AN EASY, EFFICIENT PTC-MEDIATED SYNTHESIS OF 2-SUBSTITUTED-6-CHLOROQUINOXALINES AND ANTIBACTERIAL ACTIVITY

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ABSTRACT
An easy and efficient synthetic protocol has been developed for the synthesis of 2-substituted-6-Chloro-quinoxalines by the reaction of 2,6-Dichloroquinoxaline with different alcohols, thiols and amine using Tri Ethyl Benzyl Ammonium Chloride (TEBAC). The newly synthesized compounds characterized by spectral data such as IR, 1H&13C-NMR, and mass spectrometry. The compounds were screened for their in vitro antibacterial activity against four different organisms, resulted in the compounds showed good antibacterial activities.

Keywords: 6-Chloroquinoxaline, Phase Transfer Catalysts, Thiols, Amine, Antibacterial Activity.

INTRODUCTION
Quinoxalines are an important class of heterocyclic compounds due to their importance in the fields such as medical, industrial and synthetic organic chemistry. Quinoxaline derivatives are exhibit a variety of biological activities including antifungal, antioxidant, antibacterial, anticancer and antidiabetic. Quinoxaline derivatives are also applicable in the macrocyclic receptor, electroluminescent, organic semiconductor and DNA cleavage study. Echinomycin, Neomycin and Actinoleucin are marketed antibiotic drugs which structure consists of quinoxaline core moiety and many synthetic quinoxaline derivatives are used to treat tuberculosis. The activities of the quinoxaline compound are enhanced by introducing a different substitution pattern. Also, many heterocyclic compounds with heteroatom substitution on their ring system sulfur usually are a good antifungal agent. Halogens are played an important role in drug designing over the last 30 years. In modern drug discovery, the inclusion of a chlorine atom into a drug molecule is most significant due to that distribution in a fatty and aqueous medium. Given the above facts, we have planned to synthesis 2-substituted-6-Chloro-quinoxalines (Scheme-1).

EXPERIMENTAL
Material and Methods
All Reagent grade chemicals are used in this work. Chemicals were purchased from Sigma-Aldrich Chemicals Co. and Spectrochem Pvt. Ltd. These reagent grade chemicals were used without further purification. Solvents were dried according to standard methods. Melting points were determined in open capillary tubes and are uncorrected. IR spectrum was taken on a JASCO FT-IR-4100 using KBr pellet method. 1H&13C-NMR spectra were recorded on a JEOL 400 MHz and BRUKER 500 MHz spectrometers in CDCl3 using TMS as an internal reference, J in Hertz. MS was performed using SHIMADZU LC-2010EV spectrometer.

General Procedure for the Synthesis of 2-Substituted 6-Chloroquinoxaline Derivatives (3a-f)
To a stirred solution of 2,6-Dichloroquinoxaline (1) (0.085 mol), R-X (2a-e) (0.085 mol) in N, N-Dimethyl formamide (25 mL) was added TEBAC (0.0085 mol) and K2CO3 (0.093 mol) at room
temperature and heated to 70-75°C and stirred for 6-7 hrs. After completion of the reaction (monitored by TLC), the reaction mixture filtered, the filtrate was added to ice-cold water and the compound extracted with ethyl acetate (2 x 25 ml). The organic layer dried over Na₂SO₄ and concentrated under reduced pressure to obtained crude product, then the product purified by column chromatography using silica gel using 25% Ethyl acetate in n-hexane to afford pure 2-Substituted 6-Chloroquinoxaline derivatives (3a-e).

6-Chloro-2-((6-chloropyridin-3-yl)methoxy)quinoxaline (3a)
Pale Yellow solid, Yield: 84%; mp 149-151°C; IR spectrum, ν, cm⁻¹: 3043, 2924, 1590, 1488, 1384, 828; ¹H NMR spectrum, δ, ppm (J,Hz): 5.53 s (2H), 7.37 d (J = 8.3Hz, 1H, C₆H₅), 7.63 – 7.66 dd (J = 9.0, 2.8Hz, 1H, C₆H₅), 7.78 d (J = 9.0Hz, 1H, C₆H₅), 8.03 d (J = 8.3, 2.8Hz, 1H, C₆H₅), 8.03 d (J = 2.1Hz, 1H, C₆H₅), 8.52 s (1H, C₆H₅), 8.58 d (J = 2.1Hz, 1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 156.6, 151.6, 149.9, 140.2, 139.5, 139.0, 138.6, 132.6, 131.2, 130.8, 128.4, 128.2, 124.3, 64.9; ESI-MS: Found 306.03 [M + H]+ and calculated for C₁₄H₉Cl₂N₃O: 306.15

Scheme-1: Synthesis of 2-Substituted 6-chloroquinoxaline derivatives (3a-f)
6-Chloro-2-(((6-chloropyridin-3-yl)methyl)thio)quinoxaline (3b)
Pale Yellow solid, Yield: 88%; mp 127-129°C; IR spectrum, ν, cm⁻¹: 3048, 2931, 1604, 1585,1536, 1461, 828, 781; ¹H NMR spectrum, δ, ppm (J,Hz): 4.50 s (2H), 7.27 m (1H, C₆H₅), 7.65 – 7.68 dd (J = 2.3, 8.9Hz, 1H, C₆H₅), 7.76 – 7.78 dd (J = 2.5, 8.2Hz, 1H, C₆H₅), 7.89 d (J = 8.9Hz, 1H, C₆H₅), 8.01 d (J = 2.3Hz, 1H, C₆H₅), 8.56 d (J = 2.5Hz, 1H, C₆H₅), 8.58 s (1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 154.7, 150.5, 150.3, 145.2, 141.0, 140.5, 139.4, 134.1, 132.5, 128.9, 128.4, 124.2, 30.1; ESI-MS: Found 322.20 [M + H]+ and Calculated for C₁₄H₁₂Cl₂N₂S: 322.21

2-Chloro-5-(((6-chloroquinoxalin-2-yl)thio)methyl)thiazole (3b)
Off-White solid, Yield: 85%; mp 107-109°C; IR spectrum, ν, cm⁻¹: 3086, 2947, 2958, 1606, 1573, 1529, 1305, 1054, 824; ¹H NMR spectrum, δ, ppm: (J,Hz): 5.63 s (2H), 7.69 m (2H, C₆H₅), 7.84 d (J = 8.8Hz, 1H, C₆H₅), 8.05 d (J = 2.3Hz, 1H, C₆H₅), 8.50 s (1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 155.9, 153.8, 142.1, 140.0, 139.3, 138.0, 134.4, 132.5, 131.2, 128.1, 128.0, 59.9; ESI-MS: Found 311.98 [M + H]+ and Calculated for C₁₂H₁₁C₁₂N₂OS: 312.17.

2-Chloro-5-(((6-chloroquinoxalin-2-yl)oxy)methyl)thiazole (3c)
Off-White solid, Yield: 88%; mp 138 – 140°C; IR spectrum, ν, cm⁻¹: 3054, 2985, 1603, 1536, 1477, 1087, 823; ¹H NMR spectrum, δ, ppm: (J,Hz): 4.64 s (2H), 7.53 s (1H, C₆H₅), 7.70 – 7.72 dd (J = 2.4, 8.6Hz, 1H, C₆H₅), 7.95 d (J = 9.0Hz, 1H, C₆H₅), 8.05 d (J = 2.8Hz, 1H, C₆H₅), 8.60 s (1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 154.4, 152.1, 145.2, 140.8, 140.4, 137.3, 134.3, 131.6, 128.9, 128.5, 25.8; ESI-MS: Found 273.95 [M + H]+. Calculated for C₁₂H₁₂Cl₂N₂S₂: 282.24.

2-(Benzylxoy)-6-chloroquinoxaline (3e)
Pale Yellow solid, Yield: 83%; mp 97- 100°C; IR spectrum, ν, cm⁻¹: 3043, 2924, 2853, 1603, 1565, 1009, 878; ¹H NMR spectrum, δ, ppm: (J,Hz): 5.52 s (2H), 7.35 – 7.43 m (3H, C₆H₅), 7.52 d (J = 7.6Hz, 2H, C₆H₅), 7.62 – 7.64 dd (J = 2.4, 8.6Hz, 1H, C₆H₅), 7.80 d (J = 9.0Hz, 1H, C₆H₅), 8.02 d (J = 2.8Hz, 1H, C₆H₅), 8.52 s (1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 157.3, 140.7, 139.3, 138.9, 136.1, 132.1, 131.0, 128.7, 128.6, 128.5, 128.2, 68.5; ESI-MS: Found 271.03 [M + H]+. Calculated for C₁₃H₁₁ClN₂O: 270.71.

Procedure for the synthesis of compound (3f)
A mixture of 2, 6-Dichloro Quinoxaline (1) (0.125 mol) and 2,3-Dimethylaniline (2f) (0.125 mol) were taken in N,N-Dimethylformamide (25 mL) at room temperature. Then the reaction mixture was heated to 100°C and was maintained for 4.5 hrs. Then the crude product purified by column chromatography using silica gel using 5% Ethyl acetate in n-hexane to afforded 6-Chloro-N-(2,3-dimethylphenyl)quinoxalin-2-amine (3f) as off white solid.

6-chloro-N-(2,3-dimethylphenyl)quinoxalin-2-amine (3f)
Reddish brown solid, Yield: 86%; mp 138 - 140°C; IR spectrum, ν, cm⁻¹: 3199, 3054, 2920, 1618, 1585, 1471, 1260, 821; ¹H NMR spectrum, δ, ppm (J,Hz): 2.24 s (3H, CH₃), 2.36 s (3H, CH₃), 6.67 br s (1H), 7.11 d (J = 7.6Hz, 1H, C₆H₅), 7.16 – 7.19 t (J = 7.7Hz, 1H, C₆H₅), 7.44 d (J = 7.7Hz, 1H, C₆H₅), 7.53 – 7.55 dd (J = 9.0, 2.4Hz, 1H, C₆H₅), 7.63 d (J = 9.0Hz, 1H, C₆H₅), 7.90 d (J = 2.2Hz, 1H, C₆H₅), 8.34 s (1H, C₆H₅); ¹³C NMR spectrum, δ, ppm: 151.0, 140.2, 138.5, 138.1, 136.3, 131.5, 130.9, 130.3, 128.0, 127.9, 127.5, 126.4, 122.6, 20.7, 14.2; ESI-MS: Found 284.05 [M + H]+ and Calculated for C₁₆H₁₄ClN₂O: 283.76.

RESULTS AND DISCUSSION
A model reaction of 2,6-dichloro-quinoxaline with 6-chloropyridin-3-yl)methanol using K₂CO₃ was employed under the conventional stirring method (Scheme-2) at 50-55°C in DMF medium. The expected product was yield in very low and the reaction not completed, even extending reaction time up to 24 hrs. This experiment result encouraged us to explore new ideas for better reaction conditions to get good yields. The reaction temperature increased to 70-75°C to obtained better yield (80%) in 12 hrs. While
doing further optimization study it was observed that introducing of phase transfer catalyst such as Tri Ethyl Benzyl Ammonium Chloride (TEBAC) makes the completion of the reaction within 4 hrs with increased yields at 70-75°C (Table-1). The scope of this protocol was extended to a variety of novel substituted alcohols and thiols (Table-1, entries 1-5). After making derivatives with phenols and thiols, we thought to introduce nitrogen atom in the second position of Quinoxaline. To explore this chemistry and the activity of the amino derivative, we tried a reaction with 2,3-dimethylaniline, DMF as solvent without any base obtained afford corresponding substituted derivative (Table-1, entry 6). The newly synthesized six compounds were successfully characterized by spectral data such as IR, 1HNMR, 13CNMR and mass spectrometry.

Table-1: Optimization Reaction Condition for Preparation of 3a

| Entry | Reaction Condition | Reaction Time (hr) | Yield(%)\(^a\) |
|-------|--------------------|--------------------|----------------|
| 1     | RT                 | 24                 | 28             |
| 2     | 50-55°C            | 24                 | 52             |
| 3     | 60-65°C            | 18                 | 65             |
| 4     | 70-75°C            | 12                 | 76             |
| 5     | 80-85°C            | 12                 | 80             |
| 6     | 100°C              | 12                 | 78             |
| 7     | TEBAC, 60-65°C     | 8                  | 69             |
| 8     | TEBAC, 70-75°C     | 4                  | 84             |
| 9     | TEBAC, 80-85°C     | 4                  | 82             |

\(^a\) isolated yield

Scheme-2: Optimization Reaction Condition for Preparation of 3a

Biological Activity

In vitro antimicrobial activity of the compounds (3a-f) were evaluated using Agar well-diffusion method against four different organisms two Gram-positive bacteria and two Gram-negative bacteria such as *S. aureus* (ATCC-6538), *B. subtilis* (ATCC-6633), *E. coli* (ATCC-11229) and *P. aeruginosa* (ATCC-29213). Chloramphenicol was used as a standard drug. The antibacterial study results in a zone of inhibition mentioned in Table-2. The antibacterial results show that the replacement of 6-chloropyridine ring by 2-chlorothiazol group and 2,3-dimethylaniline, activity has been enhanced against all tested bacteria. The compounds 3c, 3d and 3f showed better activity compared to the standard drug against tested all organisms (Table-2). The compounds 3c and 3d showed maximum antibacterial activities that may responsible for the 2-chlorothiazide group.

Table-2: Antibacterial Activity of 2-Substituted 6-Chloro-Quinoxaline Derivatives (3a-f)

| Compound | Gram-positive Strains | Gram-negative Strains |
|----------|-----------------------|-----------------------|
|          | *Bacillus subtilis*   | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Escherichia coli* |
| 3a       | 12.0                  | 14.0                  | 13.0                  | 11.0               |
| 3b       | 11.0                  | 12.0                  | 14.0                  | 13.0               |
| 3c       | 22.0                  | 23.0                  | 24.5                  | 21.0               |
| 3d       | 24.0                  | 23.0                  | 23.0                  | 22.0               |
| 3e       | 10.0                  | 11.5                  | 12.0                  | 10.0               |
| 3f       | 23.0                  | 24.0                  | 25.0                  | 22.0               |
| Chloramphenicol | 25.0                  | 24.0                  | 22.0                  | 20.0               |
CONCLUSION
We have successfully synthesized novel 2-Substituted-6-chloroquinoxalines by using a phase transfer catalyst. The present method is economically feasible because the products can be obtained through a facile, easy and efficient. The compounds showed 3c and 3d showed promising antibacterial activity and the remained all compounds showed moderate activity.

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REFERENCES
1. R.V. Ghadage and P.J. Shirote, Journal of Chemical and Pharmaceutical Research, 3(5), 260(2011)
2. Mao Zhang, Zhi-Cheng Dai, Shao-Song Qian, Jun-Yan Liu, Yu Xiao, Ai-Min Lu, Hai-Liang Zhu, Jian-Xin Wang and Yong-Hao Ye, Journal of Agricultural and Food Chemistry, 62(40), 9637(2014), DOI: 10.1021/jf504359p
3. A.K. Parhi, Y.Z. Zhang, K.W. Saionz, P. Pradhan, M. Kaul, K. Trivedi, D.S. Pilch and E.J. LaVoie., Bioorganic & Medicinal Chemistry Letters, 23, 4968(2013), DOI:10.1016/j.bmcl.2013.06.048
4. A.M.S.E. Newahie, Y.M. Nissan, N.S.M. Ismail., D.A. Abou, El. Ella, S.M. Khojah, and K.A.M. Abouzid, Molecules, vol. 24, 1175(2019), DOI:10.3390/molecules24061175
5. M. Santivanez-Veliz, S. Perez-Silanes, E. Torres and E. Moreno-Viguri, Bioorganic & Medicinal Chemistry Letters, 26, 2188(2016), DOI:10.1016/j.bmcl.2016.03.066
6. J.L. Sessler, H. Maeda, T. Mizuno, V.M. Lynch and F. Hiroyuki, Journal of the American Chemical Society, 124, 13474(2002), DOI:10.1021/ja0273750
7. S. Chakraborty, S. Goswami, C. K. Quah and B. Pakhira, Royal Society Open Science, 5(6), 180149(2018), DOI:10.1098/rsos.180149
8. P. Ramalingam, K. Rajendran, K. Sunil Kumar and Y. Padmanabha Reddy, International Journal of Medicinal Chemistry, 6471352, 8(2016), DOI:10.1155/2016/6471352

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