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An Efficient synthesis and Biological activity of Quinoxaline-2-Carboxylic acid and its derivatives

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ABSTRACT

Condensation of ortho phenyldiamine with acetic acid and form 2-tetrahydroxy butyl quinoxaline which further react with hydrogen peroxide and solid sodium hydroxide form quinoxaline-2-carboxylic acid. Ethyl 3-hydroxyquinoxaline2-carboxylate reacts with $POCl_3$ and to form ethyl-3- chloroquinoxaline2-carboxylate. Ethyl-3- chloroquinoxaline-2-carboxylate reacts with sodium hydroxide, alcohol, sodium methoxide and form 3-ethoxyquinoxaline-2-carboxylic acid, 3-amino quinoxaline-2-carboxylic acid, 3-methoxy quinoxaline-2-carboxylic acid, with good yield. The structure of the compounds had been established on the basis of IR, and ^1H NMR, spectral data.

Keywords: Quinoxaline-2-carboxylicacid,Ethyl-3-chloro quinoxaline-2-carboxylate, 3-chloroquinoxaline-2-carboxylic acid, Ligands.

INTRODUCTION

Quinoxaline-2-carboxylic acid is a fragment of the antibiotic echinomycin and triostin. Echinomycin chemistry of these drugs with metal ions of biological and pharmaceutical importance is of considerable interest [1]. Streptomyces eachinates cultured on a maltose minimal salts medium normally produces a single antibiotic echinomycin containing two quinoxaline-2-carbonyl chromophores. Quinoxaline-2-carboxylic acid is a fragment of the antibiotic echinomycin and triostin, a powerful selective inhibitor of nucleic acid synthesis. The chromophore moiety of quinoxaline-2-carboxylic acid plays an important biogenetic role in the synthesis of antibiotic triostion [2]. Quinoxaline-2-carboxylic acid derivative quinacillin is semi-synthetic antibiotic related to penicillin [3,5]. Several quinoxaline-2-carboxylic acid derivatives show inhibiting effect on the growth of staphylococcus aureus bacteria [4,6], anti-tuberculosis activity [5,7]. The quinoxaline-2-carboxylic acid skeleton is a relatively unexploited heterocyclic compound in drug discovery [6,8].

Thioquinox is a derivative of quinoxaline-2-carboxylic acid exhibits fungicidal property [7,9]. Quinoxaline-2-carboxylic acid and its 3-chloro and 3-hydroxy derivative were used as analytical reagents [8,], any drugs posses modified pharmacological and toxicological properties when administered in the form of metallic complexes. Probably the most widely studied citation in this respect is $Cu^{2+,}$ since as host of low molecular weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rhenmatoid, gastric, ulcer and cancer [9-11]. Quinoxaline-2-carboxylic acid derivatives are

known to be effective antibacterial agents. However, they have low solubility at neutral pH values, a characteristic which can adversely affect their suitability for parenteral administration. Quinoxaline-2-carboxylic acid can act as potential bidentate ligands [12-19].

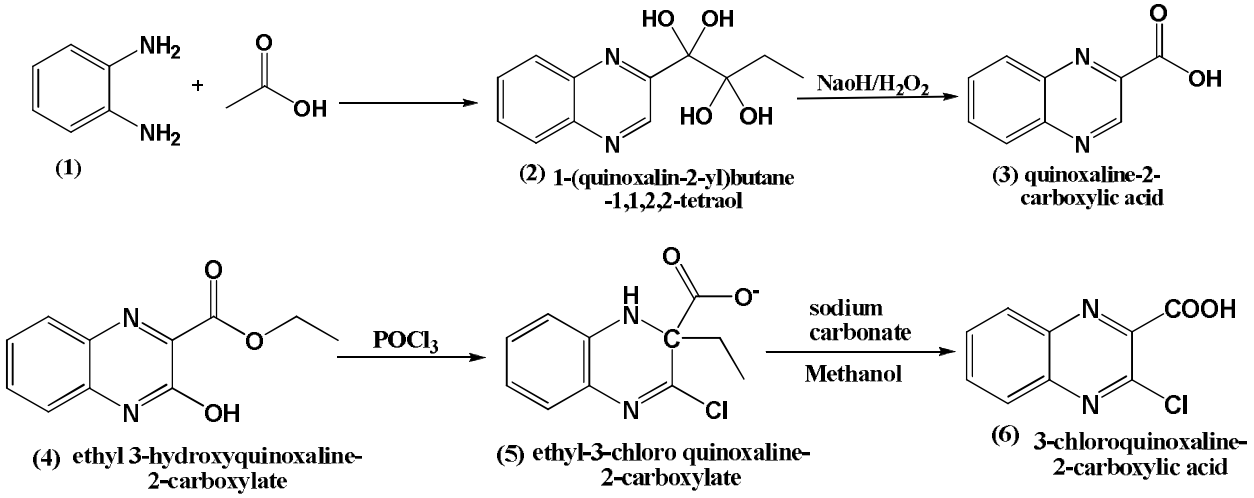
MATERIALS AND METHODS

Experimental: Melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on Schimadzu FTIR-8400S. ^{1}H-NMR spectra were recorded in $CDCl_3$ on Bruker DRX300 MHz spectrometer using TMS as internal reference with their values expressed in ppm. Purity of all the synthesized compounds were routinely checked by TLC on silica gel G in the solvent system (9:1, benzene: methanol).

General methods for the preparation of compounds

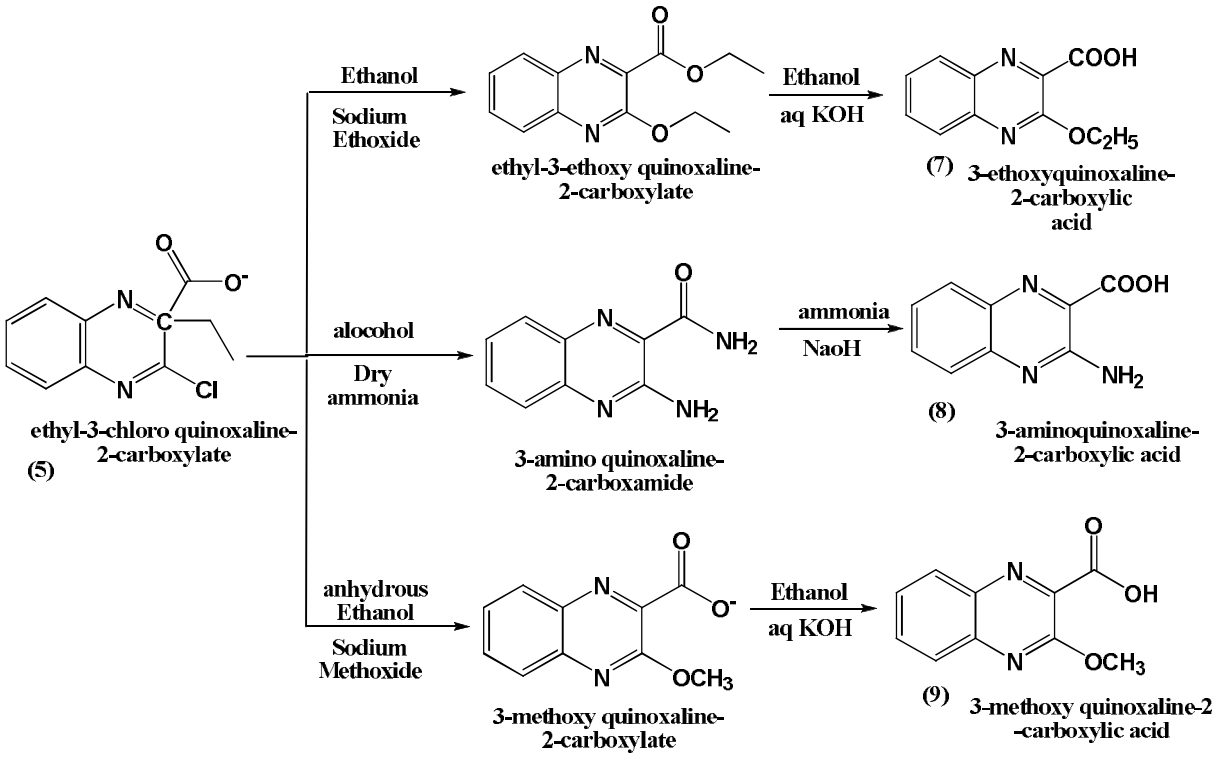
Preparation of quinoxaline-2-carboxylic acid (3): - A mixture of 200g and 60g of ortho phenyldiamine in 200 ml of distilled water and 60 ml of acetic acid was gently boiled for 2h. The product obtained was 2-tetrahydroxybutyl quinoxaline. Quinoxaline-2-carboxylic acid was obtained by treating 10 g of 2-tetrahydroxy butyl quinoxaline suspended in 600 ml of 6 %hydrogen peroxide with 24 g solid sodium hydroxide below 80^{0}C for 1h and then recrystallized from distilled water to give pale yellow crystals of quinoxaline 2-carboxylic acid m.p is 210^{0}C.
IR(KBr)cm^{-1} absorption bands 1595cm^{-1}, 3220cm^{-1}, 3418 cm^{-1}, 1760cm^{-1}, are assignable to (=C=C), (=NH), (O-H), (C=O), and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δ ppm: 8.17-7.7[4H, m Ar-H], 8.7[S, H (pyridine)],11.0[S, H (acid)]; m/z ; 174.04(100%), 175.05 (9.9%). Found Chemical Formula from data: $C_9H_6N_2O_2$. Anal. Calc. is C: 62.07, H: 3.47, N: 16.09, O: 18.37.

Preparation of Ethyl-3-chloro quinoxaline-2-carboxylate (5): - A mixture of ethyl-3-hydroxy quinoxaline-2-carboxylate (10.9 g) and phosphorus oxychloride 50 ml was heated at 110-120^{o}C for 30 minutes and the excess phosphorous oxychloride removed under vacuum. The dark green color viscous residue was poured on to crushed ice (600 g). The mixture is neutralized by the addition of ammonia and extracted with ether and dried with alkali free sodium sulphate. The resulting extract was evaporated and the residue distilled under vacuum at 100mm.The chloro ester distilled as light pink oil and solidified to mass of nearly 10.5 g. it was obtained in form of white needles, having m.p-42^{o}C.



(1) (2) 1-(quinoxalin-2-yl)butane -1,1,2,2-tetraol (3) quinoxaline-2-carboxylic acid

(4) ethyl 3-hydroxyquinoxaline-2-carboxylate (5) ethyl-3-chloro quinoxaline-2-carboxylate (6) 3-chloroquinoxaline-2-carboxylic acid



Scheme-1

IR(KBr)cm^{-1} absorption bands 1595cm^{-1}, 3220cm^{-1}, 3418 cm^{-1}, 1760cm^{-1}, 1625cm^{-1}, are assignable to (=C=C), (=NH), (O-H), (C=O), (C=N) and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δppm: 8.17-7.7[4H, m Ar-H], 8.7[S, H (pyridine)], 11.0[S, H (acid)]; m/z ; 237.04(100%), 239.04(32.1%), 238.05(12.1%) . Found Chemical Formula from data: $C_{11}H_{10}ClN_2O_2$. Anal. Calc. found is C; 55.59; H; 4.24; Cl; 14.92; N; 11.79; O; 13.46.

Preparation of 3-chloroquinoxaline-2-carboxylic acid (6):- A solution of ethyl-3-chloro quinoxaline-2-carboxylate and 0.5 g sodium carbonate in methanol was refluxed for 6 h and then evaporated to dryness under reduced pressure. The residue was dissolved in 20ml water and the solution acidified to congo red by the addition of hydrochloric acid. The Precipitated 3-chloro acid was collected (1.8g) and recrystallized from distilled water to give 3-chloroquinoxaline-2-carboxylic acid, m.p 148^{0}C.
IR(KBr)cm^{-1} absorption bands 3490 cm^{-1}, 1710cm^{-1},1725cm^{-1} ,1610cm^{-1}, 2465cm^{-1}, 1970cm^{-1} are assignable to (O-H), (=NH), (C=O), (=C=C) and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δppm: 8.17-7.7[4H, m Ar-H], 11.0[S, H (acid)]; m/z: 208.00 (100.0%), 210.00 (32.0%), 209.01 (9.9%), 211.00 (3.3%) ; Found Chemical Formula from data: $C_9H_5ClN_2O_2$, C; 51.82; H; 2.42; Cl; 17.00; N; 13.43; O; 15.34.

Preparation of 3-ethoxyquinoxaline-2-carboxylic acid (7):- A solution of ethyl-3-chloro quinoxaline-2-carboxylate (28 g) in anhydrous ethanol (11 ml) was added to hot solution of sodium ethoxide in ethanol and the mixture was refluxed for two hrs, cooled and filtered. The filtrated was evaporated to a small, bulk, distilled with water and extracted with ether. The dried extract was evaporated and the residue distilled at 0.001 mm at bath temperature of 180^{0}C. The reaction yielded ethyl-3-ethoxy quinoxaline-2-carboxylate as colorless solid with m.p-52^{0}C. A solution of ethyl 3-ethoxy quinoxaline-2-carboxylate (1g) in 20 ml ethanol and in 10% aqueous potassium hydroxide of 70 ml was refluxed for 3 hrs, concentrated, diluted with water and acidified using Congo red as pH indicator with diluted hydrochloric acid, yielded colorless needless which were recrystallized from distilled water, m.p-122^{0}C.

IR(KBr)cm^{-1} absorption bands 3490 cm^{-1}, 3430 cm^{-1}, 1625cm^{-1}, 1595cm^{-1}, 2410cm^{-1}, 1940cm^{-1} are assignable to (O-H), (C=N), (C=O), (=C=C) and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δppm: 8.17-7.7[4H, m Ar-H], 11.0[S, H (acid)]; 4.39[S, H, Methoxy]; m/z: 218.07 (100.0%), 219.07 (12.8%); Found Chemical Formula from data: $C_{11}H_{10}N_2O_3$. Anal. Calc. found is C; 60.55; H; 4.62; N; 12.84; O; 22.00.

Preparation of 3-aminoquinoxaline-2-carboxylic acid (8):- A mixture of ethyl-3-chloro quinoxaline-2-carboxylate (4g) and alcohol (120 ml) saturated at 0^0C with dry ammonia was heated with stirring in a autoclave at 150-160^{0}C for 8 hrs. When the reaction mixture was cooled the bright yellow needles (1.5g) were separated and recrystallized from 70 % acetic acid to give 3-amino quinoxaline-2-carboxamide as fine bright yellow needles. The 3-amino quinoxaline-2-carboxamide was refluxed for 4 hrs with 10 % sodium hydroxide solution (50 ml) on refluxation ammonia evolved. After standing and recrystallization from glacial acetic acid to give 3-aminoquinoxaline-2-carboxylic acid in small yellow needles having m.p-263^{0}C.
IR(KBr)cm^{-1} absorption bands 3418 cm^{-1}, 3220cm^{-1},1705cm^{-1} ,1600cm^{-1}, 2485cm^{-1}, 1970cm^{-1} are assignable to (O-H), (=NH), (C=O), (=C=C) and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δppm: 8.17-7.7[4H, m Ar-H], 11.0[S, H (acid)]; 4.0[S, H, aromatic]; m/z: 189.05 (100.0%), 190.06 (9.9%), 190.05 (1.1%); Found Chemical Formula from data: $C_9H_7N_3O_{2.}$ Anal. Calc. found is C; 57.14; H; 3.73; N; 22.21; O; 16.92.

Preparation of 3-methoxy quinoxaline-2-carboxylic acid (9):- A solution of ethyl-3-chloro quinoxaline-2-carboxylate (28 g) in anhydrous ethanol (11 ml) was added to hot solution of sodium methoxide in methanol and the mixture was refluxed for two hrs, cooled and filtered. The filtrated was evaporated to a small, bulk, distilled with water and extracted with ether. The dried extract was evaporated and the residue distilled at 10 minutes at bath temperature of 140^{0}C. The reaction yielded ethyl-3-methoxy quinoxaline-2-carboxylate as colorless solid with m.p-85^{0}C. A solution of ethyl 3-ethoxy quinoxaline-2-carboxylate, 1g in 20 ml ethanol and in 10% aqueous potassium hydroxide of 70 ml was refluxed for 3 hrs, concentrated, diluted with water and acidified using Congo red as pH indicator with diluted hydrochloric acid , yielded colorless needless which were recrystallized from distilled water, m.p-125^{0}C.
IR(KBr)cm^{-1} absorption bands 3430 cm^{-1}, 1625cm^{-1},1760cm^{-1} ,1595cm^{-1}, 2410cm^{-1}, 1940cm^{-1} are assignable to (O-H), (C=N), (C=O), (=C=C) and due to hydrogen bonds respectively; ^{1}HNMR(300 MHz, $CDCl_3$+DMSO-d_6)δppm: 8.17-7.7[4H, m Ar-H], 11.0[S, H (acid)]; 4.06[S, H, methyl]; m/z: 204.05 (100.0%), 205.06 (11.0%), 206.06 (1.2%); Found Chemical Formula from data: $C_{10}H_8N_2O_3$. Anal. Calc. found is C; 58.82; H; 3.95; N; 13.72; O; 23.51.

Assessment of Antibacterial Activity: In this study, two bacterial strains and agar well diffusion method were used to determine the antibacterial activity of the organic compounds. The agar cultures of *E. coli* and *B. subtilis* Microbial type culture collection and zene bank (MTCC Chandigarh) were prepared to assess the organic compounds inhibitory effects. 50 ml of nutrient broth medium were measured into an Erlenmeyer flask; two flasks were prepared for each examined sample. The flasks including agar medium were sterilized in an autoclave at 121ºC for 15 min. For antibacterial tests, bacterial cultures were grown at 35 ºC for 22 h by inoculation in Nutrient Broth (S.P Biotech Jaipur) Petri dishes with 10 ml of Nutrient Agar were prepared, previously inoculated with 100 µl of the culture suspension (1%, containing 106–107 cfu/ml). The wells (5.0 mm in diameter) were cut from the agar at sterile condition. 1 mg of organic compound was dissolved in 1ml of deionised water (1:1 w/v) and added into the wells of agar plates directly at different concentrations (10, 20, ml) and same volume of deionised water was used as a control. The inoculated plates were incubated for 24h at 35 °C. At the end of the incubation period, inhibition zones formed on the medium and the diameter of the inhibition zone was measured and recorded as mean diameter (mm). The results were compared with that of standard streptomycin and ampicillin.

Assessment of Antifungal Activity: A fungal strain was used in this study. The fungal strain cultures of *Aspergillus.niger* Microbial type culture collection and zene bank (MTCC Chandigarh) was grown in agar agar powder (MTCC Chandigarh) at 22 ºC for 5 days. In 1000 ml agar agar powder add 1000 ml water were added to different flasks containing 25 ml sterile agar agar at 43–45 ºC and poured into petridishes. The agar was allowed to solidify at 4 ºC for 1h. A hole was bored in the centre of the sterile agar agar by sterile corkborers (+¼ 5mm) and young spores of each fungus at 7 days age were transferred into the hole. The plates were then incubated at 22 ºC for 7 days. The inhibition effects of the extracts were calculated at the 5th and 10th day as follows: *I =(C- T)/C x 100* Where I is inhibition (%), C the colony diameter (mm) of the fungus on the control plates, and T is the colony diameter (mm) of the fungus on the plates with organic compounds, **3and 5-9,** concentrations

RESULTS AND DISCUSSION

Screening of anti-bacterial activity: The screening results of antibacterial activity and antifungal activity of the compounds **(3, 5-9)** 1 **mg mL^{-1}** concentration (table1,2).
The antibacterial activity was evaluated against two pathogenic strains (*E.coli* and *B.subtilis*). The zone of inhibition and activity index were determined by comparison with the standard drug *Ampicillin* and *streptomycin*. The outcome of this study is presented in table-1, 2. The antibacterial screening against *E.coli* showed that amongst the compounds (3-6) the compound (6) displayed highest activity in mg/ml and all compounds, and in (7-9), (7) displayed highest activity in *B.subtilis.* The antifungal activity was evaluated against a pathogenic strains (*A.niger).*The zone of inhibition and activity index were determined by comparison with the standard drug *Flucanazole*.

Table 1- Results of screening antibacterial activity of products **quinoxaline-2-carboxylic acid** (3, 5-9) of in mg mL^{-1}.

Tested Bacteria	**Products of Quinoxaline-2-carboxylic acid**	**Reference Streptomycin/ Ampiciilin Diameter(mm) 1 mg mL^{-1}/ 1 mg mL^{-1}**		**Zone of inhibition in mg/ml Diameter(mm) 1 mg mL^{-1} / 1 mg 2mL^{-1}**	
E .coli	Compound-3	13	–	17	12
E .coli	Compound-5	–	25	20	15
E.coli	Compound-6	–	21	24	18
B. Septalus	Compound-7	16	–	35	27
B. Septalus	Compound-8	18	–	22	20
B. Septalus	Compound-9	22	–	24	22

The outcome of this study is presented in table-2 (mg mL^{-1}). The antifungal screening against *A.niger* showed that amongst the compounds (3,5-9), the compound (7) exhibited highest activity in **mg mL^{-1}**. The compound (8) showed lowest activity amongst all the compounds. The remaining compounds showed only moderate activity.

Table 2- Results of screening antifungal activity of products **Quinoxaline-2-carboxylic acid** (3, 5-9) mg mL^{-1}

Tested Bacteria	**Products of Quinoxaline-2-carboxylic acid**	**Reference Flucanazole Diameter(mm) 1 mg mL^{-1}**	**Zone of inhibition in mg mL^{-1} Diameter(mm) 1 mg mL^{-1} / 1 mg $2mL^{-1}$**	
A. Niger	Compound-3	**21**	**29**	**26**
A. Niger	Compound-5	**20**	**30**	**22**
A. Niger	Compound-6	**20**	**21**	**19**
A. Niger	Compound-7	**15**	**33**	**25**
A. Niger	Compound-8	**15**	**24**	**21**
A. Niger	Compound-9	**14**	**27**	**24**

The antibacterial activity was evaluated against two pathogenic strains (*E.coli* and *B.subtilis*). The zone of inhibition and activity index were determined by comparison with the standard drug *Ampicillin* and *streptomycin*. The outcome of this study is presented in table-1, 2. The antibacterial screening against *E.coli* showed that amongst the compounds (3-6), the compound (6) displayed highest activity in **mg mL^{-1}**and all compounds, and in (7-9), (7) displayed highest activity in *B. subtilis*. The antifungal activity was evaluated against a pathogenic strains (*A.niger)*.The zone of inhibition and activity index were determined by comparison with the standard drug *Flucanazole*. The outcome of this study is presented in table-2 (**mg mL^{-1}**). The antifungal screening against *A.niger* showed that amongst the compounds (3,5-9), the compound (7) exhibited highest activity in mg mL^{-1}. The compound (8) showed lowest activity amongst all the compounds. The remaining compounds showed only moderate activity.

APPLICATIONS

Quinoxaline-2-carboxylic acid derivatives are known to be effective antibacterial agents. They were assessed for antibacterial activity. Many compounds showed moderate activity.

CONCLUSIONS

In conclusion, several quinoxaline-2-carboxylic acid derivatives (**2-9)** was synthesized which exhibit many important activities as antibacterial, anti-fungal, and fungicidal property The study provided an elegant method for the synthesis of quinoxaline-2-carboxylic acid analogues of its derivatives .

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