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SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF SUBSTITUTED TETRAZOLO QUINOXALINES CONTAINING PTHALAZINE ANALOGUES

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ABSTRACT:
Synthesis, characterization, anti-microbial activity of some novel substituted tetrazolo quinoxalines containing pthalazine analogues was reported. Majority of the compounds found to be active and few of them exhibited strong *in vitro* anti-microbial activity against *S. aureus*, *E. coli*, *C. albicans* and *A. niger.*

KEY WORDS:
Substituted-4-hydrazinyl tetrazolo quinoxaline, Pthalic anhydride, Alkyl halides /aryl halides, K_2CO_3, Acetone, Anti-microbial activity.

INTRODUCTION

During the last two decades, there is a growing interest in the synthesis of several phthalazines as promising drug candidates for the treatment of cancer. The latter research efforts have led to the synthesis of several leading phthalazines with different cellular and enzymatic targets. phthalazine nucleus has been emerged as a promising and an attractive scaffold in the development of potent antitumor agents. In addition, 1,4-disubstituted phthalazines have been emerged as promising and attractive antitumor agents. For example, 1-piperazinyl-4-substitutedphthalazines have been reported as active cytotoxic agents against A549, HT-29 and MDAMB-231[1,2], whereas 1-anilino-4-(arylsulfanylmethyl) phthalazines showed an interesting cytotoxic activity against Bel-7402 and HT-1080[3]. Moreover, Novartis identified a new class of Hedgehog (Hh) pathway inhibitors which acts via antagonism of the smoothened receptor with a main structure of 1-piperazinyl-4-benzyl phthalazine[4]. Nitrogen-containing heterocyclic compounds have received much attention as shown by the numerous studies published on their applicability in different areas, especially as drugs. 1,2-Phthalazines are examples of nitrogen heterocycles that possess exciting biological properties. They form the structural profile for several biologically active compounds and hence they are considered important key elements. Phthalazines are an example of nitrogen

containing heterocycles that possess exciting biological properties. Phthalazine-1(2*H*)-one bearing a substitution at C-4 represent key intermediates in the synthesis of various compounds with highly interesting pharmacological properties, such as the blood platelet aggregation inhibitor MV-5445[5] are more selective inhibitors of the cGMP-inhibited phosphordiesterase (PDE)[6]. Some of the phthalazinone derivatives have found application in clinical medicine[7] due to their pronounced anti-convulsant[8], cardiotonic[9], anti-microbial[10] anti-tumor[11], anti-hypertensive[12], anti-thrombotic[13], anti-diabetic[14], anti-trypanosomal[15], anti-inflammatory[16] and vaso-relaxant activities[17].

Experimental:

Melting points were determined by using a syntax apparatus. IR spectra were recorded of solids or neat films on a Perkin-Elmer Spectrometer. The ^{13}CNMR and ^{1}HNMR spectra were recorded at 100MHz and 400MHz respectively. The NMR chemical shifts were recorded in δ ppm, by using dimethylsulphoxide as the internal reference and as a solvent. Mass spectra were recorded by the ESI–MS. Elemental analyses were performed on a Perkin-Elmer 240 CHN analyzer.

General procedures:

Substituted 2-(tetrazolo[*1,5-a*] -quinoxalin-4-yl) -2,3-dihydrophthalazine-1,4-diones: **3 (a-d)**

A mixture of substituted 4-hydrazinyl tetrazolo*[1,5-a]*quinoxaline(0.01mol), phthalic anhydride (0.01mol) in absolute ethanol (10mL) and a catalytic amount of glacial acetic acid was refluxed for 3-4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, poured into crushed ice, the solid separated was filtered, washed with dilute sodium bicarbonate solution (5%) and re-crystallized with methanol.

IR (KBr,cm^{-1}): 1564 (C=N), 1593 (C-N), 1673, 1741(C=O), 3303 (-NH); ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 7.58-7.80 (m, 4H, Ar-H), 7.98-8.18 (m, 4H, Ar-H), 10.80 (br, s, -NH); MS (m/z): 332 (M^{+}+H).

General procedure:

N-substituted-3-(tetrazolo[1,5-*a*] quinoxalin-4-yl)-2,3-dihydrophthalazin-1,4-diones: **4 (a-p)**

To a stirred solution of substituted 2-(tetrazolo[*1,5-a*]-quinoxalin-4-yl)-2,3-dihydro phthalazine-1,4-diones **(a-d)**(0.01mol), anhydrous potassium carbonate (0.03mol) was added slowly at room temperature. To this added slowly a solution of corresponding aliphatic/ aromatic halides (0.01mol) then heated up to reflux for 5-6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, cooled to room temperature, then poured onto ice-water, solid separated filtered, washed with water and re-crystallized from ethanol.

2-(2-bromoethyl)-3-(tetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4a)

Yield: 63%; m.p: 268-270^{0}C; IR (KBr, cm^{-1}): 1565 (C=N), 1593 (C-N), 1673, 1703 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 7.78-8.10 (m, 8H, Ar-H), 4.60 (t, 2H, -CH$_2$), 4.78 (t, 2H, -CH$_2$).^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm) 27.2, 52.5, 121.8, 125.5, 126.4, 127.7, 128.8, 129.2, 130.3, 131.5, 133.4, 134.1, 138.3, 140.9, 144.2, 158.7, 162.0, 165.0.MS (m/z): 438 (M^{+}+H): Anal.Calcd for $C_{18}H_{12}N_7BrO_2$: C: 49.33.; H: 2.76.; N: 22.37.Found: C: 49.30.; H: 2.72.; N: 22.32.

2-(prop-2-yn-1-yl)-3-(tetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4b) :

Yield: 58%; m.p:255-257^{0}C; IR (KBr, cm^{-1}): 1512 (C=N), 1593 (C-N), 1670, 1712 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δppm):δ 7.61-7.88 (m, 4H, Ar-H), 8.02-8.20 (m, 4H, Ar-H), 3.79 (s, 2H, -CH$_2$),2.78 (s, 1H, -CH).

^{13}CNMR (100MHz, DMSO-d_6, δ ppm): 32.4, 70.1, 76.2, 124.2, 125.3, 126.3, 127.2, 127.5, 127.6, 128.2, 129.4, 132.3, 133.2, 134.8, 136.4, 141.4, 161.2, 162.4, 163.4.MS (m/z): 370 (M^++H). Anal.Calcd for $C_{19}H_{11}N_7O_2$: C: 61.79.; H: 3.00.; N: 26.55. Found: C: 61.72.; H: 2.96.; N: 26.48.

2-(prop-2-en-1-yl)-3-(tetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4c) :

Yield: 58 %: m.p:288-290^{0}C; IR (KBr, cm^{-1}): 1518 (C-N). 1597 (C=N), 1674, 1712 (C=O). ^{1}HNMR (400MHz, DMSO-d_6,δppm) δ 7.78-7.88 (m, 4H, Ar-H),8.40-8.60 (m, 4H, Ar-H), 3.80 (d, 2H, -CH_2), 5.22 (d ,2H, -CH_2), 5.80 (m, 1H, -CH). ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): 42.5, 115.8, 124.5, 125.2, 126.2, 127.6, 128.2, 128.6, 129.2, 129.2, 132.3, 133.5, 136.9, 137.4, 140.5, 141.8, 160.4, 161.5, 162.4. MS (m/z): 371 (M^++H).Anal.Calcd for $C_{19}H_{13}N_7O_2$: C: 61.45.; H: 3.53.; N: 26.40. Found: C: 61.40.; H: 3.52.; N: 26.32.

2-benzyl-3-(tetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4d) :

Yield: 61%; m.p: 311-312^{0}C;IR(KBr,cm^{-1}):1524(C=N), 1636 (C-N), 1656, 1755, (C=O).^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 4.20 (s, 2H, -CH_2), 6.85-6.94 (m, 5H, Ar-H),7.50-7.58 (m, 4H, Ar-H),7.72-7.78 (m, 4H, Ar-H).^{13}CNMR (100MHz, DMSO-d_6, δppm): 45.7, 124.4, 125.8, 126.4, 126.7, 127.0, 127.6, 128.2, 128.6, 128.6, 129.3, 129.4, 129.4, 129.5, 132.3, 133.2, 134.8, 142.4, 142.5, 143.7, 160.5, 162.8, 163.8. MS (m/z): 422 (M^++H). Anal.Calcd for $C_{23}H_{15}N_7O_2$: C: 65.55.; H: 3.59.; N: 23.27. Found: C: 65.50.; H: 3.52.; N: 23.23.

2-(2-bromoethyl)-3-(7-methyltetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4e) :

Yield: 68 %; m.p: 285-287^{0}C; IR (KBr,cm^{-1}):1514 (C=N), 1595 (C-N), 1602, 1736 (C=O). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 2.39 (s, 3H, -CH_3), 3.60-3.72 (t, 2H, -CH_2), 3.80-3.92 (t, 2H, -CH_2), 7.60-7.62 (d, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.92 (d, 1H, Ar-H), 8.08-8.20 (m, 4H, Ar-H).^{13}CNMR (100MHz, DMSO-d_6, δppm) 25.2, 27.2, 53.6, 120.0, 124.5, 125.9, 127.0, 128.3, 129.2, 130.2, 132.2, 135.3, 136.3, 137.1, 138.3, 142.2, 158.7, 162.0, 164.1: MS (m/z): 453 (M^++H). Anal.Calcd for $C_{19}H_{14}BrN_7O_2$: C: 50.46.; H: 3.12.; N: 21.68. Found: C: 50.42.; H: 3.09.; N: 21.64.

2-(7-methyltetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-yn-1-yl)-2,3-dihydrophthalazine-1,4-dione (4f) :

Yield: 63 %; m.p: 266-268^{0}C; IR (KBr,cm^{-1}): 1520 (C=N), 1592 (C-N), 1665, 1735 (C=O). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ2.35 (s, 3H,-CH_3), 2.82(s, 1H,-CH), 4.02 (s, 2H, -CH_2), 7.5 (d, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.92 (d, 1H, Ar-H), 7.98-8.15 (m, 4H, Ar-H). ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): 25.4, 33.5, 69.4, 79.3, 125.4, 126.6, 127.7, 127.9, 128.0, 128.8, 129.2, 132.3, 134.4, 133.8, 134.2, 136.9, 143.1, 160.9, 162.4, 163.5.MS (m/z): 384 (M^++H). Anal.Calcd for $C_{20}H_{13}N_7O_2$: C: 62.66.; H: 3.42.; N: 25.58. Found: C: 62.63.; H: 3.38.; N: 25.52.

2-(7-methyltetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-en-1-yl)-2,3-dihydrophth-alazine-1,4-dione (4g) :

Yield: 61%; m.p: 301-303 ^{0}C; IR (KBr, cm^{-1}): 1516 (C=N), 1598 (C-N), 1665, 1720 (C=O). ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 4.18 (d, 2H, -CH_2), 5.18 (d, 2H,-CH_2), 5.78-5.80 (m, 1H, -CH), 7.58-7.60 (d, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 8.10-8.22 (m, 4H, Ar-H).

^{13}CNMR (100MHz,DMSO-d_6,δppm): 25.6, 45.2, 115.6, 125.9, 126.2, 127.8, 128.4, 128.6, 128.8, 129.7, 132.2, 132.3, 133.5, 134.4, 134.6, 137.8, 142.2, 159.2, 162.1, 163.2. MS (m/z): 386 (M^++H). Anal.Calcd for $C_{20}H_{15}N_7O_2$: C: 62.33.; H: 3.92.; N: 25.44. Found: C: 62.30.; H: 3.86.; N: 25.34.

2-benzyl-3-(7-methyltetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4h):

Yield: 57 %; m.p: 321-313 ^{0}C; IR (KBr,cm^{-1}): 1518 (C=N), 1643 (C-N), 1672, 1715, (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 2.76 (s, 3H, -CH$_3$), 4.42 (s, 2H,-CH$_2$), 6.85-6.90 (m, 5H, Ar-H), 7.48-7.52 (m, 4H, Ar-H), 7.82 (d, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.10 (d, 1H, Ar-H).^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm) 23.5, 47.3, 125.3, 126.9, 126.9, 127.1, 127.6, 127.9, 127.4, 127.3, 128.8, 128.1, 129.1, 128.6, 132.2, 132.5, 133.8, 134.5, 137.4, 141.2, 142.5, 159.0, 161.2, 162.9 : MS (m/z): 436 (M$^+$+H). Anal.Calcd for $C_{24}H_{17}N_7O_2$: C: 66.20.; H: 3.94.; N: 22.52. Found: C: 66.18.; H: 3.93.; N: 22.48.

2-(2-bromoethyl)-3-(7,8-dimethyltetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4i) :

Yield: 64%; m.p: 314-316 ^{0}C; IR (KBr,cm^{-1}): 1512 (C=N), 1638 (C-N), 1662, 1732 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 2.62 (s, 3H, -CH$_3$), 2.72 (s, 3H,-CH$_3$), 4.62 (t, 2H, -CH$_2$), 4.72 (t, 2H, -CH$_2$), 7.68-7.72 (m, 4H, Ar-H), 7.88 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H). ^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm): 21.4, 22.1, 26.2, 51.5, 125.7, 126.9, 127.2, 127.6, 128.8, 129.8, 132.4, 132.6, 133.8, 136.4, 140.1, 144.2, 147.9,158.2, 160.2, 162.3.MS (m/z): 466 (M$^+$+H). Anal.Calcd for $C_{20}H_{16}BrN_7O_2$: C: 51.52.; H: 3.46.; N: 21.03. Found: C: 51.48.; H: 3.42.; N: 21.06.

2-(7,8-dimethyltetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-yn-1-yl)-2,3-dihydro phthalazine-1,4-dione (4j):

Yield: 65%; m.p: 255 ^{0}C; IR (KBr, cm^{-1}): 1546 (C=N), 1642 (C-N), 1663, 1736 (C=O); ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 2.62 (s, 3H, -CH$_3$), 2.76 (s, 3H,-CH$_3$), 2.78(s, 1H, -CH), 4.12 (s, 2H, -CH$_2$), 7.82 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.92-7.98 (m, 4H, Ar-H). ^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm):21.4, 22.5, 37.2, 69.7, 70.4, 124.8, 125.7, 127.2, 128.2, 128.6, 128.8, 129.4, 132.3, 133.7, 134.6, 141.2, 142.8, 146.8, 159.0, 161.4, 162.8. MS (m/z): 398 (M$^+$+H). Anal.Calcd for $C_{21}H_{15}N_7O_2$: C: 63.47.; H: 3.80.; N: 24.67. Found: C: 63.45.; H: 3.78.; N: 24.62.

2-(7,8-dimethyltetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-en-1-yl)-2,3-dihydrophthalazine-1,4-dione (4k):

%Yield: 58 %; m.p 325-327 ^{0}C; IR (KBr, cm^{-1}): 1520 (C=N), 1644 (C-N), 1672, 1724 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 2.60 (s, 3H, -CH$_3$), 2.76 (s, 3H,-CH$_3$), 4.16 (d, 2H, -CH$_2$), 5.20 (d, 2H, -CH$_2$), 7.79-7.88 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.94-7.96 (m, 4H, Ar-H).^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm): 21.5, 22.2, 45.2, 115.7, 124.9, 125.6, 127.6, 128.2, 128.8, 129.4, 132.3, 133.5, 133.5, 134.1, 134.7, 140.2, 142.4, 146.7, 160.2, 161.4, 163.5. MS (m/z): 400 (M$^+$+H). Anal.Calcd for $C_{21}H_{17}N_7O_2$: C: 63.15.; H: 4.29.; N: 24.55. Found: C: 63.10.; H: 4.27.; N: 24.52.

2-benzyl-3-(7,8-dimethyltetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione(4l)

%Yield: 58 %; m.p 352-354 ^{0}C; IR (KBr, cm^{-1}): 1524 (C=N), 1673 (C-N), 1678, 1728 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 2.66 (s, 3H, -CH$_3$), 2.74 (s, 3H,-CH$_3$), 4.42 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.72-7.76 (m, 4H, Ar-H), 8.10-8.15 (m, 4H, Ar-H).

^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm):22.1, 22.2, 46.2, 125.3, 125.8, 126.1, 127.3, 127.6, 128.6, 128.9, 129.4, 131.2, 132.7, 132.9, 133.7, 134.2, 135.6, 140.2, 141.3, 142.2, 143.5, 146.5, 159.3, 160.2, 163.6. MS (m/z): 450 (M$^+$+H). Anal.Calcd for $C_{25}H_{19}N_7O_2$: C: 66.81.; H: 4.26.; N: 21.81. Found: C: 66.79.; H: 4.22.; N: 21.77.

2-(2-bromoethyl)-3-(7-nitrotetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4m) :

Yield: 61%; m.p 251-253 ^{0}C; IR (KBr, cm^{-1}): 1518 (C=N), 1592 (C-N), 1674, 1742 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 4.60 (t, 2H, -CH$_2$), 4.78 (t, 2H,-CH$_2$), 7.62-7.68 (m, 4H, Ar-H), 7.88-7.92 (dd, 2H, -CH$_2$), 7.98 (s, 1H, Ar-H). ^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm): 27.1, 51.4, 117.9, 123.4, 127.6, 128.8, 128.9, 129.5, 132.3, 133.4, 139.2, 139.2, 140.9, 141.4, 148.5, 158.7, 162.1, 163.8. MS (m/z): 484 (M$^+$+H). Anal.Calcd for $C_{18}H_{11}BrN_8O_4$: C: 44.74.; H: 2.29.; N: 23.19. Found: C: 44.72.; H: 2.22.; N: 23.12.

2-(7-nitrotetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-yn-1-yl)-2,3-dihydrophthal-azine-1,4-dione (4n) :

Yield: 57 %; m.p: 317-319 ^{0}C; IR (KBr, cm^{-1}): 1514 (C=N), 1590 (C-N), 1678, 1738 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm) δ 2.84 (s, 1H, -CH), 3.82 (s, 2H, -CH$_2$), 7.64-7.70 (m, 4H, Ar-H), 7.88-794 (dd, 2H, Ar-H), 7.98 (s, 1H, Ar-H).^{13}CNMR (100 MHz, DMSO-d$_6$, δppm): 35.8, 71.4, 78.1, 118.7, 121.4, 127.6, 128.5, 128.8, 129.4, 130.5, 132.3, 133.8, 139.5, 140.3, 142.4, 149.6, 160.2, 163.3, 163.8. MS (m/z): 415 (M$^+$+H). Anal.Calcd for $C_{19}H_{10}N_8O_4$: C: 55.08.; H: 2.43.; N: 27.04. Found: C: 55.02.; H: 2.38.; N: 26.94.

2-(7-nitrotetrazolo[1,5-a]quinoxalin-4-yl)-3-(prop-2-en-1-yl)-2,3-dihydrophthalazine-1,4-dione (4o) :

Yield: 65%; m.p: 247-249^{0}C; IR (KBr, cm^{-1}): 1524 (C=N), 1592 (C-N), 1672, 1732 (C=O), ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 4.12 (d, 2H, -CH$_2$), 5.22 (d, 2H,-CH$_2$), 5.82-5.84 (m, 1H, -CH), 7.60-7.75(m, 4H, Ar-H), 7.84-7.92 (dd, 2H, Ar-H), 8.02 (s, 1H, Ar-H). ^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm): 43.6, 115.7, 118.3, 121.5, 127.6, 128.2, 128.8, 129.6, 130.5, 132.3, 133.8, 138.2, 139.4, 140.2, 141.1, 148.5, 159.0, 163.1, 163.5.MS (m/z): 417 (M$^+$+H). Anal.Calcd for $C_{19}H_{12}N_8O_4$: C: 54.81.; H: 2.91.; N: 26.91. Found: C: 54.78.; H: 2.87.; N: 27.98.

2-benzyl-3-(7-nitrotetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (4p) :

Yield: 51%; m.p: 255 ^{0}C; IR (KBr, cm^{-1}): 1520 (C=N), 1594 (C-N), 1672, 1732 (C=O). ^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm): δ 4.62(s, 2H, -CH$_2$), 7.48-7.58 (m, 5H, Ar-H), 7.62-7.74 (m, 4H, Ar-H), 7.80 (s, 1H, Ar-H), 7.88-7.92 (dd, 2H, Ar-H), ^{13}CNMR (100MHz, DMSO-d$_6$, δ ppm): 45.4, 117.9, 121.4, 125.4, 126.8, 127.0, 127.6, 128.2, 128.6, 128.7, 129.5, 129.9, 130.5, 132.5, 133.2, 138.2, 138.4, 141.1, 141.7, 149.5, 160.8, 163.3, 163.8. MS (m/z): 467 (M$^+$+H). Anal.Calcd for $C_{23}H_{14}N_8O_4$: C: 59.23.; H: 3.03.; N: 24.02. Found: C: 59.20.; H: 2.96.; N: 23.92.

EVALUATION OF ANTI-MICROBIAL ACTIVITY

The agar Disc-diffusion method[18] was used to evaluate anti-microbial activity of the synthesized compounds was dissolved in DMSO to 10 µg/mL and 20 µg/mL concentration. 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 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 *B.Subtilis)* and two Gram negative bacteria (*E.coli* and *B.proteus)* 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 and **Flucanazole** was used as reference standard during the screening of anti-fungal activity.

RESULTS AND DISCUSSIONS

CHEMISTRY

The reaction sequences employed for the synthesis of intermediates and target compounds are shown in the S**cheme 1**. The starting material 4-hydrazinyl tetrazolo *[1,5-a]* quinoxalines **2 (a-d)** were used in the present study, which were prepared following a previously reported literature procedure. To a stirring solution of 4-hydrazinyltetrazolo[1,5-*a*]quinoxaline and pthalic anhydride were heated to 60-80^{0}C in ethanol for 3 h**,** to give substituted 2-(tetrazolo[1,5-*a*]-quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-diones **3(a-d).** These compounds **3(a-d)** treated with a different types of aliphatic halides (or) aromatic halides to furnish the corresponding *N*-substituted-3-(tetrazolo*[1,5-a]* quinoxalin-4-yl) -2,3-dihydro phthalazine-1,4-diones **4(a-p)**. The Structures of the all newly synthesized compounds were characterized on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass data. IR spectra of 2-(2-bromoethyl) -3-(tetrazolo*[1,5-a]*quinoxalin-4-yl) -2,3-dihydrophthalazine-1,4-dione **4a** showed absorption at ῡ 1565 C=N, 1593 C-N and 1673, 1703 due to C=O. The spectral values for all the compounds and C H N analyses are given in the experimental part.

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 well diffusion assay method.

The zone of inhibition values was 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 *in vitro*. **4a, 4e, 4i** and **4m** exhibited excellent activity against *S. aureus, E. coli* and *C. albicans, A. niger.* The zone of inhibition values was given in **Table-2.** The biological activity is varied due to the presence of halogen substituents in aliphatic Bromo compound is responsible for higher activity.

Scheme:

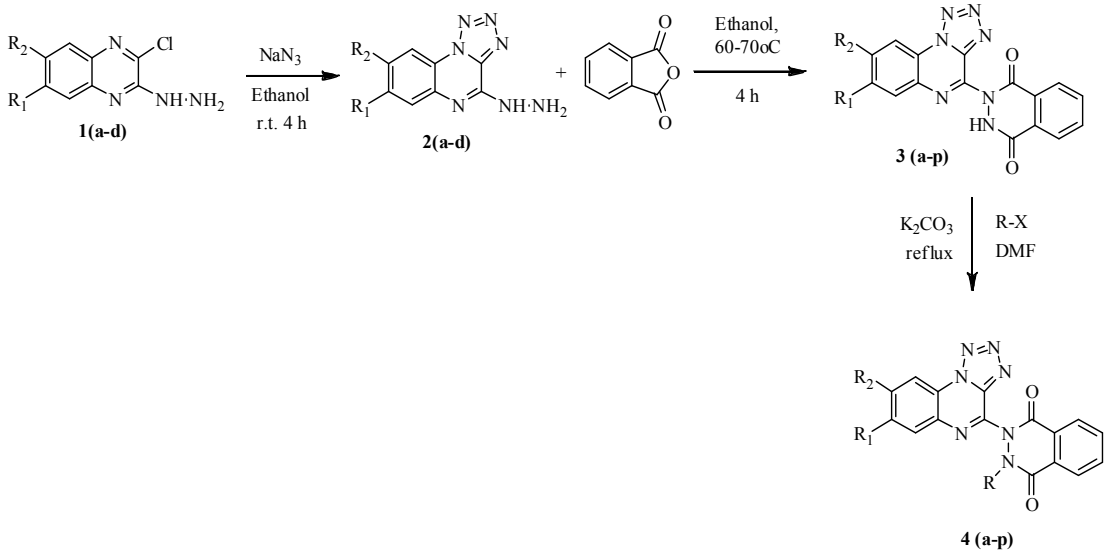


Figure 1: Synthetic Route for *N*-substituted-3-(tetrazolo[1,5-*a*] quinoxalin-4-yl)-2,3-dihydrophthalazin-1,4-diones.

Table-1

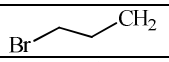
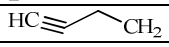
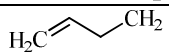
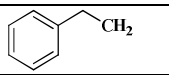
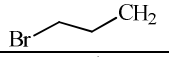
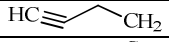
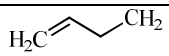
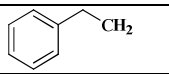
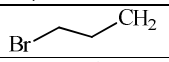
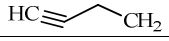
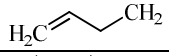
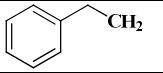
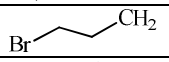
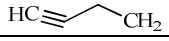
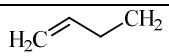
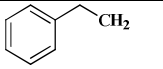
S. No	**Compd.**	**R_1**	**R_2**	**R**
1.	a	-H	-H	
2.	b	-H	-H	
3.	c	-H	-H	
4.	d	-H	-H	
5.	e	$-CH_3$	-H	
6.	f	$-CH_3$	-H	
7.	g	$-CH_3$	-H	
8.	h	$-CH_3$	-H	
9.	i	$-CH_3$	$-CH_3$	
10.	j	$-CH_3$	$-CH_3$	
11.	k	$-CH_3$	$-CH_3$	
12.	l	$-CH_3$	$-CH_3$	
13.	m	$-NO_2$	-H	
14.	n	$-NO_2$	-H	
15.	o	$-NO_2$	-H	
16.	p	$-NO_2$	-H	

Table-2. Anti-microbial activity of substituted substituted tetrazolo quinoxalines containing pthalazine analogues **4 (a-p)**

S.No	Compd. No	Concen µg/mL	Anti-bacterial		Anti-fungal	
			Zone of inhibition in mm			
			S.*aures*	E.*Coli*	C.*albicans*	A.*niger*
1.	4a	10	16	14	12	12
		20	22	18	16	14
2.	4b	10	04	07	08	05
		20	08	11	11	08
3.	4c	10	10	09	06	04
		20	12	14	08	07
4.	4d	10	05	12	07	-
		20	11	13	10	-
5.	4e	10	12	16	14	13
		20	22	20	18	16
6.	4f	10	13	08	10	04
		20	14	11	12	08
7.	4g	10	10	-	-	12
		20	18	-	-	14
8.	4h	10	-	04	10	05
		20	-	12	14	08

9.	4i	10	15	08	14	16
		20	20	20	16	18
10.	4j	10	04	-	-	06
		20	08	-	-	10
11.	4k	10	05	-	05	-
		20	10	05	08	-
12.	4l	10	-	-	10	06
		20	10	05	08	-
13.	4m	10	09	10	14	12
		20	18	15	17	14
14.	4n	10	08	05	10	08
		20	13	08	12	10
15.	4o	10	07	06	09	09
		20	12	11	10	10
16.	4p	10	06	09	05	-
		20	13	10	08	-
	Ciprofloxan	05	27	22		
	Flucanazole	05			24	21

Conclusion:

In conclusion, the present work demonstrated a convenient method for the synthesis of biologically active novel substituted tetrazolo quinoxalines containing pthalazine analogues. All the compounds were screened for anti-microbial activity. Among all the synthesized compounds, **4a, 4e, 4i** and **4m** showed significant anti-bacterial activity against both gram positive and negative bacterial strains and also anti-fungal activity than all other synthesized compounds. These results suggest that the synthesized compounds can be good candidates for future investigations.

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