SYNTHESIS, CHARACTERISATION, AND EVALUATION OF AZETIDINE-2-ONE DERIVATIVE

1. ABSTRACT

Antibacterial, antifungal, anticancer, antitubercular, anti-HIV, analgesic, anti-inflammatory, and ulcerogenic activities have all been documented for azetidinone derivatives.

The reaction of Schiff base((Z)-N (4-dimethylamino)benzylidene)pyrimidine-2-amine) with chloroacetyl chloride yielded a novel chemical called Azetdine-2-one derivative. Fourier Transform Infrared Spectroscopy and Proton Nuclear Magnetic Resonance Spectroscopy were used to confirm the chemical structures of the produced substances. The antimicrobial activity shows that the compound showed mild Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*

KEYWORDS: Azetidine-2-one derivative , Chloroacetyl chloride, Schiff Base.

2. INTRODUCTION

Antibacterial[1-2] and antifungal[1-2] properties have been observed for Azetidinone and Thiazolidinone derivatives. Pyridine derivatives have been shown to have antibacterial properties[3]. As a result, it was expected that molecules having both chemical moieties would result in compounds with biologically intriguing properties. The Schiff bases were exposed to an addition reaction with chloroacetyl chloride in the presence of 1,4dioxane and triethylamine in order to synthesise azetidinone derivatives. IR and 1HNMR were performed to assess the chemical structures of the synthesised compounds. The synthesised compounds were tested for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [4,5]

3. MATERIALS AND METHODS :

The melting points were measured in an open capillary tube and are unadjusted. The compounds' IR spectra were acquired using KBr pellets on a Win-Bommen B-104 IR Spectrophotometer. Using DMSO-d6 as a solvent, 1H-NMR spectra were acquired on the Bruker A VIII 500 MHz NMR Facility. Chemical changes from

tetramethylsilane are reported in parts per million downfield (Me4Si). TLC on precoated aluminium sheets (Silica gel 60 F254) utilising benzene and alcohol as mobile phases and visualised by iodine vapours was used to assess the purity of the compounds.

General Methods of Synthesis of Azetidine-2-one derivative (A2)[6,7,8,9,10]

For the production of Azetidine-2-one derivative, the previously synthesised Schiff base was used. At room temperature, dropwise additions of chloroacetyl chloride (0.01mol) to a combination of Schiff base (0.01mol) and triethylamine (0.02mol) in dioxane (25ml) were made. The mixture was stirred for 8 hours and then set aside for 3 days at room temperature. The contents were poured over crushed ice, and the resulting precipitate was filtered, washed with a 10% w/v sodium bicarbonate solution, vacuum dried, and recrystallized with 100% ethanol.

4. SYNTHETIC SCHEME

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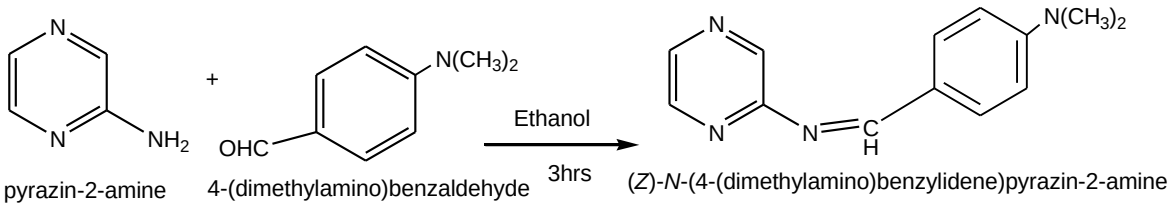
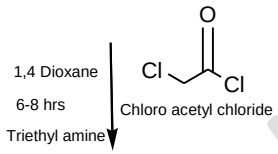
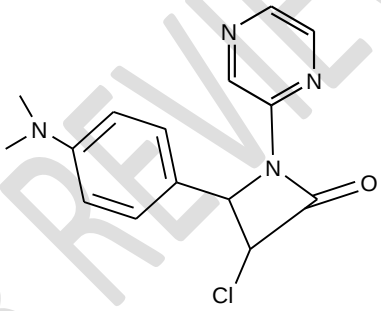


pyrazin-2-amine 4-(dimethylamino)benzaldehyde (*Z*)-*N*-(4-(dimethylamino)benzylidene)pyrazin-2-amine





3-chloro-4-(4-(dimethylamino)phenyl)-1-(pyrazin-2-yl)azetidin-2-one
Compound A2

IR (KBr) cm^{-1}: 1119(CH-N), 1400 (CH bending), 1638(C=O amide), 3423(NH)

3423 (w), 2796(w), 2688(w), 2502(w), 2264(w), 2089(m) 1638(s), 1400(s), 1119(s), 748(w), 603(w)

1H-NMR

2.5 (s, 1H; CH), 3.0 (s.CH methyl), 3.37 (s,CH methyl), 6.77-6.78(m, CH benzene), (DMSO-d6) : 7.67-7.69(m; CH Pyrazine).

Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against gram(+) bacteria (Staphylococcus aureus and gram(-) bacteria (Escherichia coli) using Nutrient agar medium.

4.1 Cup Plate method [11]

Inoculate a previously liquefied medium appropriate for the test with the required quantity of a microbe suspension, add the suspension to the medium at 40 to 50oC, and immediately pour the inoculated medium onto Petri plates to a depth of 3-4mm. Place the dishes or plates on a level surface to ensure that the layers of a medium are uniform in thickness. The prepared dishes must be stored in such a way that no major growth or death of the test organism occurs before they are utilised, and that the agar surface does not get contaminated.

A metal borer is used to create cavities in the agar plates. The cavities that are created must be consistent throughout the dish. In sterile cavities formed in the agar medium, apply the solutions to the surface of the solid media. When these are employed, the volume of solution delivered to each cavity must be uniform and almost fill those holes. Allow the dishes to remain at room temperature or at 4°C for 1-4 hours as a pre-incubation diffusion interval to reduce the impact of timing differences between the applications of different solutions. The plates are then incubated for 24 hours at 371°C to test for antibacterial activity. For the plates where the zone of inhibition was seen, the diameter of the zone of inhibition was measured. As illustrated, the average inhibition area in millimetres (mm) was determined and compared to the norms and the results are shown in the Table 1 below.

TABLE-1- Antibacterial activity Zone of Inhibition in mm

		Antibacterial activity	
S.No	Compound	*Staphylococcus aureus* (Gram+ve)	*Escherichia coli* (Gram -ve)
		Zone of Inhibition in mm	Zone of Inhibition in mm
1	Standard 100 µg/ml	40mm	36mm
2	Sample A2 100 µg/ml	20mm	25mm

2	Sample A2 200 µg/ml	22mm	27mm

The antibacterial activity of the Synthesised compound A2 was performed using ciprofloxacin as standard. The concentration of ciprofloxacin used was 100 µg/ml and the concentration of synthesises A2 used was 100 µg/ml and 200 µg/ml respectively. The antibacterial activity was done against *Staphylococcus aureus*(Gram +ve) and *Escherichia coli*(Gram –ve). The compound A2 showed mild activity against both the organisms.

5. SUMMARY AND CONCLUSION

The present work describes the synthesis of azetidinone derivative along with their antibacterial activity The azetidinone derivatives were prepared by the method of S.Ramachandran et al. The reaction completion was confirmed by TLC and the synthesized compounds were purified by recrystallization. The structures of the synthesized compounds were assigned based on the spectral data. The infrared, nuclear magnetic resonance spectra of these azetidinone compounds showed the expected frequencies and signals. The antibacterial activity of the Synthesised compound A2 was performed using ciprofloxacin as standard. The concentration of ciprofloxacin used was 100 µg/ml and the concentration of synthesized A2 used was 100 µg/ml and 200 µg/ml respectively. The antibacterial activity was done against Staphylococcus aureus(Gram +ve) and Escherichia coli (Gram –ve). The compound A2 showed mild activity against both the organisms.

6.REFERENCES

1. G.S.Singh, B.J.Molotsi; Synthesis of 2-azetidinones from 2-diazo-1, 2-diarylethanones and N-(2-thienylidene)imines as possible antimicrobial agents Il Farmaco., 2005; 60: 727-730.
2. V. P. M.Rahman, S.Mukhtar, W. H.Ansari, G.Lemiere; Synthesis, stereochemistry and biological activity of some novel long alkyl chain

substituted thiazolidin-4-ones and thiazan-4-one from 10-undecenoic acid hydrazide Eur.J.Med.Chem.,2005; 40:173-184.

3. D.S.Mehta and V.H.Shah, Synthesis and biological activity of 2-azetidinones, 4- thiazolidinones, 5-imidazolinones having benzthiazole moiety.Ind.J.Hetero.Chem. 2001:11;139-144.
4. S. Ramachandran, Binoy Varghese Cheriyan, M. Vijey Aanandhi , Activities of thiazolidine-4-one and azetidine-2-one derivatives- a review, sent on July 9th 2020 Research, Research J. Pharm. and Tech, 2021;14(8):4509-4512
5. S. Ramachandran, Vimehsya N, K.Yogeshwaran, Binoy Varghese Cheriyan, M. Vijey Aanandhi , Molecular docking studies as antidepressant agents, synthetic techniques, antimicrobial screening of azetidine-2-one derivatives- A review**,**Research J. Pharm. and Tech, 2020;13(11):5524-5528
6. S.Ramachandran, A Vignesh, Manigandan S, G.Moganapriya, Binoy Varghese Cheriyan, M.Vijey Aanandhi, Synthesis, Characterisation, Antimicrobial evaluation of 2-amino pyrazine schiff base derivative, High Technology Letters,2021;27(6):446-452
7. S. Ramachandran, M. Vijey Aanandhi , M. Tamilselvam, V. Sursha, K. T. Vigneshwara,Synthesis, Characterization, Antimicrobial Evaluation of 2-Amino pyrimidine Schiff base derivative,Research J. Pharm. and Tech.(online) 12(4): April 2019,1907-1909.
8. Ramachandran, P.Shanmugapandiyan, C.N.Nalini, Synthesis & Antimicrobial evaluation of N-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl) isonicotinamide derivatives in International Journal of Pharmaceutical sciences and Research, 2011;2(06); 1564-1568.
9. S.Ramachandran, S.Nandini, M.Noorul Hudha, Omer Abdul Gader, Otieno Winston Churchill, M.Rajasheik Nasrudeen Shah, Dr.R.Sundhararajan Synthesis, Characterization, Antimicrobial evaluation of Azetidinone derivative in World Journal of Pharmacy and Pharmaceutical Sciences,2014,3(6),1466-1470
10. S.V.More, D.V.Dongarkhadekar, R.N.Chavan, W.N. Jadhav, S.R.Bhusare, R.P.Pawar; Synthesis and antibacterial activity of new Schiff bases, 4-thiazolidinones and 2- azetidinones, J.Indian.Chem.Soc., 2002;79:768-769.

11. Indian Pharmacopoeia 2007, Government of India. Ministry of health and Family welfare, Volume 1, Pg 50