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RESEARCH ARTICLE

Synthesis and Antimicrobial Activity of Some Quinoxaline Derivatives

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Abstract: A series of quinoxaline derivatives were synthesized by doing molecular modifications at C-3 methyl group of 2-hydroxy-3-methylquinoxaline nucleus. The synthesis was initiated by bromination followed by attachment of *p*-hydroxy bezaldehydes to 3-methyl group to synthesize 3(4-formyl phenoxymethyl)-quinoxaline-(1*H*)-2-one as new intermediate and condense it with substituted aromatic amines to afford the synthesis of Schiff bases of 3-methylquinoxalin-2(1*H*)-one. The new compounds (GG1, GG2, GG3, GG4 and GG5) have been synthesized by treating *o*-phenylene-diamine with ethyl pyruvate to yield 3-methylquinoxaline 2-one, which on bromination afforded the synthesis of 3-bromomethyl quinoxaline-2-one. Finally 3-bromomethyl quinoxaline-2-one was treated with 4-hydroxy benzaldehyde to yield the synthesis of 3-(-4-formyl phenoxy methyl)-quinoxalin 2(1*H*)-one (GG1), which was treated with different substituted aromatic amines to yield the synthesis of 3-((4-(substituted-phenylimino)-methyl)-phenoxy)-methyl)quinoxalin-2(1*H*)-one (GG2,GG3,GG4 and GG5). The structures of pure compounds were characterized on the basis of IR and ^{1}H-NMR spectral analysis. All the synthesized compounds were evaluated for antimicrobial activity.

Keywords: 2-Hydroxy-3-methylquinoxaline, 3-Bromomethyl quinoxaline-2-one, *p*-Hydroxy bezaldehydes, 3-((4-(substituted-phenylimino)-methyl)-phenoxy)-methyl)quinoxalin-2(1*H*)-one, antimicrobial activity

Introduction

Compounds having Quinoxaline nucleus are reported to exhibit a broad spectrum of biological activity such as antibacterial[1-3], antifungal[4,5], antiviral[6], anticancer[7], antituberculosis[8], antimalarial[9] and anti-inflammatory properties[10]. As no work regarding attachment of *p*-hydroxy bezaldehydes to 3-methyl group has been reported it was thought worthwhile to synthesize 3(4-formylphenoxymethyl)-quinoxalin-(1*H*)-2-one as new intermediate and condense it with substituted aromatic amines to afford the synthesis of Schiff bases of 3-methylquinoxalin-2(1*H*)-one. A series of 3-methyl quinoxalin-2-one derivatives (GG1, GG2, GG3, GG4 and GG5) have been synthesized in the hope of getting better drugs through analogs based drug designing. The structures of pure compounds were characterized on the basis of IR, Mass and ^{1}H NMR spectral analysis. All the synthesized compounds were evaluated for antimicrobial activity.

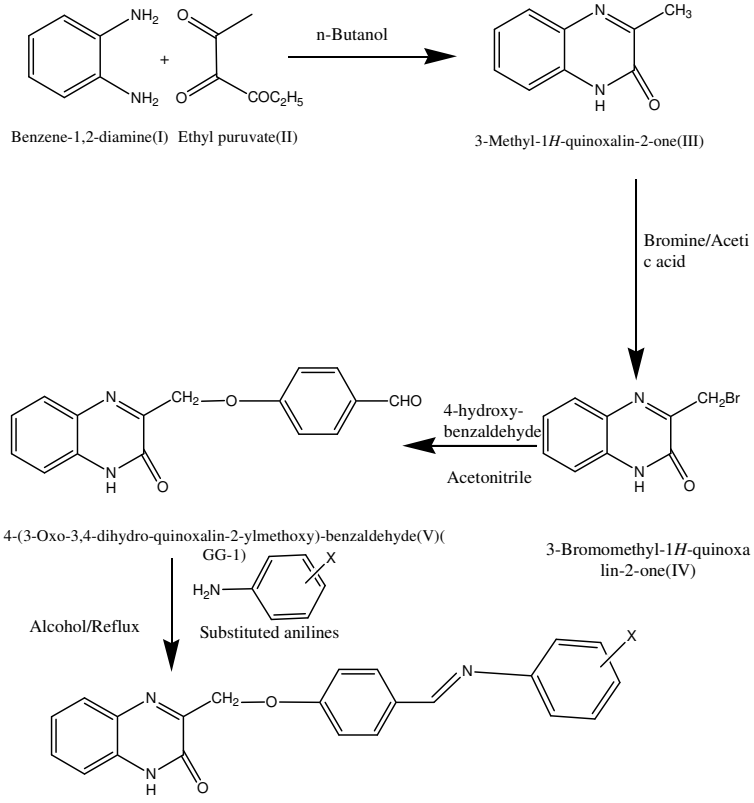
Experimental

All recorded melting points were determined on a laboratory melting point apparatus using the capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G on micro slide glass plates and spots were detected under iodine vapor. IR spectra were recorded in KBr disk on a Perkinelmer FTIR-spectrophotometer and ^{1}H NMR spectra on a Bruker advance II 400 NMR spectromete using TMS as an internal standard. All chemical shift values were recorded as δ (ppm).

Results and Discussion

Synthesis

The chemical synthesis (Scheme 1) was initiated by treating *o*-phenylenediamine (I) with ethyl pyruvate (II) to yield 3-methylquinoxaline 2-one (III), which on bromination afforded the synthesis of 3-bromomethyl quinoxaline-2-one (IV). Finally 3-bromomethyl quinoxaline-2-one was treated with 4-hydroxy benzaldehyde in acetonitrile and the mixture was refluxed to yield the synthesis of 3-(-4-formyl phenoxy methyl)-quinoxalin 2(1*H*)-one(V)GG-1 as new intermediate compound, which was treated with different substituted aromatic amines to yield the synthesis of 3-((4-(substituted-phenylimino)-methyl)-phenoxy)-methyl)quinoxalin-2(1*H*)-one(GG-2-GG-5). The synthesized compounds were purified by recrystallization from appropriate solvents. The structures of all the compounds were elucidated on the basis of IR, Mass and ^{1}H NMR spectral data.



Scheme 1. X=H, 2-CH_3, 3-CH_3, 4-CH_3

The synthesis of compounds I-IV was validated on the basis of literature physicochemical properties. The compound (V) the first intermediate showed IR absorption band at 1663.71 cm^{-1} for C=O stretching (aldehyde) and at 1112.11 cm^{-1} for C-O-C (Ether linkage). Its ^{1}H NMR spectrum exhibited a singlet at (δ) 9.87due to an aldehyde proton and a singlet at 4.03(**δ**) due to CH_2 group. This indicated that a free aldehyde function was present which could be reacted with substituted aromatic amines to form Schiff bases. The spectral data of the compounds (GG-2-GG-5) showed characteristic IR absorption band at about 1285-1316 cm^{-1} for C=N group present in Schiff compounds.^{1}H NMR spectral data were also in agreement with the structures showing a singlet at about **(δ)** 8.303-8.414 for HC=N and at about (δ) 4.074-4.214 for $–CH_2$- suggesting the attachment of substituted aromatic amines to 3-bromomethyl quinoxaline-2-one replacing the bromo group. The IR and ^{1}H NMR spectral data of other substituents and the groups were all found in agreement with the structures of the synthesized compounds to confirm the chemical characterization.

Antimicrobial activity

Antibacterial activity

The antibacterial activity was determined by the disc diffusion method at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antibacterial activity against microorganisms such as *Bacillus subtilis* (Gram positive), *Escherichia coli*, (Gram negative), using ciprofloxacin as standard antibacterial. The results of activity, presented in the Table 1, suggested that the compounds GG-1,GG-3 and GG-5 were highly active against both gram positive and gram negative bacteria whereas other were moderately active when compared to standard Ciprofloxacin. The increase in activity may be attributed to enhancement of lipophilicity due to incorporation of aromatic benzene ring and substituent methyl group at para position.

Antifungal activity

The antifungal activity was assayed by sabouraud dextrose agar media plate disc diffusion method at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antifungal activity against microorganisms such as *Asperagellus niger* and *Candida albicans.* The results of activity, presented in the Table 1, suggested that the compounds GG-1, GG-3 and GG-5 were highly active whereas other were moderately active when compared to standard Fluconazole. The increase in activity may be attributed to enhancement of lipophilicity due to incorporation of aromatic benzene ring and substituent methyl group at para position.

Table 1. Antifungal activity

Compound 50 µg/disc	Zone of inhibition, mm			
	B.subtilis	*E. coli*	*A. niger*	*C. albicans*
GG-1	17	19	18	18
GG-2	14	14	13	14
GG-3	16	18	16	17
GG-4	12	12	13	13
GG-5	15	16	15	16
Ciprofloxacin	25	25		
Fluconazole			23	22
Solvent control DMSO	-	-	-	-

Synthesis of 3-methyl quinoxalin 2-ol

o-Phenylenediamine (10.8 g, 0.1 M) was dissolved in 300 mL of *n*-butanol with warming. Ethyl pyruvate (11.6 g (15 mL), 0.10 M) was dissolved in 100 mL of *n*-butanol separately & was added to the former solution with constant stirring. The solution was set a side for 30 min & then it was refluxed for 1 h on water bath. On cooling the crystals that separated were collected by filteration washed with n-hexane & were purified by recrystallization from ethanol to yield colorless, needle shaped crystals of 2-hydroxy 3 methyl quinoxaline. Yield was 80%, and Melting point 246 ^{o}C [lit mp 245 ^{o}C][11].

Synthesis of 3-bromomethyl quinoxalin-2-one

2-Hydroxy-3- methyl quinoxaline (6.4 g) and anhydrous sodium acetate (2.8 g) were dissolved in warm acetic acid (120 mL); a solution of bromine (6.2 g) in acetic acid (20 mL) was added gradually, with constant stirring, and the mixture was heated on steam bath for 15 min. The crystals, which were separated on cooling, were collected by filtration. The Yield was 70% and melting point 222-224 ^{0}C(Decomposition)[12].

Synthesis of 3-(4-formylphenoxymethyl) quinoxaline2 (1H)-one (GG1)

4-Hydroxy benzaldehyde (0.0025 M) was dissolved in sufficient quantity of acetonitrile, then 0.2 g of anhydrous potassium carbonate was added and the mixture was refluxed with stirring for 1 hour then (0.0025 M) 3-bromomethylquinoxalin-2-one was added and the mixture was further refluxed with stirring for 30 hours, monitoring the progress of reaction by TLC. After the completion of reaction the mixture was filtered and washed with acetonitrile. The filtrate was distilled off to dryness added by 1% sodium hydroxide solution to disintegrate the solid and to dissolve unreacted 4-hydroxy benzaldehyde if any. The crude product was filtered, washed with distilled water to remove alkali. The product was purified by recrystallization from ethanol. Yield was 40% and melting point 164-166 ^{0}C.

3-((4-(Substituted phenylimino) methyl) phenoxy) methyl) quinoxalin-2(1H)-one

The compounds were synthesized by the general procedure in which an equimolar quantity of mixture of 3-(4-formylphenoxymethyl) quinoxaline-2 (1*H*)-one (0.001 M) and substituted anilines (0.001 M) was refluxed in sufficient amount of ethanol in the presence of 2-3 drop of glacial acetic acid. The progress of reaction was monitored by TLC, after the completion of reaction the mixture was cooled to yield the crude product. The crude product was filtered washed with cooled ethanol and purified by recrystallization to afford the synthesis of the required products. The summary of physical data is depicted in Table 2.

Table 2. Physical data of the synthesized compounds

S. No	Compound Code	Reaction Time h	M.P. ^{o}C	Yield %	Mol. Formula	R_f Value	Solvent system
1.	GG-1	30	165-166	40	$C_{16}H_{12}N_2O_3$	0.64	*n*-hexane:ethyl acetate(1:2)
2.	GG2	6	141-142	55.5	$C_{22}H_{17}N_3O_2$	0.76	*n*-hexane:ethyl acetate(1:2)
3.	GG3	5	147-148	52.0	$C_{23}H_{19}N_3O_2$	0.82	*n*-hexane:ethyl acetate(1:2)
4.	GG4	7	137-138	54.2	$C_{23}H_{19}N_3O_2$	0.78	*n*-hexane:ethyl acetate(1:2)
5.	GG5	6	143-144	50.3	$C_{23}H_{19}N_3O_2$	0.80	*n*-hexane:ethyl acetate(1:2)

Spectral data of the synthesized compounds

3-(4-Formylphenoxymethyl) quinoxalin-2(1H)-one(GG1)

IR (KBr cm^{-1}): 3435.28(N-H stretching), 2931.09 (-CH_2 stretching), 1663.71 (C=O stretching (aldehyde), 1600.71(C=O Stretching- amide), 1112.11(C-O-C stretching). ^{1}H NMR ($CDCl_3$ **:** δ) 4.032(s,2H,-CH_2), 6.923-7.754 (m, 8H,Ar-H), 8.134(s,1H, -NH),9.875(s,1H, -CHO)

3-((4-(Phenylimino) methyl)phenoxy)methyl)quinoxalin-2(1H)-one(GG2)

IR (KBr cm^{-1}): 3374.59 (N-H stretching), 2902.67 (-CH_2 stretching), 1601.13(C=O Stretching- amide), 1314.30(-CH=N- stretching), 1109.22 (C-O-C stretching). ^{1}H NMR ($CDCl_3$ **:** δ) 4.074 (s,2H,-CH_2), 6.812-7.625 (m, 13H,Ar-H), 8.182(s,1H, -NH),8.385 (s,1H, -CH=N-).

3-((4-((P-tolylimino)methyl)phenoxy)methyl)quinoxalin-2(1H)-one (GG3)

IR (KBr cm^{-1}): 3432.92 (N-H stretching), 2910.52(-CH_3 stretching), 2920.17 (-CH_2 stretching), 1604.15 (C=O Stretching- amide), 1285.70 (-CH=N- stretching), 1109.50 (C-O-C stretching). ^{1}H NMR ($CDCl_3$ **:** δ) 2.333(t,3H, (-CH_3),4.244 (s,2H,-CH_2), 7.710-6.819 (m, 12H,Ar-H), 8.095 (s,1H, -NH),8.385 (s,1H, -CH=N-).

3-((4-((O-tolylimino) methyl) phenoxy) methyl) quinoxalin-2(1H) - one (GG4)

IR (KBr cm^{-1}): 34021.29 (N-H stretching), 2914.21 (-CH_3 stretching), 2924.21 (-CH_2 stretching), 1601.76 (C=O Stretching- amide), 1318.01 (-CH=N- stretching), 1109.71 (C-O-C stretching).^{1}H NMR ($CDCl_3$ **:** δ) 2.416 (t,3H, (-CH_3), 4.074 (s,2H,-CH_2), 7.745-6.845 (m, 12H,Ar-H), 8.135 (s,1H, -NH),8.362 (s,1H, -CH=N-).

3-((4-((M-tolylimino)methyl)phenoxy)methyl)quinoxalin-2(1H)-one (GG5)

IR (KBr cm^{-1}): 3378.45 (N-H stretching), 2852.32 (-CH_3 stretching), 2921.39 (-CH_2 stretching), 1601.35 (C=O Stretching- amide), 1316.17 (-CH=N- stretching), 1110.72 (C-O-C stretching). ^{1}H NMR ($CDCl_3$ **:** δ) 2.351 (t,3H, (-CH_3), 4.214 (s,2H,-CH_2), 7.735-6.819 (m, 12H,Ar-H), 8.095 (s,1H, -NH), 8.415 (s,1H, -CH=N-).

Antibacterial activity

The antibacterial activity was assayed by agar plate disc diffusion method[13] at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antibacterial activity against gram positive microorganisms such as *Bacillus subtilis* and gram negative *Escherichia coli,* strains. Each test compounds were dissolved in dimethyl sulphoxide (DMSO) to get required concentration. The disc (6 mm in diameter) was impregnated, air dried and placed on the agar medium, previously seeded with 0.2 mL of broth culture of each organism for 18 h. The plates were incubated at 37 ^{0}C for 24 h and the inhibition zones were measured in mm. Discs impregnated with DMSO were used as a control and ciprofloxacin discs as antibacterial reference standard.

Antifungal activity

The antifungal activity[14] was assayed by sabouraud dextrose agar media plate disc diffusion method at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antifungal activity against microorganisms such as *Asperagellus niger* and *Candida albicans*. Each test compound was dissolved in dimethyl sulphoxide (DMSO) to get required concentration. The disc (6 mm in diameter) was impregnated; air dried and placed

on the sabouraud dextrose agar media, previously seeded with 0.2 mL of broth culture of each organism for 18 h. The plates were incubated at 22 ^{0}C for 48 h and the inhibition zones were measured in mm. Discs impregnated with DMSO were used as a control and Fluconazole discs as antifungal reference standard.

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