Heterocyclic Letters
Vol. 9| No.4|427-435|Aug –Oct |2019
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS AND ANTI MICROBIAL ACTIVITY OF TETRAZOLO QUINOXALINE CONTAINING PYRAZOLE ANALOGUES

Gouthami Dasari[2], Rajitha Sontireddy[2] and Srinivas Bandari[1].*

[1]Research Center, Department of Chemistry, Chaitanya Postgraduate College (Autonomous), Hanamkonda, Warangal. 506001, TS, India.
[2]Department of Chemistry, Vaagdevi Degree and Postgraduate College, Hanamkonda,Warangal. 506001, TS, India
*Corresponding Author: Mobile: 91-7396264954, e-mail: bandarisrinivas2005@gmail.com *,*

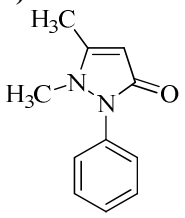
Abstract:
Pyrazoles have been the recent target of numerous methodologies, mostly due to their prevalence as scaffolds in synthesis of bioactive compounds and reactions in different media. Pyrazole and their derivatives are found to have profound biological activity. In the present work some novel substituted 5-methyl-2-(tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-ones **4(a-h)** and substituted 1-(tetrazolo[1,5-*a*] quinoxalin-4-yl) pyrazolidin-3,5-diones **5(a-h)** have been synthesized. These derivatives are synthesized by treating 4-hydrazinyl tetrazolo[1,5-*a*]quinoxalines with ethylaceto acetate and diethyl malonate in acetic acid solution. All the synthesized compounds were characterized by IR, ^{1}H-NMR and Elemental Analysis. All the newly synthesized derivatives were evaluated for anti-microbial activity on different micro-organisms (*E.coli, S. aureus, A.niger, C.albicans)* at the concentration of 10 µg/mL and 20 µg/mL by using agar disc-diffusion method. The activity was measured in terms of zone of inhibition and compared with standard drug ciprofloxacin for antibacterial and Flucanazole for antifungal activity.

Keywords:
4-Hydrazinyl tetrazolo quinoxalines, ethyl aceto acetate, diethyl malonate, acetic acid, Anti-microbial activity.

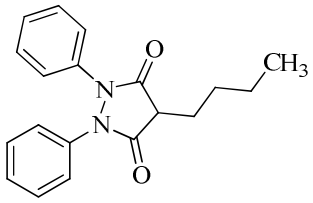
Introduction:

The synthesis and chemical transformations of pyrazoles attract a wide interest for they are extensively employed in pharmaceutical industry, agriculture, and in new materials production. Nowadays considerable attention is given to the development of new methods for the preparation of pyrazole derivatives since the presence of pyrazole ring in organic molecules gives rise to versatile physiological activity of many compounds of both synthetic and natural origin. Pyrazole derivatives have a long history of application in agrochemicals such as herbicides and insecticides and in pharmaceutical industry. Antipyrine[i] **(1)** is the one of the earliest synthetic drugs and is named after its antipyretic properties. Phenyl

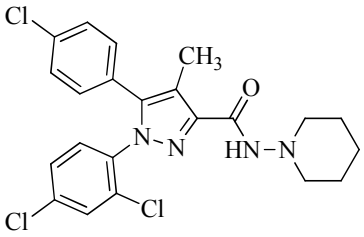
Butazolidine[ii] **(2)** another pyrazolone is a powerful anti-inflammatory drug used in rheumatic conditions. The derivatives of pyrazole have been reported to shown a broad spectrum of biological activities including anti-microbial[iii,iv], anti-inflammatory[v,vi], antituberculosis[vii,viii], antiviral[ix,x], hypoglycemic[xi,xii], anti-tumor [xiii,xiv], and anti-hypertensive[xv,xvi]. Due to its wide range of biological activity, pyrazoles have received a considerable interest in the field of drug discovery and therefore pyrazole ring constitutes a relevant synthetic target in pharmaceutical industry. In fact, such a heterocyclic moiety represents the core structure of a number of drugs **(3, 4)**.



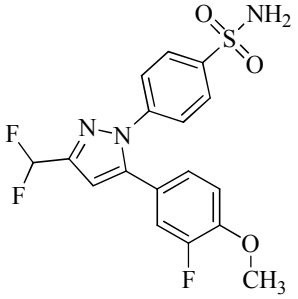
1 Antipyrine



2 Phenyl Butazolidine



3 Rimonabant



4 Deracixib

Very few pyrazole derivatives are naturally occurring may be due to the difficulty of living organisms to construct the N-N bond. Owing to the wide spread applications, synthesis and biological activity evaluation of pyrazoles and their derivatives have been a subject of intensive investigations as revealed by enormous literature covering the subject.

Materials and methods:

Melting points were determined by the capillary tube method, and the thermometer was uncorrected. The progress of reaction was monitored by thin layer chromatography on silica gel plates. Elemental analysis was measured by using of Perkin Elmer 2400 CHN elemental analyzer. ^{1}HNMR were recorded on a Brucker WM-400MHz using TMS as an internal slandered, samples was dissolved in DMSO-d_6. FT-IR Spectra were obtained as KBr discs on Perkin Elmer FT-IR240-Spectrometer. ^{13}CNMR spectra were recorded on a Brucker WM-100MHz using DMSO-d_6. Mass spectra were recorded on a JEOL.SX-102 [ESI-MS]. Elemental analysis was performed with a Carlo-Erba 1106 Elemental analysis instrument.

General procedure for the synthesis of substituted 2-hydrazinyl-3-chloro quinoxalines 2(a-h):

To a stirred solution of substituted 2,3-dichloroquinoxaline **1(a-h)** (0.025mol), triethyl amine (0.025 mol) and hydrazine hydrate (0.030 mol) in methanol (25mL) were added slowly, than stirred at room temperature for 3 h. The solid mass obtained was filtered and re-crystallized from ethanol.

General procedure for the synthesis of substituted 4-hydrazinyl tetrazolo*[1,5-a]* quinoxa- lines 3(a-h):

Substituted 2-hydrazinyl-3-chloroquinoxalines **2(a-h)** (0.05mol) was added to a mixture of ethanol (50 mL), sodium azide (0.15mol) and dimethyl formamide (2 mL) were heated to

reflux for 3-4 h. The reaction was monitored by TLC. The solid was separated filtered, washed with water and re-crystallised from ethanol to furnish the desired compounds.

General procedure for the synthesis of substituted 5-methyl-2-(tetrazolo [1,5-*a*] quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-ones 4(a-h):

To a solution of 4-hydrazinyl tetrazolo[1,5-*a*]quinoxaline **3(a-h)** (0.01 mol), in glacial acetic acid (10 mL), ethyl acetoacetate (0.01 mol) was added slowly and refluxed for 3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, poured into ice cold water and extracted with chloroform (3x10 mL). The organic layers were collected, washed with brine solution (3x10 mL), dried over anhydrous Na_2SO_4 and concentrated under *vaccuo* to get corresponding compounds, than purified by recrystallization with ethanol.

5-methyl-2-(tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-one (4a):

Yield: 65%; m.p.255-257 ^oC; IR (KBr, cm^{-1}): 1641 (C-N), 1740 (C=O), 1674 (C=N); ^{1}HNMR (400MHz, DMSO-d_6, δppm): δ 1.61(s, 3H, CH_3), 2.39 (s, 2H, -CH_2), 7.43-7.62 (m, 4H, Ar-H). ^{13}CNMR (100MHz, DMSO-d_6,δ ppm): 24.3, 44.8, 124.1, 125.7, 126.7, 132.0, 137.1 139.0, 145.1, 162.8, 159.7, 173.8. MS (m/z): 268 (M+H). Anal. Calculated for $C_{12}H_9N_7O$: C: 53.93.; H: 3.39.; N: 36.69. Found: C: 53.88.; H: 3.35.; N: 36.98.

5-methyl-2-(7-methyl tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-one (4b) :

Yield: 63%; m.p. 268-270 ^oC; IR (KBr, cm^{-1}): 1708(C=O), 1630(C-N), 1598(C=N). ^{1}HNMR (400MHz, DMSO-d_6,δ ppm): 1.70 (s, 3H, CH_3), 2.38 (s, 2H, CH_2), 2.72 (s, 3H, CH_3), 7.41(d, 1H, Ar-H), 7.58 (s, 1H, Ar-H), 7.63 (d, 1H, Ar-H).^{13}C NMR (100MHz, DMSO-d_6,δ ppm) 24.3, 26.8, 43.8, 123.7, 125.7, 127.3, 132.1, 133.4, 137.1, 143.7, 160.1, 162.1, 171.1. MS (m/z): 283 (M+H). Anal. Calculated for $C_{13}H_{11}N_7O$: C: 55.51.; H: 3.94.; N: 34.86. Found: C: 55.49.; H: 3.90.; N: 34.82.

2-(7-nitrotetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4c) :

Yield: 55%; m.p. 272-274 ^oC; IR (KBr, cm^{-1}): 1720 (C=O), 1643 (C-N), 1677 (C=N). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ1.60 (s, 3H, CH_3), 2.40 (s, 2H, CH_2), 8.38 (d, 1H, Ar-H), 8.64(d, 1H, Ar-H), 9.06 (s, 1H, Ar-H):^{13}C NMR (100MHz, DMSO-d_6,δ ppm) 26.3, 43.8, 118.0, 124.1, 130.2, 138.8, 139.9, 141.5, 149.5, 160.2, 163.7, 172.8. MS (m/z): 313 (M+H). Anal. Calculated for $C_{12}H_8N_8O_3$: C: 46.12.; H: 2.58.; N: 35.89. Found: C: 46.05.; H: 2.52.; N: 35.46.

2-(7,8-dimethyltetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4d) :

Yield: 71%; m.p. 265-267 ^oC; IR (KBr, cm^{-1}) 1607 (C-N), 1648 (C=N). 1710 (C=O). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm) δ 1.75 (s, 3H, CH_3), 2.22 (s, 2H, CH_2), 2.61 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.50 (s,1H, Ar-H), 7.62 (s, 1H, Ar-H), ^{13}C NMR (100MHz, DMSO-d_6, δ ppm) 24.6, 24.9, 26.3, 43.1, 125.7, 126.6, 134.6, 135.1, 141.1, 143.8, 147.3, 159.9, 161.2, 173.5. MS (m/z): 295 (M+H). Anal. Calculated for $C_{14}H_{13}N_7O$: C: 56.94.; H: 4.44.;N: 33.20. Found: C: 56.88.; H: 4.40.; N: 33.28.

2-(7-nitro-8-methyltetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4e) :

Yield: 65%; m.p. 286-288 ^oC; IR (KBr, cm^{-1}):1623 (C-N), 1663 (C=N), 1720 (C=O). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): 1.66 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.82 (s, 2H, CH_2), 8.45(s, 1H, Ar-H), 8.70 (s, 1H, Ar-H). ^{13}C NMR (100MHz, DMSO-d_6, δ ppm): 24.2, 25.6, 46.3, 122.0, 123.4, 129.2, 130.2, 138.2, 141.5, 151.0, 151.4, 162.3, 173.6. MS (m/z): 327 (M+H). Anal. Calculated for $C_{13}H_{10}N_8O_3$: C: 47.86.; H: 3.09.; N: 34.34. Found: C: 47.80.; H: 2.98.; N: 34.32.

2-(7,8-dinitrotetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4f) :

Yield: 47 %; m.p. 292-294 ^{o}C; IR (KBr, cm^{-1}): 1616 (C-N), 1656 (C=N), 1738 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 1.65 (s, 3H, CH_3), 2.76 (s, 2H, CH_2), 7.82 (s, 1H, Ar-H), 9.72 (s, 1H, Ar-H). ^{13}C NMR (100MHz, DMSO-d$_6$, δppm): 25.1, 44.4, 125.2, 127.4, 139.2, 141.2, 143.2, 144.5, 145.1, 162.4, 167.7, 173.8 (N=C).MS (m/z): 358 (M+H). Anal. Calculated for $C_{12}H_7N_9O_5$: C: 40.34.; H: 1.98.; N: 35.29. Found: C: 40.30.; H: 1.92.; N: 35.22.

2-(7-methoxyltetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4g):

Yield: 69 %; m.p. 274-276 ^{o}C; IR (KBr, cm^{-1}): 1614 (C-N), 1665 (C=N) 1718 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 1.62 (s, 3H, CH_3), 2.45 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 7.92 (s, 1H, Ar-H), 7.95 (d, 1H, Ar-H),8.10 (d,1H, Quin-H), ^{13}C NMR (100MHz, DMSO-d$_6$,δ ppm): 25.2, 42.7, 58.2, 101.3, 118.6, 129.3, 131.8, 134.3, 142.2, 159.8, 161.4, 163.5, 173.8. MS (m/z): 298 (M+H). Anal.Calculated for $C_{13}H_{11}N_7O_2$: C: 52.52.; H: 3.73.; N: 32.98. Found: C: 52.49.; H: 3.70.; N: 32.94.

2-(7-chlorotetrazolo[1,5-*a*]quinoxalin-4-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (4h) :

Yield: 58%; m.p. 254-256 ^{o}C; IR (KBr, cm^{-1}):1654 (C-N), 1685 (C=N), 1725(C=O). ^{1}HNMR (400MHz, DMSO-d$_6$,δ ppm): δ 1.72 (s, 3H, CH_3), 2.38 (s,2H,CH_2), 8.14 (d, 1H, Ar-H), 8.18 (d,1H, Ar-H), 8.32 (s, 1H, Ar-H).^{13}C NMR (100MHz, DMSO-d$_6$,δ ppm): 25.3, 44.5, 128.1, 132.1, 134.7, 135.2, 138.2, 141.5, 142.1, 159.5, 162.2, 172.1. MS (m/z): 302 (M+H). Anal. Calculated for $C_{12}H_8ClN_7O$: C: 47.77.; H: 2.67.; N: 32.50. Found: C: 47.75.; H: 2.66.; N: 32.49.

General procedure for the synthesis of Substituted 1-(tetrazolo[1,5-*a*]quinoxalin-4-yl) Pyrazolidine-3,5-dione 5(a-h):

To a solution of 4-hydrazinyl tetrazolo[1,5-*a*]quinoxaline 3(a-h) (0.01 mol) in glacial acetic acid (10 mL), diethyl malanoate (0.01 mol) was added slowly and refluxed for 4-5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, poured into ice cold water and extracted with chloroform (3x10 mL). The organic layers were collected, washed with brine solution (3x10 mL), dried over anhydrous Na_2SO_4 and concentrated under *vaccuo* to get corresponding compounds, than purified by recrystallization with ethanol.

1-(tetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5a):

Yield: 67 %; m.p. 245-247 ^{o}C; IR (KBr, cm^{-1}): 1716 (C=O), 1635(C=N), 1657 (C-N), 3389 (-NH).^{1}HNMR (400MHz, DMSO-d$_6$, ,δ ppm): δ 3.10 (s,2H,CH_2). 7.92-7.98 (m, 2H, Ar-H),8.00-8.10 (dd, 2H, Ar-H), 10.20 (br, s, 1H, NH). ^{13}C NMR (100MHz, DMSO-d$_6$, δppm): 47.8, 125.7, 126.0, 128.4, 132.0, 137.1, 139.0, 145.1, 162.1, 165.1, 171.1. MS (m/z): 271 (M+H). Anal. Calcd for $C_{11}H_7N_7O_2$: C: 49.07.; H: 2.62.; N: 36.42. Found: C: 48.98.; H: 2.59.; N: 36.35.

1-(7-methyl tetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5b):

Yield: 61 %; m.p.251-253 ^{o}C; IR (KBr,cm^{-1}): 1652(C-N), 1610 (C=N), 1732 (C=O), 3357 (-NH).^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm) δ 2.21 (s, 3H, CH_3), 3.10 (s, 2H, CH_2), 6.78 (d, 1H, Ar-H), 7.20 (d, 1H, Ar-H), 7.58 (s, 1H, Ar-H),10.40 (br, s, 1H, -NH). ^{13}C NMR (100MHz, DMSO-d$_6$, δppm): δ 28.3, 47.6, 124.3, 125.8, 126.1, 134.2, 135.3, 138.1, 144.02, 160.7, 166.33, 170.2.MS (m/z): 284 (M+H). Anal.Calculated for $C_{12}H_9N_7O_2$: C: 50.88.; H: 3.20.; N: 34.62. Found: C: 50.86.; H: 3.17.; N: 34.59.

1-(7-nitro tetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5c):
Yield: 58 %; m.p. 268-270 ^{o}C; IR (KBr, cm^{-1}): 1658 (C-N), 1620 (C=N), 1735(C=O), 3334 (-NH). ^{1}HNMR (400MHz, DMSO-d$_{6,}$ δ ppm): δ3.58 (s, 2H, -CH$_2$), 8.40 (d,1H, Ar-H), 8.78 (d, 1H, Ar-H), 9.20 (s, 1H, Ar-H), 11.16 (br, s, 1H, -NH); ^{13}C NMR (100MHz, DMSO-d$_{6,}$ δ ppm): 47.8, 118.3, 122.8, 129.5, 138.2, 141.1, 143.2, 148.5, 163.8, 165.8, 173.8.MS (m/z): 315 (M+H). Anal. Calcd for $C_{11}H_6N_8O_4$: C: 42.05.; H: 1.92.; N: 35.66. Found: C: 41.96.; H: 1.88.; N; 35.72.

1-(7,8-dimethyltetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5d):
Yield: 65%; m.p. 271-273 ^{o}C; IR (KBr, cm^{-1}): 1665(C-N), 1648 (C=N), 1708 (C=O), 3398 (-NH).^{1}HNMR (400MHz, DMSO-d$_{6,}$δ ppm): δ 2.46 (s, 3H, CH$_3$),2.72 (s,3H,CH$_3$), 3.58 (s, 2H, CH$_2$), 7.80 (s, 1H, Quin-H), 7.98 (s, 1H, Quin-H),10.38 (br, s, 1H, NH).^{13}C NMR (100MHz, DMSO-d$_{6,}$ δppm): δ 27.2, 27.7, 47.2, 124.7, 125.6, 128.5, 134.7, 139.1, 143.1, 147.2,159.3, 165.1, 169.5.MS (m/z): 298 (M+H). Anal. Calculated for $C_{13}H_{11}N_7O_2$: C: 52.52.; H: 3.73.; N: 32.98. Found: C: 52.48.; H: 3.57.; N: 32.94.

1-(7-nitro-8-methyltetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5e) :
Yield: 52 %; m.p. 283-285 ^{o}C; IR (KBr, cm^{-1}): 1678 (C-N), 1662 (C=N), 1728 (C=O), 3334 (-NH). ^{1}HNMR (400MHz, DMSO-d$_{6,}$ δ ppm): δ 2.42 (s, 3H, CH$_3$), 3.60 (s, 2H, -CH$_2$), 7.90 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 10.64 (br, s, 1H, -NH); ^{13}C NMR (100MHz, DMSO-d$_{6,}$ δ ppm): δ 29.2, 49.1, 121.9, 128.2, 129.1, 137.8, 138.1, 139.4, 150.5, 162.6, 166.8, 170.4.MS (m/z): 329 (M+H). Anal. Calculated for $C_{12}H_8N_8O_4$: C: 43.91.; H: 2.46.; N: 34.14. Found: C: 43.89.; H: 2.40.; N: 34.10.

1-(7,8-dinitrotetrazolo[1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5f):
Yield: 48 %; m.p 278-280 ^{o}C; IR (KBr, cm^{-1}): 1664 (C-N), 1612 (C=N), 1748(C=O), 3434 (-NH). ^{1}HNMR (400MHz, DMSO-d$_{6,}$ δppm): δ 3.80 (s, 2H, CH$_2$), 9.40 (s, 1H, Ar-H), 9.50 (s,1H, Ar-H), 11.25 (br, s, 1H, NH).^{13}C NMR (100MHz, DMSO-d$_{6,}$ δppm): δ 50.2, 124.4, 126.3, 136.1, 136.9, 141.8, 143.1,143.8, 165.8, 166.7, 172.1.MS (m/z): 360 (M+H). Anal. Calcd for $C_{11}H_5N_9O_6$: C: 36.28.; H: 1.40.; N: 35.09. Found: C: 36.24.; H: 1.38.; N: 35.01.

1-(7-methoxy tetrazolo [1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5g):
Yield: 58%; M.p 268-270 ^{o}C; IR (KBr, cm^{-1}):1605(C-N), 1664(C=N), 1720 (C=O), 3361(-NH). ^{1}HNMR (400MHz, DMSO-d$_{6,}$ δppm) δ 3.52 (s, 2H, -CH$_2$), 3.90 (s, 3H, -OCH$_3$), 7.62 (s,1H, Ar-H),7.92(d,1H, Ar-H), 8.04 (d,1H, Ar-H), 10.90 (br, s, 1H, NH). ^{13}C NMR (100MHz, DMSO-d$_{6,}$ δppm) δ 49.2, 57.3, 100.8, 116.4, 117.6,129.4, 131.8, 133.8, 143.8, 162.2, 168.8, 170.3. MS (m/z): 300 (M+H). Anal. Calculated for $C_{12}H_9N_7O_3$: C: 48.16.; H: 3.63.; N: 32.76. Found: C: 48.14.; H: 3.60.; N: 32.72.

1-(7-chloro tetrazolo [1,5-*a*]quinoxalin-4-yl)pyrazolidine-3,5-dione (5h):
Yield: 52%; m.p. 254-256 ^{o}C; IR (KBr, cm^{-1}):1648 (C-N), 1678 (C=N), 1738 (C=O), 3430 (-NH). ^{1}HNMR (400MHz, DMSO-d$_{6,}$ δppm): δ 3.52 (s, 2H, CH$_2$), 7.85 (d, 1H, Ar-H), 8.08 (d, 1H, Ar-H), 8.15 (s, 1H, Ar-H).^{13}C NMR (100MHz, DMSO-d$_{6,}$ δppm): 49.8, 123.5, 127.6, 129.3, 133.5, 136.8, 140.3, 142.8, 163.2, 165.8, 170.8, 163.2. MS (m/z):304 (M+H). Anal. Calculated for $C_{11}H_6ClN_7O_2$: C: 43.51.; H: 1.99.; N: 32.29. Found: C: 43.48.; H: 1.92.; N: 32.25.

Evaluation of antimicrobial activity

The newly synthesized compounds **4(a-h)** and **5(a-h)** were screened for in vitro anti-bacterial and anti-fungal activity against various Gram positive bacteria *S. aures* and Gram negative bacteria *E. Coli* and anti-fungal activity carried out on *C.albicans, A. niger*. The agar Disc-diffusion method[xvii] was used to evaluate anti-microbial activity. The compounds were dissolved in DMSO to 10 µg/mL and 20 µg/mL concentration solutions used. The compounds were placed aseptically on Muller-Hinton Agar for the both Gram positive and

Gram negative bacteria and Saboround dextrose agar for fungi and bacteria incubated for 24 h at 37^{o}C. At the end of the incubation period, the diameter of the growth of inhibition zones was measured. Two Gram positive bacteria *S. aureus* and Gram negative bacteria *E. coli* were used in the test method. Ciprofloxacin was used as the reference compound during the screening of anti-bacterial activity. *C. albicans and A. niger* was used in the test.

Results and discussions

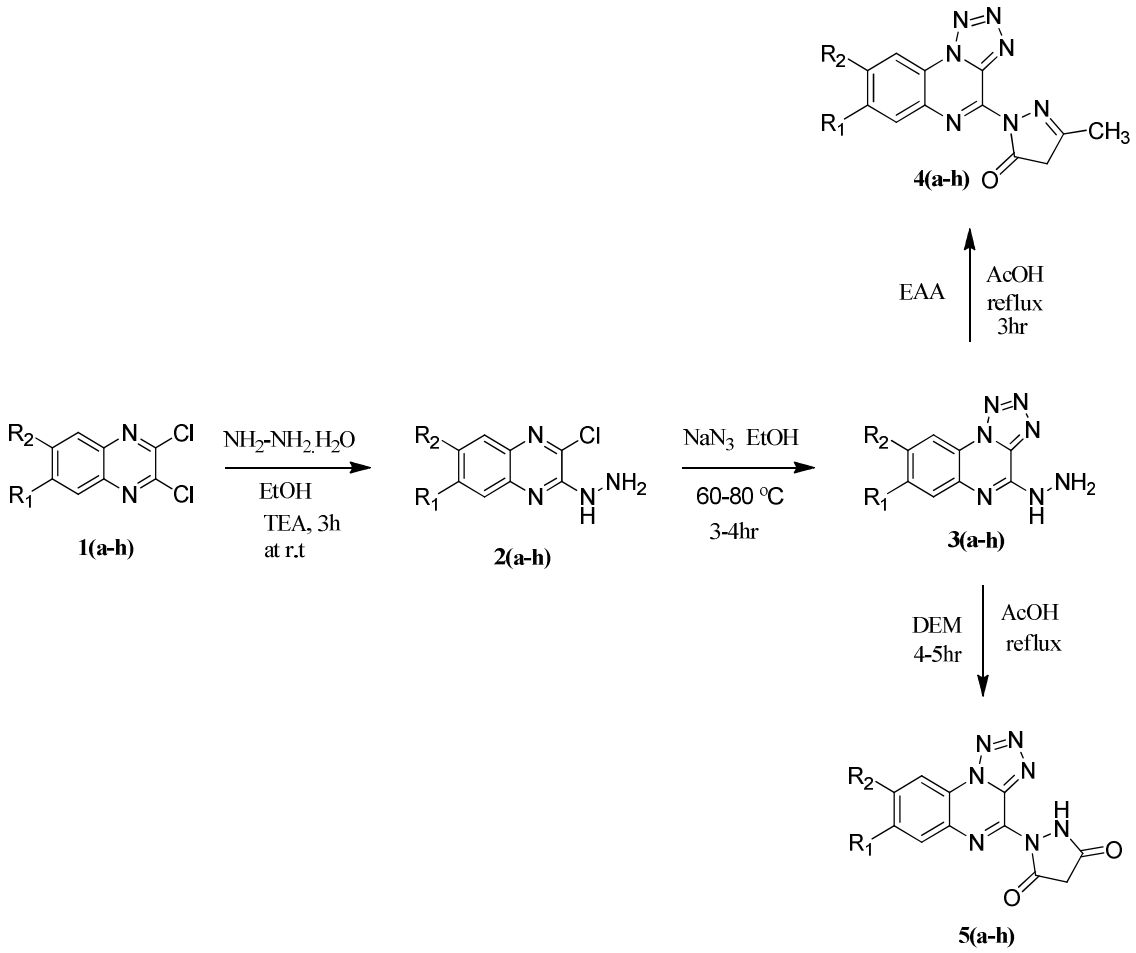
Chemistry

A series of novel substituted 5-methyl-2-(tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-ones **4(a-h)** and substituted 1-(tetrazolo[1,5-*a*] quinoxalin-4-yl) pyrazolidin-3,5-diones **5(a-h)** have been carried out by synthetic sequence illustrated in **Scheme-1.** The preparation of starting compounds 2,3-dichloroquinoxaline according to reported procedure [xviii]**.**To a stirred solution of 2,3-dichloroquinoxalines **1(a-h)** in methanol, triethylamine (0.01mol), hydrazine hydrate (0.01mol), and a catalytic amount of dimethylformamide were added slowly than, stirred at room temperature for 3 h to afforded 2-chloro-3-hydrazinyl quinoxalines **2(a-h)**. These intermediates were added to a mixture of ethanol and sodium azide then heated to 100 ^{0}C for 3 h to give corresponding 4-hydrazinyl tetrazolo[1,5-*a*]quinoxalines **3(a-h)** in good yields. The title compounds were obtained from the compounds **3(a-h)** were treated with ethyl aceto acetate and diethyl malonate in acetic acid solution heated to 100 ^{0}C for 3-4 h afforded the corresponding substituted 5-methyl-2-(tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-ones **4(a-h)** and substituted 1-(tetrazolo [1,5-*a*]quinoxalin-4yl)pyrazolidin-3,5-diones **5(a-h)** in good yields. Structures of the all the newly synthesized compounds were characterized on the basis of elemental analysis, IR, ^{1}H NMR, ^{13} C NMR and Mass spectral data. The synthesized compounds were also screened for their anti-microbial activity.

Biological activity:

The newly synthesized compounds **4(a-p)** were evaluated for their *in vitro* anti-microbial activity against *S. aureus* as Gram-positive, *E. coli* as Gram-negative bacteria and *C. albicans* and *A. niger* as fungi by agar disc diffusion assay method. The zone of inhibition values were determined and compared with Ciprofloxan and Flucanazole as standard drugs. The investigation of anti-fungal and anti-bacterial screening data revealed that some of the newly synthesized compounds showed potent activity against **4e** and **5e** was noticed significant anti-bacterial activity against *E. Coli* (16 and 18 mm) *S. aureus* (18, 17 mm) at 20µg/ml respectively. The compounds **4f, 4h, 4f** shown notable zone of inhibition against both bacterial strains tested. The antifungal screening data revealed that all the tested compounds showed very poor activity against tested fungal strains. The anti-fungal activity results shows that the compounds **4e** showed moderate anti-fungal activity against *C. albicans* and *A. niger* at 10 and 20 µg/mL concentrations respectively with the zone of inhibitions (02, 05, 03, 03 mm).

Scheme:



S. No	Compd.	R_1	R_2
1.	a	-H	-H
2.	b	$-CH_3$	-H
3.	c	$-NO_2$	-H
4.	d	$-CH_3$	$-CH_3$
5.	e	$-NO_2$	$-CH_3$
6.	f	$-NO_2$	$-NO_2$
7.	g	OCH_3	-H
8.	h	-Cl	-H

Table: 2. Anti-microbial activity result of substituted tetrazolo quinoxalines containing Dihydro pyrazole and Pyrazolidinone analogues at 10 and 20µg/ mL.

S.No	Compds	Concen µg/ml	Anti-bacterial Zone of inhibition in mm		Anti-fungal Zone of inhibition in mm	
			S. aureus	E.Coli	C. albicans	A. niger
1.	4a	10	04	05	-	-
		20	05	07	-	-
2.	4b	10	06	08	01	-
		20	13	10	01	-
3.	4c	10	07	05	01	-
		20	10	08	01	-
4.	4d	10	06	04	01	01
		20	11	08	03	01
5.	4e	10	10	10	02	03
		20	18	16	05	03
6.	4f	10	09	10	01	01
		20	17	13	02	01
7.	4g	10	09	07	02	-
		20	12	09	02	-
8.	4h	10	08	10	-	-
		20	15	14	-	-
9.	5a	10	03	03	-	-
		20	05	06	-	-
10.	5b	10	05	04	-	-
		20	11	07	-	-
11.	5c	10	05	06	-	-
		20	07	09	-	-
12.	5d	10	05	03	01	-
		20	09	06	01	-
13.	5e	10	10	11	03	01
		20	17	18	05	03
14.	5f	10	08	07	02	01
		20	14	12	02	01
15.	5g	10	06	07	-	-
		20	08	09	-	-
16.	5h	10	07	06	-	-
		20	13	09	-	-
17.	Ciprofloxacin	5	21	19	-	-
18.	Flucanazole	5	-	-	12	14

Conclusion:

We have synthesized a series of substituted 5-methyl-2-(tetrazolo[1,5-*a*]quinoxalin-4-yl)-2,4-dihydro-3*H*-pyrazol-3-ones **4(a-h)** and substituted 1-(tetrazolo[1,5-*a*] quinoxalin-4-yl) pyrazo- lidin-3,5-diones **5(a-h)** derivatives. The bioassay results revealed that most of the synthesized compounds exhibited good anti-microbial activity. The compounds **4e** and **5e** was noticed significant anti-bacterial activity against *E.Coli* and *S. aureus* respectively and the compounds **4f, 4h, 4f** shown notable activity against both bacterial strains tested. Compounds **4e** showed moderate anti-fungal activity against *C. albicans* and *A. niger* remaining compounds does not shows activity against tested fungal strains.

Acknowledgements

The authors are thankful to the chairman Dr. Ch. V. Purushottam Reddy and Dr. K. Veeravenkataiah, Prinicipal of chaitanya postgraduate college (Autonomous), Hanamkonda, Warangal, for providing research facilities.

References:

i. Sultan, baytas.; Nazan, inceler.; Khatereh Fattahpour mavaneh.;Mecit Orhan uluda,G.; Nurettin abacio glu,; Mehtap, gokc E.***Turk J Chem*****,** 2012,36, 734 –748.

ii. Dhareshwar, Sundeep S.; Stella, Valentino J. ***Journal of Pharmaceutical Sciences***, 2010, 99, 2711-2723.

iii. Dabholkar, VN.; Ansari, FY.***J Serb Chem Soc***,2009,74, 1219-1218.

iv. Youssef, M M.; Mohammed, S F.; Kotb, E R.; Salma, M A. ***World J Chem***, 2009,4, 149-156.

v. Ailwadi, S.; Jyoti, Yadav M.; Pathak, D. ***Der Pharma Chemica***. 2011, 3, 215-222.

vi. Sarangan, S.; Somashekhara, ST. ***Journal of Indian Chemical Society***, 1976, 53 (4), 426-7.

vii. Kai arene Jensen.; Henriksen, L. ***Acta Chemica Scandinavica***, 1968, 22, 1107-1128.

viii. Singaravel, Mohana b.; Sarkkarai, A. ***J Chem Pharm Res***, 2011, 3 (1), 402-413.

ix. Pattan, SR Pattan.; Rabara, PA.; Jayashri, S Pattan.; Bukitagar ,AA; Wakale,VS.; Musmade, DS. ***Indian Journal of Chemistry***, 2009,48B, 1453-1456.

x. Gamal, A Idrees.; Omar, M Aly.; Gamal El-Din, AA.; Abuo Rahma, Radwan, MF. ***Eur J Med Chem,*** 2009,44, 3973–3980.

xi. Priyanka, kamaria.; Kawathekar, N.; Prerna chaturvedi. ***E-Journal of Chemistry***, 2011, 8 (1), 305-311.

xii. David, L Smith.; Arlington, A Forist.; William, E Dulin. ***J Med Chem***, 1965,1, 350–353.

xiii. Peng Cheng, LV.; Huan Qiu, LI.; Juan Sun.; Yang Zhou.; Hai Liang Zhu. ***Bioorg Medi Chem*****,** 2010,18 (13), 4606–4614.

xiv. Michael, S Christodoulou.; Sandra Liekens, Konstantinos, M Kasiotis.; Serkos, A Harou- tounian. ***Bioorg Medi Chem*****,** 2010,18(12), 4338–4350.

xv. Kuczyński, L.; Mrozikiewicz, A Banaszkiewicz.; WPoreba, K W. ***Polish journal of pharmacology and pharmacy***, 1979,31(3), 217-25.

xvi. Ikno, AY Terno.; Yoshiyaru, S Katsunori.; Hatsno, K Masaru.; Osamu, U Mitahiko. ***Chem Pharm Bull***, 1987**,**35, 3235-3253.

xvii. Annonymous. ***Phytopathology***.1947, 37, 991.

xviii. Philips, M A. ***J Chem Soc***, 1928, 2393.

Received on August 24,2019.