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SYNTHESIS OF SOME NEW 4-OXO-AZETIDINE DERIVATIVES FROM SCHIFF BASE DERIVATIVES CONTAINING PYRIDINE MOIETY AS ANTIMICROBIAL AGENTS

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ABSTRACT

A new series of 4-oxo-azetidine (4a-0) from Schiff base derivatives containing pyridine moiety are synthesized. The Schiff's base on treatment with various aromatic aldehydes in ethanol in presence of a few drops of glacial acetic acid to afford Schiff base derivatives (3a-o).The Schiff base derivatives on treatment with dioxane and triethylamine afforded targeted compounds (4a-0).The structure of all synthesized compounds has been established on the basis of their spectral (IR, H^1&C^{13} NMR and Mass) and analytical data. The purity of the compounds was confirmed by TLC. All the synthesized compounds were evaluated for their in vitro antimicrobial activity against fungi, Gram positive and Gram negative bacteria. Some of the compounds exhibited moderate activity when compared with reference standard Ciprofloxacin and Miconazole.

KEYWORDS: **4-oxo-azetidines, Schiff base, Pyridine and antimicrobial activity.**

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INTRODUCTION

Schiff base belong to a widely used group of organic intermediates for production of speciality chemicals like pharmaceutical or rubber additives[1] and as amino protective groups in organic syntheses[2-3]. They also have used in medicinal and polymer chemistry[4]. Azetidinone skeleton is well established as the pharmacophore of β-lactam antibiotics. β-lactam antibiotics as the most widely employed class of antibiotics[5]. The important and structural diversity of biologically active β-lactam antibiotics led to the development of many novel methods for the construction of appropriately substituted azetidine with attendant control of functional group and stereochemistry. Azetidine derivatives are reported to show a variety of antimicrobial[6-8],antitubercular[9], anticonvulsant[10] and anti-inflammatory activities[11]. So we decided to do syntheses of a new series 4-oxo-azetidine derivatives with several substitutions.

EXPERIMENTAL WORK

The melting points were determined by open capillaries on an electric melting point apparatus and are uncorrected. The purity of the compound was confirmed by TLC silica gel precoated plates (0.25mm,60 F254,MERCK) using ethyl acetate and ethanol (2:3). The IR Spectra were recorded on Perkin Elmer BXF1, FTIR Spectrophotometer using KBr disc method H^1 and C^{13} Spectra were recorded on Bruker AMX,400MHz and using TMS as an internal standard. Chemical shifts are described as singlet (s), doublet (d), broad(b) and multiplet (m), FAB Mass spectra were recorded on angilent 1100 ESI-MASS (Turbo spray) spectrometer. Elemental analysis was carried out using Carlo Erba 1108 Elemental Analyser.

MATERIALS AND METHODS

A mixture of 0.1M (15.1gm) of ethyl nicotinate and 0.2M (10gm) of hydrazine hydrate with 50% ethanol taken in round bottomed flask and then refluxed for 16hrs.Then the reaction mixture concentrated to half volume and poured it into the crushed ice. The reaction was identified by TLC using silica gel (100-200 #, Merck) and ethyl acetate: ethanol (2:3) as mobile phase. The white precipitate was separated and recrystallised from ethanol. It was confirmed by spectral data; IR (Cm^{-1}); N-H, C=O and C=N observed at 3325, 1661 and 1587 respectively, H^1NMR (δ ppm); N-H singlet proton at 9.58, NH_2 singlet, two protons at 4.581and the pyridine hydrogens observed in between 7.42 – 8.98.

SYNTHESES OF COMPOUNDS (3a-o)

All the compounds were prepared by using the procedure of Praveen Kumar et al[12].A mixture of compound 2 (1.47gm, 0.001M) and substituted benzaldehydes (0.001M) were dissolved in absolute ethanol (40ml) by the addition of a few drops of glacial acetic acid and refluxed for 6hrs. The reaction was identified by TLC using silica gel (100-200 #, Merck) and ethyl acetate: ethanol (2:3) as mobile phase. Then the reaction mixture poured into a ice cold water.

SYNTHESES OF COMPOUNDS (4a-o)

A mixture of compound (3a-o) (0.01 mole) in dioxane (10ml) and triethyl amine (0.025 mole) was added to chloroacetylchloride (0.025 mole) drop wise at 5- 10^0 C. Then the reaction mixture was poured into crushed ice. The reaction was identified by TLC using silica gel (100-200 #, Merck) and ethyl acetate: ethanol (2:3) as mobile phase. .The solid separated was dried and it is crystallized from ethanol.

4a: N- (3-chloro-2(4-fluro phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 220-222^{0}C; yield (%): 56; Rf: 0.38; Molecular formula: $C_{15}H_{11}N_3O_2ClF$; Molecular weight: 319.5; IR (cm^{-1}):3242 (N-H, str.), 1651 (C=O cyclic, str.), 1707 (C=O, str.), 1512 (C=N, str.), 786 (C-Cl, str.), 1092 (C-F, str.); H^1 NMR (δ ppm): 8.95 (1H, s, N-H), 4.85 (1H, s, Azetidine-C_4-H), 5.35 (1H, s, Azetidine-C_3-H), 7.26-8.40 (8H, m, aromatic protons); C^{13}NMR (δppm):180 (acyclic C=O), 188 (cyclic C=0), Pyridine (153 -C_2, 149.1-C_3,

137.7-C_4, 135.6-C_5, 135.1-C_6), Benzene (131.3-C_1, 129.5 C_2&C_6, 127.7-C_3&C_5, 137.2-C_4); Mass (m/z): 319 (M+); Elemental analysis: calcd.(found):C: 56.33(56.46) H: 3.44 (3.46) N: 13.14 (13.27).

4b: N- (3-chloro-2(4-bromo phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 270-272^{0}C; yield (%): 59; Rf: 0.48; Molecular formula: $C_{15}H_{11}N_3O_2ClBr$; Molecular weight: 380.5; IR (cm^{-1}): 3243 (N-H, str.), 1721 (C=O, str.), 1705 (C=O cyclic, str.), 1646 (C=N, str.), 698 (C-Br, str.), 778 (C-Cl, str.); H^1 NMR (δ ppm): 8.93 (1H, s, N-H), 4.45 (1H, s, Azetidine C_4-H), 5.50 (1H, s, Azetidine C_3-H), 7.26-8.40 (8H,m ,aromatic protons); Elemental analysis: calcd.(found): C: 48.5 (49.2) H:2.96 (2.90) N: 11.33 (11.48).

4c: N- (3-chloro-2(4-methyl phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 180-182^{0}C; yield (%): 63; Rf: 0.38; Molecular formula: $C_{16}H_{14}N_3O_2Cl$; Molecular weight: 315.5; IR (cm^{-1}): 3244 (N-H str.), 1702 (C=O, cyclic str.), 1724 (C=O, str.), 3116 (C=C, str.), 1646 (C=N,str.), 763 (C-Cl, str.); H^1 NMR (δ ppm): 8.80 (1H, s, N-H), 5.11 (1H,s, Azetidine C_4-H), 5.65 (1H,s, Azetidine-C_3-H), 7.22-8.35 (8H,m ,aromatic protons),2.4(3H,s,-CH_3) Elemental analysis: calcd.(found): C: 63.64 (63.74) H: 4.64 (4.58) N: 9.28 (9.34).

4d: N- (3-chloro-2(4-hydroxy phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 264-266^{0}C; yield (%): 56; Rf: 0.49; Molecular formula: $C_{15}H_{12}N_3O_3Cl$; Molecular weight: 317.5; IR (cm^{-1}) :3244 (N-H, str.), 1703 (C=O, cyclic str.), 1725(C=O, str.), 3117(OH, str.), 2977 (C=C, str.),1646(C=N, str.), 762 (C-Cl); H^1 NMR (δ ppm): 8.75 (1H, S, N-H), 4.90 (1H,s, Azetidine- C_4-H), 5.39 (1H, s, Azetidine-C_3-H), 5.89 (1H, s, Ar-OH), 7.26-8.40(8H, m , aromatic protons); Elemental analysis: calcd.(found): C: 59.30 (59.34) H:3.95 (3.90) N: 9.22 (9.34).

4e: N- (3-chloro-2(2-hydroxy phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 256-258^{0}C; yield (%): 49; Rf: 0.65; Molecular formula: $C_{15}H_{12}N_3O_3Cl$; Molecular weight: 317.5; IR (cm^{-1}): 3006 (N-H, str.), 1607 (C=O, cyclic,str.), 2943 (OH, str.), 1708 (C=O, str.), 1499 (C=N, str.), 826 (C-Cl, str.); H^1 NMR (δ ppm): 8.90 (1H, s, N-H), 3.45 (1H,s,Azetidine-C_4-H),4.90(1H,s,Azetidine-C_4-H),5.35(1H,s,Ar-OH),7.32-8.50 (8H,m,aromatic protons), Elemental analysis: calcd.(found): C: 59.30 (59.34) H: 3.95 (3.90) N: 9.22 (9.34).

4f: N- (3-chloro-2(4-nitro phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p:278-280^{0}C; yield (%): 74; Rf: 0.56; Molecular formula: $C_{15}H_{11}N_4O_4Cl$; Molecular weight:346.5; IR (cm^{-1}): 3466 (N-H, str.), 1716 (C=O, str.), 1218 (C-N, str.), 1622 (C=O, cyclic, str.),780 (C-Cl, str.), 1455 (NO_2, anti) , 1304 (NO_2, syn); H^1 NMR (δ ppm): 8.90 (1H, s, N-H), 2.30 (1H, s, azetidine-C_4-H), 4.80 (1H,s,Azetidine-C_3-H), 7.30-8.70 (8H,m,aromatic protons), Elemental analysis: calcd (found): C: 54.13 (54.21) H:3.30 (3.42) N: 12.64 (12.74).

4g: N- (3-chloro-2(2-hydroxy 5-nitrophenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 290-292^{0}C; yield (%): 62; Rf: 0.36; Molecular formula: $C_{15}H_{11}N_4O_5Cl$; Molecular weight: 362.5; IR (cm^{-1}): 3359 (N-H, str.), 1700 (C=O, str.), 3220 (free OH, str.),1643(C=O, cyclic str.),(3107 bonding OH, str.),1588 (C=N, str.), 818 (C-Cl), 1456 (NO_2, assym.),1376 (NO_2,sym.); H^1 NMR (δ ppm): 8.70 (1H, S, N-H), 3.90 (1H, Azetidine-C_4-H), 4.98 (1H,s,Azetidine-C_3-H), 7.10-8.10 (7H,m ,aromatic protons), 5.42 (1H,s, Ar-OH); Elemental analysis: calcd.(found): C: 51.6 (52.1) H: 3.15 (3.21) N: 12.05 (12.14).

4h: N- (3-chloro-2(4-N,N-dimethyl amino phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 192-194^{0}C; yield(%): 49; Rf: 0.69; Molecular formula: $C_{17}H_{17}N_4O_2Cl$; Molecular weight:344.5; IR (cm^{-1}): 3244 (N-H, str.), 1702 (C=O, cyclic, str.), 3115 (=C-H, str.), 1725 (C=O, str.), 2977 (C-H, str.) ,1646 (C=N, str.), 779 (C-Cl); H^1 NMR (δ ppm): 8.70 (1H, s, N-H), 2.25 (1H, s, Azetidine- C_4-H), 4.80 (1H, s, Azetidine-C_3-H), 7.12-8.30 (8H, m, aromatic protons), 3.45 (6H, s, Methyl protons); Elemental analysis: calcd.(found): C: 59.21 (59.34) H: 4.93 (4.90) N: 16.25 (16.34).

4i: N- (3-chloro-2(4-chloro phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 136-138^{0}C; yield(%): 67; Rf: 0.53; Molecular formula: $C_{15}H_{11}N_3O_2Cl_2$; Molecular weight: 336; IR (cm^{-1}): 3243 (N-H, str.), 1647 (C=O, cyclic, str.),1707 (C=O, str.), 3117 (=CH, str.), 463 (C=N, str.), 784 (C-Cl, str.); H^1 NMR (δ ppm): 8.95 (1H, s, N-H), 4.09 (1H, Azetidine-C_4-H), 5.15 (1H, s, Azetidine-C_3-H), 7.15-8.80 (8H, m, aromatic protons); Elemental analysis: calcd.(found): C: 53.57 (53.64) H: 3.27 (3.30) N: 12.5 (12.57).

4j: N- (3-chloro-2(3-bromo phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 320-322^{0}C; yield(%): 54; Rf: 0.63; Molecular formula: $C_{15}H_{11}N_3O_2ClBr$; Molecular weight:380.5; IR (cm^{-1}): 3242 (N-H, str.), 1649 (C=O cyclic, str.),1705 (acyclic C=O, str.), 661 (C-Br, str.), 1511 (C=N, str.), 787 (C-Cl, str.); H^1 NMR (δ ppm): 8.90 (1H, s, N-H), 4.61(1H,s,Azetidine-C_4-H),5.56(1H,s,Azetidine-C_3-H), 7.30-8.40 (8H, m, aromatic protons); Elemental analysis: calcd.(found): C: 48.54 (48.63) H: 2.96 (2.90) N: 11.33 (11.43).

4k: N- (3-chloro-2(2-chloro phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 98-110^{0}C; yield(%): 51; Rf: 0.39; Molecular formula: $C_{15}H_{11}N_3O_2Cl_2$; Molecular weight: 336; IR (cm^{-1}): 3258 (N-H, str.),1610 (C=O, cyclic, str.),1701(C=O, str.),3104 (= CH,str.), 1500 (C=N, str.), 829 (C-Cl, str.); H^1 NMR (δ ppm): 8.90 (1H, s, N-H), 4.95 (1H, C_3-H), 5.45 (1H, s, C_2-H), 7.11-8.30 (8H,m ,aromatic protons); Elemental analysis: calcd.(found): C: 53.57 (53.54) H: 3.27 (3.20) N: 12.53 (12.63)

4l: N- (3-chloro-2(2-methoxy phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 165-167^{0}C; yield(%): 46; Rf: 0.49; Molecular formula:$C_{16}H_{14}N_3O_3Cl$; Molecular weight:331.5; IR (cm^{-1}): 3244 (N-H, str.), 1706 (C=O, str.), 3116 (=CH, str.), 1647 (C=O, cyclic, str.), 1289 (C-O-C), 2977 (C-H, str.),1461 (C=N, str.), 785 (C-Cl, str.): H^1 NMR (δ ppm): 8.80 (1H, s, N-H), 5.08 (1H, s, C_3-H), 5.70 (1H, s, C_2-H) 7.22-8.40 (8H, m, aromatic protons), 2.40 (3H, s, OCH_3); Elemental analysis: calcd.(found): C: 57.91 (57.84) H: 4.21 (4.30) N: 12.66 (12.74).

4m: N- (3-chloro-2(2,4-dimethoxy phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 170-172^{0}C; yield(%): 59; Rf: 0.37; Molecular formula: $C_{17}H_{16}N_3O_4Cl$; Molecular weight: 361.5; IR (cm^{-1}): 3255 (N-H, str.), 1703 (C=O, str.),1637 (C=O cyclic, str.), 1288 (C-O-C, str.),1594 (C=N, str.), 762 (C-Cl); H^1 NMR (δ ppm): 8.80 (1H, s, N-H), 4.29 (1H, s, azetidine-C_4-H), 6.63 (1H, s, Azetidine-C_3-H), 7.30-8.30 (7H, m, aromatic protons), 3.04 (6H, s, Methoxy); Elemental analysis: calcd.(found): C: 56.43 (56.34) H: 4.42 (4.50) N: 11.64 (11.59).

4n: N- (3-chloro-2(3,4,5-trimethoxy phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 210-212^{0}C; yield (%): 59; Rf: 0.49; Molecular formula: $C_{18}H_{18}N_3O_5Cl$; Molecular weight: 391.5; IR (cm^{-1}): 3242 (N-H, str.), 1707 (C=O, str.), 1226 (C-N, str.), 1651 (C=O, cyclic, str.), 1283 (C-O-C), 1512 (C=N, str.), 786 (C-Cl, str.); H^1 NMR (δ ppm): 8.90 (1H, s, N-H), 4.15 (1H, s, Azetidine-C_4-H), 5.10 (1H, s, Azetidine-C_3-H), 7.22-8.70 (6H, m , aromatic protons), 3.29 (9H, s, methoxy); Elemental analysis: calcd.(found): C: 55.17 (55.23)H: 4.59 (4.68)N: 10.72 (10.79).

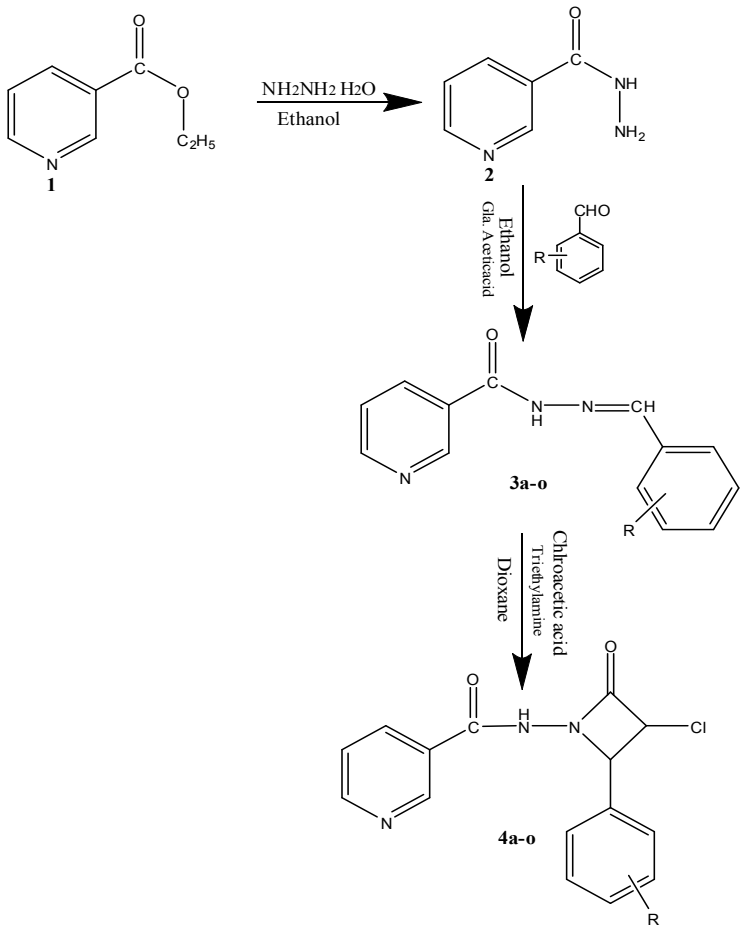
4o: N- (3-chloro-2(2-nitro phenyl) 4-oxo-azetidine-1-yl) nicotinamide

m.p: 280-282^{0}C; yield (%) : 78; Rf: 0.58; Molecular formula: $C_{15}H_{11}N_4O_4Cl$; Molecular weight: 346; IR (cm^{-1}): 3242 (N-H), 1461 (NO_2 , anti ,str.),1387 (NO_2, syn, str.), 3113 (=C-H, str.), 1707 (acyclic C=O, str.), 1511 (C=N, str.), 787(C-Cl, str.), 1650 (cyclic C=O, str.); H^1 NMR (δ ppm): 8.85 (1H, s, N-H), 4.80 (1H, s, Azetidine-C_4-H), 5.25 (1H, s, Azetidine-C_3-H), 7.32-8.40 (8H, m, aromatic protons); Elemental analysis: calcd.(found): C: 60.23 (60.34)H: 3.86 (3.90)N: 16.21 (16.14).

Antimicrobial activity

The In vitro antimicrobial activity study was carried out against 48 hr old cultures of Four bacterial and two fungal species by cup plate method[13] at 50 µg and 100µg concentrations by using Ciprofloxacin and Miconazole as standards. The inhibition zone measure in mm

and was compared with the standard drug after 24hr of incubation at 25˚C for antimicrobial activity and 48 hr at 30˚C for antifungal activity. The results are reported in Table.1 and Table.2.



Scheme

Table 1
Antibacterial activity of Synthesized compounds (4a-o)

S.NO	Cpd. Code	G +ve				G -ve			
		B. Subtilis		B. Pimilis		P. Vulgaris		E.Coli	
		50µg/ml	100µg/ml	50µg/ml	100µg/ml	50µg/ml	100µg/ml	50µg/ml	100 µg/ml
1.	4a	21	30	24	32	23	34	19	22
2.	4b	19	25	19	22	21	29	18	24
3.	4c	20	20	19	21	21	25	10	14
4.	4d	22	35	20	32	24	28	21	24
4.	4e	18	30	16	26	22	32	19	31
6.	4f	19	31	20	23	24	38	16	20
7.	4g	18	22	23	29	25	39	14	18
8.	4h	20	36	20	24	19	23	18	30
9.	4i	15	23	10	16	15	18	10	12
10.	4j	14	19	20	26	19	22	12	14
11.	4k	21	23	25	27	18	20	15	18
12.	4l	18	31	19	30	19	29	16	28
13.	4m	21	35	20	26	19	31	22	26
14.	4n	18	29	24	30	16	28	18	30
15.	4o	19	26	24	30	18	31	17	29
Std.	Ciprofloxacin	28	-	32	-	30	-	24	-
Ctrl.	DMSO	-	-	-	-	-	-	-	-

Table 2
Antifungal activity of Synthesized compounds (4a-o)

S.NO	Cpd. code	Aspergillus Niger		Candida Albicans	
		50 µg/ml	100 µg/ml	50 µg/ml	100µg/ml
1.	4a	24	30	18	24
2.	4b	21	28	19	28
3.	4c	19	25	20	23
4.	4d	20	25	21	27
5.	4e	24	28	22	30
6.	4f	18	23	22	30
7.	4g	16	22	18	24
8.	4h	16	28	18	21
9.	4i	22	26	20	24
10.	4j	18	21	22	26
11.	4k	20	24	19	23
12.	4l	19	26	22	24
13.	4m	20	25	21	26
14.	4n	18	22	20	24
15.	4o	17	21	18	24
Standard	Miconazole	30	-	27	-
Control	DMSO	-	-	-	-

RESULTS AND DISCUSSION

Schiff base containing pyridine moiety and their Azetidinone derivatives were synthesized. Thin layer chromatography was performed on pre-coated silica gel glass plates using chloroform: ethanol (9:1) solvent systems to ascertain the purity of these compounds. The compounds gave single spots. The structure of synthesized compounds was confirmed by infrared spectroscopy, H^1 NMR spectroscopy and Mass spectroscopy. Infrared spectroscopy showed the characteristic absorption bands of C=N stretching and C=O vibration of these compounds. The H^1 NMR spectra of the synthesized compounds show chemical shifts, which are characteristics of the anticipated structure of compounds. All the targeted compounds showed comparable antimicrobial activities when compared with reference standard.

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