 °C;^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.29 (tdd, *J* = 7.2, 5.8, 2.3 Hz, 1H), 7.22 (ddd, *J* = 9.2, 7.4, 1.9 Hz, 1H), 7.11 – 7.01 (m, 2H), 6.15 (dd, *J* = 7.5, 0.9 Hz, 1H), 6.03 (d, *J* = 7.5 Hz, 1H), 3.74 – 3.66 (m, 1H), 3.04 (dt, *J* = 9.6, 6.6 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.6 Hz, 1H), 2.02 – 1.90 (m, 1H), 1.82 (s, 1H), 1.86 – 1.65 (m, 2H). Anal. Calcd for: $C_{14}H_{14}N_2$ (296.72); Calculation (C, 56.67; H, 4.76; N, 9.44%); found (C, 56.65; H, 4.72; N, 9.41%). MS (EI) *m*/*z*: 296.72 (M^+), 297.86 (M+1). IR (KBr, cm^{-1}): v= 3412 (-NH), 1679(C=O), 1325 (C-N).

3-chloro-4-(4-fluorophenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4h

The product was obtained as Orange crystals, mp: 161-163°C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.18 – 7.10 (m, 2H), 7.12 – 7.03 (m, 2H), 6.22 (dt, *J* = 7.5, 1.0 Hz, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 3.74 – 3.66 (m, 1H), 3.04 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.09 (ddt, *J* = 13.0, 8.6, 7.8 Hz, 1H), 1.93 (ddt, *J* = 13.0, 8.6, 7.7 Hz, 1H), 1.82 (s, 1H), 1.88 – 1.68 (m, 2H). Anal.

Calcd for: $C_{14}H_{14}N_2$ (296.72); Calculation (C, 56.67; H, 4.76; N, 9.44%); found (C, 56.64; H, 4.73; N, 9.40%). MS (EI) *m*/*z*: 296.72 (M^+), 297.76 (M+1). IR (KBr, cm^{-1}): v= 3415 (-NH), 1672(C=O), 1349 (C-N).

3-chloro-4-(2-chlorophenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4i

The product was obtained as white needles, mp: 231–233°C; ^{1}H NMR ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.43 (dd, *J* = 7.5, 2.0 Hz, 2H), 7.27 (td, *J* = 7.5, 2.0 Hz, 2H), 7.16 (td, *J* = 7.5, 2.0 Hz, 2H), 6.94 (ddd, *J* = 7.5, 2.0, 1.0 Hz, 2H), 6.55 (dd, *J* = 6.8, 1.0 Hz, 2H), 5.85 (d, *J* = 6.8 Hz, 2H), 3.69 (t, *J* = 8.7 Hz, 2H), 3.04 (dt, *J* = 9.5, 6.7 Hz, 2H), 2.81 (dt, *J* = 9.5, 6.7 Hz, 2H), 2.14 (ddt, *J* = 13.2, 8.8, 7.8 Hz, 2H), 1.97 (ddt, *J* = 13.1, 8.7, 7.8 Hz, 2H), 1.81 (s, 2H), 1.89 – 1.70 (m, 4H). Anal. Calcd for: $C_{14}H_{14}Cl_2N_2O_2$ (313.18); Calculation (C, 53.69; H, 4.51; N, 8.94 %); found (C, 53.67; H, 4.48; N, 8.92 %). MS (EI) *m*/*z*: 313.18 (M^+), 314.26 (M + 1). IR (KBr, cm^{-1}): v= 3408 (-NH), 1660(C=O), 1325 (C-N).

3-chloro-4-(4-chlorophenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 6j

The product was obtained as yellow needles, mp: 271–274 °C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.39 – 7.33 (m, 2H), 7.22 – 7.16 (m, 2H), 6.24 (dt, *J* = 7.5, 1.0 Hz, 1H), 5.86 (d, *J* = 7.5 Hz, 1H), 3.70 (t, *J* = 8.6 Hz, 1H), 3.04 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.09 (ddt, *J* = 13.0, 8.6, 7.8 Hz, 1H), 1.93 (ddt, *J* = 13.0, 8.6, 7.7 Hz, 1H), 1.82 (s, 1H), 1.88 – 1.68 (m, 2H). Anal. Calcd for: $C_{14}H_{14}Cl_2N_2O_2$ (313.18); Calculation (C, 53.69; H, 4.51; N, 8.94 %); found (C, 53.66; H, 4.47; N, 8.91 %). MS (EI) *m*/*z*: 313.18 (M^+), 314.26 (M + 1). IR (KBr, cm^{-1}): v= 3406 (-NH), 1665(C=O), 1336 (C-N).

3-chloro-4-(2,3-dichlorophenyl)-1-((S)-pyrrolidine-2-carbonyl)azetidin-2-one 4k

The product was obtained as yellow needles, mp: 273–276 °C; ^{1}H NMR ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.29 – 7.20 (m, 2H), 6.81 (ddd, *J* = 7.1, 2.3, 1.0 Hz, 1H), 6.60 (dd, *J* = 6.9, 0.9 Hz, 1H), 5.85 (d, *J* = 6.9 Hz, 1H), 3.69 (t, *J* = 8.7 Hz, 1H), 3.04 (dt, *J* = 9.5, 6.7 Hz, 1H), 2.81 (dt, *J* = 9.5, 6.7 Hz, 1H), 2.14 (ddt, *J* = 13.0, 8.6, 7.8 Hz, 1H), 1.96 (ddt, *J* = 13.0, 8.6, 7.7 Hz, 1H), 1.81 (s, 1H), 1.89 – 1.70 (m, 2H). Anal. Calcd for: $C_{14}H_{13}Cl_3N_2O_2$ (347.62) ;Calculation (C, 48.37; H, 3.77; N, 8.06 %); found (C, 48.33; H, 3.74; N, 8.03%). MS (EI) *m*/*z*: 347.62 (M^+), 348.72 (M+1). IR (KBr, cm^{-1}): v= 3415 (-NH), 1675(C=O), 1340 (C-N).

RESULT AND DISCUSSION

Synthesis of 3-chloro-4-phenyl*L*-1-((*S*)-pyrrolidine-2-carbonyl)azetidin-2-one 4a has been obtained by condensation reaction of (*S,Z*)-N-benzylidenepyrrolidine-2-carboxamide 3a with chloroacetyl chloride and TEA in 1,4-dioxane as solvent. The compound 3a has been synthesized by one pot condensation of *L*-prolinamide, aromatic aldehydes and catalytic amount of acetic acid in methanol.Scope of the reaction was studies for the synthesis of 3-chloro-4-phenyl*L*-1-((*S*)-pyrrolidine-2-carbonyl) azetidin-2-one 4a–k. The products of the reaction were fully characterized by ^{1}H NMR, Mass, Elemental analysis and IR. The synthesized compounds were characterized on the basis of the spectral and analytical studies.The IR spectra of compounds 4a–k are observed a broad band in the region 3415-3392 cm^{-1}due to the –NH- group. The C=O group is observed as a strong and sharp band at 1679-1658 cm^{-1}in all compounds. The C-N (*β*-lactam ring) observed at their usual positions at 1389-1260. Similarly, all these compounds were purified by column chromatography and characterized on the basis of spectral studies.The aromatic protons show muliplet signals in the range of 7.34-7.13 ppm. The –Ph-CH- proton of the *β*-lactam ring shows the splitting pattern of doublet of triplets at 6.26 ppm due to its chirality. On the other hand, the doublet pattern of the –C*L*-CH- of the *β*-lactam ring appears at 5.86 ppm. The –CH- proton of the *L*-prolinamide appears as a triplet at 3.70. The other -CH_2-protons of the *L*-prolinamide appears as multiplets at 2.81, 2.10 and in the range between 1.99-1.68.dt denotes ‘doublet of triplets’ where as ddt denotes ‘doublet of doublet of triplets’.

Biological activity

Antibacterial activity: The minimum inhibitory concentration (MIC) of the tested compounds 4a–k is shown in (Table 2). Most of the compounds tested, exhibited considerable activities against four bacterial species, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes.[22,23] Azetidinone compounds 4c exhibited excellent at 62.5 µg/mL; 4a, 4b, 4e, 4f and 4i exhibited a good activity at 100-125 µg/mL against Escherichia coli as compared to Ampicillin (MIC=100 µg/mL). Compound 4c exhibited excellent activity against Pseudomonas aeruginosa at 100 µg/mL; 4b, 4d, 4e, 4f and 4i exhibited good activity as compared to Ampicillin (MIC= 100-125 µg/mL).

Table 2.
Antimicrobial activity data of synthesized compounds

Entry	*E. Coli* MTCC 443	*P. Aeruginosa* MTCC 1688	*S. Aureus* MTCC 96	*S. Pyogenus* MTCC 442
4a	125	200	500	200
4b	100	125	200	250
4c	62.5	100	200	250
4d	200	125	250	125
4e	125	125	200	500
4f	125	125	200	100
4g	250	250	200	250
4h	200	200	125	125
4i	125	125	100	250

4k	200	250	125	100
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Standard deviation	± 5	± 5	± 5	± 5
Control (DMSO)	-	-	-	-

Compounds 4h, 4i and 4k exhibited very good activity at 100–125 µg/mL; 4b, 4c, 4d, 4e, 4f and 4g exhibited good activity at 200–250 µg/mL against Staphylococcus aureus as compared to Ampicillin (MIC= 250 µg/mL). Compounds 4d, 4f, 4h and 4k exhibited good activity against Streptococcus pyogenes at 100 µg/mL as compared to Ampicillin (MIC= 100 µg/mL).

Table-3.
Antifungal activity (MIC) of synthesis compounds

Compound No.	***C. Albicans* MTCC 227**	***A. Niger* MTCC 282**	***A. Clavatus* MTCC 1323**
4a	>1000	500	500
4b	>1000	>1000	1000
4c	1000	1000	500
4d	250	500	>1000
4e	1000	500	500
4f	500	>1000	>1000
4g	>1000	>1000	500
4h	500	500	>1000
4i	250	>1000	500
4j	500	500	>1000
4k	250	500	500
Griseofulvin	500	100	100
Control (DMSO)	-	-	-

Antifungal activity

The minimum inhibitory concentration (MIC) of the tested compounds 4a–k is shown in (Table 3). Compounds 4d, 4f, 4h, 4i, 4j and 4k exhibited very good activity at 250–500 µg/mL against Candida albicansas compared to Griseofulvin (MIC= 500 µg/mL); their MIC values were in the range between (100–500 µg/mL).All the screened compounds were less active against Aspergillus niger and Aspergillus clavatus. The other compounds tested showed less activity against the fungal species.

CONCLUSION

In conclusion, we have described a simple approach for the synthesis of azetidinone derivatives of *L*-prolinamide 4a–k. Synthesiszed compounds were screened for antibacterial, antifungal and anti tubercular activity. Compounds 4a, 4b, 4c, 4e, 4f and 4i were possessed excellent activity comparable to ampicillin for different four species. Compounds 4d, 4i and 4k showed good activity of 250 µg/ml comparable to griseofulvin. Compounds bearing fluoro, chloro, methoxy and methyl derivatives are more effective to inhibit the both bacterial and fungal species. Present work will be useful for understanding antimicrobial activity of Azetidinone derivatives of *L*-prolinamide.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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