Antimicrobial and antinociceptive profile of some novel pyrazole derivatives

Sandip Sen, Pradip Bhaumik, Biswabara Roy, T S Easwari, Ratna Choudhury, Arunabha Mallik & Biplab De*

1IMT College of Medical Sciences, O. Pocket, Ganga Nagar, Meerut 250 001, India
2Department of Medicine, Agartala, Govt. Medical College, Agartala 799 006, India
3Translam Institute of Pharmaceutical Education and Research, Meerut 250 001, India
4Henry Derozio Academy, Kunjaban, Agartala 799 006, India
5Bansal College of Pharmacy, Anand Nagar, Bhopal 462 021, India
6Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala 799 005, India
E-mail: biplab_32@yahoo.co.in

Received 25 May 2015; accepted (revised) 22 January 2016

A new series of 4-(3-methyl-1H-pyrazol-4-yl)-1-phenylazetidin-2-ones 6a-j have been prepared by intermolecular cyclization of Schiff bases 5a-j and monochloro acetyl chloride in the presence of a base catalyst triethylamine. The 1H and 13C NMR data show the presence of E isomer of Schiff bases 5a-j. Structures of all the synthesized compounds have been confirmed by spectral analysis. The biological evaluation of the compounds like antimicrobial, analgesic have been performed. From the biological investigation, it is found that out of all the synthesized compounds 6a, 6b, 6d, 6f and 6j show potent antimicrobial and anti-nociceptive activity. The SAR study confirms the presence of azetidine-2-one nucleus along with pyrazole nucleus essential for the activity. However, in particular, efficacy depends on substitution at phenyl ring, attached to the azetidine-2-one nucleus.

Keywords: Antimicrobial, analgesic, azetidine-2-one, pyrazole-4-carboxaldehyde

Pyrazole and its derivatives have attracted a great deal of attention from medicinal chemists due to the wide range of pharmacological activities associated with them. In particular, they were proven as antitumor, antibacterial and antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents. Some of these compounds also showed anti-inflammatory, anti-diabetic, anesthetic and analgesic properties. Another heterocyclic azole azetidin-2-one, also known as the β-lactam monocyclic ring, was reported to exhibit interesting biological activities such as antimicrobial, anti-tubercular, carbonic anhydrase inhibitors, local anesthetics, anti-inflammatory, anthelmintic, anticonvulsant, hypoglycemic activity. The studies showed that microbial infection induces inflammation through immune cell recruitment. Inflammatory pain during infection has been thought to be triggered by the action of immune-derived proteins (for example, cytokines and growth factors), lipids (for example, prostaglandins) and other mediators such as amines, potassium and protons on receptors expressed by nociceptors. Keeping these in mind, our present study was designed to synthesize some 4-(3-methyl-1H-pyrazol-4-yl)-1-phenylazetidin-2-one moieties by cyclization of unsymmetrical imines in the presence of chloro acetyl chloride and a base catalyst to create potent compounds. These compounds might have the potency to overcome associated problems of microbial infection and pain.

Results and Discussion

The Schiff bases 5a-j were obtained from the reaction between the electrophilic carbon atom of 3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde and the nucleophilic nitrogen atom of substituted aromatic amines. The intermolecular [2+2] cyclization of unsymmetrical imines resulted in the formation of azetidine-2-one derivatives 6a-j (Scheme 1). The reactions occurred in the presence of triethylamine as the base catalyst. Tertiary amine counts balancing between nucleophilic character and basicity, which were considered as the key factor for efficient completion of the reaction. The substitutions on aromatic amine have great significance on yield value. The substitution with electron donating group at para and meta position of the ring increases the percentage yield whereas it decreases in case of ortho substitution due to steric effect.


The physical and analytical data were recorded for the synthesized compounds. The formation of desired compounds was established from elemental analysis (CHN analysis) and by correlating the structural characteristics. To provide the correct assignment of structure, spectral analysis was performed. During previous investigations by NMR techniques, it was concluded that aldimines exist completely in *E* configuration. It was also reported that compounds which have C=N double bond prefer *E* geometrical isomer in DMSO-*d<sub>6</sub> and *Z* isomer can be preferred in less polar solvents. The compounds 5a-j under investigation were soluble in DMSO-*d<sub>6</sub>. Similarly δ value at 7.75-7.39 (s, CH<sup>⁻</sup>=N) and 160.1-165.1 (13CH=N) indicated *E* conformation for respective imines. The IR peaks in KBr pellets gave bands at 1755.67-1690.61, 1681.67-1624.73, 1321.33-1210.33 and 1280.11-1150.11 cm⁻¹ indicating presence of C=O, C=N, C-N and C-C groups in the synthesized compounds. The characteristic ¹H NMR peaks at δ 4.85-4.81, 3.49-3.41 and 3.24-3.15 were indicative of the presence of azetidine-2-one nucleus in compounds 6a-j respectively. The ¹³C NMR spectral data also supported the proposed structures by showing characteristic peaks. From the mass spectra, it was found that primary molecular ion peaks complied with the calculated molecular mass of the synthesized compounds.

It has been reported that pyrazoline<sup>8-10</sup> and 2-azetidinones<sup>2</sup> possess analgesic, anti-inflammatory and antimicrobial activities. Microbial infections produce pain by unknown molecular mechanisms, although they are presumed to be secondary to immune activation. Bacteria induce calcium flux and action potentials in nociceptor neurons *via* N-formylated peptides and the pore-forming toxin α-haemolysin, through distinct mechanisms. Specific ablation of Nav1.8-lineage neurons, which include nociceptors, abrogated pain during bacterial infection and concurrently increased local immune infiltration and lymphadenopathy of the draining lymph node. Thus, bacterial pathogens produce pain by directly activating sensory neurons that modulate inflammation, an unsuspected role for the nervous system in host-pathogen interactions<sup>11</sup>. Based on this fact, the present study was aimed to investigate the antimicrobial and antinociceptive property of the some novel pyrazoline derivative, where beta-lactam rings are present. The antimicrobial activity was determined by paper disc diffusion technique against different strains of bacteria and fungi at 100 µg, 150 µg, and 250 µg/mL as shown in the Table I. From the antimicrobial data (zone of inhibition), it was found that compounds 6a, 6b, 6d, 6f, and 6j show greater antimicrobial activity in comparison to standards, whereas compound 6e and 6i show
moderate activity. The compounds were able to exhibit antibacterial activity probably by inhibition of cell wall synthesis due to the presence of beta-lactam rings but associated antifungal activity might be due to azole nucleus by inhibiting ergosterol biosynthesis.

The analgesic activity of the synthesized compounds 7a-j were determined by the acetic acid-induced writhing tests in mouse (Table II), tail flick (Table III), tail immersion (Table IV) and by the formalin-induced pain method (Table V). It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists and non-thermal tests to opioid κ-agonists. Aspirin produces analgesia through inhibition of prostaglandin synthesis. The abdominal contraction response induced by acetic acid is used for peripherally acting analgesics and such a response was thought to involve local peritoneal receptors. The tail immersion and tail flick tests caused a profound and dose-related analgesia in the treated mice, which are behavioral methods that have been developed to study nociception in animals. The data generated in the present study suggests that the involvement of both κ and μ opioid receptor are distinct in the analgesic activity of compounds. The formalin-induced pain as an experimental model of analgesia is useful for elucidation of the mechanism of pain and analgesia. Drugs that act centrally, such as the narcotics, inhibit both phases of formalin-induced pain while peripherally acting drugs can only inhibit the late phase.

From the results it was found that compounds 6a, 6b, 6d, 6h, and 6j show more potent analgesic activity in comparison to standards (aspirin and morphine sulphate) which might be due to the attachment of groups at para position on phenyl ring, whereas bi-substitution at para and meta substitution in compounds 6d, 6f result in moderate activity in comparison to standard drugs. From the above discussion, it was confirmed that the presence of azetidine-2-one nucleus along with pyrazole nucleus increases the potency of compounds. However, in particular, efficacy depends on substitution at phenyl ring, which was attached to the azetidine-2-one nucleus. Compounds with para substitution were potent, whereas compounds bi-substituted at para and meta position show moderate activity. The ortho-substituted compounds could not show significant activity. It was also observed that electron-
withdrawing substituents at para or meta position decreased the potency of the compounds.

Material and Method

All the chemicals used for the synthesis were of reagent grade of Sigma-Aldrich and Merck Laboratory. The solvents were purified by standard laboratory procedure and free from atmospheric oxygen. The melting points of synthesized compounds were determined by the open capillary method and are not corrected. The IR spectra were recorded in KBr pellets on a Shimadzu 8201 PC FTIR spectrophotometer. Both $^1$H and $^{13}$C NMR were observed in DMSO-$d_6$ by using Bruker 500 MHz NMR spectrometers, where TMS was used as the internal standard. Mass of each compound was analyzed by the ESI-mass method using Thermo Finnigan mass spectrophotometer. Elemental analysis were carried out using Vario (GmbH) EL III Elemental Analyzer. TLC was performed in the pre-coated plastic sheet of silica gel g/UV-254 of 0.2 mm thickness.

Experimental Section

Procedure for synthesis of 3-methyl-1, 2-dihydropyrazol-5-one, 3

One mol equivalent hydrazine hydrate was taken in a 500 mL beaker. To that 1 mol equivalent of ethyl acetoacetate was also added and the mixture allowed to warm at 100°C on the water bath for 3 h. Cooling
of the mixture to RT resulted in the formation of solid crystals. Washing was carried out with ether followed by purification by recrystallization from hot ethanol\textsuperscript{17}. Yield 93\%, m.p.143-44\degree C. IR (KBr): 3380.14 (N-H, Str); 1780.14 (=CO, Str); 1341.22 cm\textsuperscript{-1} (-CH\textsubscript{3}, str);

\textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}, 500 MHz): \(\delta\) 8.11 (s, 1H, -NH of pyrazole), 5.18 (s, 1H, Ar-H of Pyrazole), 2.15 (s, 1H, -CH\textsubscript{3} of pyrazole).

\textsuperscript{13}C NMR (DMSO-\textit{d}\textsubscript{6}): m/z 110.03 (M+).

Procedure for synthesis of 3-methyl-1H-pyrazole-4-carbaldehyde, 4

1 Mol of 3-methyl-1, 2-dihydropyrazol-5-one 3 and 3 mol of DMF along with 1 mol of POCl\textsubscript{3} were mixed together. During the addition of POCl\textsubscript{3}, the temperature of reaction mixture was maintained at 0-5\degree C. After completion of addition, the mixture was refluxed for 6 h, and then basified with sodium hydroxide solution. The reaction mixture was cooled overnight. After addition of crushed ice, the crystalline product was filtered and purified by recrystallization from hot ethanol\textsuperscript{18}. Yield 83\%, m.p.166-67\degree C. IR (KBr): 3326.14 (N-H, Str); 2815.14 (-CHO, Str); 1341.22 cm\textsuperscript{-1} (-CH\textsubscript{3}, str);

\textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}, 500 MHz): \(\delta\) 10.11 (s, 1H, -NH of pyrazole), 8.52 (s, 1H, -CHO of pyrazole), 7.82 (s, 1H, Ar-H of pyrazole), 2.79 (s, 3H, -CH\textsubscript{3} of pyrazole); 1\textsuperscript{3}C NMR (DMSO-\textit{d}\textsubscript{6}): \(\delta\) 184.1, 145.4, 141.4, 111.1, 13.6; ESI-MS: m/z 110.03 (M+).
Synthesis of N-((3-methyl-1H-pyrazol-4-yl)methylene) benzenamine, 5a-j

The reaction mixture of 2 g of 3-methyl-1H-pyrazole-4-carbaldehyde 4 in 50 mL of ethanol was stirred for 30 min under nitrogen atmosphere. 1 mL glacial acetic acid was added, followed by addition of 2 g of substituted aromatic amines and refluxed for 1 h. On addition of crushed ice, the crystalline product was filtered, dried at RT and then purified by recrystallization from hot ethanol.

4-Chloro-N-((3-methyl-1H-pyrazol-4-yl) methyl) benzenamine, 5a: Yield 98%. m.p.91-94°C. IR (KBr): 2927.34 (-CH, str), 1435.14 (-CH3, def), 1681.67 (C=N, str), 764.21 cm⁻¹ (C-Cl, str); ¹H NMR (DMSO-d6): δ 13.7 (s, 1H, -NH), 7.52 (s, 1H, -N=CH-), 7.66-7.64 (d, 1H, J = 10 Hz, Ar-H), 7.15 (s, 1H, Ar-H), 6.8-6.78 (d, 2H, J = 10 Hz, Ar-H), 2.79 (s, 3H, -CH3); ¹³C NMR (DMSO-d6): δ 160.1, 147.1, 145.5, 143.3, 142.2, 135.7, 133.2, 126.7, 126.7, 105, 11; ESI-MS: m/z 253.02 (M+). Anal. Calcd for C11H8Cl2N2: C, 51.99; H, 3.52; N, 16.51%.

4-Chloro-N-((3-methyl-1H-pyrazol-4-yl) methyl) benzenamine, 5a: Yield 96%. m.p.88-88°C. IR (KBr): 2927.14 (-CH3, str); 1513.02 (C-NO2, str), 1436.24 (-CH3, def), 1680.67 (C=N, str), 764.21 cm⁻¹ (C-Cl, str); ¹H NMR (DMSO-d6): δ 13.7 (s, 1H, -NH), 7.49 (s, 1H, -N=CH-), 7.21-7.19 (d, 2H, J = 10 Hz, Ar-H), 7.11-7.09 (d, 1H, J = 10Hz, Ar-H), 2.39 (s, 3H, -CH3); ¹³C NMR (DMSO-d6): δ 165.1, 146.2, 143.5, 142.2, 135.7, 133.2, 132.2, 121.7, 121.7, 105, 11; ESI-MS: m/z 264.04 (M+). Anal. Calcd for C11H8ClN3O2: C, 49.92; H, 3.43; N, 21.17. Found: C, 49.89; H, 3.39; N, 21.1%.

3. 4-Difluoro-N-((3-methyl-1H-pyrazol-4-yl) methyl)benzenamine, 5f: Yield 98%. m.p.82-83°C. IR (KBr): 2924.14 (-CH3, str), 1433.24 (-CH3, def), 1680.67 (C=N, str), 1111.21 cm⁻¹ (C-F, str); ¹H NMR (DMSO-d6): δ 13.7 (s, 1H, -NH), 7.65 (s, 1H,-N=CH-), 7.45 (s, 1H, Ar-H), 7.21-7.19 (d, 2H, J = 10 Hz, Ar-H), 7.11-7.09 (d, 1H, J = 10Hz, Ar-H), 2.49 (s, 3H, -CH3); ¹³C NMR (DMSO-d6): δ 164.1, 148.2, 143.5, 140.2, 136.7, 133.2, 133.2, 126.7, 126.7, 105, 11.5; ESI-MS: m/z 566.01 (M+). Anal. Calcd for C13H8F2N3O2: C, 59.22; H, 3.90; N, 14.80. Found: C, 59.49; H, 3.86; N, 21.1%.

2-Fluoro-N-((3-methyl-1H-pyrazol-4-yl) methyl)benzenamine, 5g: Yield 69%. m.p.80-81°C. IR (KBr): 2927.14 (-CH3, str), 1534.34 (C-NO2, str) 1433.24 (-CH3, def), 1680.67 (C=N, str), 1160.21 cm⁻¹ (C-F, str); ¹H NMR (DMSO-d6): δ 13.5 (s, 1H, -NH), 8.2-8.18 (d, 2H, J = 10 Hz, Ar-H), 7.75 (s, 1H,-N=CH-), 7.45 (s, 1H, Ar-H), 7.21-7.19 (d, 2H, J = 10 Hz, Ar-H), 2.49 (s, 3H, -CH3); ¹³C NMR (DMSO-d6): δ 160.1, 145.5, 143.3, 141.4, 136.2, 123.2, 118.7, 117.2, 105, 60.1, 11.5; ESI-MS: m/z 248.07 (M+). Anal. Calcd for C13H8F2N3O2: C, 59.23; H, 3.65; N, 22.57. Found: C, 53.11; H, 3.56; N, 22.41%.
Synthesis of 4-(3-methyl-1H-pyrazol-4-yl)-1-phenylazetidin-2-one, 6a-j

N-(3-methyl-1H-pyrazol-4-yl)benzylamine 6a-j (2 g) was placed in 100 mL round bottom flask and stirred under nitrogen atmosphere followed by addition of 1,4-dioxane (20 mL) as solvent. Triethylamine was used as base catalyst (0.5 mL) and the mixture stirred for 1 h. The resulting solutions were cooled at 0-5°C and 2 mL of chloroacetyl chloride was added. During the addition of chloroacetyl chloride, the internal temperature was not allowed to rise over 8°C. After refluxing the mixture for 8 h, the mixture was left to cool to RT overnight. The mixtures were neutralized with 5% sodium bicarbonate solution after addition of ice. The isolated compounds were washed with cold water and then with 50% ethyl acetate in petroleum ether after which the product was dried. The purification of compounds was carried out by column chromatography over silica gel (230 mesh) using benzene and chloroform (8:2) as the mobile phase.

1-(4-Chlorophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6a: Yield 68%. m.p.111-12°C. IR (KBr): 2950.34 (-CH3, str), 1755.67 (C=O, str), 1465.14 (-CH3, def), 1210.33 (C-N, str), 1233 (C-C, str), 764.21 cm\(^{-1}\) (C-Cl, str); \(^1\)H NMR (DMSO-d6): δ 13.5 (s, 1H, -NH), 8.21-7.19 (d, 2H, J = 10 Hz, Ar-H), 7.15 (s, 1H, Ar-H), 6.76-6.87 (d, 2H, J = 10Hz, Ar-H), 4.85 (s, 1H, CH-N), 3.49 (s, 1H, CH2-C=O), 3.24 (s, 1H, CH2-C=O), 2.79 (s, 3H, -CH3); \(^13\)C NMR (DMSO-d6): δ 170.9, 147.1, 145.5, 141.4, 132.8, 130.2, 123.7, 105.5, 45, 45, 11; ESI-MS: m/z 261.07 (M+). Anal. Calcd for C12H12ClN2O: C, 59.66; H, 4.62; N, 16.06. Found: C, 59.46; H, 4.49; N, 15.82%.

1-(4-Bromophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6b: Yield 66%. m.p.113-14°C. IR (KBr): 2951.24 (-CH3, str), 1690.61 (C=O, str), 1460.14 (-CH3, def), 1300.33 (C-N, str), 1180.11 (C-C, str), 664.21 cm\(^{-1}\) (C-Br, str); \(^1\)H NMR (DMSO-d6): δ 12.5(s, 1H,-NH), 7.21-7.19 (d, 2H, J = 10 Hz, Ar-H), 7.17 (s, 1H, Ar-H), 6.81-6.79 (d, 2H, J = 10Hz, Ar-H), 4.81 (s, 1H, CH-N), 3.47 (s, 1H, CH2-C=O), 3.22 (s, 1H, CH2-C=O), 2.70 (s, 3H, -CH3); \(^13\)C NMR (DMSO-d6): δ 170.7,147.2,145.1,141.3,132.8,129.2,129.2,123.7,105.7,45.2,45.2,11.3; ESI-MS: m/z 305.02 (M+). Anal. Calcd for C12H12BrN2O: C, 51.00; H, 3.95; N, 13.72. Found: C, 49.66; H, 3.59; N, 13.42%.

4-(3-Methyl-1H-pyrazol-4-yl)-1-(4-nitrophenyl)azetidin-2-one, 6c: Yield 76%. m.p.116-17°C. IR (KBr): 2949.24 (-CH3, str), 1700.61 (C=O, str), 1515.02 (C=O, str), 1375.14 (-CH3, def), 1311.33 (C-N, str), 1182.11 cm\(^{-1}\) (C-C, str); \(^1\)H NMR (DMSO-d6): δ 13.5 (s, 1H,-NH), 7.23-7.21 (d, 2H, J = 10 Hz, Ar-H), 7.19 (s, 1H, Ar-H), 6.80-6.78 (d, 2H, J = 10Hz, Ar-H), 4.83 (s, 1H, CH-N), 3.41 (s, 1H, CH2-C=O), 3.22 (s, 1H, CH2-C=O), 2.70 (s, 3H, -CH3); \(^13\)C NMR (DMSO-d6): δ 170.5,147.3,144.1,139.3,132.7,126.2,126.2,121.7,121.7,107.7,44.2,44.2,12.3; ESI-MS: m/z 272.09 (M+). Anal. Calcd for C13H12N4O3: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.00; H, 4.19; N, 20.32%.

1-(3,4-Dichlorophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6d: Yield 68%. m.p.118-19°C. IR (KBr): 2948.24 (-CH3, str), 1720.61 (C=O, str),
1471.14 (d, 2H, J = 10 Hz, Ar-H), 7.11-7.09 (d, 1H, J = 10Hz, Ar-H), 4.83 (s, 1H, -CH2-C=O), 2.70 (s, 3H, -CH3); 13C NMR (DMSO-d6): δ 170.3, 147.2, 140.1, 137.3, 132.7, 132.5, 123.2, 121.7, 121.7, 105.7, 47.2, 47.2, 11.3; ESI-MS: m/z 296.15 (M+). Anal. Caled for C13H11Cl2N2O: C: 52.72; H: 3.74; N: 14.19. Found: C: 52.32; H: 3.52; N: 13.71%.

1-(4-Chloro-3-nitrophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6e: Yield 63%. m.p.110-111°C. IR (KBr): 2948.24 (-CH2=C=O), 1720.61 (C=O, str), 1525.02 (C=NO2, str), 1471.14 (-CH3, def), 1321.33 (C-N, str), 1180.11 (C-C, str), 764.21 cm⁻¹ (C-Cl, str); 1H NMR (DMSO-d6): δ 12.3 (s, 1H, -NH), 7.35 (s, 1H, Ar-H), 7.72-7.18 (d, 2H, J = 10 Hz, Ar-H), 7.11-7.09 (d, 1H, J = 10Hz, Ar-H), 4.85 (s, 1H, CH2-C(O)-O), 2.69 (s, 3H, -CH3); 13C NMR (DMSO-d6): δ 168.0, 137.3, 132.8, 125.2, 125.2, 121.7, 121.7, 105.7, 47.2, 47.2, 11.3; ESI-MS: m/z 306.15 (M+). Anal. Caled for C13H11ClF2N3O: C: 56.95; H: 4.10; N: 14.23. Found: C: 56.52; H: 3.71; N: 13.89%.

1-(3,4-Difluorophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6f: Yield 63%. m.p.113-115°C. IR (KBr): 2948.24 (-CH2=C=O), 1720.61 (C=O, str), 1471.14 (-CH3, def), 1321.33 (C-N, str), 1201 (C-F, str), 1180.11 cm⁻¹ (C-C, str); 1H NMR (DMSO-d6): δ 12.37 (s, 1H, -NH), 7.32 (s, 1H, Ar-H), 7.23-7.21 (d, 2H, J = 10 Hz, Ar-H), 7.11-7.09 (d, 1H, J = 10Hz, Ar-H), 4.83 (s, 1H, CH2-N), 3.48 (s, 1H, CH2-C=O), 3.15 (s, 1H, CH2=C=O), 2.89 (s, 3H, -CH3); 13C NMR (DMSO-d6): δ 169.0, 146.5, 141.3, 138.8, 132.8, 127.2, 123.2, 121.7, 121.7, 105.7, 47.2, 47.2, 11.3; ESI-MS: m/z 263.15 (M+). Anal. Caled for C13H11F2N3O: C: 59.31; H: 4.21; N: 12.97. Found: C: 59.11; H: 4.12; N: 12.51%.

1-(2-Fluoro-5-nitrophenyl)-4-(3-methyl-1H-pyrazol-4-yl)azetidin-2-one, 6g: Yield 69%. m.p.80-81°C. IR (KBr): 2927.14 (-CH3, str), 1720.61 (C=O, str), 1534.34 (C=NO2, str), 1321.33(C-N, str), 1433.24 (-CH3, def), 1280.11(C-C, str), 1160.21 cm⁻¹ (C-F, str); 1H NMR (DMSO-d6): δ 13.5 (s, 1H, -NH), 8.2-8.18 (d, 2H, J = 10 Hz, Ar-H), 7.45 (s, 1H, Ar-H); 7.21-7.19 (d, 2H, J = 10Hz, Ar-H), 4.81 (s, 1H, CH2-N), 3.43 (s, 1H, CH2=C=O), 2.49 (s, 3H, -CH3); 13C NMR (DMSO-d6): δ 170.2, 149.5, 143.3, 136.8, 132.7, 123.2, 121.2, 119.7, 119.7, 107.7, 47.2, 47.2, 11.3; ESI-MS: m/z 290.08 (M+). Anal. Caled for C13H11F2N3O: C: 53.79; H: 3.82; N: 19.3. Found: C: 53.51; H: 3.56; N: 19.00%.

**4-(3-Methyl-1H-pyrazol-4-yl)-1-(4-(trifluoromethyl)phenyl)azetidin-2-one, 6h:** Yield 75%. m.p.123-24°C. IR (KBr): 2927.14 (-CH3, str), 1720.61 (C=O, str), 1433.24 (-CH3, def), 1150.11 (C-C, str), 1160.21 cm⁻¹ (C-F, str); 1H NMR (DMSO-d6): δ 13.7 (s, 1H, -NH), 7.21-7.18 (d, 2H, J = 15 Hz, Ar-H), 7.15 (s, 1H, Ar-H), 6.8-6.78 (d, 2H, J = 10Hz, Ar-H), 4.81 (s, 1H, CH2-N), 3.43 (s, 1H, CH2-C=O), 3.15 (s, 1H, CH2-C(O)-O), 2.69 (s, 3H, -CH3); 13C NMR (DMSO-d6): δ 170.3, 149.6, 147.1, 139.7, 133.7, 124.3, 123.7, 121.5, 120.3, 120.3, 107.7, 47.2, 47.2, 11.3; ESI-MS: m/z 257.07 (M+). Anal. Caled for C13H11F2N3O: C: 65.36; H: 5.88; N: 16.33. Found: C: 65.16; H: 5.52; N: 16.58%.

**Biological Activity**

**Test microorganism and medium**

For the determination of antimicrobial activity gram-positive bacteria _Staphylococcus aureus_ ATCC12600, _Bacillus subtilis_ ATCC 11775,
Enterobacter cloacae ATCC13047 and the fungi Candida albicans ATCC 90028, and Aspergillus niger ATCC1027 were used. Bacterial strains were cultured overnight at 37°C in LB-agar broth and fungal strains were cultured overnight at 30°C in cornmeal agar media. Test strains were suspended in nutrient agar to give the final density of 5×10^5 cf/mL.

Screening for antimicrobial activity (Zone of inhibition assay)
Antimicrobial activity of the compounds was determined by disc diffusion method. Sterilized 10% nutrient agar (20 mL) was poured into each sterile Petri dish after mixing the culture of micro-organism at a concentration of 150 µL and the plate allowed to solidify. Standard and test compounds were dissolved in DMSO to prepare a stock solution of 1000µg/mL. From the stock solution, the concentration of 100, 150, 200 µg/mL were made for amoxicillin, streptomycin, nystatin and test compounds respectively. Whatman filter paper was sterilized and dipped in test compounds, standards, and solvent control respectively. Discs were placed on agar plates and incubated at 37°C for 24 h for bacterial and 30°C for 24 h for fungi and the zone of inhibition was measured in millimeter. Each study was performed in triplicate and the average value is depicted in the table.

Animals
Albino mice of either sex weighing between 18-25 g were taken for analgesic activity. Animals were maintained under the standard environmental condition at temperature of 22±2°C and 45-50% relative humidity for 24 h each of dark and light cycle with proper diet. All the studies were carried out according to protocol approved by Institutional Animal Ethical Committee (IAEC) of Bansal College of Pharmacy (Reg. no-1252/ac/10/CPCSEA, Ref. no-BCP/IAEC/12/02).

Acute toxicity study
The acute oral toxicity study was carried out according to OECD guideline no 423 in albino mice. The doses were fixed at 10 mg/kg (p.o) to 100 mg/kg (p.o) and contain 5 animals in each group. The mortality and general behavior were under observation for 14 days. The test compounds were nontoxic in the dose of 50 mg/kg body weight.

Analgesic Activity
Writhing Tests
Mice of either sex with a weight between 20 and 25 g were used. 0.1 mL of a 0.6% solution of acetic acid was administered intraperitoneally to the mice. The animals consisted of 12 groups, 5 in each group. Group-1 was considered as control, Group-2 was used for morphine sulfate (5 mg/kg) as standard and group 3 to 12 were used for the test compounds. The mice were placed individually in glass beakers and 5 min was allowed to elapse. The mice were then observed for a period of 10 min and the number of writhes was recorded for each animal. The time period with the greatest percent of inhibition is considered the peak time. A dose range was reserved for interesting compounds or those which inhibit writhing to the extent of more than 70%. Compounds with less than 70% inhibition were considered to have minimal activity.

Haffner’s Tail Clip
Male albino mice with a weight between 18 and 25 g were used. The animals consisted of 12 groups with 5 in each group. Group-1 was considered as control, Group-2 was used for morphine sulfate (5 mg/kg) as standard and group 3 to 12 were used for test compounds. The test compounds were administered 15 min prior to testing. An artery clip was applied to the root of the tail approximately 1 cm from the body to induce pain. The animal quickly responded to this noxious stimulus by biting the clip. The reaction time of the test animals which were greater than the cut-off time was called a positive response and indicated the presence of analgesic activity. The length of time until response indicated the period of greatest activity after dosing.

Tail immersion Test
Albino mice of either sex 25-30 g were used for the study. The animals consisted of 12 groups 5 in each group. Group-1 was considered as control, group-2 was used for morphine sulfate (5 mg/kg) as standard drug and group-3 to 12 were used for the test compounds. The animals were allowed time for adopting to the environment before the study. The tails were marked 5 cm above and immersed in hot water kept at exactly 55°C. The tail withdrawal time (in sec) was noted down before and after.
administration of a drug. The cut off time was 10 sec and 1-5 sec for treated and untreated animals respectively. If tail withdrawal time was more than 6 sec, it indicated positive response.

**Formalin test**
Formalin test was carried out by the method of Hunskaar et al. The animals have consisted of 12 groups, 5 in each group. Group-1 was considered as control, Group-2 and 3 were used for aspirin (100 mg/kg) and morphine sulfate (5 mg/kg) as standard respectively and group-4 to 13 were used for the test compounds. After 30 min treatment of all tested compounds (15 min treatment for morphine), 20 µL of 2.5% formalin was injected subcutaneously in hind paw of rats. The time spent in licking the injected paw in the early phase (0–5 min) and late phase (15–30 min) was recorded.

**Statistical Analysis**
The results are shown in different tables as Mean ± SEM and comparison between standard and test compounds have been made by one-way ANOVA followed by Dunnett's test. Values of p ≤ 0.001 are considered as significant.

**Conflicts of interest**
There is no conflict of interest.

**Acknowledgement**
The authors thank IIMT College of Medical Sciences, Regional Institute of Pharmaceutical Sciences and Technology, Govt. of Tripura, AIRF, JNU, New Delhi and Arbro Lab, New Delhi.

**References**