Synthesis of some new tetrazolo-[1,5-α]quinazolino[2,3-c]imidazolo-
[4,5-b]quinoxaline derivatives as antimicrobial agents

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Treatment of 6,7-dimethoxy-2-chloroquinazolin-4-amine 1 and
7,8-dimethoxy tetrazolo-[1, 5-a]-quinazolin-5-amine 4 with 2, 3-
dichloroquinoxaline 2a-e in glacial acetic acid /DMF afford
the corresponding substituted 6,7-dimethoxy-2-chloroquinazolino[3,4-
c]-imidazo-[4,5-b]-quinazolines 3a-e and 7,8-dimethoxytetrazolo-
[1,5-a]-quinazolin-5-amine 4 with 2, 3-dichloroquinoxalines 2a-e
The chemical structures of the newly synthesized compounds
have been characterized by IR, NMR, mass spectral and CHN
analysis. All the title compounds are subjected to
in vitro antibacterial testing against two pathogenic strains and antifungal
screening against two fungi. Among the tested compounds, 5b
and 5c show significant antibacterial and antifungal activities.
Also the compound 3b show significant antifungal activity against
Candia albicans.

Keywords: Tetrazole, amino quinazoline, quinoxaline, sodium
azide, antimicrobial

Quinoxalines are a big family of heterocyclic compounds, which have shown broad variety of
biological activities12. The ever growing resistance to antibiotics has led to continuous screening for new
biologically effective substances of natural or synthetic origin. Quinoxalines, the derivatives of
benzopyrimidine, are the compounds used in the pharmaceutical industry, in medicine and agriculture
because of their wide spectrum of biological activity such as analgesic3, anti-inflammatory4, anticonvulsant3,
anticancer5, antidiabetic7, antihypertensive8 and many others. Out of the wide substitution patterns known,
4-aminoquinozines are useful as fungicides9,10, anti-inflammatory11,12, anti-cancer13,14, anti-microbial
and anti-hypertensive agents15,16. The compounds like
6-substituted benzimidazole-[1,2-c]-quinazoline and
5-ethyl-2,3-dihydroimidazo-[1,2-c]-quinazoline7,18
shows bronchodilatory activity.

Compounds containing the quinoxaline nucleus exhibit a broad spectrum of biological activity such as
antiviral19, anti-inflammatory, anti-protozoal20, antihelmintic21, anti-cancer22, anti-malarial activities23. Some of quinoxalines exhibited good anti-oxidant,
antioxidative and analgesic activities24-28. This biological importance prompted us to synthesize some
new heterocyclic derivatives having imidazoquinazoline and quinoxaline moiety starting from
2,3-dichloroquinoxaline in acetic acid media, in order to search for better antimicrobial activity.

Results and Discussion
The reaction sequences employed for synthesis of intermediates and target compounds 2a-e are shown
in Schemes I and II. The starting materials used in the present study were prepared following a
previously reported literature procedure29. Cyclisation of these 2,3-dichloroquinoxalines 2a-e with 6,7-di-
methoxy-2-chloroquinazolin-4-ylamine 1 reuffling in

Notes
antimicrobial activity
The agar disc-diffusion method30 was used for the
screening of in vitro antimicrobial activity. The
anti-microbial activity of the synthesized compounds
3a-e and 5a-e were screened against Staphylococcus
aureus and Escherichia coli using nutrient agar
medium. The antifungal activity of the compounds
was tested against Candida albicans and Aspergillus
niger using Sabouraud dextrose agar medium.

The minimum inhibitory concentration (MIC) study was
carried out using micro dilution susceptibility method31.
Ciprofloxacin was used as a standard
antibacterial drug and Flucanazole was used as a
standard antifungal drug. The observed data on the
antimicrobial activity of compounds and control drugs
are given in Table I.
The investigation of antibacterial screening (Table I) revealed that some of the newly synthesized compounds showed moderate to good inhibition at 25-100 µg/mL in DMSO. Amongst all the compounds, compounds 5b, 5c showed excellent antibacterial activity against E. coli (MIC: 25 µg/mL) and S. aureus (MIC: 25 µg/mL). Compounds 3b and 3d displayed good activity against S. aureus (MIC: 50 µg/mL). Compounds 3a, 3e, 5a and 5e exhibited moderate activity against S. aureus and E. coli.

The investigation of antifungal screening (Table I) revealed that some of the newly synthesized compounds showed moderate to good inhibition at 25-100 µg/mL in DMSO. Amongst the tested
compounds, compounds 5b and 5c showed excellent inhibitory growth against C. albicans (MIC: 25 µg/mL) and A. niger (MIC: 25 µg/mL) respectively. Compound 3b showed excellent activity against C. albicans whereas compounds 3d, 3e and 5d exhibited good activity against A. niger. Remaining compounds showed moderate to least activity against both bacteria and fungi.

Biological protocol

Antimicrobial activity

Preliminary antimicrobial activities of compound 3a-e and 5a-e were tested by Agar disc diffusion method. Sterile filter paper discs (6 mm diameter) were carefully placed on the agar culture plates that had been previously incubated separately with the microorganisms. The plates were incubated at 37°C and the diameter of the growth inhibition zones were measured after 24 hr in case of bacteria and after 48 hr in case of fungi. The MICs of the compound assays were carried out using micro dilution susceptibility method. Ciprofloxacin was used as reference for antibacterial activity agent. Flucanazole was used as reference for anti fungal agent. The test compounds, Ciprofloxacin and Flucanazole were dissolved in DMSO at concentration of 800 µg/mL and two fold serial dilution of the solution was prepared (400, 200, 100, ..., 6.25 µg/mL). The microorganism suspensions were inoculated into the corresponding wells. The plates were incubated at 37°C for 24 hr and 48 hr for bacteria and fungi respectively. The minimum inhibitory concentration (MIC: µg/mL) of the compounds were recorded as the lowest concentration of each chemical compounds in the tubes without turbidity (i.e. no growth) of inoculated bacteria/fungi.

Experimental Section

Melting points were measured in open capillary and are uncorrected. Column chromatography was performed using silica gel (100-200 mesh size) purchased from Thomas Baker and Thin Layer Chromatography (TLC) was carried out using aluminium sheets pre-coated with silica gel 60F254 purchased from Merck. IR spectra (KBr) were obtained using Baker WM-4(X) spectrometer (577 model). 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Bruker WM-400 spectrometer in DMSO-d6 with TMS as an internal standard. Mass spectra (ESI) were recorded on a Jeol SX-102 spectrometer. CHN analysis was carried out on a Carlo Erba EA 1108 automatic analyzer. Combustion analyses were found to be within the limits of permissible error. The chemicals and solvents used were of commercial grade and used without further purification unless otherwise stated.

General procedure for the synthesis of substituted 2-chloro-6,7-dimethoxyquinazolin-2,3-c-imidazo-[4,5-b]-quinoxalines, 3a-e

A mixture of 2-chloro-6,7-dimethoxy-4-aminoquinazoline (0.01 mol) 1 and 2,3-dichloro quinoxalines (0.01 mol) 2a-e in glacial acetic acid 10 mL containing 0.2 mL of DMF as a catalyst was refluxed for 10 hr (monitored by TLC). The reaction mixture was cooled and the deposited solid was filtered, dried and re-crystallized from DMF/ MeOH 1:4 to furnish compounds 3a-e.

2-Chloro-6, 7-dimethoxyquinazolin-[2,3-c]-imidazo-[4,5-b]-quinoxaline, 3a. Yield: 85%; m.p. 262-64°C; IR (KBr): 1524, 1572, 1618 cm⁻¹; 1H NMR (DMSO-d6, 400 MHz): δ 3.82 (s, 3H,-OCH3), 3.88 (s, 3H,-OCH3), 7.06-7.14 (m, 4H, Ar-H), 7.88 (s, 1H, Ar-H),8.02 (s, 1H, Ar-H); 13C NMR (DMSO-d6, 100 MHz): δ 56.2, 56.6, 107.2, 107.8, 114.2, 129.8, 130.2, 142.6, 143.8, 143.9, 144.6, 147.2, 153.4, 154.8, 162.4; MS: m/z 366 (M+1). Anal. Calcd for C17H16ClN3O2: C, 59.11; H, 3.31; N, 19.15. Found: C, 59.05; H, 3.28; N, 19.12%.

2-Chloro-6, 7-dimethoxyquinazolin-[2,3-c]-imidazo-[4,5-b]-6-methylquinoxaline, 3b. Yield: 79%; m.p. 272-74°C; IR (KBr): 1522, 1570, 1613 cm⁻¹; 1H NMR (DMSO-d6, 400 MHz): δ 2.26 (s, 3H,-CH3), 3.82 (s, 3H,-OCH3), 3.86 (s, 3H,-OCH3), 6.89-6.92 (s, 2H, Ar-H), 7.00-7.02 (s,1H, Ar-H), 7.79 (s,1H, Ar-H), 7.92 ( s, 1H, Ar-H); 13C NMR (DMSO-d6, 100 MHz): δ 26.4, 56.2, 56.5, 106.4, 106.8, 115.2, 129.2, 129.8, 130.8, 139.4, 141.2, 142.8, 143.8, 144.4, 145.2, 147.2, 153.4, 156.8, 163.2; MS: m/z 380 (M+1). Anal. Calcd for C18H17ClN3O2: C, 60.09; H, 3.72; N, 18.44. Found: C, 59.92; H, 3.68; N, 18.40%.

2-Chloro-6,7-dimethoxyquinazolin-[2,3-c]-imidazo-[4,5-b]-6-chloroquinoxaline, 3c. Yield: 80%; m.p. 287-88°C; IR (KBr): 1522, 1570, 1624 cm⁻¹; 1H NMR (DMSO-d6, 400 MHz): δ 3.84 (s, 3H,-OCH3), 3.86 (s, 3H,-OCH3), 7.12 (s, 1H, Ar-H ), 7.18 (s,1H, Ar-H), 7.25 (s,1H, Ar-H), 7.92 ( s, 1H, Ar-H), 7.98 (s, 1H, Ar-H); 13C NMR (DMSO-d6, 100 MHz): δ 56.2, 56.5, 106.8, 107.4, 112.4, 129.4, 131.4, 133.6, 142.4, 144.2, 145.2, 146.3, 147.4, 148.6, 154.2, 156.4, 162.6; MS: m/z 399 (M+1). Anal. Calcd for C18H17Cl2N3O2:
C, 54.02; H, 2.77; N, 17.50. Found: C, 53.92; H, 2.72; N, 17.48%.

2-Chloro-6,7-dimethoxyquinazolin-[2, 3-c]-imidazo-[4,5-b]-6-bromoquinoxaline, 3d. Yield: 75%; m.p. 264-66°C; IR (KBr): 1522, 1564, 1618 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 3.84 (s, 3H,-OCH\(_3\)), 3.86 (s, 3H,-OCH\(_3\)), 7.14 (s, 1H, Ar-H), 7.20 (s,1H, Ar-H), 7.42 (s,1H, Ar-H), 7.93 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H); \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): δ 56.2, 56.5, 107.4, 107.8, 112.9, 120.4, 131.4, 131.8, 134.2, 141.4, 144.2, 145.7, 146.2, 147.8, 149.0, 154.2, 156.4, 162.9; MS: \(m/z\) 443 (M+1). Anal. Calcld for C\(_{18}\)H\(_11\)Cl Br N\(_3\)O\(_2\): C, 48.62; H, 2.49; N, 15.75. Found: C, 48.60; H, 2.40; N, 15.68%.

2-Chloro-6,7-dimethoxyquinazolin-[2,3-c]-imidazo-[4,5-b]-6-nitroquinoxaline, 3e. Yield: 78%; m.p. 270-72°C; IR (KBr): 1522, 1568, 1624 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 3.98 (s, 3H,-OCH\(_3\)), 4.02 (s, 3H,-OCH\(_3\)), 7.16 (s, 1H, Ar-H), 7.76 (s,1H, Ar-H), 7.92 (s,1H, Ar-H), 8.21 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H); \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): δ 56.2, 56.6, 106.4, 107.8, 117.0, 124.2, 127.4, 132.4, 142.2, 144.2, 148.2, 147.8, 150.2, 151.5, 153.2, 156.7, 164.6; MS: \(m/z\) 411 (M+1); Anal. Calcld for C\(_{18}\)H\(_11\)ClN\(_2\)O\(_2\): C, 52.63; H, 2.70; N, 20.46. Found: C, 52.60; H, 2.66; N, 20.38%.

Procedure for the synthesis of 7, 8-dimethoxytetrazolo-[1,5-a]-quinazolin-5-ylamine, 4

Compound 6,7-dimethoxy-2-chloro-4-aminoquinazoline 1 (0.001 mol) was dissolved in ethanol (10 mL) and to this sodium azide (0.0012 mol) was added. Then the reaction mixture was refluxed for 3 hr. After completion of the reaction (monitored by TLC), the reaction mixture was poured into water, the solid separated was filtered, washed with water, dried and purified by column chromatography (2:8 CHCl\(_3\):EA) to furnish the desired compounds 5a-e.

A solution of compound 7, 8-dimethoxytetrazolo-[1,5-a]-quinazolin-5-amine 4 (0.001 mol) and substituted 2,3-dichloroquinoxaline 2a-e (0.001 mol) in glacial acetic acid containing 0.2 mL of DMF as a catalyst was stirred at reflux temperature for 12-15 hr. After completion of the reaction (monitored by TLC), the reaction mixture was poured into water, the solid separated was filtered, washed with water, dried and purified by column chromatography (2:8 CHCl\(_3\):EA) to furnish the desired compounds 5a-e.

7,8-Dimethoxytetrazolo-[1, 5-a]-quinazolin-[2,3-c]-imidazo-[4,5-b]-quinoxaline, 5a. Yield: 64%; m.p. 282-84°C; IR (KBr): 1522, 1570, 1618 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 3.90 (s, 3H,-OCH\(_3\)), 3.96 (s, 3H,-OCH\(_3\)), 7.72-7.86 (m, 4H, Ar-H), 8.10 (s, 1H, Ar-H) 8.12 (s, 1H, Ar-H); \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): δ 56.17, 56.59, 97.5, 100.4, 106.2, 130.2, 130.6, 141.6, 142.6, 144.9, 152.2, 152.6, 162.6; MS: \(m/z\) 373 (M+1). Anal. Calcld for C\(_{18}\)H\(_{12}\)N\(_5\)O\(_2\): C, 58.06; H, 3.25; N, 30.09. Found: C, 58.01; H, 3.20; N, 30.02%

7,8-Dimethoxytetrazolo-[1, 5-a]-quinazolin-[2,3-c]-imidazo-[4,5-b]-6-methyl quinoxaline, 5b. Yield: 68%; m.p. 274-76°C; IR (KBr): 1524, 1576, 1618 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 2.32 (s, 3H,-CH\(_3\)), 3.82 (s, 3H,-OCH\(_3\)), 3.86 (s, 3H,-OCH\(_3\)), 7.62 (s, 1H, Ar-H ), 7.72-7.72 (m, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H); \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): δ 26.2, 56.6, 56.2, 100.4, 102.4, 112.0, 115.2, 129.6, 130.4, 131.4, 134.8, 140.4, 142.2, 144.0, 144.2, 144.9, 154.2, 156.4, 162.5; MS: \(m/z\) 387 (M+1). Anal. Calcld for C\(_{19}\)H\(_{13}\)N\(_5\)O\(_2\): C, 59.06; H, 3.65; N, 29.00. Found: C, 59.02; H, 3.61; N, 28.94%.

7,8-Dimethoxytetrazolo-[1, 5-a]-quinazolin-[2, 3-c]-imidazo-[4,5-b]-6-chloro quinoxaline, 5c. Yield: 72%; m.p. 292-94°C; IR(KBr): 1522, 1572, 1624 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 3.82 (s, 3H,-OCH\(_3\)), 3.88 (s,3H,-OCH\(_3\)), 7.48 (s,1H,Ar-H), 7.64 (s,1H, Ar-H ), 7.76 (s, 1H, Ar-H), 8.08 (s,1H,Ar-H), 8.12 (s,1H, Ar-H); \(^13\)C NMR (DMSO-\(d_6\), 100 MHz): δ 56.57, 56.15, 106.4, 108.2, 112.3, 115.7, 129.4, 131.4, 133.4, 141.4, 141.9, 142.4, 142.8, 147.2, 144.7, 154.2, 156.4, 162.2; MS: \(m/z\) 407 (M+1). Anal. Calcld for C\(_{18}\)H\(_{11}\)ClN\(_5\)O\(_2\): C, 53.15; H, 2.73; N, 27.55. Found: C, 53.10; H, 2.68; N, 27.52%.

7,8-Dimethoxytetrazolo-[1, 5-a]-quinazolin-[2, 3-c]-imidazo-[4,5-b]-6-bromo quinoxaline, 5d. Yield: 65%; m.p. 298-300°C; IR (KBr): 1532, 1572, 1624 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): δ 3.82 (s,
3H, -OCH₃), 3.88 (s, 3H, -OCH₃), 7.46 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.76 (m, 1H, Ar-H), 8.10 (s, 1H, Ar-H); ¹³C NMR (DMSO-d₆, 100 MHz): δ 56.6, 56.2, 107.2, 108.4, 113.0, 116.2, 119.2, 129.8, 131.4, 134.6, 141.4, 141.7, 144.2, 144.8, 145.8, 152.8, 156.2, 162.8; MS: m/z 451 (M+1). Anal. Calcd for C₁₈H₁₁BrN₅O₆: C, 47.87; H, 2.46; N, 24.79%. Found: C, 47.76; H, 2.47; N, 24.80.

Conclusion

In summary, a series of novel 6,7-dimethoxy-2-chloroquinazo[3,4-c]-imidazo-[4,5-b]-6-nitroquinoxalines 3a-e and 7,8-dimethoxytetrazolo-[1,5-a]-quinazolino[2,3-c]-imidazo-[4,5-b]-quininoxalines 5a-e have been synthesized and characterized by spectral and elemental analyses. All the newly synthesized compounds were screened for their in vitro antimicrobial activity. Among the screened samples 5b and 5c showed significant antibacterial and antifungal activities compared to other tested samples.

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References