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Antimicrobial activities of some substituted quinoxalin-2(1*H*)-one derivatives

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ABSTRACT

In an effort to develop potent anti-microbial agents, we have synthesized some substituted quinoxalin-2(1H)-one derivatives by Phillip's condensation mechanism. Final derivatives were screened for their in- vitro anti-bacterial activity against range of Gram positive and Gram negative and also for their anti-fungal activity against a strain of Candida albicans species. All the compounds were characterized by IR and ^{1}H NMR spectroscopic data. It was found that all the selected compounds exhibit wide anti-microbial activity. Amongst these compounds; compound IIIb was highly active against E. coli. The compounds IIId and compound IIIc were highly active against P.aerogenosa and S. Aureus. The compounds IIIc and IIId were highly active against C. albicans.

Keywords: Quinoxaline, Anti-microbial, Phillip's 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 presence 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], molecular iodine as a catalyst [16], manganese octahedral molecular sieves[17], task-specific ionic liquid[18], from PEG [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.

EXPERIMENTAL SECTION

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 $CDCl_3$ 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:

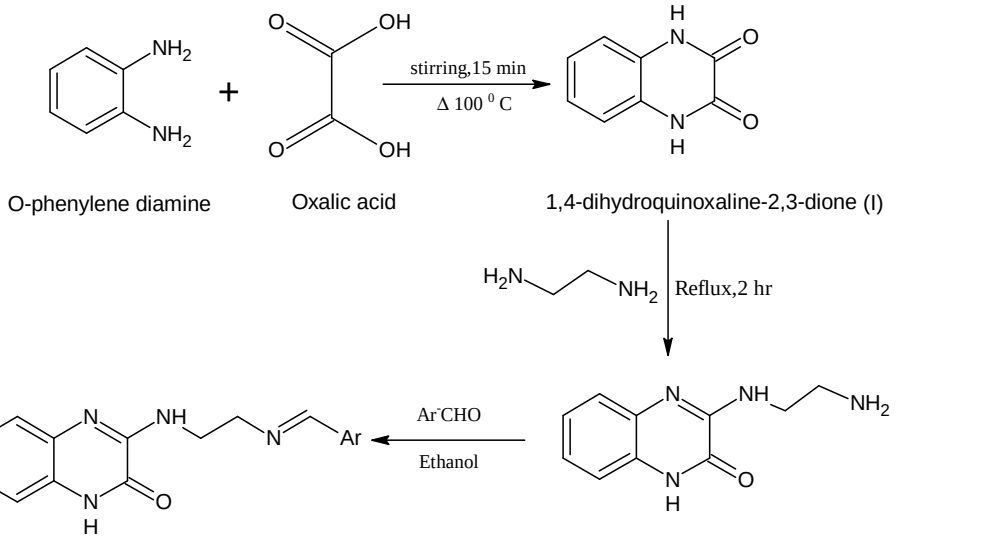
- Nutrient Agar Medium (Research Lab, Poona)
- Mac Conkey's Agar Medium (Research Lab, Poona)
- Sabouraud's Agar Medium (Micro Master Laboratories, Thane)
- Dimethylsulfoxide [DMSO] (Research Lab Fine Chem Industries, Mumbai)

Bacerial strains utilized:

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

Fungal strain utilized - *Candida albicans*

Scheme for synthesis



Steps involved in the synthesis are as follows;

I. Synthesis of 1,4-dihydroquinoxaline-2,3-dione (I)
II. Synthesis of 3-[(2-aminoethyl)amino] quinoxalin-2(1H)-one (II)
III. 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.

Table No.1 list of aromatic aldehyde used

Compounds	Aromatic aldehyde
III $_a$	C_6H_5-CH_2CH=CH CHO
III $_b$	3 Cl - C_6H_4 CHO
III $_c$	$(CH_3)_2$N C_6H_4 CHO
III $_d$	3, 4 Cl- C_6H_3 CHO
III $_e$	OH $C_{12}H_8$ CHO

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

A mixture 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.

No. 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: - Toluene: Acetone (4:5)*

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; ^{1}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. = 280^0C, molecular formula ($C_{17}H_{14}N_4OCl_2$); IR: 3416, 3060, 2951, 2840, 1694, 1617, 1551, 1494, 1385, 1373, 806, 831, 791, 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. = 272^0C, 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 which were *Staphylococcus aureus, Bacillus amylase* and two Gram-ve bacteria *Escherichia coli, Pseudomonas aeurogenosa* also against one fungal stain *Candida albicans.* The antibacterial activity was compared with standard drugs viz. Ampicillin and antifungal activity was compared with Fluconazole.

All the synthesized compounds, that is quinoxaline derivatives were dissolved in dimethylsulfoxide (DMSO) so as to get the concentrations of 50 ug/ml and 500 ug/ml. Ampicillin and Fluconazole were separately dissolved in DMSO and used as standard drug for antibacterial & antifungal screening respectively.

Nutrient agar medium, Sabouraud's dextrose medium and Mac Conkey's agar medium were sterilized in autoclave at 15 lbs pressure (121^0C) for 15 minutes. Petri plates, Whatmann filter paper No. 41, cotton swabs were sterilized in oven at 160^0C for two hours.

Preparation of Standard Inoculum suspension: By using 24/48 hr old cultures of bacteria / fungi from the slant, suspensions of bacteria / fungi were made separately in sterile normal saline solution (0.85% NaCl in distilled water) in aseptic condition, to get moderate turbidity. The turbidity of each solution was compared and adjusted with the turbidity of solution resulting by mixing 0.5 ml of 1.175% of barium chloride and 99.5 ml of 0.36% ammonium sulphate (NH_2SO_4).

Inoculation of suspension of bacteria & fungi on culture media*:* Sterile, non-toxic cotton swab were dipped in to the standardized inoculum turbidity as adjusted as to obtained confluent growth on the Petri plate) and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between streaking. Then the streaked inoculums were allowed to dry for 5-15mins with lid in place.

Preparation of Discs: paper discs were prepared by punching whatman filter paper (no. 41). Discs were then dipped in solutions containing synthesized drug at conc.50 ug/ml and 500 ug/ml in DMSO. Standard drugs Ampicillin and Fluconazole (50 ug/ml, 500ug/ml in DMSO) were prepared and discs were dipped in them too. The discs were then removed from solutions using sterile forceps and paced separately on petri-plates containing solid media. Then petri-plates

were kept in refrigerator for 30 min. to allow proper drug diffusion. The plates were then removed and bacteria inoculated plates were incubated at 37^0C for 24 hrs while fungi inoculated plates were incubated at 37^0C for 48 hrs. Petri-plates were observed for zones of inhibition, which was reported (in mm) given in Table No 3.

Table No. 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

RESULTS AND DISCUSSION

In the current research work, we aimed to synthesize some novel substituted quinoxalines. The aforementioned compounds were prepared according to the synthetic process illustrated in scheme. The structural elucidation of the synthesized compounds was carried out with the help of IR spectroscopy and ^{1}H NMR spectroscopy. Screening of the *in vivo* anti-microbial activity of the 3-{[2-({(E)-[substituted) phenyl] methylidene} amino) ethyl] amino} quinoxalin-2(1H)-one Allowed us to identify interesting anti-microbial candidates based on their potency, making them valid new leads for synthesizing new compounds that might improve the previously methods of synthesis.

In the present investigation, different aromatic aldehyde was used in this scheme and the physicochemical properties of synthesized derivatives are summarized in Table No. 1 and 2 respectively. 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, absence of metal catalyst.

Synthesized derivatives were evaluated for anti-microbial activity. The anti-microbial activity of synthesized compounds tested against different strains of bacteria and fungi; results are shown in Table No. 3. It can be concluded that all the compounds have displayed maximum activity against *P.aerogenosa*. The compound IIIb was highly active against *E. coli.* The compounds IIId and compound IIIc were highly active against *P.aerogenosa* and *S. Aureus.* Compounds IIIc and IIId were highly active against *C.albicans*. Therefore it may be concluded from results that anti-bacterial activity may be due to the presence of electro negative functionality in the molecule.

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