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Synthesis and Antifungal Evaluation of Substituted 3-Benzylquinoxaline Derivatives

***[1]V.P.Devmurari, [2]N.P Jivani**
[1]Jodhpur National University, Department of Pharmaceutical Science, Jhavar Road, Narnadi, Jodhpur.
[2]Smt R.B.Patel Mahila Pharmacy College, Atkot, Gujarat, India

Abstract
In the present study, seven substituted 3-benzylquinoxalines were synthesized from substituted phenyl pyruvic acid and o-phenylenediamine. All the synthesized compounds were structurally elucidated by IR, Mass, NMR spectroscopy. Antifungal activity of the synthesized compound was analyzed against a three fungus by agar well diffusion method. All the Compounds showed good antifungal activity against the test fungus.

Key words
3-benzyl quinoxalines, Antifungal activity, agar well diffusion method.

Introduction
Quinoxalines are an important class of antibiotics that bind to DNA and thereby modify its biological activities[1,2]. So, synthetic quinoxaline derivatives can be good lead for future antifungal agent. In present study synthetic quinoxaline derivatives have been synthesized and evaluated for their antifungal potential against selected bacterial strains.

Material and Method

The melting points were taken in a remi M.P.apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 881 and FTIIR 8201 PC Shimadz spectrophotometer and values are expressed in cm^{-1}. NMR spectra were recorded on Bruker WM-200 spectrometer. The chemical shifts are expressed in ppm using TMS as an internal standard. Mass spectra were recorded on JEOL JMS-D-3000 spectrometer and are reported in the form of m/z and FAB on SX-102 instrument. All the reactions were monitored by thin layer chromatography over pre-coated silica gel plates, using UV lamp, iodine vapors or $KMnO_4$ spray as developing agents. A series of 3-substituted benzylquinoxalines were synthesized by general procedure[3,5]. Substituted phenylpyruvic acids were prepared from reported methods. O-Phenylenediamine was purchased from SD fine chemicals.

***Corresponding Author:**
viraldev1985@gmail.com

General procedure

Equimolar amount of substituted phenyl pyruvic acid and o-phenylenediamine were dissolved in ethanol and refluxed for 3 hour. Crude product was washed with ethanol and dried. Physical characterization of the synthesized compounds is given in Table 1.

3-Benzylquinoxalin-2-one

IR (KBr, cm^{-1}): 3322.75 (Secondary amide stretch.), 1499.6 (NH bend.), 1660.41 (C=O (I) stretch.), 1559.17 (C=O (II)), 664.358 (N-H wagging), 1296.89 (C-N stretch.), 2962.13 (C-H stretch.).

Mass m/z: 237.2 (M^++1)

^{1}H NMR $CDCl_3$, δ = 7.167 – 7.329 (m , 5H , phenyl) , δ = 7.401 – 7.525 (m , 3H , $H_{5/6/7}$) , δ = 7.823 – 7.863 (d ,1H , J=8.04 Hz , H_8) δ = (s , 2H , benzyl – CH_2).

3-(4-Chlorobenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3316 (Secondary amide stretch.), 1483.96 (NH bend.), 1661.37 (C=O (I) stretch.), 1556.27 (C=O (II)), 660.5(N-H wagging), 1294 (C-N stretch.), 2970.8(C-H stretch.).

Mass (*m/z* [M+1]): 271.08

^{1}H NMR (DMSO-d_6, δ, ppm): 12.43 (s, 1*H*, -NH), 7.26 -7.72 (m, 8*H*, Aromatic protons), 4.12 (s, 2*H*, Methylene proton)

3-(4-Methoxybenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3311.18 (Secondary amide stretch.), 1509 (NH bend.), 1660.41 (C=O (I) stretch.), 1605.45 (C=O (II)), 684.60 (N-H wagging), 1246.75 (C-N stretch.), 2959.23 (C-H stretch.)

3-(2-Nitrobenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3314.07 (Secondary amide stretch.), 1557.2 (NH bend.), 1668.12 (C=O (I) stretch.), 1519.63 (C=O (II)), 659.53 (N-H wagging), 1294 (C-N stretch.), 2938.98 (C-H stretch.).

3-(4-Nitrobenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3426.89 (Secondary amide stretch.), 1347.3 (NH bend.), 1596.77 (C=O (I) stretch.), 1516.74 (C=O (II)), 652.78 (N-H wagging), 1187.94 (C-N stretch.), 2932.23 (C-H stretch.).

3-(4-Methylbenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3311.18 (Secondary amide stretch.), 1509 (NH bend.), 1660.41 (C=O (I) stretch.), 1605.45 (C=O (II)), 684.60 (N-H wagging), 1246.75 (C-N stretch.), 2959.23 (C-H stretch.)

3-(2-Chlorobenzyl) quinoxalin-2-one

IR (KBr, cm^{-1}): 3302.5 (Secondary amide stretch.), 1428.03 (NH bend.), 1661.37 (C=O (I) stretch.), 1609.31 (C=O (II)), 659.53 (N-H wagging), 1201.43 (C-N stretch.).

Antifungal Evaluation

In the present research work, the activity spectrum of all the synthesized compounds was analyzed by agar well diffusion method in triplicate [6-12]. Digital colony counter (Toshiba, EIE-1901) was used for inoculum preparation. Antibiotic zone reader (EIE Instruments) was used to measure diameters of inhibition zones. For the antifungal assay, 3 fungal strain, *Candida albicans* (ATCC 90028), *Saccharomyces cerevisiae* (ATCC 14884) and *Aspergillus fumigatus* (ATCC 90906) were used. Inoculum size was adjusted to 1 to 2 × 10^4 CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Stock solution of 10000µg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 6000µg/ml, 4000µg/ml, 2000µg/ml and 1500µg/ml solutions were prepared from which 100 µl solution was taken for assay. Fluconazole (25 µg/ml) was used as a standard. All the dilutions were done by Water for Injection (WFI). 20 % v/v WFI in DMSO was used as a control. 20 % WFI in DMSO was used as a control.

Result and discussion

Structural elucidation of the synthesized compound was done by IR, Mass & NMR Spectroscopy. Result showed that compound 2, 4, 5 possess good antifungal activity against test fungal strains. The results of the study were interpreted by mean diameter of inhibition zone in mm and given in table 2.

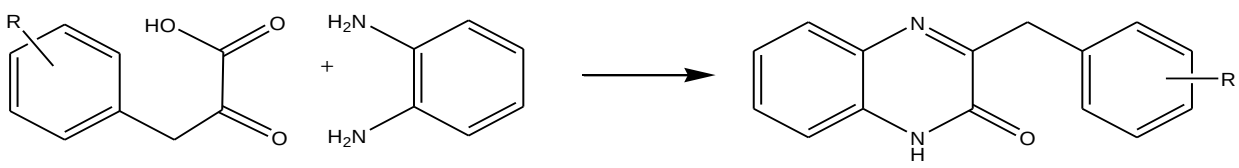

Table 1: physical data of the synthesized compounds

Sr. No.	Sr. No.	Molecular formula	Mol. Weight	R_f	M.P.(°C)
1	3-Benzylquinoxalin-2-one	$C_{15}H_{12}N_2O$	236.26	0.70	154-156
2	3-(4-Chlorobenzyl) quinoxalin-2-one	$C_{15}H_{11}ClN_2O$	270.06	0.75	192-194
3	3-(4-Methoxybenzyl) quinoxalin-2-one	$C_{16}H_{14}N_2O_2$	266.29	0.77	138-140
4	3-(2-Nitrobenzyl) quinoxalin-2-one	$C_{15}H_{11}N_3O_3$	281.26	0.72	142-144
5	3-(4-Methylbenzyl) quinoxalin-2-one	$C_{16}H_{14}N_2O$	250.30	0.61	156-158
6	3-(4-Nitrobenzyl) quinoxalin-2-one	$C_{15}H_{11}N_3O_3$	281.26	0.74	166-168
7	3-(2-Chlorobenzyl) quinoxalin-2-one	$C_{15}H_{11}ClN_2O$	270.06	0.83	175-177

Table 2: Zone of inhibition of synthesized compounds against test fungus

	Zone of Inhibition (mm)											
	Candida albicans				***Saccharomyces cerevisiae***				***Aspergillus fumigatus***			
	150 µg/well	200 µg/ well	400 µg/ well	600 µg/ well	150 µg/we ll	200 µg/ well	400 µg/ well	600 µg/ well	150 µg/ well	200 µg/ well	400 µg/ well	600 µg/ well
STD	**18. 0 ± 0.14**	25.0 ± 0.25	30.3 3 ± 0.45	36. ± 0.80	13. 0 ± 0.25	20.7 ± 0.61	24.00 ± 0.92	28. ± 0.76	13.40 ± 0.22	17.5 3 ± 0.47	20.7 3 ± 0.23	24.6 7± 0.63
1	**4.0 ± 0.89**	7.0 ± 0.56	8.0 ± 0.33	10.0 ± 1.2	6.0 ± 0.35	8.00 ± 0.42	9.00 ± 0.40	12.0 ± 0.61	1.0± 0.38	3. ± 0.25	5.55 ± 0.89	8.33 ± 0.63
2	**7 ± 0.95**	09 ± 0.23	11 ± 0.75	12.0 ± 0.55	8.0 ± 0.29	9.0 ± 0.61	13. ± 0.31	16.33 ± 0.42	2.40 ± 0.40	6.23 ± 0.78	8.82 ± 0.24	12.0 ± 0.66
3	**5.0 ± 0.35**	8.0 ± 0.86	9.0 ± 0.75	10 ± 1.3	4.0 ± 1.02	5.0 ± 0.20	6.33 ± 0.31	10.0 ± 0.81	3.33 ± 0.52	5.0 ± 0.25	6.20 ± 0.36	8.0 ± 0.48
4	**6 ± 0.23**	9 ± 0.66	10± 0.98	13 ± 0.45	4.0 ± 0.33	7.0 ± 0.88	8.33 ± 0.45	12.33 ± 0.12	2.33 ± 0.33	6.20 ± 0.29	8.33 ± 0.78	10.4 7± 0.46
5	**9 ± 0.72**	13 ± 0.75	16 ± 0.72	18 ± 0.95	5.00 ± 0.40	6.0 ± 0.31	8.67 ± 0.42	10.33 ± 0.31	10.33 ± 0.35	12.3 3 ± 0.40	14.9 3 ± 0.66	16.6 0 ± 0.45
6	**5.0 ± 0.71**	6.80 ± 1.35	8.20 ± 0.62	9.51 ± 0.95	4.0 ± 0.42	6.0 ± 0.23	7.0 ± 0.85	11.0 ± 0.69	2.33 ± 0.77	5 ± 0.23	8.33 ± 0.58	12.0 0 ± 0.74
7	**3.0±0.8 8**	5.0± ± 0.55	7.56 ± 0.48	3.23 ± 0.95	2.00 ± 0.15	4.20 ± 0.44	5.33 ± 0.78	8.0 ± 0.56	6.33 ± 0.33	10.8 3 ± 0.42	12.0 7 ± 0.55	14± 0.96

References

1. Giovanni U, Andrew H J, Wang J, Gary. J Q, *Nucleic Acids Research,*, 1985, 13,104.
2. Esther M, Ana N, Luque F J, *Nucleic Acids Research*, 2005, 33, No. 6214–6224.
3. Pratt Y T, The Quinoxalines – in The Chemistry of Heterocyclic Compounds, R. C. Elderfield Ed. John Wiley and sons INC., New York, 1957. Vol. 6, 455-495.
4. Hinsberg O , *Liebigs, Ann. Chem,* 1887, 237, 1228.
5. Carta A, Piras S, *Mini Reviews In Medicinal Chemistry*, 2006, 6, 1179-1200.
6. Mishra P K, Sarma P K, Singhai P K and Singh.U P, *Mycobiology*, 2007, 35(2), 72-75.
7. Mohammed I A, SubrahmanyamE V S, Hareesh A R, Kowti R, *Int J Pharm Sci Bio*, 2010,1(2),80-85.
8. Drobnica L, Zemanova M, Nemec P, Kristian A P, Martvon, Vodska E Z, *Applied Microbiology*, 1968, 16(4), 582-587.
9. Mendoza L, Modak B, Torres R and Cotoras M, *J. Chil. Chem. Soc*., 2008, 53, 1447-1449.
10. Brownlee G and Woodbine M, *Brit. J. Pharmacol*., 1948, 3, 305.
11. Muthumani P, Neckmohammed L, Meera R, Venkataraman S, Chidambaranathan N, Devi P, Kumar C A S, *Int J Pharm Biomed Res*, 2010, 1(3), 78-86.
12. Zhang J W, Li S K and Jun Wu W J, *Molecules*, 2009, 14, 273-278.

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