SYNTHESIS, ANTIMICROBIAL ACTIVITY AND DOCKING STUDIES OF 1-METHYL-3-(QUINOXALIN-2-YL) PYRROLIDIN-2-ONE

K. Srivani^{1,2}, E. Laxminarayana³ and M. Thirumala Chary^{1,✉}

¹Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad, Telangana -500 085 India

²S R University, Ananthasagar, Hasanparthy, Warangal, Telangana 506371

³Sreenidhi Institute of Science and Technology (Autonomous) Yamnampet, Ghatkesar, Hyderabad- 501301, Telangana, India

✉Corresponding Author: mtcharya@yahoo.com

ABSTRACT

The preparation of 1-methyl-3-(quinoxalin-2-yl)pyrrolidin-2-one was described in a simple manner followed by green procedures, title compound was thoroughly characterized by IR, ¹HNMR and mass spectra data. The resulted compounds **5**, **6** and **7** screened for biological activity and docking studies results are presented.

Keywords: Phosphorous oxychloride, diethylmalonate, Lithium chloride, 2-bromo-1,1-dimethoxyethane

RASĀYAN J. Chem., Vol. 14, No.1, 2021

INTRODUCTION

Quinoxalines exhibits a wide variety of biological activities, because of this the study of quinoxalines became more interesting. The biological activities are antibacterial¹⁻⁶, antifungal⁷⁻⁸, anticancer⁹⁻¹¹, antitubercular¹², antileishmanial¹³, antimalarial¹⁴⁻¹⁵ and antidepressant activities¹⁶⁻¹⁷. The compounds like quinoxalin-2-ones and quinoxaline-2,3-diones have been reported to the activities like antimicrobial¹⁸⁻¹⁹, potent antithrombotic²⁰, analgesic and anti-inflammatory²¹⁻²² activities. Few synthetic quinoxalines are included in various antibiotics such as actinomycin, echinomycin and levomycin. Some known drugs which contain quinoxaline moiety are Brimonidine, alleviate glaucoma symptoms.²³

Brimonidine

Quinoxalines have a wide range of applications in DNA cleaving agents, dyes, organic semiconductors, dihydroannulenes, cavitands, and efficient electroluminescent and used as main components for the synthesis of anion receptor. In continuous to our earlier work²⁴ and due to this wide range of applications of quinoxalines derivatives in various fields, we synthesized and characterized the novel title derivative of quinoxaline.

RESULTS AND DISCUSSION

The final compound was prepared as outlined in Scheme-1; The quinoxalin-2-ol **1** was reacted with POCl₃ for 18 h at 80°C and obtained good yields of compound **2**, which was then treated with diethyl malonate in presence of DMF, NaH at room temperature to get compound **3** with 80% of yield. Compound **3** was treated with LiCl in DMSO at room temperature to give compound **4** with 79% yield. When compound **4** reacted with 2-bromo-1,1-dimethoxyethane at 0 °C for 4 h in presence of NaH, DMF,

it afforded compound **5** with 90 % of yield. Compound **5** is reacted with HCl to form compound **6**, which undergoes self-condensation to give title compound **7**.

Antibacterial Activity

The synthesized compounds (**5**, **6** and **7**) were screened for their antibacterial activity by agar diffusion method²⁵. Among the compounds tested (**5**, **6**, **7**) showed prominent antibacterial activity compared with that from other compounds **5g** noticed the highest zone of inhibition 16mm against gram-negative strain *protius vulgaris*. *Proteus vulgaris* is a Gram-negative bacteria with rod-shaped and nitrate-reducing that inhabits the intestinal tracts of humans and animals. These bacteria are present in water, soil, and fecal matter. This bacteria causes wound infections and other species of the same bacterial type are known to cause urinary tract infections. All the gram-negative strains are more susceptible to tested compounds. The zone of inhibition was represented in mm (Table-1).

Docking Studies

The docking server software was used for docking studies to find out the biointeractions between the ligand and the protein 5-cox oxidoreductase. The compound synthesized was docked to protein 5-cox oxidoreductase. The protein is known for its role in inflammation. The estimated free energy of binding is -4.89 kcal/mol which is shown in Table-2. The docking pose of the ligand with the protein 5-cox oxidoreductase showing the binding of the amino acids ASN68, TYR55, SER38, GLN42, PRO35, LYS166 (Fig.-1). Decomposed interaction energies of different hydrogen, hydrophobic and other bonds in kcal/mol are shown in Table-3 which shows the presence of hydrogen, halogen, polar, hydrophobic and other bonds that are involved in this interaction. The interaction of different carbon and nitrogen atoms binding to different amino acids is shown in Table-4. Figure-2 representing the 2D plot of the interaction between enzyme and the ligand showing clearly the nearness of the amino acids of the enzyme towards the ligand. Docking observations of the compound in the present scheme indicates that the compound acts as an anti-inflammatory agent.

EXPERIMENTAL

Chemicals and reagents used in the reaction were obtained from Finar, solvents used are of grade LR. Merck AL silica gel 60 F₂₅₄ plates were used for thin-layer chromatography (TLC) and these plates were imaged under UV light. ¹H NMR spectra and ¹³C NMR spectra were recorded with a Varian Mercury plus 400 MHz instrument in DMSO-*d*₆. By taking TMS as an internal standard Chemical shift are reported in δ (ppm). Assuming the first-order behavior ¹H-NMR coupling constants and chemical shifts of compounds were determined. Multiplicity is indicated by the following: d (doublet), t (triplet), s (singlet), m (multiplet), br (broad), q (quartet),; the list of coupling constants (*J*) resembles the order of multiplicity assignment. Mass spectra were recorded by using Shimadzu LCMS-QP 1000 mass spectrometer. Open glass capillaries on a Stuart SMP30 apparatus was used to determine the melting points and are uncorrected. The reactions of the scheme were done under an inert atmosphere.

Scheme-1: Synthesis of 1-Methyl-3-(quinoxalin-2-yl)pyrrolidin-2-one

Diethyl 2-(quinoxalin-2-yl)malonate (3)

To a suspension of NaH (1.5eq) in THF (10 Vol) a solution of diethyl malonate (1 eq.) in DMF (5vol) was added dropwise and stirred the reaction for 1 hr at the same temperature. Then at 0°C 2-chloroquinoxaline (2) was added, the reaction mixture was allowed to come to room temperature then stirred at 70°C for 18 h. TLC technique was used to know the progress of the reaction. The reaction mixture was reduced with sat. NH₄Cl solution was discharged into ice water and then EtOAc was used for extraction. The extract was washed with water, brine solution, dried over anhydrous Na₂SO₄ and evaporated the solvent to afford crude product. Purification of crude product was done by column chromatography; 50% EtOAc in Petroleum ether was used to extract diethyl 2-(quinoxalin-2-yl) malonate (3) as a white solid.

¹H NMR (DMSO-d₆, 400MHz): δ = 8.92 (s, 1H), 8.10 (d, 2H, *J* = 7.8 Hz), 7.75 (t, 2H, *J* = 7.8 Hz), 4.61 (s, 1H), 4.18 (q, 4H), 1.28 (t, 6H); MS: m/z, 288.9 (M⁺+H).

Ethyl 2-(quinoxalin-2-yl)acetate (4)

Diethyl 2-(quinoxalin-2-yl) malonate (3) in DMSO (5 vol) mixture was added to LiCl (1.2 eq) at room temperature. Then reaction was heated for 16 h at 120 °C. After completion of the reaction, the product was identified by TLC. The reaction was quenched with sat. NH₄Cl solution. The reaction mixture was poured into ice water, extracted with EtOAc, combined extracts were washed by water, NaCl solution, dehydrated over anhy. Na₂SO₄ and the solvent were evaporated to afford crude product. Purification of crude product was done by column chromatography; the required product was extracted with 50% EtOAc in Pet ether to get ethyl 2-(quinoxalin-2-yl) acetate (4) as solid.

¹H NMR (DMSO-d₆, 400MHz): δ = 8.98 (s, 1H), 8.11 (d, 2H, *J* = 7.8 Hz), 7.76 (t, 2H), 4.20 (q, 2H), 3.61 (d, 2H), 1.26 (t, 3H); MS: m/z, 216.9 (M⁺+H).

Ethyl 4-ethoxy-4-methoxy-2-(quinoxalin-2-yl)butanoate (5)

To a suspension of NaH (1.5 eq.) in DMF (5 vol) at 0°C was added solution of ethyl 2-(quinoxalin-2-yl)acetate (4) in DMF (2vol) as dropwise. This was stirred for 1 h, and then cooled to 0 °C. To the resulting mixture, 2-bromo-1,1-dimethoxyethane (1.2 eq.) was added and stirred at room temperature for 6 h. The reaction mixture was quenched with sat. NH₄Cl solution, transferred into ice-cold water, extracted with EtOAc. The extract was rinsed with water, NaCl solution, dried over anhy. Na₂SO₄ and evaporated the solvent to get the crude product. Purification of crude product was done by column chromatography; the required product was eluted with Pet ether with 50% EtOAc to afford ethyl 4-ethoxy-4-methoxy-2-(quinoxalin-2-yl)butanoate (5) as white solid.

¹H NMR (DMSO-d₆, 400MHz): δ = 8.97 (s, 1H), 8.12 (d, 2H, *J* = 8.0 Hz), 7.78 (t, 2H, *J* = 8.0 Hz), 4.42 (m, 2H), 4.10 (q, 2H), 3.81 (t, 1H), 3.60 (s, 6H), 2.31 (m, 2H), 1.12 (t, 3H); MS: m/z, 305.0 (M⁺+H).

Ethyl 3-formyl-2-(quinoxalin-2-yl)propanoate (6)

To a ethyl 4-ethoxy-4-methoxy-2-(quinoxalin-2-yl)butanoate (5) 1N HCl (5 vol) solution was added at 0°C and stirred the reaction for 4 h. The reaction mixture was quenched with sat. NH₄Cl solution, transferred into ice-cold water, extracted with EtOAc. The combined extract was washed with brine solution, water and dried over anhy. Na₂SO₄ then the solvent was evaporated to get the crude product. Column chromatography was used to purify the Crude product; the required product was extracted with 50% EtOAc in Pet ether to get ethyl 3-formyl-2-(quinoxalin-2-yl) propanoate (6) as off-white solid.

¹H NMR (DMSO-d₆, 400MHz): δ = 9.79 (s, 1H), 8.71 (s, 1H), 7.84 (d, 2H, *J* = 7.8 Hz), 7.70 (m, 2H), 4.20 (m, 2H), 4.20 (t, 2H), 3.90 (m, 1H), 2.98 (m, 2H), 1.21 (t, 3H); MS: m/z, 258.9 (M⁺+H).

Table-1

Compound	5	6	7	Gentamycin
<i>gram-positive</i>				
<i>Bacillus subtilis</i>	11	9	8	19
<i>Bacillus cereus</i>	10	11	7	14
<i>S. aureus</i>	8	11	10	16

Gram-negative				
<i>E.coli</i>	9	11	13	13
<i>Proteus vulgaris</i>	14	16	11	20
<i>k.pneumonia</i>	6	11	7	15

1-methyl-3-(quinoxalin-2-yl)pyrrolidin-2-one (7)

To a stirred solution of ethyl 3-formyl-2-(quinoxalin-2-yl)propanoate(6) (1 eq.) in DCM (10 Vol) an amine (B) (1 eq.) was added and stirred the reaction for 18 h at room temperature. The reaction mixture was poured into ice-cold water and extracted with EtOAc. The extract was washed with water, brine solution, dried over anhy. Na₂SO₄ and evaporated the solvent to obtain a crude product. Crude product purification was observed by column chromatography; the required product was eluted with 50% EtOAc in Pet ether to obtain 1-methyl-3-(quinoxalin-2-yl)pyrrolidin-2-one (7) as a white solid.

¹H NMR (DMSO-d₆, 400MHz): δ= 8.72 (s, 1H), 8.18 (d, 2H, *J* = 7.8 Hz), 7.70 (m, 2H), 3.92 (m, 1H), 3.61 (m, 2H), 2.78 (s, 1H), 2.21 (m, 1H), 2.12 (m, 1H); MS: *m/z*, 228.0 (M⁺+H).

Fig.-1 Interaction of Ligand and Protein

Fig.-2 Docking of Ligand
Table-2: Free Energy of Binding

Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolecular Energy	Frequency	Interaction Surface
-4.65 kcal/mol	389.99 uM	-5.50 kcal/mol	-0.05 kcal/mol	-5.55 kcal/mol	50%	414.46 l

Table-3: Decomposed Interaction Energies in kcal/mol

Hydrogen Bonds	Polar	Other
SER38 (O)	ASN68 (O)	PRO40 (O)
TYR55 (O)	GLU67 (O)	

Table-4: Interaction of Ligand and Protein

Hydrogen Bonds	Polar	pi-pi	Cation-pi	Other
N1 (O) – SER38 [2.64] – (CB, OG)	H5 (O) – SER38 [3.77] – (OG)	C1 (O) – TYR55 [3.20] – (CD1, CE1)	H12 (O) – TYR55 [3.00] – (CE1, CZ)	C4 (O) – SER38 [3.61] – (OG)
O1 (O) – SER38 [3.41] – (CB, OG)	N2 (O) – TYR55 [3.69] – (OH)	C2 (O) – TYR55 [3.77] – (CD1)		C5 (O) – SER38 [3.41] – (OG)
N4 (O) – TYR55 [2.82] – (OH)	H12 (O) – TYR55 [2.01] – (OH)	C6 (O) – TYR55 [3.26] – (CD1, CE1)		C7 (O) – SER38 [3.37] – (OG)
N3 (O) – TYR55 [2.78] – (OH)	N4 (O) – GLU67 [3.40] – (OE2)			H12 (O) – PRO40 [3.21] – (CB)
	N4 (O) – ASN68 [2.98] – (ND2, OD1)			N2 (O) – TYR55 [3.29] – (CE1)
	N3 (O) – ASN68 [3.02] – (ND2, OD1)			N4 (O) – GLU67 [3.56] – (CD)
	H12 (O) – ASN68 [3.06] – (ND2, OD1)			C10 (O) – GLU67 [3.26] – (CD, OE1, OE2)
				C11 (O) – GLU67 [3.26] – (CD, OE1, OE2)
				C12 (O) – GLU67 [3.37] – (OE1)
				C13 (O) – GLU67 [3.45] – (OE1)
				C14 (O) – GLU67 [3.43] – (OE1)
				C15 (O) – GLU67 [3.33] – (OE1)
				C17 (O) – GLU67 [3.76] – (OE2)
				N4 (O) – ASN68 [3.63] – (CG)
				N3 (O) – ASN68 [3.60] – (CG)
				H12 (O) – ASN68 [3.57] – (CG)

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[RJC-5993/2020]