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Synthesis and Antimicrobial Activity of New Pyridine Containing Substituted Phenyl Azetidine-2-One Derivatives

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Abstract

The most important nitrogen containing heterocycles of pyridine containing substituted phenyl azetidine-2-ones have found pharmacological application such as antibiotics and these compounds also have practical importance. The structure of the new derivatives was confirmed by the spectral data and elemental analyses. Out of five new derivatives, three were revealed mild to moderate activity compared with Streptomycin & Fluconazole as a reference standard. Among this new series, 3-chloro-1-(4-fluoro phenyl)/(4-chloro phenyl)-4-(pyridine-3-yl) azetidine-2-one **(4a & b)** were found most activity.

Keywords

Antibacterial, Antifungal, Azetidin-2-One, Pyridine

1. Introduction

Recently, our group newly synthesized derivatives of (4-substituted phenyl)-4-(pyridine-3-yl) azetidin-2-one **(4a-e)** which showed inhibitory activity against antibacterial and anti fungal. The several studies have been made to investigate the structure activity relation (SAR) of these synthesized derivatives of (4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)** showing several different activities.

Due to our experience of working with para position for generating antibacterial, and the role of para position in the "C" ring moiety of phenyl derivatives, we synthesized 10 compounds presenting substituents with different electronic properties (F, Cl, Br, CF_3, OCH_3).

Heterocyclic compounds include many of the biochemical materials essential to life. Many naturally occurring antibiotics are heterocyclic compounds. Mod-

ern society is dependent on synthetic heterocycles for us as drugs.

The newly synthesized derivatives of pyridine contain azetidine-2-one to identify the pyridine system as a group for the development of new antimicrobial agents such as pharmaceutically important pyridine derivatives including the tuberculostat, anti-AIDS, vasodilator used for treating urinary tract, analgesic, anti-inflammatory and an anesthetic [1]-[8].

Heterocycles possessing azetidin-2-one ring were found to show various types of biological activities such as anti-bacterial, anti-fungal [9] [10] [11], anti-convulsing [12] and antimicrobial [13] [14] [15].

2. Experimental

2.1. Materials and Methods

All the chemicals used in the present investigation were purchased from Sigma-Aldrich chemical company, Inc.USA. And used without further purification.

2.2. Instruments

Thin Layer Chromatography was performed on aluminium sheet of silica gel 60F254, E-Merk, Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected. IR spectra were measured using Nexus, 470-670-760 spectrophotometer FT IR, spectrometer spectrum 8400 s, using KBr pellets for solid compounds and neat liquid compounds between KBr plates. NMR spectra were measured at 24˚C on a Joel 400 MHz spectrometer using deuterium locking $^{13}C(^{1}H)$-NMR observation frequency 75 MHz, ^{1}H-NMR,observation frequencies, 400 MHz.

3. Microbial Assay (Agar Well Diffusion Method)

Nutrient agar (Bacto-beef extract 2.5 g; peptone 5 g; sodium chloride 6 g; and distilled water 1000 mL) was used for bacteria growth and Asthana and Hawker's (Glucose 6 gr; potassium nitrate 4 g; KH_2PO_4 2.25 g; hydrated magnesium sulphate 1.25 g and distilled water 1000 mL) media which are used for fungi growth. The media chemicals present study purchased from Merck**.** The standard bacterial and fungal strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), and Chandigarh, India. The pure bacterial cultures were maintained on Nutrient Agar Media (NAM) for bacterial and fungal culture on potato dextrose agar (PDA).

These newly synthesized 3-chloro-1-(4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)** derivatives were performed by antimicrobial activity according to Agar well diffusion method is preferred to be used in this study since it was found to be better than the disc diffusion method suggested by Parekh *et al.* [16] and also recommended by the National Committee for Clinical Laboratory (NCCLS, 1993). The newly synthesized compounds were used at the con-

centration of 2 mg/mL dimethyl sulfoxide as a solvent [17]. A standardized 1 to 2×10^7 cfu/mL 0.5 MC Farland standard was introduced onto the surface of a sterile agar plate and evenly distributed inoculums by using a sterile glass spreader. Simultaneously, 6 mm wells were cut from the plate using a sterile cork borer. 50 µl solution at a concentration of 2.5 mg/mL of the compounds was introduced into well and incubated at 35˚C for 24 hours, the inhibition zones were measured with a ruler and compared with the control well containing only 1 mg/mL in dimethyl sulfoxide of streptomycin as the standard. The antifungal assay of the compounds was carried out by agar well diffusion method as described by Magaldi *et al.* [18] 6 mm diameter open wells punched with a sterile cork borer on cultured plates with test organisms before incubated. The wells were filled with 50 µl solution at a concentration of 2.5 mg/mL of the compounds at 32˚C. After 72 hours, the zones of inhibition were measured and compared with those of the control dimethyl sulfoxide and the standard Fluconazole at a concentration of 1 mg/mL.

Antibacterial assay

The antibacterial activity of 3-chloro-1-(4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)** were screened against the *Staphylococcus aureus* (*MTCC*-3160) and *Bacillus subtilis* (*MTCC*-441) (gram + ve) and *Escherichia coli* (MTCC-1652) organisms. Here Streptomycin is tested as reference compound to compare the activity.

Antifungal assay

Antifungal activity of 3-chloro-1-(4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)** were screened against *Pseudomonas aeruginosa* (MTCC-467) (gram-ve), *Aspergillus niger* (MTCC-282) and *Penicillium rubrum*, our isolate. Here Fluconazole is tested as reference compound to compare the activity. The anti-bcterial and anti-fungal activity of **(4a-e)** were shown in Table 1.

Table 1. Anti-bcterial and anti-fungal activity 3-chloro-1-(4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)**.

Entry	COMP	R	Zone of inhibition (mm) 50 µL for well					
			[1]Anti-bacterial activity			[2]Anti-fungal activity		
			S.a	B.s	E.c	P.a	A.n	P.r
1	4a	*F	17	18	16	17	16	15
2	4b	*Cl	18	17	14	16	14	14
3	4c	Br	15	13	10	11	13	12
4	4d	CF_3	12	13	10	07	09	05
5	4e	OCH_3	11	07	09	10	04	06
Std	Streptomycin		25	20	20	23	-	-
Std	Fluconazole		-	-	-	-	18	15

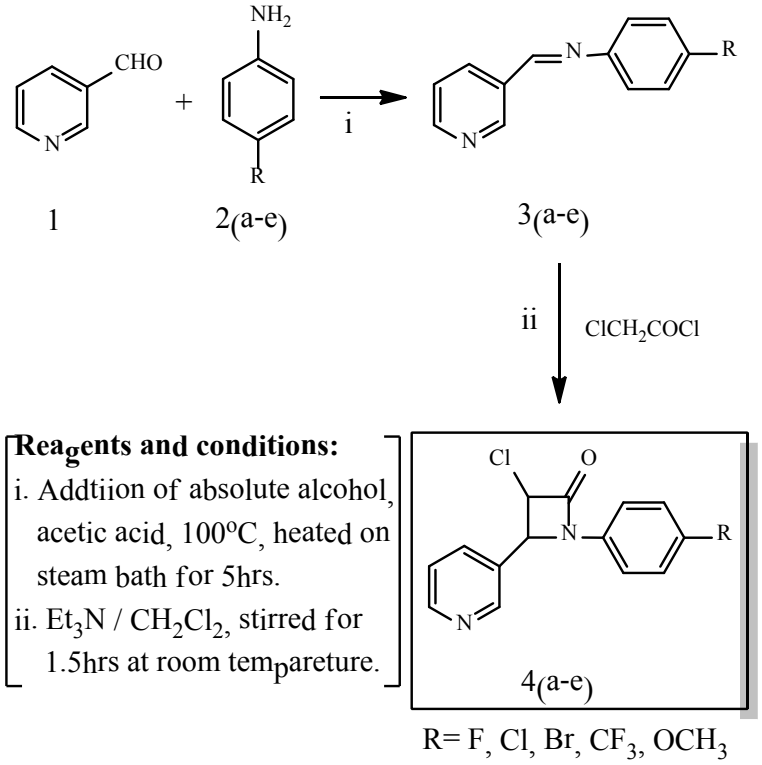
[1]S.a: *Staphylococcus aureus*, B.s: *Bacillus subtilis*, E.c: *Escherichia coli*, P.a: *Pseudomonas aeruginosa*. [2]A.n: *Aspergillus niger*, P.r: *Penicillium rubrum*. *Indicates more activity.

4. Results and Discussion

Scheme 1 shows the synthetic route for preparing of (4-substituted phenyl)-4-(pyridin-3-yl) azetidin-2-one **(4a-e)** group by reaction mixture of schiff base **(3a-e)** with monochloro acetyl chloride. Schif base obtained by condensation of nicotinaldehyde and substituted aniline. The anti-bacterial and anti-fungal activities of **(4a-e)** were shown in Table 1. The compounds **(4a-e)** were tested for antimicrobial activity. Amongst all the tested compounds **4a** & **4b** exhibited higher activity than other which may be due to the presence of electron withdrawing substituents increases the activity when compared with electron donating substituents.

4.1. General Method for Preparation of Schiff Bases 3(a-e)

The reaction mixture of nicotinaldehyde **(1)** (1.00 mmol) and 4-fluoroaniline **(2a)**/4-chloro/4-bromo/4-(trifluoromethyl)/4-methoxy-aniline **(2b-e)** (1.00 mmol) were refluxed in ethanol (20 ml) for 1-5 hours. After cooling, the products of Schiff bases **(3a-e)** were dissolved by adding water and the crude product **3a** or **(3b-e)** was precipitated after the reaction mixture was neutralized with potassium carbonate. The precipitate was filtered, washed with water and recrystallised from aqueous EtOH and dried under vacuum over night. **3a:** 71% yield, mp 161˚C, **3b:** 70% yield, mp 158˚C, **3c:** 68% yield, mp 160˚C, **3d:** 74% yield, mp 168˚C, **3e:** 70% yield, mp 162˚C.



Scheme 1. Synthetic pathway of 3-chloro-1-(4-substituted phenyl)-4-(pyridin-3-yl)azetidin-2-one **(4a-e)**.

4.2. General Method for Synthesis of 3-Chloro-1-(4-Substituted Phenyl)-4-(Pyridin-3-Yl) Azetidin-2-One (4a-e)

The mixture of corresponding Schiff bases **(3a/b/c/d or e)** (10 mmol) with appropriate monochloroacetyl chloride (10 mmol) in presence of triethyl amine (2 mmol) in dichloromethane (20 ml) at room temperature. The reaction mixture was boiled under reflux with stirring for 1.5 hours and left at room temperature. At the end of the reaction was neutralized with excess of $NaHCO_3$. The collected precipitate washed with water, dried in vacuum, purified by thin layer chromatography using cyclohexane and ethyl acetate (9:1) solvent mixture as a mobile phase. Pour the content on crushed ice. The dried product was azetidine-2-one **(4a/b/c/d or e)** recrystallized with absolute alchohol.

The structures of these newly synthesized compounds of **(4a/b/c/d and e)** were established by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and microanalytical data.

5. Experimental Section

The IR, ^{1}HNMR, ^{13}CNMR and Mass spectral data of the synthesized compounds 4a/b/c/d and e:

1) 3-chloro-1-(4-fluoro phenyl)-4-(pyridine-3-yl) azetidine-2-one (4a) MF: $C_{14}H_{10}ClFN_2O$. The product was synthesized according to general procedure **4.2** to afford the target compound as a white solid 0.52 g (68%).

IR (KBr 4000 - 400 cm^{-1}): 3052 (stretching of Ar-H), 2895 (CH), 1690 (C=O of azetidinone), 1556 (C-N) and 720 cm^{-1} (C-Cl).

^{1}H-NMR (400 MHz, DMSO-d6): δ_{PPM} 5.16 (d, 1H, CH), 5.44 (d, 1H, CH), 7.25 - 7.29 (m, 4H, of C_6H_5 J = 7.26), 7.38 (q, 1H, CH of C_6H_4N J = 8.59), 7.86 (d, 1H, CH of C_6H_4N), 8.45 (d, 1H, CH of C_6H_4N) and 8.59 (s, 1H, CH of C_6H_4N).

^{13}C-NMR (75 MHz, DMSO-d6): δ_{PPM} 162.9, 162.2, 148.4, 146.9, 140.8, 135.5, 133.1, 123.4, 123.2, 123.2, 115.7, 115.7, 68.1 & 62.0 corresponding to C_1 to C_{14} respectively. MS 276.05.

2) 3-chloro-1-(4-chloro phenyl)-4-(pyridine-3-yl) azetidine-2-one (4b) MF: $C_{14}H_{10}Cl_2N_2O$. The product was synthesized according to general procedure **4.2** to afford the target compound as a yellow precipitate 0.48 g (66%).

IR (KBr 4000 - 400 cm^{-1}): 3055 (stretching of Ar-H), 2890 (CH), 1690 (C = O of azetidinone), 1550 (C-N) and 720 cm^{-1} (C-Cl).

^{1}H-NMR (400 MHz, DMSO-d6): δ_{PPM} 5.16 (d, 1H, CH), 5.44 (d, 1H, CH), 7.34 - 7.54 (m, 4H, of C_6H_5 J = 7.26), 7.38 (q, 1H, CH of C_6H_4N J = 8.59), 7.86 (d, 1H, CH of C_6H_4N), 8.45 (d, 1H, CH of C_6H_4N) and 8.59 (s, 1H, CH of C_6H_4N).

^{13}C-NMR (75 MHz, DMSO-d6): δ_{PPM} 162.2, 148.4, 146.9, 140.8, 137.6, 133.5, 133.3, 129.0, 129.0, 125.6, 125.6, 123.4, 68.1 & 62.0 corresponding to C_1 to C_{14} respectively. MS 292.02.

3) 1-(4-bromo phenyl)-3-chloro-4-(pyridine-3-yl) azetidin-2-one (4c) MF: $C_{14}H_{10}BrClN_2O$. The product was synthesized according to general procedure **4.2** to afford the target compound as a yellow solid 0.55 g (64%).

IR (KBr 4000 - 400 cm^{-1}): 3052 (stretching of Ar-H), 2888 (CH), 1696 (C = O of azetidinone), 1555 (C-N) and 720 cm^{-1}(C-Cl).

^{1}H-NMR (400 MHz, DMSO-d6): δ_{PPM} 5.16 (d, 1H, CH), 5.44 (d, 1H, CH), 7.38 (q, 1H, CH of C_6H_4N J = 8.59), 7.86 (d, 1H, CH of C_6H_4N), 8.13-8.20 (m, 4H, of C_6H_5 J = 7.26), 8.45 (d, 1H, CH of C_6H_4N) and 8.59 (s, 1H, CH of C_6H_4N).

^{13}C-NMR (75 MHz, DMSO-d6): δ_{PPM} 162.2, 148.4, 146.9, 140.8, 138.5, 136.7, 136.7, 133.5, 131.8, 131.8, 123.4, 122.3, 68.1 & 62.0 corresponding to C_1 to C_{14} respectively. MS 337.96.

4) 3-chloro-4-(pyridine-3-yl)-1-(4-(trifluoromethyl) phenyl) azetidine-2-one (4d) MF: $C_{15}H_{10}ClF_3N_2O$. The product was synthesized according to general procedure **4.2** to afford the target compound as a yellow precipitate 0.60 g (70%).

IR (KBr 4000 - 400 cm^{-1}): 3058 (stretching of Ar-H), 2890 (CH), 1695 (C = O of azetidinone), 1561 (C-N) and 720 cm^{-1}(C-Cl).

^{1}H-NMR (400 MHz, DMSO-d6): δ_{PPM} 5.16 (d, 1H, CH), 5.44 (d, 1H, CH), 6.85 - 7.67 (m, 4H, of C_6H_5 J = 7.26), 7.38 (q, 1H, CH of C_6H_4N J = 8.59), 7.86 (d, 1H, CH of C_6H_4N), 8.45 (d, 1H, CH of C_6H_4N) and 8.59 (s, 1H, CH of C_6H_4N).

^{13}C-NMR (75 MHz, DMSO-d6): δ_{PPM} 162.2, 148.4, 146.9, 142.8, 140.8, 133.8, 133.8, 133.5, 132.1, 125.3, 125.3, 124.1, 123.4, 68.1 & 62.0 corresponding to C_1 to C_{15} respectively. MS 326.04.

5) 3-chloro-1-(4-methoxy phenyl)-4-(pyridine-3-yl) azetidine-2-one (4e) MF: $C_{15}H_{13}ClN_2O_2$. The product was synthesized according to general procedure **4.2** to afford the target compound as a yellow precipitate 0.66g (74%).

IR (KBr 4000 - 400 cm^{-1}): 3065 (stretching of Ar-H), 2980 (CH), 1692 (C = O of azetidinone), 1560 (C-N) and 720 cm^{-1} (C-Cl).

^{1}H-NMR (400 MHz, DMSO-d6): δ_{PPM} 3.83 (s, 3H, CH_3), 5.16 (d, 1H, CH), 5.44 (d, 1H, CH), 7.04 - 7.38 (m, 4H, of C_6H_5 J = 7.26), 7.38 (q, 1H, CH of C_6H_4N J = 8.59), 7.86 (d, 1H, CH of C_6H_4N), 8.45 (d, 1H, CH of C_6H_4N) and 8.59 (s, 1H, CH of C_6H_4N).

^{13}C-NMR (75 MHz, DMSO-d6): δ_{PPM} 162.2, 158.9, 148.4, 146.9, 140.8, 133.5, 131.8, 123.4, 119.0, 119.0, 114.5, 114.5, 68.1, 62.0 & 55.8 corresponding to C_1 to C_{15} respectively. MS 288.07.

6) The microanalytical data and m.pts. Data are listed in Table 2, for the synthesized compounds (4a/b/c/d and e).

Table 2. Microanalytical data and M.P. data for the synthesized compounds **4a/b/c/d & e**.

Comp No	R	MW	Mp (˚C)	Yield (%)	Found (Calc) (%)		
					C	H	N
4a	F	276.69	154 - 156	68	60.70 (60.77)	3.59 (3.64)	10.05 (10.12)
4b	Cl	293.02	150 - 152	66	57.29 (57.36)	3.39 (3.44)	9.49 (9.56)
4c	Br	335.97	160 - 163	64	49.74 (49.81)	2.94 (2.99)	8.23 (8.30)
4d	CF_3	326.70	168 - 170	70	55.08 (55.15)	3.04 (3.09)	8.50 (8.57)
4e	OCH_3	288.73	162 - 164	74	62.33 (62.40)	4.49 (4.54)	9.63 (9.70)

6. Conclusion

In conclusion, we have demonstrated the synthesis of a series of novel pyridine containing substituted phenyl azetidine-2-one derivatives of **(4a-e)** involving condensation of nicotinaldehyde and 4-substituted anilines via Schiff base intermediates. Some of these compounds may serve as good pharmacological activities.

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