SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF AZITIDINONE - SPIRO DERIVATIVES

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Abstract

In these research article reports The synthesis, characterization and antimicrobial activity of Spiro derivatives, namely N-(3-Alkoxyhydroxy-2-oxo-1-azaspiro[3.5]nonan-1-yl) isonicotinamide **(6a-c)**. Spiro derivatives **(6a-c)** were synthesized by reacting N-(3-chloro-2-oxo-1-azaspiro[3.5]nonan-1-yl)isonicotinamide **(4)** with various hydroxy group containing drugs **(5a-c)**. 2-Azetidinone derivatives, Compound **(4)** was synthesized by reacting Schiff base compounds, N'-cyclohexylidene isonicotino hydrazide **(3)** with chloro acetyl chloride. The Schiff base compounds, N'-cyclo hexylideneisonicotinohydrazide **(3)** was synthesised from isonicotino-hydrazide **(1)** and cyclochexanone **(2)**. The synthesized compounds were characterized by using advanced analytical tools like NMR spectroscopy, IR spectroscopy and by Mass spectroscopy. All the newly synthesized compounds were evaluated for their Antibacterial activity.

Keywords: Spiro compound, 2-Azetidinone, Schiff base, Spectral Analysis and Antibacterial activity.

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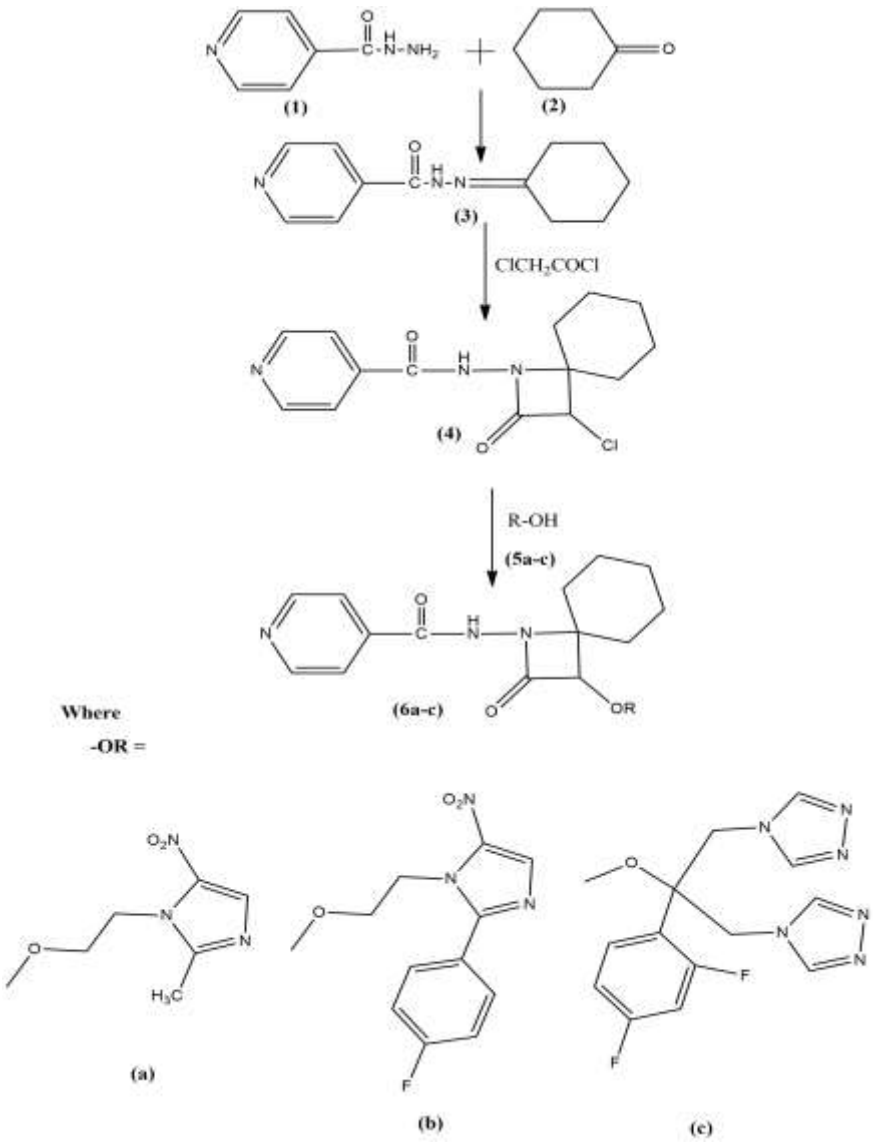
INTRODUCTION

The β-lactams or Azetidinones, are well-known nitrogen containing heterocyclic compounds. Anti-Inflammatory Activity, Anti–Leukemic activity, Antitubercular activity[1-3], Anticonvulsant Agents, anticancer activity, anti-bacterial activity, antifungal activity, etc.[4-7] The other interesting compounds say, Spiro-compounds is most important groups of compounds due to their extreme therapeutics and biological properties like, anti-viral and anti-cancer[8-10], Antimicrobial activity, Antifungal activity, etc[11-14] . Many natural and biologically active compounds and drug molecules are contains the organic Spiro-compounds as one of the most important constituent. Most recently the Spiro azetidinone derivatives have been reported based on cyclohexanone[15].By reviewing the pharma-ceutical and biological activities of Spiro compounds and Azetidinone derivatives, the present research work thought to synthesis of heterocyclic compounds which contains spiro Azetidinones with some antifungal drugs and also evaluation of these derivatives for their Antibacterial activity. The reaction scheme is shown as follow.



REACTION SCHEME – 1

MATERIAL AND METHODS

Chloro acetyl chloride and Hydroxy group containing drugs (Metronidazole, Flunidazole and Flucanozole) were procured from local market. All the other chemicals used directly without any purification. Schiff base compound, N'-cyclo hexylidene isonicotino hydrazide **(3)** was prepared by our earlier research work.[15]

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ^{1}H NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz.

Synthesis of Schiff's bases of cyclohexanone :(N-cyclohexylidene isonicotino hydrazide) This was prepared following the method reported for 4-acetamido cyclo hexanone [15]. Equimolar amounts of cyclohexanone and isoniazide are mixed into 30ml toluene and then refluxed for one day. The reaction was checked by TLC. Water was added (200ml) into resultant mixture. The organic layer collected and vacuum distilled the toluene to get compound. The yield was 80%. M.P.150-1°C. Elemental analysisfor $C_{12}H_{15}N_3O$(217 gm/mole), Calc.,%C, 66.34; %H, 6.96;%N,19.34 and Found, %C,66.3; %H, 6.9; %N, 19.3;IR (cm^{-1}): 3025-3050cm^{-1} (Aromatic C-H stretching), 3350-3280 (N-H stratching),1675 cm^{-1} (C=O stretching of Azetidinone), 1625-1650cm^{-1} (C=N);1HNMR(δ ppm/$CHCl_3$): 7.80-8.90(4H,m,Pyridine-H), 9.52 (1H, s,-NH),1.65-1.80(6H,m,-CH_2) and 2.35-2.40(4H,t,-CH_2). M^+(m/z):218.3

Synthesis of N-(3-chloro-2-oxo-1-azaspiro [3.5] nonan-1-yl)isonicotinamide (4)

The 2-Azetidinone derivatives **(4)** were prepared from respective Schiff base derivative **(3)** by reported method given in literature [16,17].

A schiff base, N'-cyclohexylideneisoni-cotino-hydrazide **(3)** (0.015 mole), Chloroacetyl chloride

(0.025 mole), and triethyl amine (Five drops) are added into RBF containing 1,4-dioxane (25 ml). The reaction mass was maintained 3 hour at reflux temperature. The solid were precipitated on cooling at 0-10°C and stirred for 5 hours, then it was filtered, dried in hot air oven at 40-45°C to give off light yellowish white crystals of N-(3-chloro-2-oxo-1-azaspiro[3.5]nonan-1-yl) isonicotinamide **(4)**. The overall yield obtained is about 76%. M. P. 172-3°C. The elemental analysis for N-(3-chloro-2-oxo-1-azaspiro[3.5]nonan-1-yl) isonicotinamide(4), $C_{14}H_{16}N_3O_2Cl$ (293.75), Elemental analysis: Calc.,%C, 57.24; %H, 5.49; %N, 14.30; %Cl, 12.07;and Found, %C, 57.2; %H, 5.4; %N, 14.2; %Cl, 12.0;IR (cm^{-1}): 3030-3050cm^{-1}(Aromatic C-H stretching),3350(N-H stratching), 1670 cm^{-1} (C=O stretching of Azetidinone),1625-1650 cm^{-1} (C=N),1080 cm^{-1} (C-Cl);1HNMR(δ ppm/$CHCl_3$): 7.80-8.90(4H,m,Pyridine-H),9.52 (1H,s,-NH),5.10 (1H,s,Azitidinone-H),1.55-1.40 (6H,m,-CH_2) and 1.70-1.60(4H,t,-CH_2). M+ (m/z): 294.2

Synthesis of N-(3-alkoxy-2-oxo-1-azaspiro [3.5] nonan-1-yl) isonicotinamide (6a-c)

The Alkoxy derivatives **(6a-c)** were prepared by following the method given in literature [18].

The sodium salt of hydroxy group containing drugs **(5a-c)** (0.2 mole) was added to N-(3-chloro-2-oxo-1-azaspiro[3.5]nonan-1-yl) isonicotinamide **(4)** (0. 15 mole) in ethyl acetate. The mixture was heated on a steam bath for appropriate time with occasional stirring. The reaction mixture was poured into ice cold 10% alkali solution. The solid product which precipitated was collected by filtration, washed with solid product with ethyl acetate water, dried and recrystallized from petroleum ether (b.p.60°-68°C). The details are given in Table-1.

Table:-1 Analytical Data Analysis of Compounds (6a-c)

Compd.	Molecular formula (Mol.wt.)	Yield	M.P.* ^0C	Elemental Analysis							
				%C		%H		%N		%X	
				Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
6a	$C_{20}H_{24}N_6O_5$ (428)	67	238-9	56.0	56.07	5.6	5.65	19.6	19.6	-	-
6b	$C_{25}H_{25}N_6O_5F$ (508)	62	234-5	59.0	59.0	4.9	4.96	16.53	16.5	3.74	3.7
6c	$C_{27}H_{27}N_9O_3F_2$ (563)	60	230-1	57.5	57.54	4.8	4.83	22.3	22.37	6.7	6.74

Uncorrected LC-MS data of 6b-508.9

ANTIBACTERIAL ACTIVITY

By agar cup plate method[19,20] the Antibacterial activity of all the compounds was studied against gram-+ve bacteria (*B.megaterium* and *S.Aureus*) and gram- -ve bacteria (*E.coli and Ps.Aeruginosa*) at a concentration of 50µg/ML. A methanol system was used as control in this method. The area of inhibition of zone measured in mm. Compounds 6c was found more toxic for microbes. All compounds found to be less or moderate active shown in Tables -2.

Table:-2 Antibacterial Activity of Compounds

Compound (Designation)	Zone of Inhibition (in mm)			
	Gram positive		Gram negative	
	B.megaterium	*S.Aureus*	*E.Coli*	*Ps.Aeruginosa*
3	9	10	10	10
4	10	11	11	11
6a	12	14	11	13
6b	14	13	12	15
6c	16	16	15	18
Tetracycline	18	19	18	21

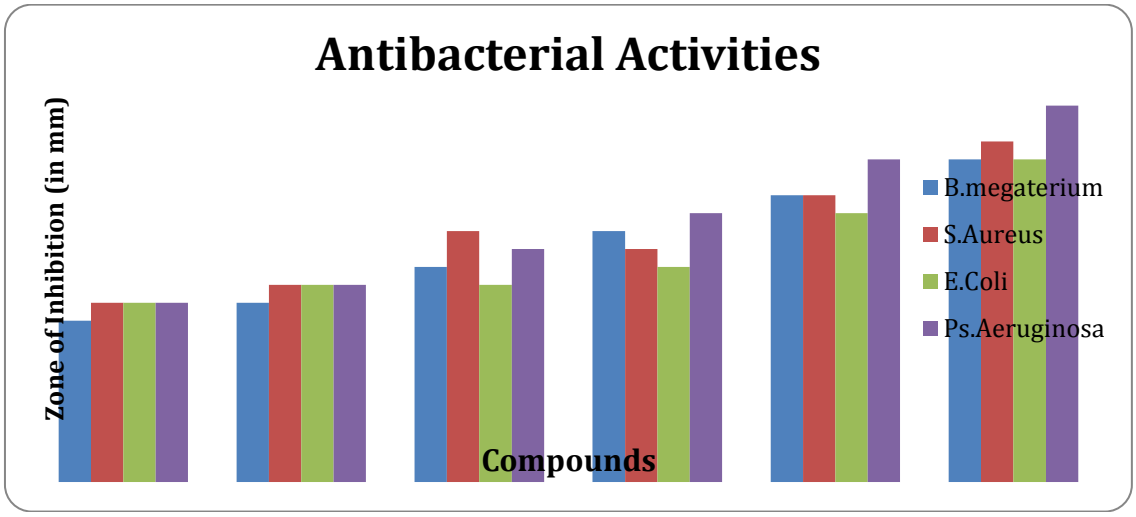


Figure 1 Antibacterial activity comparison of Compounds

RESULTS AND DISCUSSION

The IR spectra of N-(3-alkoxy-2-oxo-1-azaspiro [3.5]nonan-1-yl) isonicotinamide **(6a-c)** showing an absorption bands at 3050-3030cm^{-1} (Aromatic C-H stretching),3350(-N-H stratching), 2950, 2880(C-H stratching),1120 cm^{-1} (-O-C),1665-1670 cm^{-1} (C=O stretching of Azetidinone), 1625-1650 cm^{-1} (C=N),1080 cm^{-1} (-Cl),1555,1375 cm^{-1} (-NO_2) and 1250 cm^{-1} (C-F).

^{1}H NMR: 7.80-8.90(4H,m,Pyridine-H), 9.52 (1H,s,-NH),5.10 (1H, s, Azitidinone-H), 1.55 - 1.40 (6H,m,-CH_2) and 1.70-1.60(4H,t,-CH_2), (a) 4.10-3.90(4H,t,-CH_2),2.55(3H,s,-CH_3) and 7.85(1H,s, triazole-H),(b) 4.10-3.90(4H,t,-CH_2) and 7.90-8.00 (5H,s, Aromatic and triazole-H),(c) 4.20-4.22 (4H,s,-CH_2) and 6.65-8.70(7H,s, Aromatic and triazole-H). The C, H, N, S analysis data of all compounds are presented in Table-1.

The C, H, N, S analysis data of all compounds are presented in Table-1. LC-MS of selected samples 6b show the peak respectively at 508.9, which assign the molecular weight of compound,

The examination of elemental analytical data reveals that the elemental contents are consistence with the predicted structure shown in Scheme-1. The IR data also direct for assignment of the predicted structure.

CONCLUSION

The present study describes the synthesis and evaluation of the Antibacterial activity of Spiro-Azetidinone derivatives with pyridine. The synthesized compounds, therefore, present a new scaffold that can be used to as lead in the development of novel antibacterial agents.

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