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ANTIMICROBIAL ACTIVITY OF SOME SUBSTITUTED QUINOXALIN-2(1H)-ONE DERIVATIVES

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ABSTRACT

In this study, Some 3-substituted [(phenyl) methylidene] amino}ethyl)amino]quinoxalin-2(1H)-one have been synthesized by Phillips condensation mechanism. All the final derivatives were evaluated for antimicrobial activity *in vitro* by using Disc diffusion method. It was found that all the selected compounds exhibit wide antimicrobial activity and that compound IIId had a broad spectrum of activity.

Keywords: Quinoxaline, Anti microbial, Phillips condensation, Disc diffusion method.

Introduction

Heterocyclic compounds represent an important class of biological active molecules. Specifically those containing quinoxaline derivatives have evoked considerable attention in recent years as these are endowed. Quinoxalines are a versatile class of nitrogen containing heterocyclic compounds and they constitute useful intermediates in organic synthesis. Quinoxaline, also called a benzopyrazine, in organic chemistry, is a heterocyclic compound containing a ring complex made up of a benzene ring and a pyrazine ring and they are isomeric with cinnolenes, phthalazines and quinazolines [1]. There are a number of processes available to generate quinoxaline but generally, they are synthesized by the condensation of 1, 2-dicarbonyls with 1,2 diamines in in precence of suitable catalyst using various solvent systems.

They possess well known biological activities including AMPA/GlyN receptor antagonis [2], antihistaminic agents [3], anti-trypanosomal activity [4], anti-herps [5], antiplasmodial activity [6], Ca uptake/ Release inhibitor [7], inhibit vascular smooth muscle cell proliferation [8]. Quinoxaline derivatives constitute the basis of many insecticides, fungicides, herbicides, as well as being important in human health and as receptor antagonists. Although rarely described in nature, synthetic quinoxaline moiety is a part of number of antibiotics such as echinomycin, levomycin and actinomycin which are known to inhibit the growth of Gram-positive bacteria and also active against various transplantable tumours [9,10]. In addition, quinoxaline derivatives are reported for their application in dyes, efficient electroluminescent materials, organic semiconductors and DNA cleaving agents [11]. These are useful as intermediates for many target molecules in organic synthesis and also as synthons.

Numerous methods are available for the synthesis of quinoxaline derivatives which Extensive researches have generated numerous synthetic approaches for the construction of the skeleton of such heterocycles. Among these methods, the most widely used one relies on the condensation of aryl-1,2- diamines with aryl ketones, usually α-dicarbonyl compounds or their equivalents [12]. Recent improvements on these conditions were reported via solid-phase [13], oxidative coupling of epoxides with ene-1,2-diamines [14]. Improved methods have been reported via a condensation process catalyzed by CAN [15],

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molecular iodine as a catalyst [16], manganese octahedral molecular sieves [17], task-specific ionic liquid [18], from PEG-400 [19], from IBX [20], from PbO [21], from ZrO_2 [22], from galactose [23]. Recently, a number of catalysts have been reported for the synthesis of quinoxalines. Considering the significant applications in the fields of medicinal, industrial and synthetic organic chemistry, there has been tremendous interest in developing efficient methods for the synthesis of quinoxalines.

Materials and methods

All chemicals and solvents were procured from commercial sources, and were used without any additional purification. The chemicals were purchased From Sigma–Aldrich, Merck, Laboratory (Pune), Research Lab (Poona), Loba chemicals Pvt. Ltd. (Mumbai) etc. The reactions were monitored by thin layer chromatography (TLC) on gel glass plates. All melting points were measured in "VMP-I" melting point apparatus and were uncorrected. The infrared spectra for the synthesized compounds were recorded using JASCO-FTIR 8400 spectrophotometer using potassium bromide pellet technique. The 1H-NMR spectra were recorded on a Bruker 400 Ultrashield instrument (300 MHz), using TMS as the internal standard and with CDCl3 as the solvents; the chemical shifts are reported in ppm (δ) Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (1%) as the internal standard (CDRI Lucknow, India).

Nutient agar utilised

- Nutrient Agar Medium (Research Lab, Poona)
- MacConkey's Agar Medium (Research Lab, Poona)
- Sabouraud's Agar Medium (Micro Master Laboratories, Thane)
- Dimethylsulfoxide [DMSO] (Research Lab Fine Chem Industries, Mumbai)

Bacerial strains utilized

- *Bacillus amylase* – Gram +ve
- *Staphylococcus aureus* – Gram +ve
- *Escherichia coli* – Gram -ve
- *Pseudomonas aerogenosa* – Gram -ve

Fungal strain utilized - *Candida albicans*

Steps involved in the synthesis are as follows

1) Synthesis of 1,4-dihydroquinoxaline-2,3-dione (I)
2) Synthesis of 3-[(2-aminoethyl)amino]-3,4-dihydroquinoxalin-2(1H)-one (II)
3) Synthesis of 3-[(2-{[(E)-(substituted phenyl) methylidene]amino}ethyl)amino]quinoxalin-2(1H)-one (IIIa-e)

General procedure for synthesis 1,4-dihydroquinoxaline-2,3-dione (I)

A solution of oxalic acid dihydrate (0.238mole, 30g) in H_2O (100ml) was heated to 100 °C and conc.HCl 4.5ml was added, followed by O-phenylendiamine (0.204 mole, 22g) with s**t**irring ,temperature was maintained at 100 °C for 20 min. the mixture cooled by addition of ice. The precipitate was formed and washed with water. Recrestallization from ethanol

3-[(2-aminoethyl)amino]quinoxalin-2(1H)-one (II):

A mixture of the quinoxalindione **(I)** (0.062mole, 10.04g), ethylene diamine (1mole, 50ml,) , and water (50ml) was heated under reflux for 2h, then cooled to room temperature, the precipitate was filtered, washed with water and crystallized from 2-butanol.

3-[(2-{[(E)-substituted (phenyl) methylidene]amino}ethyl)amino]quinoxalin-2(1H)-one (III)a-e:

Amixture of 3-[(2-aminoethyl) amino] quinoxalin-2(1H)-one **(II)** and the corresponding aromatic aldehyde (0.01 mole of each) in ethanol as solvent (20ml) was refluxed for 5hr. Upon cooling the precipitate was obtained, filtered, dried and crystallized from ethanol.

Physical and spectral data of synthesized compounds

1,4-dihydroquinoxaline-2,3-dione (I)

m.p. = 300 °C, molecular formula ($C_8H_6N_2O_2$)

IR:3404,3176,3113,1682,1618,1522,1499,1426,1383,755,744; 1H-NMR ($CDCl_3$), δ ppm 8.003(s, 2H, NH), 6.978(t, 2H, CH), 6.715 (d.2H, CH)

3-[(2-aminoethyl) amino] quinoxalin-2(1H)-one (II)

m.p. = 262 °C, molecular formula ($C_{10}H_{12}N_4O$)
IR:3484,3374,3098,2968,2928,1608,1513,1494,1435,820,746;^{1}HNMR($CDCl_3$):,δppm7.711(d,2H,CH),7.590(t,2H,ArH),2.268(q,2H,CH_2),2.747(t,2H,CH_2),8.131(s,2H,NHCO),3.631(s,1H,NH),5.929(s,2H,NH_2)

3-[(2-{[(1E, 2E)-3-phenylprop-2-en-1-ylidene] amino} ethyl) amino]quinoxalin-2(1H)-one (IIIa)

m.p. = 258^{0}C, molecular formula ($C_{19}H_{18}N_4O$);
IR:3448,3417,3067,2923,1699,1610,1586,1456,1586,1456, 1427,1383,1315,739,780;H-NMR($CDCl_3$) δ ppm ; 7778 (t, 2H, Ar-H), 7.678(d, 2H, Ar-H), δ 10.694 (s, 1H, CH=N), δ 3.446 (s, 1H, NH), 9.065 (s,1H,NHCO),2.291 (q, 2H, CH_2), 2.509 (t, 2H, CH_2),6.845(d,1H,Ar-H),7.074(t,1H,Ar-H),7.310-7.549(m,2H, Ar-H),7.742-7.254(d,2H, Ar-H)

3-[(2-{[(E)-(3-chlorophenyl) methylidene] amino} ethyl) amino] quinoxalin -2(1H)-one (IIIb)

m.p. = 282^{0}C, molecular formula ($C_{17}H_{15}N_4OCl$);
IR : 3444, 3404, 3178, 3022, 2898, 1615, 1682, 1578, 1499, 1473, 1413, 1384, 754, 744, 721;^{1}H-NMR ($CDCl_3$):,δ ppm

8.095(s,1H,NH), 8.014(d,2H,CH), 7.431(t,2H,Ar-H), 3.832(s,1H,NH), 2.857(m,2H,CH_2), 2.267(t,2H,CH_2), 9.953(s,1H,-CH=N-),7.867(s,1H,Ar-H), 7.609(t,1H, Ar-H), 6.96-7.647(d,2H, Ar-H)

3-{[2-({(E)-[3,4-(dimethylamino) phenyl]methylidene} amino)ethyl]amino}quinoxalin-2(1H)-one (IIIc)

m.p. = 177^{0}C, molecular formula ($C_{19}H_{21}N_5O$);
IR: 3417, 3060, 2951, 1694, 1638, 1617, 1511, 1384, 1494, 1373, 858, 806; ^{1}H-NMR ($CDCl_3$):, δ ppm;8.602(s,1H,NH),7.608(d,2H,Ar-H),7.087(t,2H,Ar-H),3.832(s,1H,NH), 2.511(m,2H,CH_2),2.832(t,2H,CH_2),9.672(s,1H,-CH=N-),6.702(d,2H,Ar-H),6.583(d,2H,Ar-H), 3.095(s,3H,CH_3)

3-[(2-{[(E)-(3, 4-dichlorophenyl) methylidene]amino}ethyl)amino]quinoxalin-2(1H)-one (IIId)

m.p. = 280^{0}C, molecular formula ($C_{17}H_{14}N_4OCl_2$);
IR: 3416, 3060, 2951, 2840, 1694, 1617, 1551, 1494, 1385, 1373, 806, 831791,765; ^{1}H-NMR ($CDCl_3$):, δ ppm; 8.943(s,1H,NH), 7.778(d,2H,Ar-H), 7.532(t,2H,Ar-H), 3.870(s,1H,NH), 2.386(m,2H,CH_2), 2.591(t,2H,CH_2), 9.763(s,1H,-CH=N-),7.448(s,1H,Ar-H), 6.860(d,2H,Ar-H)

3-[(2-{[(E)-(1-hydroxynaphthalen-2-yl)methylidene] amino} ethyl) amino] quinoxalin-2(1H)-one (IIIe)

m.p. = 272^{0}C, molecular formula ($C_{21}H_{18}N_4O_2$);
IR: 3340, 3442, 3041, 2923, 2979, 1684, 1631, 1550, 1497, 1466, 1384, 1331, 827,802^{1}H-NMR($CDCl_3$)δppm;8.564(s,1H,NH),7.534(d,2H,Ar-H),7.711(t,2H,Ar-H); 4.062(s,1H,NH),3.484(m,2H,CH_2),2.147(t,2H,CH_2),10.739(s,1H,-CH=N-),11.566(S,1H,OH), 7.067-7.128(d,4H Ar-H), 7.908(t,2H,Ar-H)

Pharmacological evaluation

The anti microbial activity of synthesized compounds, IIIa-e was determined in vitro by disc diffusion technique. In vitro antimicrobial activity of all synthesized compounds and standard drugs have been evaluated against four strains of bacteria which include two Gram +ve bacteria such as *Staphylococcus aureus, Bacillus amylase*, two Gram-ve bacteria such as *Escherichia coli*, *Pseudomonas aeurogenosa and* one fungal stain *Candida albicans.* The antibacterial activity was compared with standard drugs viz. Ampicillin and antifungal activity was compared with Fluconazole.

Anti-bacterial activity

The purified products were screened for their antibacterial activity by using disc diffusion method. The nutrient agar, Mac conky' s agar prepared by the usual method, was inoculated aseptically with 0.5 mL of 24 h old subculture of *S. aureus, B. amylase, P. auriginosa* on nutrient agar and *E. coli* on MacConkey's respectively in separate conical flasks at 40 ^{0}C – 45 ^{0}C and mixed well by gentle shaking. About 15 mL of the contents of the flask were poured and evenly spread in petridish and allowed to set for two h. Discs of diameter 5 mm were prepared by using whatmann filter paper and sterilized. Sterilized discs soaked in drug solution of 50µg/mL and 500µg/mL and dried. The dried discs were placed on media in petri plate.

The plates were incubated at 37 ^{o}C for 24 h and the control was also maintained with DMSO in similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and recorded in Table No 3.

Anti-fungal activity

Candida albicans was employed for testing antifungal activity by disc diffusion method. The culture was maintained on sabrouds agar slants. Sterilized Sabrouds agar medium was inoculated with 48 h old 0.5 mL suspension of fungi in a separate flask. About 15 mL of the inoculated medium was evenly spread in a sterilized Petridishesh and allowed to set for 2 h. Discs of diameter of 5 mm were prepared by using whatmann filter paper and sterilized. Sterilized discs soaked in drug solution of 50µg/mL and 500µg/mL and dried. The dried discs were placed on media in Petri plate. The plates were incubated at 37^{o}C for 48 h. After the completion of incubation period, the zones of inhibition of growth in the form of diameter in mm were measured. The control was also maintained with DMSO in similar manner. The zones of inhibition are recorded in Table No. 3

Table 1. List of aromatic aldehyde used

Compounds	Aromatic aldehyde
III $_a$	C_6H_5-CH_2CH=CH CHO
III $_b$	3 Cl - C_6H_4CHO
III $_c$	$(CH_3)_2N$-C_6H_4CHO
III $_d$	3, 4 Cl- C_6H_3 CHO
III $_e$	2 OH $C_{12}H_8CHO$

Table 2. The physico -chemical data of compounds

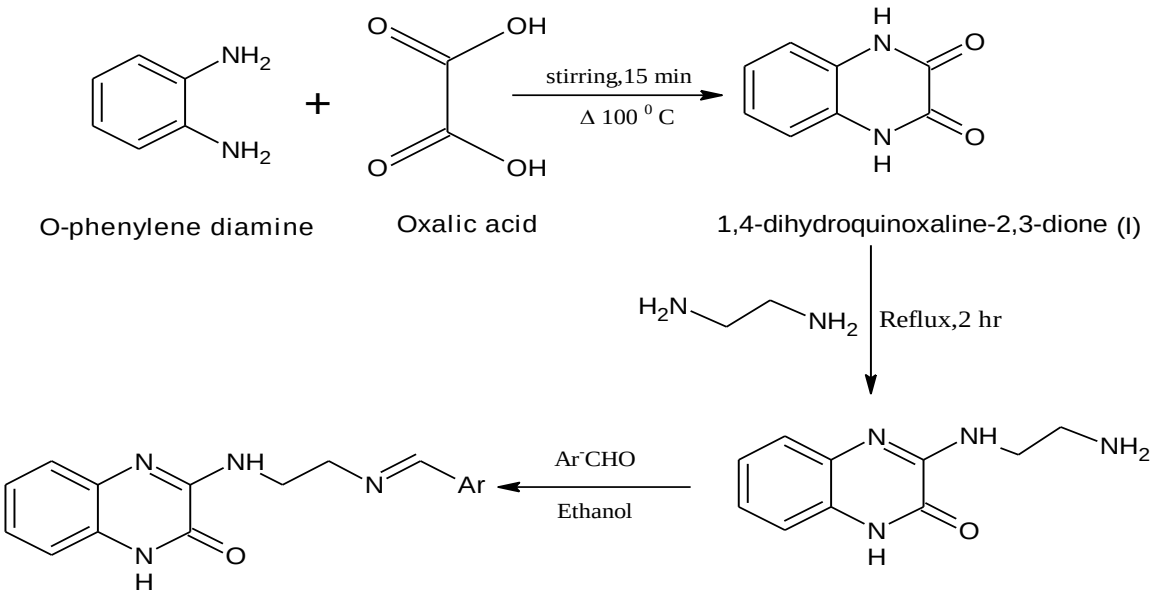
Compounds	Molecular formula	MP (^{0}C)	% Yield	*R_f
I	$C_8H_6N_2O_2$	300 ^{0}C	77 %	0.67
II	$C_{10}H_{12}N_4O$	262 ^{0}C	71 %	0.54
IIIa	$C_{19}H_{18}N_4O$	258^{0}C	71%	0.51
IIIb	$C_{17}H_{15}N_4OCl$	282^{0}C	60%	0.81
IIIc	$C_{19}H_{21}N_5O$	177^{0}C	57%	0.86
IIId	$C_{17}H_{14}N_4OCl_2$	280^{0}C	55%	0.84
IIIe	$C_{21}H_{18}N_4O_2$	272^{0}C	62%	0.90

*Mobile phase: - Tolune: Acetone (4:5)

Table 3. Anti microbial activity, presented as zone of inhibition in mm

Compound	Conc. of Test Compound (ug/ml)	Zone of Inhibition (diameter in mm)				
		B.amylase	***S. Aureus***	***E. coli***	***P.aerogenosa***	***C.albicans***
Ampicillin\ Flucanazole	500	13	14	12	14	11
	50	10	11	9	10	9
IIIa	500	9	7	8	11	8
	50	7	6	7	8	7
IIIb	500	7	9	10	8	7
	50	5	5	8	6	6
IIIc	500	8	9	9	12	9
	50	5	7	7	9	8
IIId	500	10	9	8	11	9
	50	7	6	6	9	7
IIIe	500	9	7	9	10	9
	50	8	6	6	8	6

Scheme for synthesis



RESULTS

In the present investigation, 3-[(2-{[(E)-(substituted phenyl) methylidene] amino} ethyl) amino] quinoxaline-2(1H)-one. The different aromatic aldehyde was used in this scheme and the physicochemical properties of synthesized derivatives are summarized in Table No. 1 and 2 respectively. The structural elucidation of the synthesized compounds was carried out with the help of IR spectroscopy and ^{1}H NMR spectroscopy. Some of the major Advantages of this procedure are such as the ambient conditions, Very good yields, short reaction times, and use of an inexpensive, readily available Simple work-up procedure and absence of volatile and hazardous solvents. And absence of metal catalyst. Screening of the in vitro anti-microbial activity of the novel series, Schiff's bases of substituted quinoxaline-2 quinoxaline-2(1H)-one. Allowed us to identify interesting anti-microbial candidates based on their potency.

DISCUSSION

In a continuing effort to obtain new anti microbial drug candidates, the syntheses of some 3-[(2-{[(E)-substituted (phenyl) methylidene] amino} ethyl) amino] quinoxalin-2(1H)-one by using Phillips condensation mechanism. Synthesized derivatives were evaluated for anti-microbial activity by using ampicilline as Standard drug for comparing the anti- micobial activity and flucanazole was used as standard drug for comparing the anti- fungal activity

The antimicrobial activity of tested compounds against different strains of bacteria and fungi is shown in Table No. 3. It can be concluded that all the compounds have displayed maximum activity against P.aerogenosa. The compound IIIb is highly active against E. coli. The compounds IIId and compound IIIc is highly active against P.aerogenosa and S. Aureus. The compound IIIc and IIId highly active against C. albicans. All the compounds except IIId are found to be highly active against bacterial strains. Therefore it may be concluded fro results that anti- bacterial activity may be due to the presence of electro negative functionality in the molecule.

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