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Synthesis and anti-microbial activity of novel series of Substituted 2-(Tetrazolo[1,5- *a*]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione derivatives

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ABSTRACT

*A novel and efficient synthesis of substituted 2-(Tetrazolo[1,5-a]quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione has been carried out. The substituted 4-hydrazinyl tetrazolo quinoxalines has been treated with pthalic anhydride. The structures of all the compounds were confirmed by IR, ^{1}H-NMR, elemental analysis and Mass spectroscopy. All the synthesized compounds **5(a-g)** were screened for anti-microbial activity. Among all the synthesized compounds **5a, 5c**, and **5d** and **5e, 5f** and **5g** showed moderate activity against gram-positive bacterial strains S. aureus and gram-negative bacterial strains E.coli. **5e** showed appreciable activity against C. albicans and A. niger organism.*

Key words: 4-hydrazinyl tetrazolo quinoxalines, pthalic anhydride, ethanol, anti-microbial activity.

INTRODUCTION

The ideal anti-microbial agents are selective in only targeting the microorganism but not host cells. Resistance to anti-microbial agents is now recognized as a major global public health problem. In addition, because of the increased number of immune compromised patients, Considerable attention because of their pharmacological properties and clinical applications [1,2]. Phthalazine derivatives were reported to possess anti-convulsant[3,4], cardiotonic[5], anti-tumor[6], anti-hypertensive[7], anti-thrombotic[8], anti-diabetic[9], anti-microbial[10], anti-trypanosomal[11], anti-infammatory[12], cytotoxic[13], vasorelexant[14] and vascular endothelial growth factor receptor 11(VEGFR-2) inhibitory [15]. Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives[16]. 1,4-disubstituted phthalazines have received a considerable attention as antitumor agents in the past few years[17]. A successful example is *N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin-1-amine also known as Vatalanib (PTK-787) which is VEGFR (vascular endothelial growth factor receptor) inhibitor.

In view of the above mentioned facts and in continuation of our research interest for the synthesis of biologically active heterocycles, we report here, the synthesis of novel series of phthalazinones bearing 4-hydrazinyl tetrazolo quinoxalines derivatives.

MATERIALS AND METHODS

Melting points were determined by the capillary tube method, and the thermometer was uncorrected. Mass spectra were obtained on an Agilent 1100 HPLC-MS instrument. ^{1}H NMR spectra were run in DMSO-d_6, with TMS at the internal standard, on a Bruker ARX-300 instrument operating at 400 MHz. IR spectra (KBr disks) were recorded on a Bruker IFS 55 instrument. Elemental analysis was performed with a Carlo-Erba 1106 Elemental analysis instrument.

2-(Tetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (5a):
Yield: 85%; m.p 118-120^{0}C; 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), 11.03 (br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 125.2, 125.9, 127.6, 128.1, 128.3, 128.6, 129.9, 131.2, 132.5, 134.5, 135.2, 136.6, 143.5, 162.3 162.5, 167.8; MS (m/z): 332 (M+H); Anal. Calculated for $C_{16}H_9N_7O_2$: C: 58.01.; H: 2.74.; N: 29.60. Found: C: 57.94.; H: 2.68.; N: 29.65.

2-(7-Methyl tetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (5b):
Yield: 82%; m.p: 128-130 ^{0}C; IR (KBr,cm^{-1}): 1561(C=N), 1590 (C-N), 1673, 1738 (C=O), 3298 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 2.78 (s, 3H, -CH_3), 7.50-7.65 (m, 4H, Ar-H), 7.80 (d, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 8.20 (d, 1H, Ar-H),11.08(br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 25.3, 125.3, 126.5, 127.0, 127.6, 128.1, 128.8, 129.2, 132.5, 133.0, 133.2, 134.8, 137.5, 142.8, 162.3, 163.2, 167.8; MS (m/z): 346 (M+H); Anal.Calcd for $C_{17}H_{11}N_7O_2$: C: 59.13.; H: 3.21.; N: 28.39. Found: C: 59.11.; H: 3.18.; N: 28.32.

2-(7-Chlorotetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione(5c):
Yield: 51 %; m.p: 201-203 ^{0}C; IR (KBr, cm^{-1}): 1576 (C=N), 1582 (C-N), 1679, 1732, (C=O), 3352 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 7.96-8.08 (m, 4H, Ar-H), 7.45 (d, 1H, Ar-H), 7.65 (d, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 12.40 (br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 121.8, 123.8, 127.4, 127.9, 128.5, 128.8, 129.8, 131.1, 132.5, 136.5, 137.7, 139.8, 145.2, 162.8, 163.4, 164.5; MS (m/z): 365 ($M^{+\cdot}$), 367 (M+2); Anal. Calcd for $C_{16}H_8ClN_7O_2$: C, 52.54.; H, 2.20.; N, 26.81. Found: C, 52.49.; H, 2.16.; N, 26.79.

2-(7-Bromotetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione(5d):
Yield: 49 %; m.p: 238-240 ^{0}C; IR (KBr, cm^{-1}): 1568 (C=N), 1588 (C-N), 1688, 1742, (C=O), 3362 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 7.05-8.12 (m, 4H, Ar-H), 7.90 (d, 1H, Ar-H), 7.77 (d, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 11.90 (br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 122.2, 123.5, 128.2, 128.6, 129.5, 129.8, 130.2, 131.7, 133.5, 137.9, 138.7, 140.2, 146.1, 163.8, 164.1, 166.5; MS (m/z): 410 ($M^{+\cdot}$), 412 (M+2); Anal. Calcd for $C_{16}H_8BrN_7O_2$: C, 46.85.; H, 1.97.; N, 23.90. Found: C, 46.80.; H, 1.90.; N, 23.88.

2-(7-nitrotetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione(5e):
Yield: 65%; m.p:174-176^{0}C; IR (KBr, cm^{-1}): 1562 (C=N), 1589 (C-N), 1680, 1745, (C=O), 3312 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 7.52-7.66 (m, 4H, Ar-H),7.82-7.88 (dd, 2H, Ar-H), 8.05 (s, 1H, Ar-H), 11.52 (br, s, 1H, -NH); ^{13}CNMR (100MHz,DMSO-d_6, δ ppm): δ 118.2, 122.7, 127.5, 127.8, 128.2, 128.9, 129.5, 132.5, 133.2, 138.2, 138.6, 141.3, 145.2, 162.5, 163.2, 167.9; MS (m/z): 377 (M+H); Anal.Calcd for $C_{16}H_8N_8O_4$: C, 51.07.; H, 2.14.; N, 29.78. Found: C, 50.92.; H, 2.10.; N, 29.70.

2-(7-methoxytetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (5f):
Yield: 59%; m.p:210-212^{0}C; IR (KBr, cm^{-1}): 1556 (C=N), 1578 (C-N), 1678, 1729, (C=O), 3325 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 4.05 (s, 3H, -OCH_3), 7.81-7.88 (m, 4H, Ar-H), 7.23 (d, 1H, Ar-H), 7.60(d, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 11.82 (br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 45.8, 120.2, 123.4, 128.5, 128.9, 129.1, 129.3, 129.5, 132.5, 133.2, 137.5, 138.7, 140.5, 144.2, 161.7, 162.2, 165.7; MS (m/z): 362 (M+H); Anal.Calcd for $C_{17}H_{11}N_7O_3$: C, 56.51.; H, 3.07.; N, 27.14. Found: C, 56.49.; H, 3.00.; N, 27.14.

2-(7,8-dimethyltetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione (5g):
Yield: 73%; m.p: 114-116 ^{0}C; IR (KBr, cm^{-1}): 1562 (C=N), 1590 (C-N), 1662, 1740, (C=O), 3315 (-NH); ^{1}HNMR (400MHz, DMSO-d_6, δ ppm): δ 2.60 (s, 3H, -CH_3), 2.65 (s, 3H, -CH_3) 7.43-7.62 (m, 4H, Ar-H), 7.92 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 11.23(br, s, 1H, -NH); ^{13}CNMR (100MHz, DMSO-d_6, δ ppm): δ 23.2, 24.5, 124.8, 126.5, 127.5,

127.9, 128.5, 129.1, 132.1, 132.9, 133.6, 134.2, 134.5, 140.8, 143.2, 147.1, 159.2, 161.3; MS (m/z): 360 (M+H); Anal.Calcd for $C_{18}H_{13}N_7O_2$: C, 60.16.; H, 3.65.; N, 27.29. Found: C, 60.12.; H, 3.60.; N, 27.30.

Scheme:

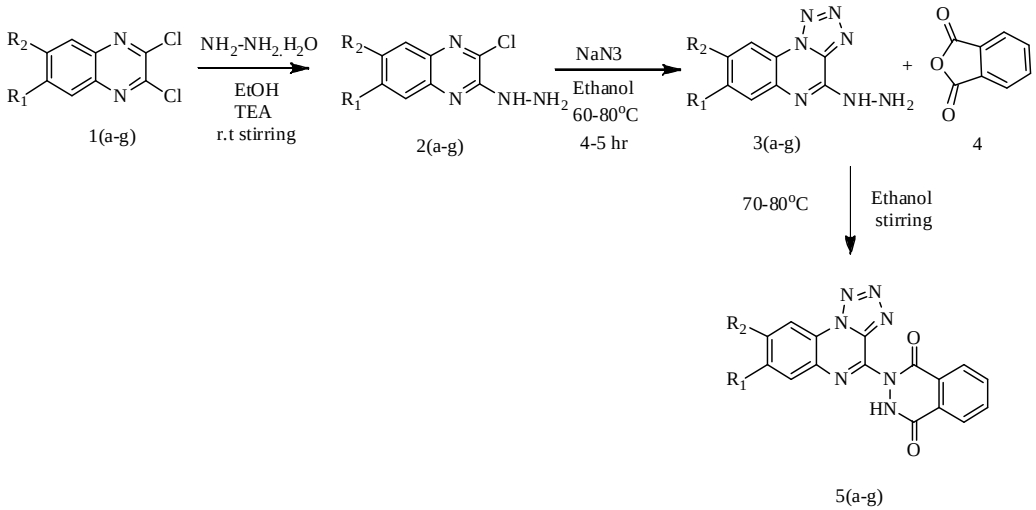

Table 1:

Sl .No.	Compd	R_1	R_2
1	A	-H	-H
2	B	$-CH_3$	-H
3	C	Cl	-H
4	D	-Br	-H
5	E	$-NO_2$	-H
6	F	$-OCH_3$	-H
7	G	$-CH_3$	$-CH_3$

Anti-microbial activity:

The Agar Disc-diffusion method[18] was used to evaluate anti-microbial activity of all the synthesized compounds. The compounds were dissolved in DMSO to 10 µg/mL and 20 µg/mL concentration solutions. The compounds were placed aseptically on Muller-Hinton Agar for the both Gram positive and Gram negative bacteria and Saboround's dextrose agar for fungi and incubated for 24 h at 37°C. At the end of the incubation period, the diameter of the growth of inhibition zones was measured. The 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 and Flucanazole was used as reference standard during the screening of anti-fungal activity[19].

RESULTS AND DISCUSSION

Chemistry:

The synthesis of substituted 2-(Tetrazolo*[1,5-a]*quinoxalin-4-yl)-2,3-dihydrophthalazine-1,4-dione illustrated in the scheme-1. The starting compound 4-hydrazinyl tetrazolo quinoxalines **3(a-g)** prepared according to previous literature methods. The hydrazine compound was treated with pthalic anhydride in ethanol at refluxing temperature. The synthesized compounds were characterized by IR, ^{1}HNMR, 13CNMR and Mass spectroscopy elemental analysis data. The IR spectrum of compounds 5(a-g) showed a characteristic absorption bands within the 1670-1742 cm^{-1} due to the presence of carbonyl groups and 3305-3362 cm^{-1} shows a characteristic absorption band due to –NH group. The ^{1}HNMR spectrum of compound **5a** shows a multiplates between 7.58-7.80 and 7.98-8.18 δ ppm due to aromatic protons and a broad singlet absorbed at 11.20 δ ppm due to presence of –NH protons.[13]C NMR spectrum of compound **5b** display a characteristic signal at δ 2.78 ppm for $-CH_3$ protons.

Biological Activity

Anti-microbial Activity:

Anti-bacterial activity results **(Table 2)** revealed that, among the synthesized compounds **5a, 5c**, and **5d** showed potent activity against gram-positive bacterial strains S. aureus, and gram-negative bacterial strains *E.coli*, on comparing with the standard drugs Ciprofloxacin . Compound **5e, 5f** and **5g** showed moderate activity against gram-positive bacterial strains *S. aureus* and gram-negative bacterial strains *E.coli*. The anti-fungal screening data revealed that all the tested compounds showed poor to moderate activity against the tested fungal strains. The anti-fungal screening results revealed that **5e** showed appreciable activity against *C. albicans* and *A. niger* organism.

Table 2

S.No	Compds	Concen µg/ml	Anti-bacterial		Anti-fungal	
			Zone of inhibition in mm			
			S. aureus	E.Coli	C. albicans	A. niger
1.	5a	10	08	09	05	04
		20	15	12	02	05
2.	5b	10	09	10	12	13
		20	10	05	01	03
3.	5c	10	12	15	01	06
		20	18	19	05	03
4.	5d	10	15	13	01	01
		20	18	16	03	01
5.	5e	10	09	10	15	17
		20	05	08	12	16
6.	5f	10	09	10	01	01
		20	18	14	02	01
7.	5g	10	10	09	02	01
		20	13	09	02	01
8.	Ciprofloxacin	5	22	25	-	-
9.	Flucanazole	5	-	-	18	21

CONCLUSION

We have synthesized new phthalazinone derivatives 5(a-g) 4-hydrazinyl tetrazolo quinoxalines reacting with pthalic anhydride in ethanol. All the synthesized compounds **5(a-g)** were screened for anti-microbial activity. Among all the synthesized compounds **5a, 5c**, and **5d** and **5e, 5f** and **5g** showed moderate activity against gram-positive bacterial strains *S. aureus* and gram-negative bacterial strains *E.coli*. **5e** showed appreciable activity against *C. albicans* and *A. niger* organism.

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