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Synthesis, Docking and Biological Evaluation of New Series of Pyrrolidine Derivatives as Potent Antibacterial Agents

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The chemical flexibility of the pyrrolidine nucleus and its ability to generate a wide range of structural variations may have a significant impact on the therapeutic effectiveness. In present study, 17 analogs of 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-amine (**7a-q**) were synthesized and characterized. All the synthesized compounds were purified by combi-flash chromatography using RediSep RF 1.5 Flash silica gel columns. The synthesized compounds were investigated for antibacterial activity utilizing the agar well diffusion method. Apart from compounds **7l**, **7o** and **7p**, antibacterial activity against *E. coli* was exhibited by almost all the compounds. All the tested compounds were effective in showing antibacterial activity against *Bacillus* and against *Aspergillus niger*, none of the drugs had antifungal efficacy. The SwissADME analysis of the synthesized compounds, the Lipinski characteristics revealed that compounds **7a**, **7b**, **7e-h**, **7j-l**, **7n** and **7o** don't violate the rule of five. Therefore, these substances might be viewed as orally accessible and pharmacologically active drug candidates. Auto DockVina was used for the purpose of molecular docking of the synthesized compounds.

Keywords: Pyrrolidine derviatives, Antimicrobial activity, SwissADME, Molecular docking.

INTRODUCTION

The increasing prevalence of bacteria that have acquired resistance to existing antibiotic therapies has prompted a heightened emphasis on the advancement of innovative pharmaceuticals to address these threats to human health. Pyrrolidine, alternatively referred to as tetrahydropyrrole, is categorized as a saturated heterocycle, distinguished by its cyclic arrangement and secondary amine moiety [1]. The incorporation of the pyrrolidine ring, a five-membered nitrogen heterocycle, is widely employed by medicinal chemists in the synthesis of therapeutic compounds intended to target many human diseases [1,2].

The pyrrolidine nucleus has garnered significant attention from the scientific community, leading to effective synthesis of pyrrolidines with various replacements by different chemical industries. These synthesized pyrrolidines have been employed as essential building blocks in the creation of innovative medications. Within this selection, the pyrrolidine, pyrrolidin-2-one, pyrrolidine-2,5-dione and prolinol scaffolds are esteemed premade ring structures that have demonstrated significant utility in the creation of innovative bioactive compounds [3-7].

Several pyrrolidine analogues, including nicotine, scalusamide, bgugaine, D-ribitol and aegyleptolidine, have been isolated from the natural sources and microorganisms. These analogues demonstrate a diverse array of biological functions [8,9]. The aforementioned attribute facilitates the ability of medicinal chemists to strategically develop compounds possessing ideal structures, hence enabling efficient binding to the ligand binding site of a certain protein target [2]. This study focuses on synthesizing a novel compounds and investigating its biological activity, taking into account the previously established facts and the significance of pyrrolidine, as well as the urgent need for an antibiotic to combat resistant bacteria. In this investigation, our focus was on examining the antibacterial activity of the compounds that is synthesized. The aim was to enhance the range of therapeutic options available for combating antibiotic resistance. Additionally, the antimicrobial activity was evaluated against one Gram-positive, one Gram-negative and one fungal strains. Further, the minimal inhibitory concentration and minimal bactericidal or fungicidal concentration

were determined. Finally, *in-silico* molecular docking simulations and toxicity predictions were also evaluated.

EXPERIMENTAL

For the synthesis of pyrrolidine analogues, only commercially available reagents and solvents (Sigma Aldrich, TCI, combi-blocks) were utilized. All reactions were monitored by TLC using Merck classic aluminum silica plates with size 20 cm × 20 cm, thickness 200 µm were detected in UV 254 nm using ninhydrine solution. All chemicals were purified using combi-flash chromatography and Teledyne ISCO's RediSep RF 1.5 Flash silica gel columns. A Bruker Avence 300 MHz and Ascend 400 MHz spectrometer was used to record the proton ^{1}H and ^{13}C NMR spectra. Using TMS as a standard reference, proton NMR chemical shifts are reported in parts per million (δ). ESI spectra were captured using ESI+ software, a 3.98 kV capillary voltage and an ESI mode positive ion trap detector on a Micro mass, Quattro LC. Only the principal peaks in the IR spectra, which were captured using a Shimadzu FT-IR 8300 spectrophotometer. The melting points were recorded in open capillaries and are uncorrected.

Synthesis of 4-bromo-2-(pyrrolidin-1-yl) benzaldehyde (2): To a solution of 4-bromo-2-fluorobenzaldehyde (**1**) (15.0 g, 73.8 mmol) in DMSO (120 mL) were added pyrrolidine (9.1 mL, 110.8 mmol) and K_2CO_3 (25.5 g, 184.7 mmol) at room temperature under argon atmosphere. The mixture was stirred at 100 ºC for 5 h. The progress of reaction was monitored by LC-MS. After completion of reaction, the reaction mixture was diluted with ice-cold water (120 mL) and extracted with dichloromethane (2 × 200 mL). The combined organic layer was washed with brine solution (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by combi-Flash column chromatography eluted using 12-15% EtOAc in hexane to afford 4-bromo-2-(pyrrolidin-1-yl)benzaldehyde (**2**) (10.3 g, 54.8%) as pale orange solid. LCMS (ESI + APCI): *m/z* = 255 $[M + H]^+$.

Synthesis of *tert*-butyl-(1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)carbamate (4): To a solution of 4-bromo-2-(pyrrolidin-1-yl)benzaldehyde (**2**) (10.0 g, 39.3 mmol) in anhydrous $CH_2CH_2Cl_2$ (DCE) (120 mL) were added *tert*-butyl pyrrolidin-3-ylcarbamate (**3**) (8.0 g, 43.3 mmol) and acetic acid (0.7 mL) at room temperature. The reaction mixture was stirred at room temperature for 4 h, followed by addition of sodium triacetoxyborohydride (STAB) (16.6 g, 78.6 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 16 h. The progress of reaction was monitored by LCMS. After the completion of reaction, the reaction mixture was concentrated under reduced pressure. The residue was diluted with ice-cold water (70 mL) and extracted with CH_2Cl_2 (2 × 120 mL). The combined organic layer was washed with brine solution (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by combi-Flash column chromatography eluted using 15% EtOAc in hexane to afford *tert*-butyl-(1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl) carbamate (**4**) (11.8 g, 70.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.21 (d, *J* = 8.0 Hz, 1H), 6.92-6.87 (m, 3H), 3.87 (brs, 1H), 3.50 (9, *J* = 4.8 Hz, 2H), 3.21 (t, *J* = 6.4 Hz, 4H), 2.70-2.66 (m, 1H), 2.45-2.42 (m, 2H), 2.23 (q, *J* = 5.2 Hz, 1H), 2.03-1.96 (m, 1H), 1.87-1.83 (m, 4H), 1.58-1.53 (m, 1H), 1.34 (s, 9H); LCMS (ESI + APCI): *m/z* =425.34 $[M + H]^+$.

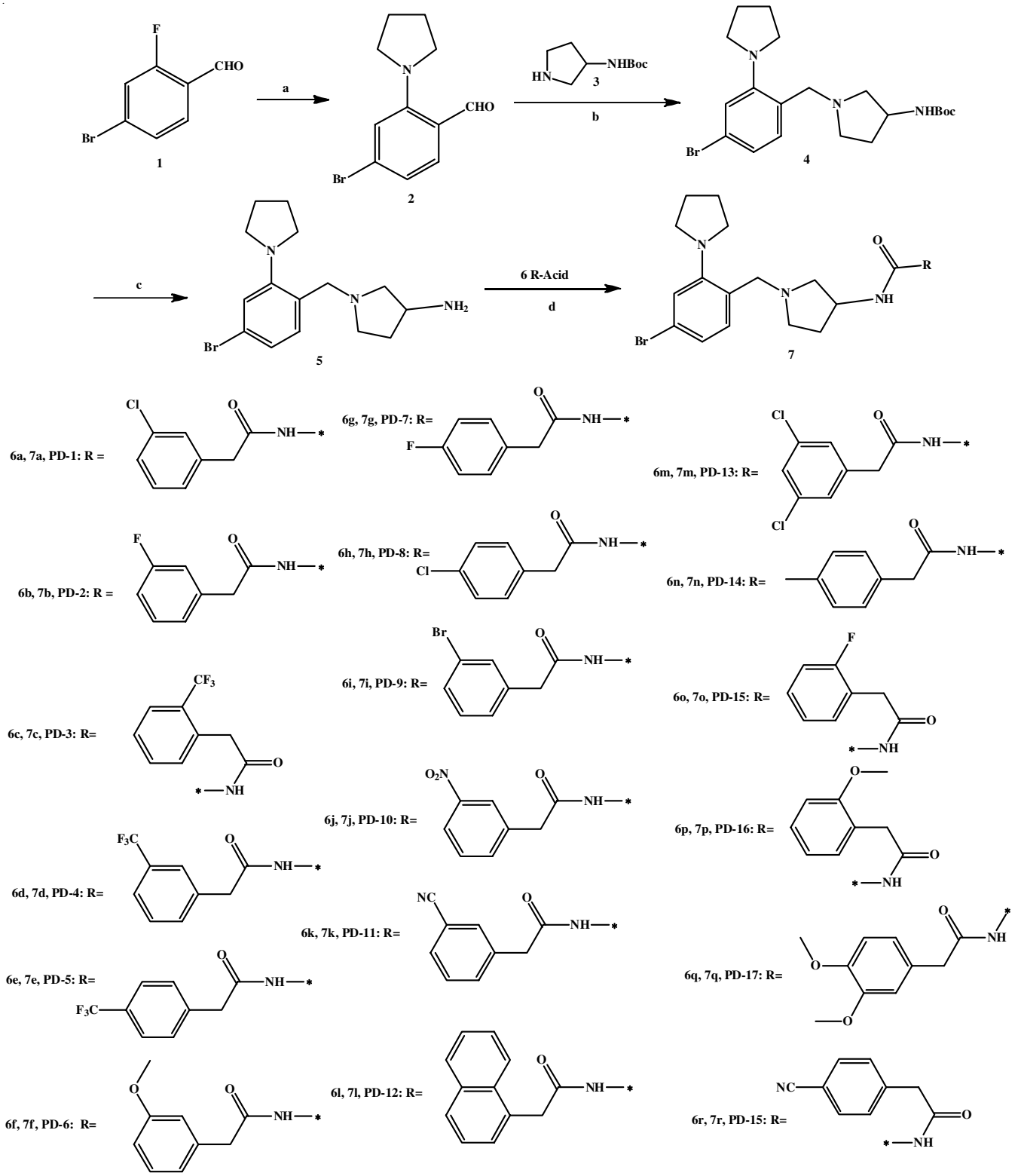
Synthesis of 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)-pyrrolidin-3-amine (5): A suspension of *tert*-butyl-(1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)carbamate (**4**) (11.5 g, 27.1 mmol) in trifluloroethanol (30 mL) was added TMSCl (6.8 mL, 54.2 mmol) at 0 ºC. The reaction mixture stirred at room temperature for 2 h. The progress of reaction was monitored by TLC and LCMS. After completion of reaction, the reaction mixture was concentrated under reduced pressure and washed with pentane to afford 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-amine (**5**) (7.7 g, 87%) as an off-white solid. LCMS (ESI + APCI): *m/z* = 325.41 $[M + H]^+$.

General procedure for the synthesis of compounds (7a-q): To a stirred solution of compound **5** (1.0 equiv.) in DCM (5.0 mL) were added compound **6** (1.1 equiv.), DIPEA (4.0 equiv.) and 50% propyl phosphonic anhydride (T3P) in EtOAc (1.5 equiv.). The reaction mixture stirred at room temperature for 16 h under nitrogen atmosphere. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture diluted with water (10 mL) and the aqueous layer was extracted with DCM (2 × 15.0 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by combi-Flash column chromatography eluting with EtOAc in hexane followed by pentane washings to obtain final compounds (**7a-o**) within range of 62-82% yield (**Scheme-I**).

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-chlorophenyl)acetamide (7a):** Yield: 83%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, *J* = 6.4 Hz, 1H), 7.30 (s, 3H), 7.22 (t, *J* = 8.4 Hz, 2H), 6.81 (t, *J* = 8.4 Hz, 2H), 4.10 (brs, 1H), 3.51 (d, *J* = 6.8 Hz, 2H), 3.40 (s, 2H), 3.20 (s, 4H), 2.61-2.57 (m, 2H), 2.37-2.29 (m, 2H), 2.09-2.07 (m, 1H), 1.84 (s, 4H), 1.58-1.55 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.51, 150.38, 139.43, 133.17, 132.70, 130.45, 129.25, 128.31, 127.11, 126.74, 121.48, 120.77, 118.20, 60.58, 56.88, 52.92, 51.27, 48.60, 42.00, 31.44, 25.26. LCMS (ESI + APCI): *m/z* = 478.10 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-fluorophenyl)acetamide (7b):** Yield: 78%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (d, *J* = 6.8 Hz, 1H), 7.35-7.23 (m, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 8.8 Hz, 3H), 6.91 (t, *J* = 8.4 Hz, 2H), 4.11 (brs, 1H), 3.51 (d, *J* = 5.6 Hz, 2H), 3.41 (s, 2H), 3.20 (s, 4H), 2.65-2.50 (m, 2H), 2.39-2.29 (m, 2H), 2.12-2.03 (m, 1H), 1.84 (s, 4H), 1.59-1.53 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.54, 163.66, 161.24, 150.38, 139.78, 139.71, 132.70, 130.49, 130.40, 127.10, 125.53, 125.50, 121.46, 120.77, 118.19, 116.21, 116.00, 113.65, 113.44, 60.58, 56.88, 52.92, 51.27, 48.59, 42.14, 31.43, 25.26. LCMS (ESI + APCI): *m/z* = 462.14 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(2-(trifluoromethyl)phenyl)acetamide (7c):** Yield: 76.7%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.61-7.57 (m, 1H), 7.46-7.1 (m, 2H), 7.23 (d, *J* = 8.0 Hz, 1H), 6.91-6.88 (m,



Scheme-I: Reagents and conditions: (a) pyrrolidine, K_2CO_3, 100 °C, 5 h (b) STAB, AA, DCE, room temp., 16 h (c) TMSCl, trifluloroethanol, room temp., 2 h (d) Acid **7a-q**, DIPEA, T3P, DCM, room temp., 16 h

2H), 4.14-4.12 (m, 1H), 3.63 (s, 2H), 3.52 (s, 2H), 3.22 (s, 4H), 2.63-2.59 (m, 2H), 2.41-2.31 (m, 2H), 2.13-2.04 (m, 1H), 1.83 (s, 4H), 1.58-1.52 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.92, 150.38, 135.05, 133.49, 132.75, 132.56, 128.01, 127.71, 127.46, 127.11, 126.27, 125.97, 125.91, 123.55, 121.43, 120.78, 118.18, 60.53, 56.95, 52.93, 51.26, 48.65, 31.39, 25.26. LCMS (ESI + APCI): *m/z* = 512.06 [M + H]$^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-(trifluoromethyl)phenyl)acetamide (7d):** Yield: 74.7%; off-white solid ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30

(brs, 1H), 7.60-7.53 (m, 4H), 7.22 (d, *J* = 8.0 Hz, 1H), 6.91-6.88 (m, 2H), 4.12 (s, 1H), 3.51 (s, 4H), 3.20 (s, 4H), 2.62-2.58 (m, 2H), 2.37-2.33 (m, 2H), 2.11-2.08 (m, 1H), 1.83 (s, 4H), 1.58-1.52 (m, 1H). LCMS (ESI + APCI): *m/z* = 512.06 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(4-(trifluoromethyl)phenyl)acetamide (7e):** Yield: 75.3%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, *J* = 6.4 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 2H),7.21 (d, *J* = 8.0 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 2H), 4.11 (s, 1H), 3.52 (s, 4H), 3.20 (s, 4H), 2.64-2.57 (m, 2H), 2.37-2.30 (m, 2H), 2.09-2.07 (m, 1H), 1.84 (s, 4H), 1.58-1.56 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.35, 150.38, 141.86, 132.71, 130.21, 127.11, 127.71, 125.48, 125.45, 121.47, 120.76, 118.19, 60.56, 56.86, 52.94, 51.27, 48.63, 42.25, 31.41, 25.25. LCMS (ESI + APCI): *m/z* = 512.06 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-methoxyphenyl)acetamide (7f):** Yield: 71.9%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (d, *J* = 6.8 Hz, 1H), 7.22-7.17 (m, 2H), 6.91-6.89 (m, 2H), 6.81-6.77 (m, 3H), 4.11 (s, 1H), 4.10 (s, 3H), 3.72 (s, 2H), 3.59-3.51 (s, 2H), 3.20 (s, 4H), 2.63-2.61 (m, 2H), 2.40-2.38 (m, 2H), 2.10-2.05 (m, 1H), 1.86-1.83 (m, 4H), 1.61-1.56 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.00, 159.56, 150.42, 138.43, 132.74, 129.62, 129.54, 121.59, 120.86, 118.27, 115.13, 112.04, 111.84, 60.51, 56.84, 55.36, 52.95, 51.30, 48.53, 42.65, 31.35, 25.25. LCMS (ESI + APCI): *m/z* = 474.10 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(4-fluorophenyl)acetamide (7g):** Yield: 77%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (d, *J* = 6.4 Hz, 1H), 7.27-7.20 (m, 3H), 7.10 (t, *J* = 8.8 Hz, 2H), 6.89 (t, *J* = 8.4 Hz, 2H), 4.11 (brs, 1H), 3.51 (d, *J* = 6.4 Hz, 2H), 3.36 (s, 2H), 3.20 (s, 4H), 2.65-2.58 (m, 2H), 2.38-2.28 (m, 2H), 2.09-2.05 (m, 1H), 1.84 (s, 4H), 1.58-1.56 (m, 1H). LCMS (ESI + APCI): *m/z* = 462.14 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(4-chlorophenyl)acetamide (7h):** Yield: 76%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, *J* = 6.8 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.26 -7.20 (m, 3H), 6.89 (t, *J* = 8.4 Hz, 2H), 4.10 (brs, 1H), 3.51 (d, *J* = 6.4 Hz, 2H), 3.37 (s, 2H), 3.20 (s, 4H), 2.62-2.57 (m, 2H), 2.39-2.28 (m, 2H), 2.09-2.07 (m, 1H), 1.86 (s, 4H), 1.58-1.55 (m, 1H). LCMS (ESI + APCI): *m/z* = 478.10 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-bromophenyl)acetamide (7i):** Yield: 76%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, *J* = 6.4 Hz, 1H), 7.42 (t, *J* = 6.8 Hz, 2H), 7.24 (t, *J* = 8.4 Hz, 3H), 6.89 (t, *J* = 8.4 Hz, 2H), 4.10 (brs, 1H), 3.53 (d, *J* = 6.4 Hz, 2H), 3.47 (s, 2H), 3.20 (s, 4H), 2.63-2.57 (m, 2H), 2.37-2.29 (m, 2H), 2.07-2.05 (m, 1H), 1.84 (s, 4H), 1.58-1.55 (m, 1H). LCMS (ESI + APCI): *m/z* = 522.06 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-nitrophenyl)acetamide (7j):** Yield: 70%; pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (d, *J* = 6.8 Hz, 1H), 8.18-8.15 (m, 2H), 7.51 (t, *J* = 8.8 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 3H), 6.91-6.88 (m, 2H), 4.12-4.10 (m, 1H), 3.56 (s, 2H), 3.52-3.50 (m, 2H), 3.47 (s, 2H), 3.22-3.19 (m, 4H), 2.62-2.58 (m, 2H), 2.40-2.29 (m, 2H), 2.11-2.08 (m, 1H), 1.86 (s, 4H), 1.58-1.53 (m, 1H). LCMS (ESI + APCI): *m/z* = 489.08 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3-cyanophenyl)acetamide (7k):** Yield: 68%; pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, *J* = 6.4 Hz, 1H), 7.69 (t, *J* = 12.0 Hz, 2H), 7.57 (t, *J* = 7.2 Hz, 1H), 7.52-7.48 (m, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 6.91-6.88 (m, 2H), 4.11 (s, 1H), 3.51-3.47 (m, 4H), 3.20 (s, 4H), 2.61-2.59 (m, 2H), 2.38-2.32 (m, 2H), 2.09-2.07 (m, 1H), 1.86 (s, 4H), 1.57-1.55 (m, 1H). LCMS (ESI + APCI): *m/z* = 469.17 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(naphthalen-1-yl)acetamide (7l):** Yield: 67%; pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (d, *J* = 6.8 Hz, 1H), 7.88-7.82 (m, 3H), 7.72 (m, 1H), 7.48 (t, *J* = 5.2 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.13 (s, 1H), 3.55-3.49 (m, 4H), 3.20 (d, *J* = 2.8 Hz, 4H), 2.64-2.57 (m, 2H), 2.38-2.33 (m, 2H), 2.09-2.07 (m, 1H), 1.83 (s, 4H), 1.61-1.58 (m, 1H). LCMS (ESI + APCI): *m/z* = 494.08 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(2,4-dichlorophenyl)acetamide (7m):** Yield: 76%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (d, *J* = 6.4 Hz, 1H), 7.56 (s, 1H), 7.36 (s, 2H),7.22 (d, *J* = 7.6 Hz, 1H), 6.92-6.88 (m, 2H), 4.13 (brs, 1H), 3.55-3.51 (m, 4H), 3.21 (s, 4H), 2.63-2.61 (m, 2H), 2.39-2.28 (m, 2H), 2.09-2.07 (m, 1H), 1.85 (s, 4H), 1.59-1.58 (m, 1H). LCMS (ESI + APCI): *m/z* = 478.10 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(*p*-tolyl)acetamide (7n):** Yield: 69%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (d, *J* = 6.8 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.12-7.06 (m, 4H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.09 (brs, 1H), 3.55-3.45 (m, 2H), 3.31 (d, *J* = 2.4 Hz, 2H), 3.21 (d, *J* = 2.1 Hz, 4H), 2.62-2.55 (m, 2H), 2.37-2.35 (m, 2H), 2.11 (s, 3H), 2.09-2.07 (m, 1H), 1.84 (s, 4H), 1.58-1.56 (m, 1H). LCMS (ESI + APCI): *m/z* = 456.15 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(2-fluorophenyl)acetamide (7o):** Yield: 77%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, *J* = 6.0 Hz, 1H), 7.28-7.22 (m, 3H), 7.15-7.12 (m, 2H), 6.92-6.88 (m, 2H), 4.12 (s, 1H), 3.51 (s, 2H), 3.44 (d, *J* = 6.8 Hz,, 2H), 3.21 (s, 4H), 2.61 (d, *J* = 6.8 Hz, 2H), 2.39-2.35 (m, 2H), 2.09-2.05 (m, 1H), 1.84 (s, 4H), 1.58-1.56 (m, 1H). LCMS (ESI + APCI): *m/z* = 462.14 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(2-methoxyphenyl)acetamide (7p):** Yield: 77%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.96 (d, *J* = 6.4 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.94-6.83 (m, 4H), 4.13 (s, 1H), 3.72 (s, 3H), 3.57-3.50 (m, 2H), 3.43 (d, *J* = 7.2 Hz, 2H), 3.21 (s, 4H), 2.61 (d, *J* = 7.2 Hz, 2H), 2.39-2.35 (m, 2H), 2.09-2.05 (m, 1H), 1.84 (s, 4H), 1.58-1.56 (m, 1H). LCMS (ESI + APCI): *m/z* = 462.14 $[M + H]^+$.

***N*-(1-(4-Bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-yl)-2-(3,4-dimethoxyphenyl)acetamide (7q):** Yield: 67%; off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (d, *J* = 6.4 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 6.90-6.83 (m, 4H), 6.73 (d, *J* = 8.4 Hz, 1H), 4.10 (s, 1H), 3.71 (s, 6H), 3.56-3.45 (m, 2H), 3.28 (s, 2H), 3.20 (s, 4H), 2.62 -2.56 (m, 2H), 2.37-2.28

(m, 2H), 2.07-2.04 (m, 1H), 1.84 (s, 4H), 1.58-1.57 (m, 1H). LCMS (ESI + APCI): m/z = 474.10 $[M + H]^+$.

Antimicrobial activity: The agar well diffusion technique was used to determine the antibacterial activity. *Escherichia coli*, a Gram-negative bacterium (MTCC2412) and *Bacillus cereus*, a Gram-positive bacteria (MTCC2128), were employed to investigate the antibacterial activity of various compounds. Ciprofloxacin was used as a positive reference. On the agar surface, 8 mm diameter wells were punctured and then filled with test compounds, each at a concentration of 100 µg. After the bacterial strains were cultivated in sterilized Mueller-Hinton (MH) broth and incubated for 18 h at 37 ºC, the radius of inhibition zone (RIZ) surrounding each well was measured in millimetres (mm) to ascertain the antibacterial activity. The experiments were done in triplicates. The antifungal properties of the compounds were assessed using *Aspergillus niger*. The selective media, Potato Dextrose Agar was used for plating these fungal cultures. The plates were incubated at 37 ºC for 72 to 96 h.

Determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC): Determination of MIC and MBC was done using the micro-broth dilution technique in 96-well plates, as per the usual methodology. A two-fold serial dilution of the compounds was prepared using the appropriate antibiotic. A 100 µL of MH broth was used to fill each well plate. A 100 µL of compound or antibiotic was taken from the stock solution and dissolved in the first well plate. Various concentrations were achieved *via* serial dilution. The stock concentration was 1.0 mg/mL. The 24 h culture's turbidity was changed to conform to 0.5 McFarland standards or 1×10^8 CFU/mL. With the exception of the antibiotic control well, the standardized suspension (100 µL) of bacteria was added to each well and they were all incubated at 37 ºC for 24 h. A 40 µL of MTT reagent (0.1 mg/mL in 1x PBS) was added to each well after the first 24 h of incubation. The MIC was determined to be the lowest concentration that did not exhibit any growth as visible from the MTT-developed blue colour. The clear wells were used to create subcultures and the MBC was determined to be the lowest concentration that yielded no increase after subculturing.

SwissADME: To be effective as a drug, a potent molecule must be concentrated enough to get to its target in the body and remain there in a bioactive state long enough for the anticipated biologic processes to occur. Early in the discovery phase, when there are a lot of possible compounds but limited access to physical samples, drug development entails gradually evaluating absorption, distribution, metabolism and excretion (ADME). The SwissADME, an online ADME prediction tool, was employed in the present experiments to investigate the pharmacokinetic and drug-like properties of the synthesized compounds. The predictive absorption for molar refractivity (MR), skin permeability coefficients (log Kp), total polar surface area (TPSA), number of rotatable bonds (nRotB), gastrointestinal (GI) absorption and CYP1A2 inhibitor were also assessed. Lipinski's rule of five (RO5) predicts the drug-likeness of the design derivatives. The rule of 5 indicates that when there are more than 5H-bond donors, 10 H-bond acceptors, a molecular weight more than 500 and a computed Log P (CLog P) greater than five, poor absorption or penetration is more likely in the context of drug development. Hence, molecules will unlikely become orally bioavailable as a drug if they pose properties more significant than the desired number.

Molecular docking: Molecular docking helps in predicting the preferred orientation of binding to the target protein in comparison to other candidate ligands when the ligand is the target are bound forming a stable complex. Docking also helps in checking the interaction between the protein and ligand at the molecular level.

UCSF Chimera: To better comprehend the molecular structure and function, structural biologists, biomedical researchers and others frequently utilize UCSF Chimera in the fields of bioinformatics and drug discovery. Chimera is an extensible programme that allows users to interactively visualize and analyze molecular structures as well as better understand the data that goes along with them, including sequence alignments, density maps, supramolecular assemblies, trajectories, docking results and information about conformational ensembles [10].

AutoDock Vina: AutoDock Vina is one of the most well-known bioinformatics software for computational docking. Autodock Vina is largely used for a variety of docking approaches, including site-specific docking, blind docking, protein-ligand docking, *etc.* In addition to docking, Autodock Vina (Autodock Tools) supports structural modifications to proteins and ligands. Using this technique, any size molecular libraries may be tested. It gives details on RMSD values, binding energy and H-bonds [11].

RESULTS AND DISCUSSION

The general strategy for the synthesis of 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-amine analogs (PD 1-17) is described in **Scheme-I**. Firstly, the commercially available 4-bromo-2-fluorobenzaldehyde (**1**) was treated with pyrrolidine in presence of potassium carbonate to obtain compound **2**. Then, compound **2** was subjected to reductive amination with *tert*-butyl pyrrolidin-3-ylcarbamate in the presence of sodium triacetoxyborohydride (STAB) and DCE as solvent yielded compound **4**. To achieve key intermediate **5**, compound **4** was reacted with trimethylsilyl chloride (TMSCl) and trifluoroethanol at room temperature. After preparing intermediate **5**, the synthesis of library of 1-(4-bromo-2-(pyrrolidin-1-yl)-benzyl)pyrrolidin-3-amine derivatives (**7a-q**) was carried out and the reaction was tested with a different type of acids yielded the corresponding products. All the synthesized compounds were purified by combi-Flash column chromatography and aracterized by spectroscopic methods.

Antimicrobial studies

Antibacterial activity: The synthesized molecules were evaluated for their antibacterial activity against two bacterial strains *viz. Bacillus cereus* (MTCC2128) (Gram-positive) and *Escherichia coli* (MTCC2412) (Gram-negative). Ciprofloxacin will use as a positive reference. The zone of inhibition, MIC and MBC was determined and tabulated (Table-1). Following incubation, when the inoculated plates were checked for the

TABLE-1
RADIUS OF INHIBITION ZONE (mm) AT 100 µg CONCENTRATION AND MIC AND MBC VALUES AT 1.0 mg/mL STOCK CONCENTRATION

Compound	Zone of inhibition (mm)	
	Escherichia coli	*Bacillus cereus*
7a	7.5	10.0
7b	5.0	5.5
7c	13.0	12.0
7d	12.5	10.0
7e	16.0	11.0
7f	8.0	12.0
7g	5.0	6.0
7h	9.0	11
7i	11.0	10.0
7j	9.0	10.0
7k	7.5	8.5
7l	No inhibition	5.0
7m	6.0	6.0
7n	12.0	11
7o	No inhibition	6.5
7p	No inhibition	6.0
7q	6.0	6.0

zone of inhibition, it was observed that compound **7e** showed a maximum radius of inhibition zone (16 mm) against *E. coli*. This was followed by compound **7c** which showed an inhibition radius of 13 mm. Compounds **7l**, **7o** and **7p** failed to show any zone of inhibition against *E. coli.* The compound showing the maximum inhibition against *Bacillus* was compounds **7f** and **7c** with the radius of inhibition zone of 12 mm each. Compound **7b** showed the minimum activity with a zone of 5.5 mm. All the tested compounds were effective in inhibiting the growth of *Bacillus* to some extent, thus showing antibacterial activity (Fig. 1). All the compounds had an approximately equal MIC and MBC against *E. coli* and *Bacillus*, with values of 0.25 and 0.5 µg/mL, respectively.

Antifungal activity: Synthesized compounds were tested for their antifungal activity against *Aspergillus niger.* Following

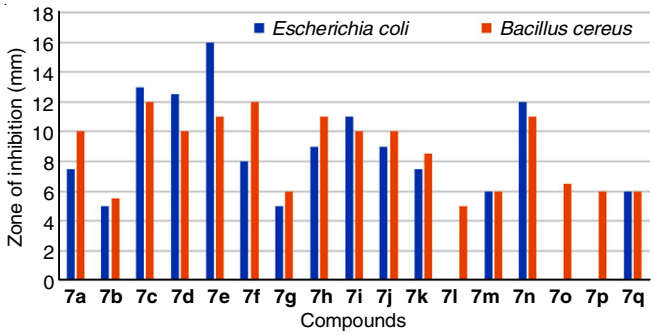


Fig. 1. Zone of inhibition of 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-amine analogs

incubation, no zone of inhibition was obtained on any of the inoculated plates.

Design and evaluation of physico-chemical properties: Lipinski parameters, drug likeness and the *in silico* ADMET screening were anticipated for the designed derivatives of 1-(4-bromo-2-(pyrrolidin-1-yl)benzyl)pyrrolidin-3-amine (Table-2). The molecular weights of the designed derivatives varied from 456.42 (compound **7n)** to 521.29 (compound **7i**). The molecular weight (MW) of compounds **7c-e** is 510.39 each and compound **7m**, **7i** and **7q** is 521.29, 511.28 and 502.44, respectively, which do not obey the rule of 5. All synthesized compounds have 2 hydrogen bond donors (HBA) and the range of hydrogen bond acceptor (HBA) is between 1 to 4. The iLOGP ranges between 3.75 to 4.79, with compound **7m** having the lowest value and compound **7l** having the highest. The molar refractivity ranged between 125.66 to 150.9 and the number of rotatable bonds varied from 7 to 9. The total prostate specific antigen (TPSA) values were below 140 for all the compounds, with the lowest being 36.68 and the highest, 60.57. All the compounds showed a high GI absorption and were brain blood barrier (BBB) permeant. All the molecules gave a positive result for Pgp substrate and a negative result for CYP1A2 inhibitor. All compounds are within the parameter range of MW ≤ 500 Da, Log P < 5, nHBD ≤ 5, nHBA ≤ 10 and TPSA < 140 Å^2,

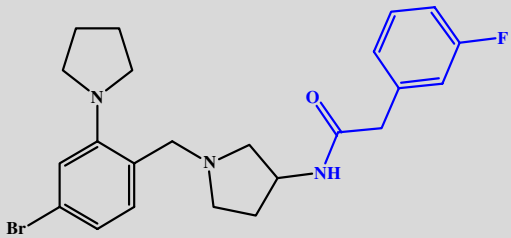
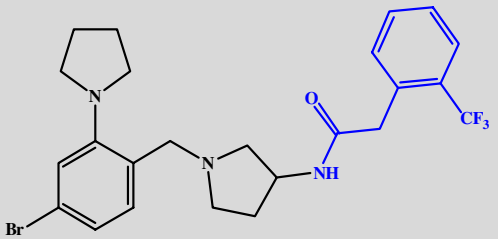
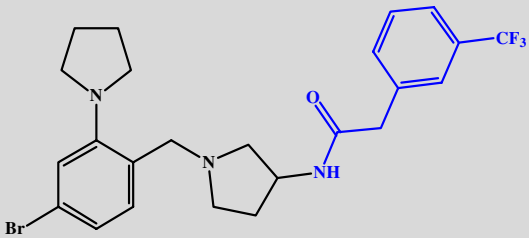
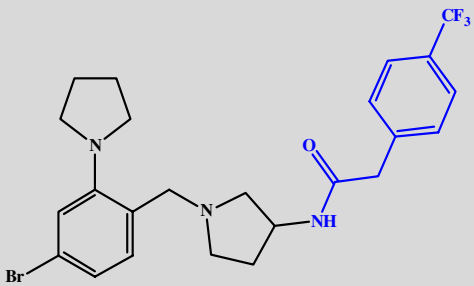
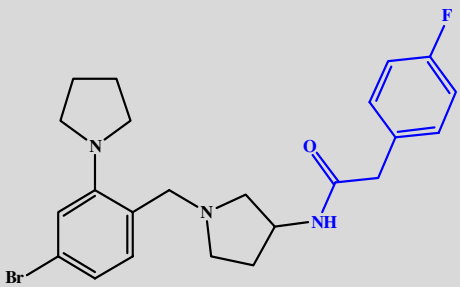
TABLE-2
LIPINSKI PROPERTIES OF THE SYNTHESIZED COMPOUNDS ANALYZED WITH SwissADME

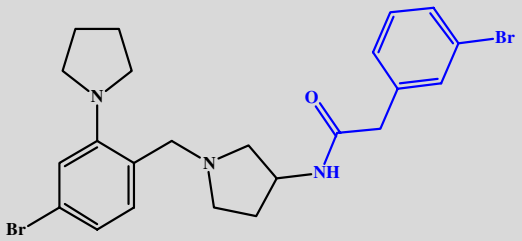
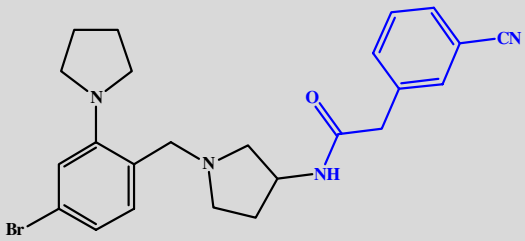
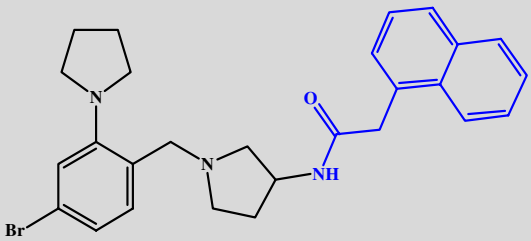
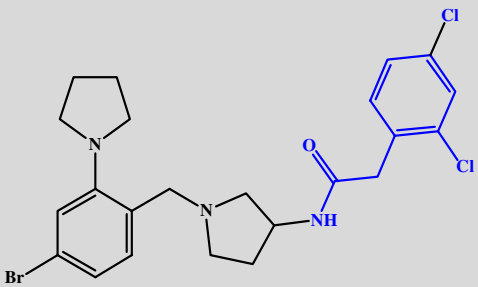
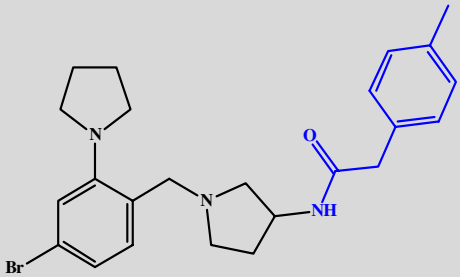
Compd.	MW	HBA	HBD	MR	TPSA	iLOGP	GI absorption	Lipinski violation	Rotatable bond	BBB permeant	Pgp substrate	CYP1A2 inhibitor
7a	476.84	1	2	130.75	36.78	4.21	High	0	7	Yes	Yes	No
7b	460.38	2	2	125.70	36.78	3.90	High	0	7	Yes	Yes	No
7c	510.39	4	2	130.74	36.78	4.16	High	1	8	Yes	Yes	No
7d	510.39	4	2	130.74	36.78	4.16	High	1	8	Yes	Yes	No
7e	510.39	4	2	130.74	36.78	4.45	High	1	8	Yes	Yes	No
7f	472.42	2	2	132.23	46.01	4.14	High	0	8	Yes	Yes	No
7g	460.38	2	2	125.70	36.78	3.98	High	0	7	Yes	Yes	No
7h	476.84	1	2	130.75	36.78	4.07	High	0	7	Yes	Yes	No
7i	521.29	1	2	133.44	36.78	4.35	High	1	7	Yes	Yes	No
7j	487.39	2	2	130.46	60.57	4.13	High	0	7	Yes	Yes	No
7k	467.40	1	2	143.25	36.78	4.40	High	0	7	Yes	Yes	No
7l	492.45	2	2	150.90	36.78	4.79	High	1	7	Yes	Yes	No
7m	511.28	1	2	130.71	36.78	3.75	High	0	7	Yes	Yes	No
7n	456.42	2	2	1254.7	36.78	4.08	High	0	7	Yes	Yes	No
7o	460.38	2	2	132.23	46.01	4.30	High	0	8	Yes	Yes	No
7p	472.42	3	2	138.72	55.24	4.53	High	1	9	Yes	Yes	No
7q	502.44	3	2	125.66	36.68	4.53	High	0	7	Yes	Yes	No

except for compounds **7c-d**, **7i**, **7m** and **7q**. When a molecule satisfies all the requirements under "rule of 5", a molecule becomes a drug like molecule. All the remaining compounds conform to Lipinski's rule of five, according to the obtained result (Table-2); consequently, these substances might be considered pharmacologically potent and orally accessible drug candidates [12]. The substances have also shown the excellent potential for blood-brain barrier (BBB) penetration (access) into the central nervous system (CNS) and good gastro-intestinal (GI) absorption. These findings indicate that these compounds have a high likelihood of rapidly entering the brain to target the desired enzyme and being efficiently absorbed by the digestive system [13].

Docking based virtual screening: After performing the SwissADME analysis, the molecular docking was performed. Further, screening was done using AutoDock Vina for precision. Out of the 17 compounds synthesized, the 10 compounds that showed the highest docking energy were compounds **7b-e**, **7g**, **7i** and **7k-n** (Table-3). Among these, the highest docking energy observed was -9.4 for compound **7l**. It was observed to have conventional hydrogen bond with ASNA:46 as contains pi-sigma bond with amino acids, ILE78 and THR165 and also

TABLE-3
DOCKING AND AMINO ACID INTERACTIONS OF THE SYNTHESIZED COMPOUNDS

Compd.	Docking energy	Structure	Amino acid interactions
7b	-8.4		MET91, VAL43, VAL120, ILE90, HIS95, ALA96, GLY119, GLU42, GLY117, ASP45, GLU50, ILE78, ASP73-van der Waals; ASP49-Attractive charge; ASN46-Conventional hydrogen bond; VAL71-Halogen (fluorine); THR165:Pi Sigma; ALA47, VAL167-Alkyl
7c	-9.1		ALA96, GLY119, GLU42, GLY117, ASP45, GLU50, ILE78, ASP73, MET166, GLN72, VAL120, MET91, ILE90, HIS95-Van der Waal; ASN46, VAL167-Conventional hydrogen bond; VAL71, VAL43-Halogen (fluorine); ALA47-Alkyl
7d	-8.8		GLN72, VAL120, MET91, ASP49, PRO79, GLY77, ASN46, ASP73, VAL44, MET166-Van der Waal; GLU50, ARG76-Attractive charge; THR165, VAL167-Conventional hydrogen bond; VAL43, VAL71-Halogen (fluorine); ARG136-Unfavourable positive-positive; ARG76-Pi-Cation; GLU50-Pi Anion; ALA47, ALA53, ILE78-Alkyl
7e	-8.7		MET91, ASP49, ASP45, GLY117, GLU42, GLY119, SER121, ALA96, HIS95, ILE90, GLU50, ASP73, ALA47, THR165-Van der Waal; PRO79-Carbon hydrogen bond; ASN46-Pi Amide Stacked; ILE78-Pi Sigma; VAL43-Halogen (fluorine); VAL120, VAL167-Alkyl
7g	-8.2		ASP45, GLU50, ASP73, VAL167, VAL43, THR165, ILE78, MET91, ILE90, HIS95, ALA96, GLY119, GLU42, GLY117-van der Waals; ASP49-Attractive charge; ASN46-Conventional hydrogen bond; ALA47, VAL120-Pi-Alkyl

7i	-8.0		GLN72, ASP73, GLU50, HIS95, ALA96, SER121, GLY119, ASP45, GLU42, GLY117, ASP49, VAL120, MET91, VAL43, VAL167-van der Waals; ASN46-Unfavourable Donor Donor; THR165-Pi-Sigma; ILE78, ALA47, VAL71, ILE90-Pi-Alkyl
7k	-8.3		THR165, ASP73, GLN72, VAL71, GLU50, ASP45, GLU45, GLU42, ASP49, GLY117, GLY119, HIS95, ALA96, SER121, MET91, VAL120, ASN46, VAL43, VAL167-Van der Waal; ALA47, ILE78, ILE90-Alkyl
7l	-9.4		MET91, VAL71, ASP73, GLU50, SER121, GLY119-van der Waals; ASN46-Conventional hydrogen Bond; ASP49-Attractive charge; ILE78, THR165-Pi Sigma; VAL120, ALA47, VAL167, VAL43, PRO79, ILE90-Alkyl
7m	-8.1		ASP49, ASN46, MET91, GLY117, GLU42, VAL120, HIS95, GLY119, ALA96, SER121, PRO79, GLU50, ILE78, ASP73, THR165-Van der Waal; ALA47, VAL167, VAL71, VAL43, ILE90-Alkyl
7n	-8.1		ASP73, ILE78, GLY77, VAL120, THR165, VAL167, MET91-Van der Waal; GLU50-Attractive charge; ARG76: Unfavourable positive positive; ASN46-Conventional hydrogen bond; ALA47, VAL71, VAL43, ILE90-Alkyl; PRO79-Pi Alkyl

contains alkyl and van der Waals bond with amino acids (Fig. 2).

This was followed by compound **7c** with the second highest docking energy of -9.1 and the conventional hydrogen bonds with ASN46 AND VAL167 were observed. The third highest docking energy was -8.8 for compound **6d** and it was found that conventional hydrogen bonds with THR165 and VAL167 were present. However, unfavourable positive-positive interactions with ARGA:136 were also observed [14]. There were no hydrogen bonds found in compound **7i**, which had the lowest docking energy of -8.0. Compounds **7e** and **7d** exhibited good docking score and also show good antibacterial properties.

Conclusion

In this work, the synthesis and characterization of novel series of pyrrolidine derivatives were conducted. All compounds were purified in combi-flash chromatography using RediSep RF 1.5 Flash silica gel columns. Except for compounds **7l**, **7o** and **7p**, rest of the newly synthesized compounds exhibited antibacterial activity against *E. coli*. Similarly, all the compounds showed good antibacterial activity against *Bacillus.* However, none of the synthesized compounds demonstrated antifungal activity towards *Aspegillus niger.* Lipinski properties of the synthesized compounds showed that only compounds **7a**, **7b**, **7e-h**, **7j-l**, **7n** and **7o** conferred the rule of five. The

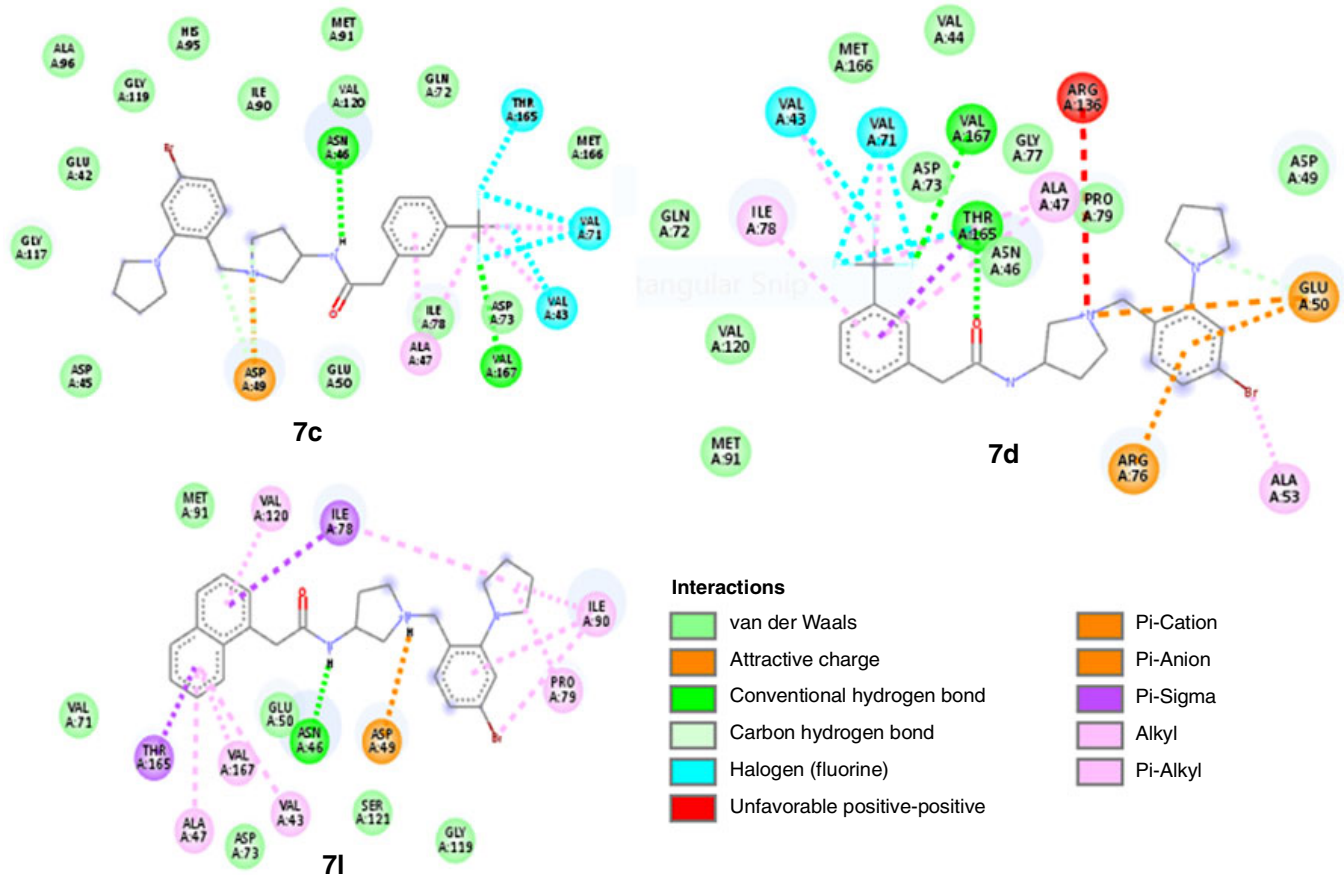


Fig. 2. 2D Ligand interaction diagrams for docking-compound **7c**, **7d** and **7l**

molecular docking was also performed and compound **7l** was shown to have the highest docking energy. Therefore, the potential for developing these pyrrolidine derivatives as effective antibacterial medicines has been expanded because of this investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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