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Design, Synthesis and Antimicrobial Activity of Amide-Linked Quinoxaline Derivatives

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Abstract

A novel series of substituted (thiouracil derivatives, 2-mercapto benzimidazole derivatives and cyclic secondary amines) -N-(2, 3-diphenylquinoxalin-6-yl) acetamide derivatives (4a-h) have been designed, synthesized and characterized. The antimicrobial (against Gram-positive, Gram-negative) and antifungal (using three clinical fungal strains) activity was tested using broth micro-dilution assay and results were indicated as the minimum inhibitory concentration (MIC). All the compounds revealed considerable antimicrobial activity, of these synthesized compounds **4a**, **4b**, **4h**, and **4k** compounds, showed strong antimicrobial activity with respect to reference standard.

Keywords: Amide, Quinoxaline derivatives, antimicrobial, broth micro-dilution

INTRODUCTION

Infectious microorganisms have caused serious risks among humans and are consistently among the foremost health crises through the world [1]. In addition to that, many bacteria that are encountered evade our immunities and became resistant to multiple antibiotics of both natural and synthetic origin [2, 3]. Such bacterial infections lead to thousands of deaths and billions of dollars in healthcare costs annually, necessitating a constant push to discover and improve strategies to counter these threats [4, 5]. Quinoxaline derivatives are an important class of heterocyclic

family [6]. The recent research literature revealed that the chemistry of quinoxaline has attracted considerable attention among world [7] due to its varied chemical reactivities [8-10] and wide spectrum of biological activities [11, 12], such as antibacterial [13, 14], antifungal [15,16], anticancer [17], analgesic [18], antimalarial [19], antitumor [20], antiamoebic [21], antiepileptic [22], anticonvulsant [23], antitubercular [24], antiproliferative [25], anti-HCV [26] and anti-inflammatory properties [27]. Furthermore acetamide linked organic moieties are found to be versatile agents, that also exhibit a wide range of bio-logical activities, such as antibacterial [28, 29], anticonvulsant agents[30], anti-inflammatory agents[31, 32], antitubercular[33], Antinociceptive Agents[34].

In the present work due to broad biological spectrum of quinoxalines, we synthesized a series of new quinoxaline derivatives linked through acetamide with various heterocyclic compounds such as uracil derivatives (4a-e), 2-mercapto benzimidazole derivatives (4f & 4g) and cyclic aliphatic secondary amines(4h-k). The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectra, and screened for their in vitro antimicrobial activity.

EXPERIMENTAL

Materials and Methods

All chemicals used in the synthesis were purchased from Sigma-Aldrich and S. D. Fine chem. Ltd. Mumbai. Thiouracil derivatives [36] and 2-mercaptobenzimidazole derivatives [37] were synthesized according to literature reports. Melting points were measured in open capillary tubes and are uncorrected. The progress of the reaction was monitored by thin layer chromatography (TLC) and the spots were exposed to iodine vapours for visulization. IR spectra were recorded on Shimadzu FTIR (KBr) – 408 spectrophotometer. The ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$/DMSO-d_6 using TMS as internal standard and are given in δ units. The LC-MS spectra were recorded on WATER, Q-TOF Micromass.

Synthesis of 6-Nitro-2, 3-diphenylquinoxaline (1)

A mixture of the Benzil 1 (6.3 g 30 mmol), 4-nitrobenzene-1,2-diamine 2 (4.59 g, 30 mmol) and molecular iodine (0.77 g 10 mol %) in DMSO (40 ml) was stirred at room temperature for 2 h. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the reaction mixture was poured onto ice cold water. Then this mixture was treated with sodium thiosulfate solution to remove excess iodine. Finally solid which separated was filtered, washed with aqueous sodium thiosulfate and with water, and then recrystallized from ethanol to afford pure 6-Nitro-2, 3-diphenylquinoxaline 1.

Yield: 91.60%; MP. 192ºC; ^{1}HNMR ($CDC1_3$, 400 MHz) δ(ppm): 7.2-7.4(m, 13H), 8.1(t, 1H), 8.2(t, 1H), 8.5(d, 1H); MS: 328.2 (M+1)

Synthesis of 2, 3-diphenylquinoxalin-6-amine (2)

Compound (1) (8.17 g 25 mmol) was dissolved in ethanol (50 mL). To this solution Palladium-carbon catalyst (10%) and hydrazine hydrate (2.5 ml 2 mmol) was added. The mixture was heated under reflux for 1.5 h. The progress of reaction was monitored by TLC. After completion of reaction the hot solution was filtered through a Whatman paper to remove Pd/C and the solvent was evaporated. The resultant crude product was purified by passing through a column packed with silica gel (60-120 mesh) and n-hexane: ethyl acetate (1:3) as eluent.

Yield: 90%; MP. 200 ºC; IR (KBr) cm^{-1}: 3448-3320 (NH_2), 3057 (CH Ar), 1637 (C=N), 1314 (C-S). ^{1}HNMR ($CDC1_3$, 400 MHz) δ(ppm): 7.03-7.79(m, 13H), 6.01 (br, 2H, -NH_2).

Synthesis of 2-Chloro-N-(2,3-diphenylquinoxaline-6-yl)acetamide (3)

To a solution of Compound (2) (5.94 g 20 mmol) in DMF 20ml was added drop wise chloro acetyl chloride (2.5 ml 1.1mmol) drop wise at room temperature and stirred at room temperature for 8 h. The progress of reaction was monitored by TLC. After completion of reaction the solution was poured over crushed ice, separated solid was filtered washed with water, dried and recrystallized from ethanol.

Yield: 92%; MP. 244-246 ºC; IR (KBr) cm^{-1}: 3284 (NH), 1676 (CO), 1600 (C-N), 1314 (C-S). ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.74(s, 1H, NH), 8.51(d, 1H, Ar), 8.02(d, 1H, Ar), 7.86(dq, 1H, Ar), 7.23-7.41(m, 10H, Ar), 4.29 (s, 2H,-CH_2).

Synthesis of substituted -N-(2, 3-diphenylquinoxalin-6-yl)acetamide derivatives (4a-h)

General method. A mixture of compound 3 (0.373 g , 1mmol), thiouracil derivative or 1H-benzo[d]imidazole-2-thiol or cyclic secondary amines (1 mmol) and 0.2 ml triethylamine in dry acetonitrile (20 ml), was refluxed for 6h. The progress of reaction was monitored by TLC. After completion of reaction the solvent was evaporated under reduced pressure, residue was treated with cold water; separated solid was filtered washed with water, dried and recrystallized from ethanol.

2-((4-(4-chlorophenyl)-5-cyano-6-hydroxypyrimidin-2-yl)thio)-N-(2,3-diphenylquinox alin-6-yl)acetamide (4a): Yield: 90.2%; mp: 230-232 ºC; IR

(KBr)cm^{-1}: 3391 (NH,OH), 3058 (CH Ar), 2212(CN), 1678(C=O), 1600 (C-N); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 11.46(s, 1H, -OH), 8.55(s, 1H, - NH), 8.05(d, 1H, Ar), 7.90(d, 1H, Ar),7.84(s, 1H, Ar), 7.82(s, 1H, Ar), 7.32-7.48(m, 13H, Ar), 3.96 (s,2H,-CH_2); ^{13}C NMR (DMSO-d6, 400 MHz) δ (ppm): 38.2(CH_2), 93.1(C-CN), 113.9(Ar), 115.2(CN), 127.5, 127.9, 128.16, 128.19, 128.3, 128.51, 128.56, 128.6, 128.7, 130.1, 130.3, 133.0, 133.3, 133.4, 133.8, 135.2, 135.5, 136.6, 136.9, 160.7, 165.4, 165.6, 166.9(Ar), 168.6 (CO), 173.5(C-OH); MS: 601.1 (M+1).

2-((4-(3-chlorophenyl)-5-cyano-6-hydroxypyrimidin-2-yl)thio)-N-(2,3-diphenylquinox alin-6-yl)acetamide (4b): Yield: 87.8%; mp: 218-220 ^{o} C; IR (KBr)cm^{-1}: 3291 (NH,OH), 3056 (CH Ar), 2983, 2210(CN), 1692(C=O), 698(C-Cl); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 11.47(s, 1H, -OH), 9.32(s, 1H, - NH), 8.62(d, 1H, Ar), 8.11(d, 1H, Ar),7.90 (t, 2H, Ar), 7.48(s, 6H, Ar), 7.32-7.44(m, 7H, Ar), 4.03 (s,2H,-CH_2); ^{13}C NMR (DMSO-d6, 400 MHz) δ (ppm): 35.4(CH_2), 89.9(C-CN), 114.9(CN), 118.9, 123.8, 126.8, 127.85, 127.87, 127.90, 128.38, 128.55, 129.13, 129.56, 129.61, 129.72, 129.87, 133.02, 137.35, 138.83, 138.84, 139.09, 140.35, 141.30, 151.07, 153.00, 165.69, 168.12(Ar), 169.85 (CO), 171.57(C-OH); MS: 601.1 (M+1).

2-((5-cyano-4-(4-fluorophenyl)-6-hydroxypyrimidin-2-yl)thio)-N-(2,3-diphenylquinoxalin-6-yl)acetamide (4c): Yield: 85.6%; mp: 310-312 ^{o} C; IR (KBr)cm^{-1}: 3285 (NH,OH), 3057(CH Ar), 2925, 2226(CN), 1659(C=O), 1251 (C-F); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.98(s, 1H, -OH), 8.8.47(s, 1H, - NH), 8.06(d, 1H, Ar), 7.80-7.89(m, 3H, Ar), 7.26-7.41(m, 11H, Ar), 7.09-7.14(m, 2H, Ar), 4.17 (s,2H,-CH_2); MS: 585.2 (M+1).

2-((5-cyano-4-hydroxy-6-(4-nitrophenyl)pyrimidin-2-yl)thio)-N-(2,3-diphenylquinoxalin-6-yl)acetamide (4d): Yield: 88.0%; mp: 250-252 ^{o} C; IR (KBr)cm^{-1}: 3374 (NH,OH), 2213(CN), 1695(C=O), 3079 (CH Ar), 1352, 1538 (NO_2); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 11.49(s, 1H, -OH), 8.8.47(s, 1H, - NH), 8.06(d, 1H, Ar), 7.80-7.89(m, 3H, Ar), 7.26-7.41(m, 11H, Ar), 7.09-7.14(m, 2H, Ar), 4.17 (s,2H,-CH_2); ^{13}C NMR (DMSO d6, 400 MHz): 35.42(CH_2), 89.83(C-CN), 114.79(CN), 119.15, 122.69, 123.83, 124.57, 127.98, 128.00, 128.50, 128.68, 129.29, 129.62, 129.66, 129.92, 134.58, 137.28, 138.64, 138.84, 140.39, 141.21, 147.53, 151.25, 153.17, 164.90, 168.27, 169.73(CO), 171.91(C-OH); MS: 612.18 (M+1).

2-((5-cyano-4-hydroxy-6-(3-nitrophenyl)pyrimidin-2-yl)thio)-N-(2,3-diphenylquinoxalin-6-yl)acetamide (4e): Yield: 89.5%; mp: 242-244 ^{o} C; IR

(KBr)cm^{-1}: 3330 (NH,OH), 3056(CH Ar), 2982, 2210(CN), 1690(C=O), 1348, 1535 (NO_2) ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 11.32(s, 1H, -OH), 8.49(s, 1H, - NH), 8.15(d, 1H, Ar), 7.49-8.02(t, 3H, Ar),7.86 (s, 1H, Ar), 7.41(t, 4H, Ar), 7.27(d, 7H, Ar), 3.90 (s,2H,-CH_2); ^{13}C NMR (DMSO d6, 400 MHz): 35.48(CH_2), 90.39(C-CN), 114.93(CN), 118.63, 123.06, 123.74, 127.84, 128.31, 128.48, 129.06, 129.53, 129.57, 137.35, 138.64, 138.81, 140.30, 141.27, 143.19, 147.93, 151.05, 152.98, 165.20, 168.10, 169.95(CO), 171.93(C-OH); MS: 612.19 (M+1).

N-(2,3-diphenylquinoxalin-6-yl)-2-((5-methyl-1H-benzo[d]imidazol-2yl)thio)aceta mide(4f): Yield: 88.7%; mp: 178-180 ^{o} C; IR (KBr)cm^{-1}: 3186 (NH), 3058(CH Ar), 2985, 1682(C=O), 1630; ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 12.46(s, 1H, -NH), 11.07(s, 1H, - NH), 8.00(d, 1H, Ar), 7.83-7.88(t, 2H, Ar),7.37-7.41 (t, 4H, Ar), 7.26-7.30(t, 8H, Ar), 6.89(d, 2H, Ar), 4.26 (s,2H,-CH_2), 2.45(s, 3H, -CH_3); ^{13}C NMR (DMSO d6, 400 MHz): 21.21(-CH3), 36.41(CH_2), 115.30, 123.58, 127.84, 128.35, 128.52, 129.12, 129.52, 129.56, 137.46, 138.75, 140.06, 151.20, 153.04, 166.99(C=O); MS: 502.22 (M+1).

2-((5-benzoyl-1H-benzo[d]imidazol-2-yl)thio)-N-(2,3-diphenylquinoxalin-6-yl)acetamide(4g): Yield: 89.6 %; mp: 142-144 ^{o} C; ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 13.04(s, 1H, -NH- imidazole), 11.01(s, 1H, - NH-), 8.56(s, 1H, Ar), 8.07(d, 1H, Ar), 7.94(d, 1H, Ar), 7.78(s, 1H, Ar), 7.74(d, 1H, Ar), 7.62(s, 2H, Ar), 7.54-7.45(m, 7H, Ar),7.37-7.32 (m, 6H, Ar), 4.44 (s,2H,-CH_2); ^{13}C NMR (DMSO d6, 400 MHz): 36.36(CH_2), 115.40, 123.61, 127.82, 128.07, 128.50, 129.14, 129.33, 129.51, 129.55, 130.46, 137.49, 138.73, 139.99, 141.21, 151.21, 153.02, 166.56(C=O); M: 592.23(M+1).

N-(2, 3-diphenylquinoxalin-6-yl)-2-morpholinoacetamide(4h): Yield: 85.7%; mp: 222-224 ^{o} C; IR (KBr)cm^{-1}: 3277 (NH), 3059(CH Ar), 2913(-CH_2-), 1689 (C=O), 1618; ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.14(s, 1H, - NH), 8.61(d, 1H, Ar), 8.05(d, 1H, Ar), 8.00-7.98(dq, 1H, Ar),7.49-7.45 (m, 4H, Ar), 7.37-7.30(m, 6H, Ar), 3.73-3.71(t, J=4.48, 4H, CH_2), 3.25(s,2H,-CH_2 amide), 2.62-2.60(t, J= 4.44, 4H); ^{13}C NMR (DMSO d6, 400 MHz): 53.21(N-CH_2), 62.15(N-$\underline{C}H_2$-C=O), 66.07(O-CH_2), 115.57, 124.12, 127.85, 127.86, 128.37, 128.91, 129.53, 137.44, 138.78, 138.80, 139.80, 141.17, 151.16, 152.96, 168.69(C=O); MS: 425.18 (M+1).

N-(2,3-diphenylquinoxalin-6-yl)-2-thiomorpholinoacetamide(4i): Yield: 84.2%; mp: 216-218 ^{o}C; IR (KBr)cm^{-1}: 3288 (NH), 3059(CH Ar), 2913(-CH_2-), 1693(C=O), 1618; ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.19(s, 1H, - NH), 8.62(d, 1H, Ar),

8.07(s, 1H, Ar), 8.05 (dq, 1H, Ar),7.48-7.44 (m, 4H, Ar), 7.39-7.32(m, 6H, Ar), 3.2(s,2H,-CH_2 amide), 2.85-2.83(t, J=4.41, 4H, CH_2), 2.62-2.60(t, J= 4.44, 4H); MS: 441.18 (M+1).

N-(2, 3-diphenylquinoxalin-6-yl)-2-(piperidin-1-yl)acetamide(4j): Yield: 86.8%; mp: 182-184 ^{o} C; IR (KBr)cm^{-1}: 3281 (NH), 3059(CH Ar), 2937(-CH_2-), 1697(C=O); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.08(s, 1H, - NH), 8.60(d, 1H, Ar), 8.06(d, 1H, Ar), 8.0-7.97 (dq, 1H, Ar),7.49-7.45 (m, 4H, Ar), 7.39-7.30(m, 6H, Ar), 3.17(s,2H,-CH_2 amide), 2.53-2.52(t, J=5.6, 4H, CH_2), 1.67-1.62(m, J=5.64, 4H), 1.47-1.46(t, J=4.64, 2H); ^{13}C NMR (DMSO d6, 400 MHz): 23.48(-CH_2-), 25.42(-CH_2-), (54. 16(N-CH_2), 62.73(N-$\underline{C}H_2$-C=O), 115.1, 124.10, 127.87, 128.38, 128.55, 128.95, 129.57, 137.41, 138.79, 138.80, 141.18, 151.14, 152.97, 168.25(C=O); MS: 423.20 (M+1).

N-(2, 3-diphenylquinoxalin-6-yl)-2-(pyrrolidin-1-yl)acetamide(4k): Yield: 85.3%; mp: 160-162 ^{o}C; IR (KBr)cm^{-1}: 3288 (NH), 3059(CH Ar), 2968(-CH_2-), 1693(C=O); ^{1}HNMR (DMSO d6, 400 MHz) δ(ppm): 10.12(s, 1H, - NH), 8.61(d, 1H, Ar), 8.06(d, 1H, Ar), 8.01-7.98 (dq, 1H, Ar),7.49-7.45 (m, 4H, Ar), 7.38-7.30(m, 6H, Ar), 3.17(s,2H,-CH_2 amide), 1.66-1.61(m, J=5.64, 4H), 1.46-1.45(t, J=4.64, 4H); ^{13}C NMR (DMSO d6, 400 MHz): 23.48(-CH_2-), 53.84 (N-CH_2), 59.62 (N-$\underline{C}H_2$-C=O), 115.61, 124.03, 127.78, 128.26, 128.43, 128.81, 129.48, 129.52, 137.46, 138.76, 138.77, 139.77, 141.22, 151.06, 152.08, 168.32(C=O); MS: 409.16 (M+1).

Antimicrobial Activity

Micro-organisms

The microbial strains of bacteria and fungi used in this study were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Four species of bacteria and three species of fungi were used for the screening process. The bacterial strains consisted of Gram-positive (including *Staphylococcus aureus* [MTCC96] and *Streptococcus pyogenus* [MTCC442]) and Gram-negative (*Escherichia coli* [MTCC443] and *Pseudomonas aeruginosa* [MTCC441]). The fungi species used were *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC282) and *Aspergillus clavatus* (MTCC1323).The bacteria were grown and maintained on Muller-Hinton agar slants (Merck), while fungi were on Sabouraud dextrose agar slants (Merck). They were maintained on agar slant at 4 °C in the Laboratory.

Broth dilution assay

In vitro screenings of antimicrobial potential of synthesized compounds were carried out using prescribed method [38, 39]. All the synthesized compounds were screened for antibacterial and antifungal activity against above selected pathogens. DMSO was used as diluent to get desired concentration of drugs to test upon standard bacterial and fungal strains. MICs of the samples were determined by tube dilution method (turbimetric method). The test organisms maintained on agar slants were recovered for testing by inoculating into Mueller–Hinton broth (MHB) and incubated at 37ºC till the concentration of the test organisms matched with the 0.5 McFarland standard (10^8 CFU/ml). Fungal cultures were inoculated into Sabouraud dextrose broth (SDB) and incubated at 28-30ºC with concentration 10^4 CFU before being used. The bacterial suspensions were diluted 1:10 in broth and 100 µl of it were used for the study. The sterilized nutrient broth (2ml) was introduced in each of the 5 test tubes and similar procedure was followed for all the samples. Each synthesized compound was diluted obtaining 2000 mg/mL concentration as a stock solution. In primary screening 2000, 1000, 500, 250 and 200 mg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5 and 6.250 mg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

RESULTS AND DISCUSSION

Chemistry:

The 6-Nitro-2, 3-diphenylquinoxaline (**1**) was prepared from condensation of benzil and 3-nitro-1,2-phenylene diamine [35], further 2, 3-diphenylquinoxalin-6-amine (**2**) was obtained by reducing NO_2 group of compound **1** in ethanol using palladium-carbon catalyst and hydrazine hydrate as a source of hydrogen. The formation of compound **2** is confirmed by the presence of broad singlet for two protons at δ 6.01 in ^{1}H NMR indicating the conversion of $–NO_2$ group of compound **1** into $-NH_2$ group in compound **2**. Compound **2** was acylated with chloroacetyl chloride to obtain product 2-Chloro-N-(2,3-diphenylquinoxaline-6-yl)acetamide (**3**). The FT-IR spectra of **3** showed characteristic absorption band at 3284 cm^{-1} for -NH- and 1676 cm^{-1} for carbonyl groups of amide. The ^{1}H NMR spectra of **3** showed a sharp singlet at δ 4.29 indicating the presence of methylene group ($-CH_2-$) and singlet at δ 10.74 for -NH-proton. The title compounds R-N-(2, 3-diphenylquinoxalin-6-yl) acetamide derivatives as shown in table 1 (**4a-k**) were synthesized by reacting compound **3** with various nucleophilic species such as thiouracil derivatives derivatives, 2-mercaptobenzimidazole derivatives and cyclic secondary amines. The structure of the

synthesized compounds was elucidated on the basis of their FT-IR, 1H NMR, ^{13}C NMR and mass spectra. The FT-IR spectra of **4a-k** showed absorption band in the range of 3200-3400 cm^{-1} for -NH- and 1680-1695 cm^{-1} for amide carbonyl (C=O) group. The absorption band around 2200 cm^{-1} for –CN group and broad peak around 3300 cm^{-1} of pyrimidine ring (**4a-e**). All the synthesized compounds **4a-k** has agreement with FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data with their proposed structures.

Antimicrobial Studies

Antibacterial activity

All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against both Gram-positive and Gram-negative bacterial strains using broth microdilution method along with varied reference standards (Gentamycin, Ampicillin, Chloroamphenicol, Ciprofloxacin and Norfloxacin). Furthermore, the results of the antibacterial activity of the compunds **4a-k** were evaluated by means of minimum inhibitory concentration (MIC) as shown in table 2. All the newly synthesized compounds showed moderate to potent inhibition against all the tested bacterial strains. Compound **4a** shows very good antibacterial activity against *S.pyogenus, S.aureus* and *E. coli*, while moderate against *P. aruginosa* with reference standard ampicillin. Compound **4h** exhibit very good activity against all tested microorganism except *P. aruginosa*, while compound **4b** shows substantial activity against *E. coli*, *S.pyogenus* and *S.aureus*. Compound **4e** exhibit strong activity against *S.aureus* only with reference standard. Compound **4k** shows excellent activity against *E. coli* and *S. aureus*, while compound **4c, 4d, 4f, 4g, 4i** and **4j** exhibits potent activity against *S. aureus* only and less antibacterial activity to other used bacterial strains than the employed standard drug. Moreover, when compared to both, Gram-positive bacteria were more susceptible than Gram-negative bacterial strains. In addition to that, all synthesized compounds revealed moderate antibacterial activity against all the tested bacterial strains. The structure activity relationship studies from the results of the antibacterial activities revealed that compound containing pyrimidine ring substituted with 4-chlorophenyl (**4a**) and mopholine derivative (**4h**) revealed broad spectrum antibacterial activity when compared with standard.

Antifungal activity

The MIC values of antifungal activity revealed that compound **4a, 4b, 4d** and **4f** exhibited good activity against *C. albicans* when compared to reference standard greseofulvin. Rest of the compounds did not exhibit comparable activity against both the fungal strains (*A. niger and A. clavatus*) as shown in table 3.

CONCLUSION

In the present studies, we synthesized a series of novel quinoxaline derivatives linked through acetamide (**4a-k**) and screened for antimicrobial activity. All the compounds revealed considerable antimicrobial activity, of these synthesized compounds **4a**, **4b**, **4h**, and **4k** compounds, showed strong antimicrobial activity with respect to reference standard. Structure activity relationship revealed that pyrimidine ring substituted with 4-chlorophenyl (**4a**) and mopholine derivative (**4h**) appears to be crucial for the observed activity. Further screening is warranted using high throughput assay to evaluate the potential of these compounds as a source of lead or precursor molecule.

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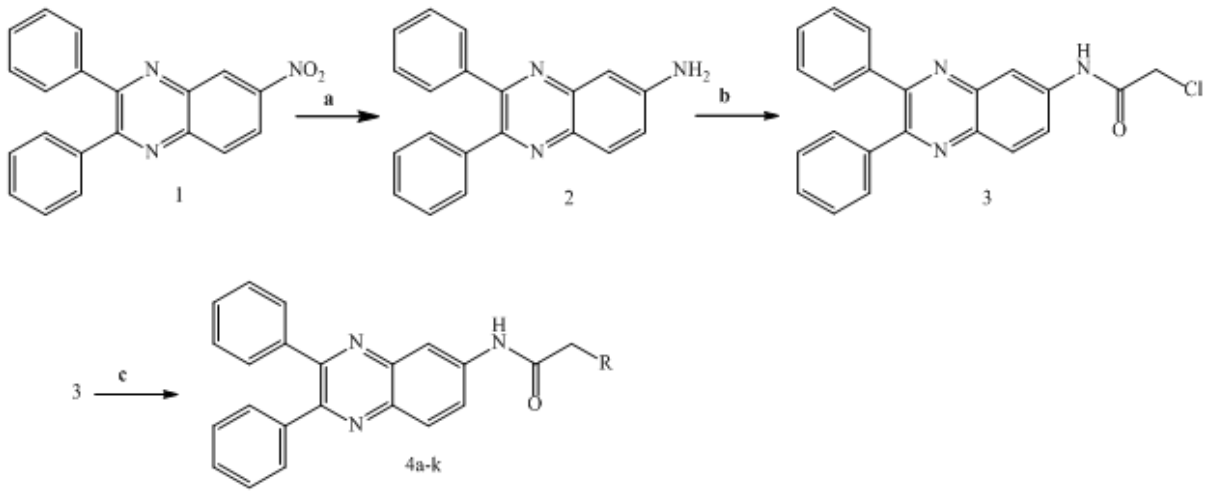
Scheme: Outline for synthesis of for N-(2, 3-diphenylquinoxalin-6-yl) acetamide derivatives.



Reagents and conditions: (a) PdC/NH_2NH_2/EtOH/ 1.5h reflux. (b) Chloroacetyl chloride/ DMF/ 6h stir/rt. (c) 4-Oxo-2-thioxopyrimidine derivatives or 1H-benzo[d]imidazole-2-thiol derivatives or cyclic secondary amines, Acetonitrile/TEA/6h/reflux

Table 1: Different substituent of -N-(2, 3-diphenylquinoxalin-6-yl) acetamide derivatives (4a-h)

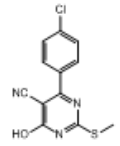
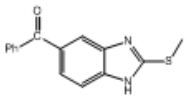
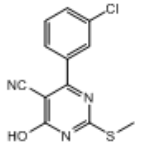
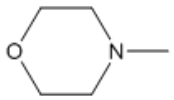
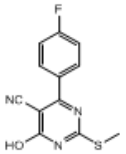
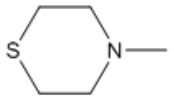
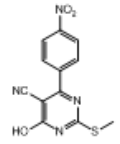
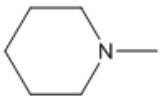
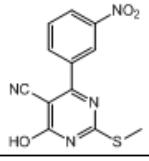
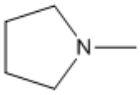
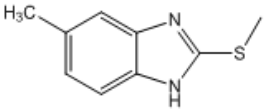
No.	R	Yield (%)	No.	R	Yield (%)
4a		90.2	4g		89.6
4b		87.8	4h		85.7
4c		85.6	4i		84.2
4d		88.0	4j		86.8
4e		89.5	4k		85.3
4f		88.7		--	

Table 2: In vitro antibacterial activities of compounds 4a-4k[a]

Compounds	**Minimum inhibitory concentration (µg/ml)**			
	Gram-negative		***Gram-positive***	
	E.coli	*P.aeuginosa*	*S. aureus*	*S. pyogenus*
4a	100	200	100	62.5
4b	62.5	200	125	200
4c	200	250	250	250
4d	250	250	100	250
4e	125	200	125	250
4f	250	125	200	125
4g	250	250	250	500
4h	100	200	100	100
4i	200	250	100	125
4j	200	250	250	250
4k	100	100	125	200
Standard				
Gent.	0.05	1	0.25	0.5
Amp.	100	--	250	100
Chlor.	50	50	50	50
Cipro.	25	25	50	50
Norflo.	10	10	10	10

[a] Gent. :Gentamycin; Amp: Ampicillin;
Chlor : Chloroamphenicol; Cipro:Ciprofloxacin;
Norflo. Norfloxacin

Table 2 In vitro antifungal activities of compounds 4a-4k[a]

Compounds	**Minimal Fungicidal Concentration (µg/ml)**		
	C. albicans	***A. niger***	***A. clavatus***
4a	500	1000	1000
4b	500	250	500
4c	1000	500	500
4d	500	>1000	>1000
4e	>1000	250	250
4f	250	>1000	>1000
4g	1000	1000	1000
4h	1000	500	500
4i	>1000	>1000	>1000
4j	>1000	>1000	>1000
4k	1000	500	1000
Standard			
Nyst.	100	100	100
Greseo.	500	100	100

[a] Nyst: Nystatin; Greseo; Greseofulvin

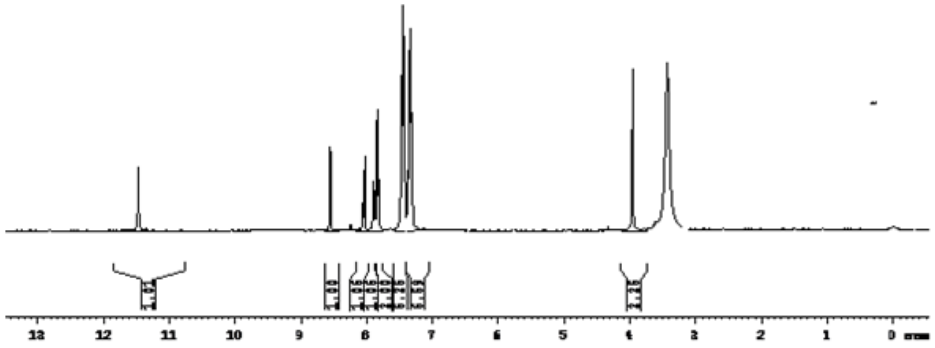


Fig.1. ^{1}H NMR spectrum of 4a.

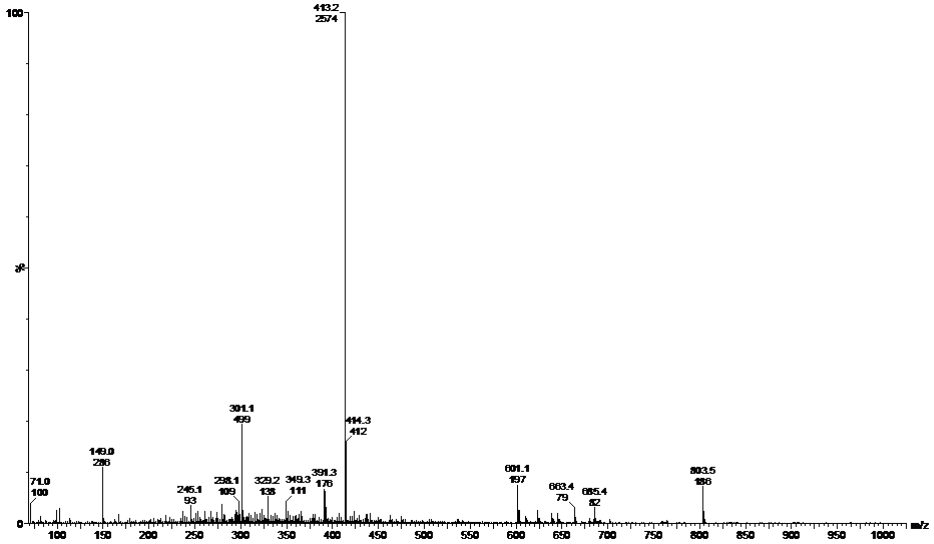


Fig. 2. Mass spectrum of 4a.

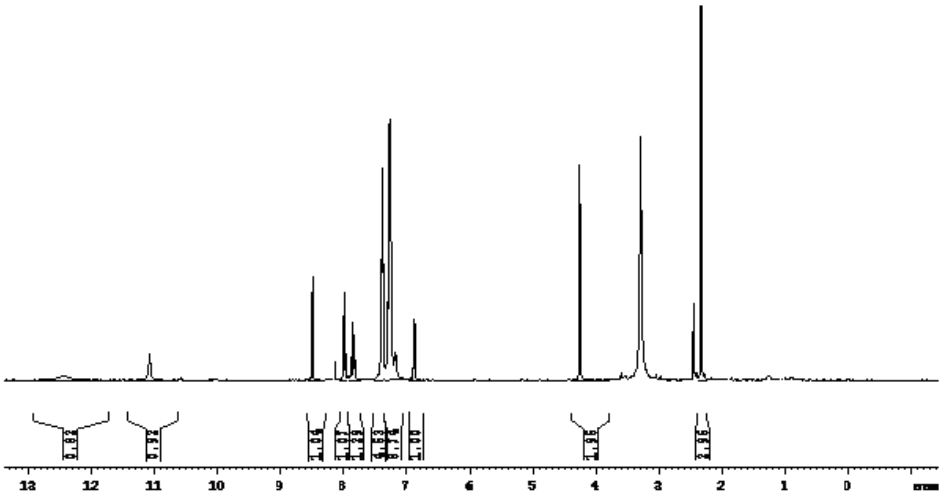


Fig. 3. ^{1}H NMR spectrum of 4f.

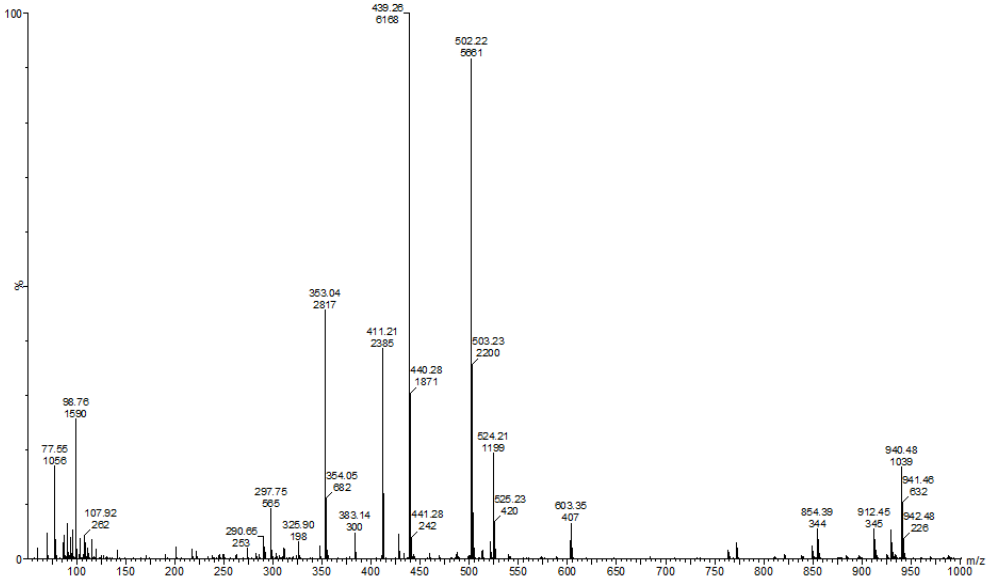


Fig. 4. Mass spectrum of 4f.

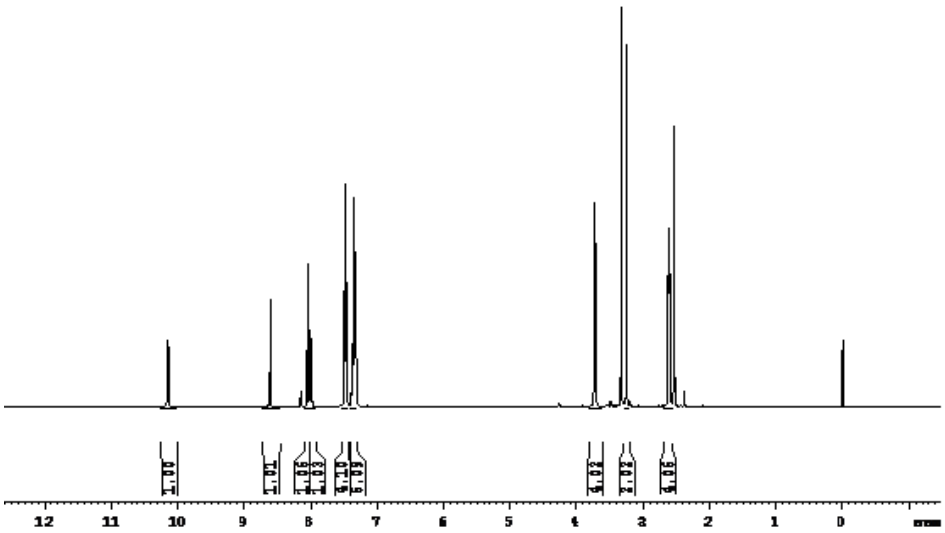


Fig. 5. ^{1}H NMR spectrum of 4h.

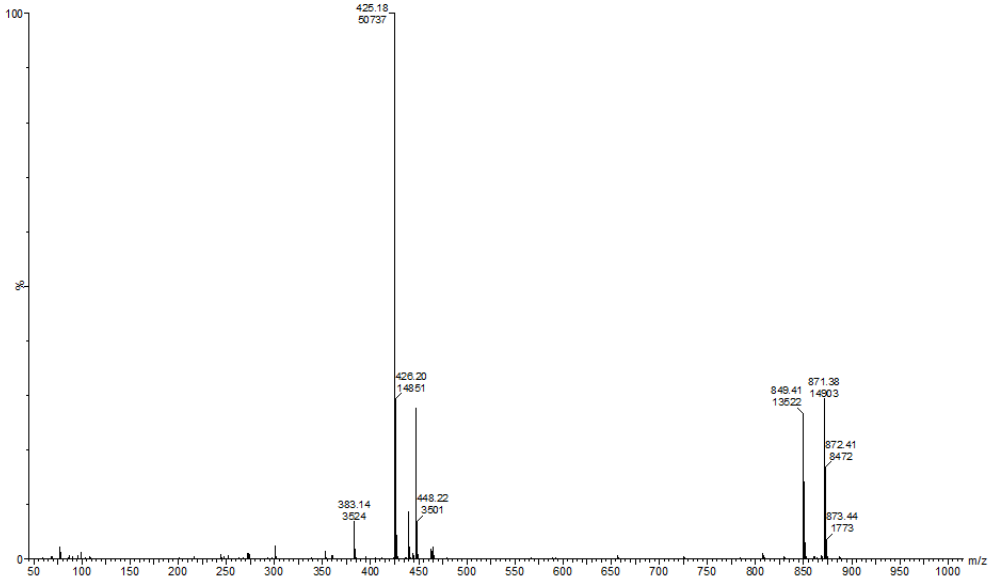


Fig. 6. Mass spectrum of 4h.