World Journal of Pharmaceutical research

Volume 3, Issue 1, 1265-1272. Research Article ISSN 2277 – 7105

SYNTHESIS OF NOVEL TETRAZOLO AND TRIAZOLO [1, 2-*e*] IMIDAZOLO [4, 5-*b*] QUINOXALINE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Article Received on
20 October2013
Revised on 24 November 2013,
Accepted on 30 December 2013

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ABSTRACT

An efficient general method has been described for the synthesis of novel tetrazolo and triazolo[1,2-*e*]imidazolo[4,5-*b*] quinoxaline derivatives **4(a-f)** and **5(a-f)** by the reaction of (1H)-tetrazol-5-amine and (3H)-1,2,3-triazol-4-amine with substituted 2,3-dichloro quinoxaline in DMF solvent and added a catalytic amount of K_2CO_3. These analogs were evaluated for their antimicrobial activity against *Bacillus Subtillis*, *Staphylococcus aureus (*Gram positive bacteria) *Escherichia Coli* (Gram Negative bacteria) and *Aspergillus niger*, *Candida albicans* (fungi).The analogs **4d**, **4f**, **5d** and **5f** were identified as potent antimicrobial agents. Structural elucidation of all the newly synthesized title compounds has been established by the spectroscopic data IR, ^{1} HNMR, ^{13} C NMR, mass and elemental analysis.

Key words: Quinoxaline, tetrazole, triazole, antibacterial strains, antifungal strains.

INTRODUCTION

Heterocyclic compounds hold a special place among pharmaceutically significant natural products and synthetic compounds. The remarkable ability of heterocyclic nuclei to serve both as biomimetics and relative pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs. Among the family of heterocyclic compounds, nitrogen containing heterocycles especially quinoxalines, tetrazoles and triazoles are gaining considerable importance owing to their varied biological properties as well as their easy economic and less time consuming preparation methods. Quinoxaline derivatives

are of special importance because of their versatile biological and pharmacological activities.[1-8] like anti-inflammatory, anticonvulsant, hypnotic, antihelmintic, hypertensive, anti bacterial agents. Tetrazoles possess diverse biological activities like antinociceptive[9] antibacterial[10], antifungal[11] anti HIV, anticancer, immunosuppressive [12] anti inflammatory, [13] antiulcer [14] and analgesic [15] activities. Some of 4-styryltetrazolo[1,5-a] quinoxaline and 1-substituted 4-styryl[1,2,4]triazolo [4,3-a]quinoxaline derivatives screened for in vivo anticonvulsant activity,[16] 7-chloro-4,5-dihydro-4-oxo-8-[1,2,4-triazol-4-yl]-1,2,4-triazolo[1,5-a]quinoxaline-2-carboxylic acid as novel selective AMPA receptor antagonists [17] these biological importance prompted us to synthesize some heterocyclic derivatives having tetrazolo and triazolo quinoxaline moieties starting from 2,3-dichloro quinoxalines.

MATERIALS AND METHODS

Melting points were recorded in open capillary and were 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 (K Br) were obtained using a Bruker WM-4(X) spectrometer (577model). ^{1}H NMR (400MHz) and ^{13}C NMR (100MHz) spectra were recorded on a Bruker WM-400 spectrometer in DMSO-d_6 with TMS as an internal standard. Mass spectra (ESI) were carried out on a JEOL SX-102 spectrometer. CHN analysis was done by the Carlo Erba EA 1108 automatic elemental analyzer. The chemicals and solvents used were of commercial grade and were used without further purification unless otherwise stated.

General procedure for the Synthesis of tetrazolo and triazolo-[1,2-e]imidazolo[4,5-b] quinoxaline derivatives 4(a-f) and 5(a-f)

A suspension of compound **1** (10 mmol) and amino tetrazole **2** (10mmol) and amino triazole **3** (10 mmol) in DMF (10mL) containing a catalytic amount of K_2CO_3 was stirred and heated to reflux for 12-15 h, approximately at 120 ^{0}C (monitored by TLC), the reaction mixture was cooled, the formed precipitate was filtered off, dried and purified by column chromatography (1:9 MeOH: Pet Ether).

Tetrazo[1,2-e]-4H-imidazo[4,5-b]quinoxaline 4a

Yield:78%, m. p. 223–224 ^{0}C; IR (KBr,cm^{-1}): 3406, 1613; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.54(m,2H,Ar-H),7.96(dd,2H,Ar-H), 11.8 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ

130.6, 131.4, 139.2, 143.8, 144.3, 146.5, MS (*m/z)* 212(M+1)+. Anal.Calcd for $C_9H_5N_7$; C, 51.19; H, 2.39; N, 46.43; Found: C, 51.24; H, 2.41; N, 46.38 %.

7-Methyl tetrazo[1,2-e]-4H-imidazo[4,5-b]quinoxaline 4b

Yield: 65%, m. p. 215–216 ^{0}C; IR (KBr,cm^{-1}): 3392, 1609; ^{1}H NMR (400MHz, DMSO-d_6): δ 2.43(s,3H,-CH_3),7.40(dd,1H,Ar-H),7.92(dd,1H,Ar-H),7.96 (s,1H,Ar-H), 12.2 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 24.2, 129.8, 130.2, 130.8, 137.2, 139.4, 144.3, 144.8, 145.8: MS (*m/z)* 226 (M+1)+. Anal.Calcd for $C_{10}H_7N_7$; C, 53.33; H, 3.13; N, 43.54; Found: C, 53.28; H, 3.19; N, 43.32 %.

7, 8-Dimethyl tetrazo[1,2-e]-4H-imidazo[4,5-b]quinoxaline 4c

Yield: 69%, m. p. 202–203 ^{0}C; IR (KBr,cm^{-1}): 3375, 1623; ^{1}H NMR (400MHz, DMSO-d_6): δ 2.20(s,6H,2x-CH_3),7.92(dd,2H,Ar-H),11.92 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 21.2, 129.2, 130.2,137.8,141.3, 144.5, 149.3: MS (*m/z)* 240 (M+1)+. Anal.Calcd for $C_{11}H_9N_7$; C, 55.22; H, 3.79; N, 40.98; Found: C, 55.32; H, 3.85; N, 41.22 %.

7-Nitro tetrazo[1,2-e]-4H-imidazo[4,5-b]quinoxaline 4d

Yield: 71%, m. p. 245–246 ^{0}C; IR (KBr,cm^{-1}): 3402, 1619; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.28(dd,1H,Ar-H),8.72(dd,1H,Ar-H),8.54(m,1H,Ar-H),13.2 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 120.2, 125.4, 137.4, 140.7, 145.3, 148.8, 149.6, 151.2: MS (*m/z)* 257 (M+1)+. Anal.Calcd for $C_9H_4N_80_2$; C, 42.20; H, 1.57; N, 43.74; Found: C, 42.28; H, 1.45; N, 43.69 %.

7-Bromo tetrazo[1,2-e]-4H-imidazo[4,5-b]quinoxaline 4e

Yield: 74%, m. p. 267–269 ^{0}C; IR (KBr,cm^{-1}): 3388, 1629; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.90(dd.1H,Ar-H), 7.92(dd,1H,Ar-H),8.18(s,1H,Ar-H),13.2 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 118.2, 131.2, 131.8, 132.5, 137.3, 141.2, 143.8, 145.8, 146.4: MS (*m/z)* 291 (M+1)+. Anal.Calcd for $C_9H_4BrN_7$; C, 37.26; H, 1.39; N, 33.80; Found: C, 37.16; H, 1.32; N, 33.72 %.

7-Chloro tetrazo[1,2-*e*]-*4H*-imidazo[4,5-*b*]quinoxaline 4f

Yield:70%,m.p.223–224^{0}C;IR(KBr,cm^{-1}):3397,1613;^{1}HNMR(400MHz,DMSO-d_6): δ 7.78 (dd. 1H,Ar-H), 7.98(dd,1H,Ar-H), 8.12 (s,1H,Ar-H),12.9 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ

129.2,131.6,133.4,137.2,141.4,143.2,144.2,146.2,147.5:MS(*m/z)*246(M+1)+.Anal. Calcd for $C_9H_4ClN_7$; C, 44.01; H, 1.64; N, 39.92; Found: C, 44.12; H, 1.55; N, 39.96 %.

Quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5a

Yield:69%, m. p. 231–232 ^{0}C; IR (KBr,cm^{-1}): 3396, 1619; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.68(s,1H,-CH), 7.72(dd,2H,Ar-H),8.10(dd,2H,Ar-H), 12.4 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 129.8, 130.5, 131.6, 132.4, 142.8, 143.1, 145.4; MS (*m/z)* 211(M+1)+. Anal.Calcd for $C_{10}H_6N_6$; C, 57.14; H, 2.88; N, 39.98; Found: C, 57.21; H, 2.82; N, 39.56 %.

7-Methyl quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5b

Yield:62%, m. p. 209–211 ^{0}C; IR (KBr,cm^{-1}): 3385, 1609; ^{1}H NMR (400MHz, DMSO-d_6): δ 2.42(s,3H,-CH_3), 7.52(dd,1H,Ar-H), 7.62(s,1H,-CH), 7.92(s,1H,Ar-H), 8.02(dd,1H,Ar-H), 12.8 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 24.8, 129.2, 130.2, 131.6, 132.4, 132.9,139.2 140.9, 142.8, 144.2, 145.3; MS (*m/z)* 225(M+1)+. Anal.Calcd for $C_{11}H_8N_6$; C, 58.92; H, 3.60; N, 37.48; Found: C, 58.89; H, 3.53; N, 37.42 %.

7,8-Dimethyl quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5c

Yield:74%, m. p. 219–221 ^{0}C; IR (KBr,cm^{-1}): 3405, 1624; ^{1}H NMR (400MHz, DMSO-d_6): δ 2.24(s,6H,2xCH_3), 7.82(s,1H,Ar-H), 7.80(s,1H,-CH), 7.86(s,1H,Ar-H), 12.8 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 22.2, 129.2, 130.8, 131.9, 141.4, 144.2, 148.8; MS (*m/z)* 239(M+1)+. Anal.Calcd for $C_{12}H_{10}N_6$; C, 60.50; H, 4.23; N, 35.27; Found: C, 60.39; H, 4.21; N, 35.19 %.

7-Nitro quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5d

Yield:78%, m. p. 254–256 ^{0}C; IR (KBr,cm^{-1}): 3402, 1623; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.72(s,1H,-CH), 8.24(dd,1H,Ar-H), 8.56(dd,1H,Ar-H),8.62(s,1H,Ar-H), 13.2 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 123.6, 128.2, 131.4, 132.5, 132.8,142.4, 146.7, 147.8, 149.4, 151.2; MS (*m/z)* 225(M+1)+. Anal.Calcd for $C_{10}H_5N_7O_2$; C, 47.07; H, 1.97; N, 38.42; Found: C, 46.98; H, 1.93; N, 38.35 %.

7-Bromo quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5e

Yield:67%, m. p. 239–241 ^{0}C; IR (KBr,cm^{-1}): 3397, 1617; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.68(s,1H,-CH), 7.92(dd,1H,Ar-H), 8.10(dd,1H,Ar-H),8.20(s,1H,Ar-H), 13.0 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 118.4, 129.6, 131.2, 131.5, 131.9,132.4, 141.4, 144.2,

144.8, 146.5; MS (*m/z)* 290(M+1)+. Anal.Calcd for $C_{10}H_5BrN_6$; C, 41.55; H, 1.74; N, 29.07; Found: C, 41.48; H, 1.69; N, 28.98 %.

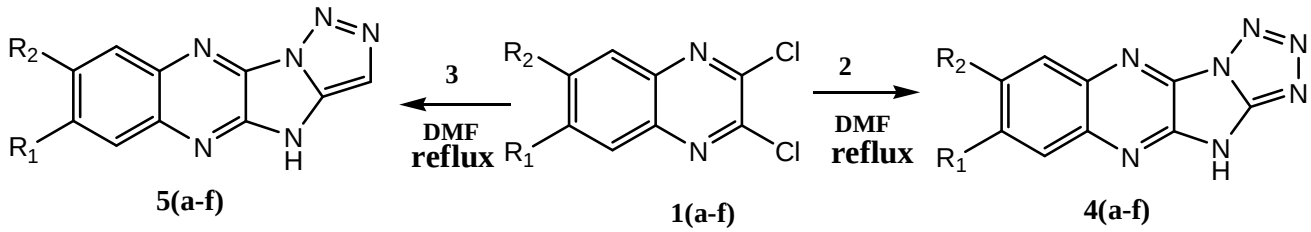
7-Chloro quinoxalino[4,5-*b*]-*4H*-imidazo[1,2-*e*][1,2,3]-triazole 5f

Yield:63%, m. p. 256–257 ^{0}C; IR (KBr,cm^{-1}): 3394, 1612; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.62(s,1H,-CH), 7.84(dd,1H,Ar-H), 7.98(dd,1H,Ar-H),8.14(s,1H,Ar-H),13.2 (br,s,1H,-NH); ^{13}C NMR (100MHz, DMSO-d_6): δ 129.8, 131.2, 131.9, 132.2, 133.6, 141.4, 143.4, 144.8, 148.8, 146.2; MS (*m/z)* 245(M+1)+. Anal.Calcd for $C_{10}H_5ClN_6$; C, 49.10; H, 2.06; N, 34.35; Found: C, 49.02; H, 2.01; N, 34.28 %.

RESULTS AND DISCUSSION

The target compounds were synthesized as outlines in **Scheme I**. The starting material 2, 3-dichloroquinoxaline **1** was prepared according to a reported procedure [18,19] and allowed to react with (1H)-tetrazol-5-amine and (3H)-1,2,3-triazol-4-amine in the presence of DMF as solvent and added a catalytic amount of K_2CO_3 at 120^o c to produce **4(a-f)** and **5(a-f)**. The structure of the all newly synthesized compounds was elucidated on the basis of their spectral (IR, ^{1}H NMR, C^{13} NMR, and mass) and elemental analyses data. The IR spectrum of **4(a-f)** and **5(a-f)** showed characteristic absorption bands within the ν=3405-3350 cm^{-1} due to the presence of -NH group. The ^{1}H NMR spectrum of compounds **4(a-f)** and **5(a-f)** showed peaks at δ 7.52 to 8.34 ppm due to aromatic protons and a broad peak obtained at δ 11.2-13.8 ppm due to the presence of -NH proton also compounds **4c** and **5c** exhibited two singlet signals at δ 2.20 ppm due to two methyl protons. Further, compounds **5(a-f)** displayed characteristic signals at δ 7.62-7.80 ppm for –CH proton for triazole ring. The ^{13}C NMR spectrum of compounds **4c** and **5c** displayed characteristic signals within δ 21.2-24.8 ppm due to two methyl groups and compounds **4b** and **5b** displayed signals at δ 24.2-24.8 ppm for methyl groups respectively. The -CH carbon of compounds **5(a-f)** showed a signals at δ 131.8- 132.4 ppm for triazole group.

Scheme I:



5(a-f) **1(a-f)** **4(a-f)**

2**:** (1H)-tetrazol-5-amine; **3**: (3H)-1,2,3-triazol-4-amine

Sl No.	Compd.	R_1	R_2
1	a	-H	-H
2	b	$-CH_3$	-H
3	c	$-CH_3$	$-CH_3$
4	d	$-NO_2$	-H
5	e	-Br	-H
6	f	-Cl	-H

ANTIMICROBIAL ACTIVITY

Compounds **4(a-f)** and **5(a-f)** were initially screened for *in vitro* antibacterial activity against Gram positive bacterial strains (*Bacillus Subtillis, Staphylococcus aureus*) and Gram negative bacteria strain (*E-Coli*) utilizing the agar diffusion assay[20]. The anti biotic drug, ampicillin was also used as positive control. Antibacterial activity screening for analogs and positive control was performed at a fixed concentration of 1000µg/mL. All compounds exhibited antibacterial activity against Gram +Ve and Gram –Ve bacterial strains with Zones of inhibition (ZOI) ranging from 20 mm to 50 mm. Compound **4f** was identified as a potent antibacterial agent against all Gram +Ve and Gram –Ve bacterial strains. Compounds **4d**, **5d** and **5f** also showed good antibacterial activity against all Gram +Ve and Gram –Ve bacterial strains compared to standard anti biotic drug, ampicillin (Table-1).

Analogs **4(a-f)** and **5(a-f)** were also examined for antifungal activity against fungal strains i.e., *Aspergillus niger* and *Candida albicans.* The antifungal drug, Ketaconozole was used as appositive control. The fungal strains were grown and maintained on sabouraud glucose agar plates. The plates were incubated at 27 ^{0}C for 72 h and resulting zone of inhibitions (ZOIs) were measured[21]. Antifungal screening for analogs and positive control was performed at a fixed concentration of 1000µg/mL. Compounds **4d** and **5f** were identified the most potent antifungal agent against all fungal strains. The remaining compounds **4f** and **5d** showed good antifungal activity compared to standard antifungal drug, Ketaconozole.

Table 1: Zone of inhibition of data for 4(a-f) and 5(a-f) against different bacteria and fungi at 1000 µg/mL concentration.

Analog	Gram positive bacteria		Gram negative bacteria	Fungi	
	B. Subtilis	S. Aureus	E. Coli	A. Niger	C. Albicans
	31	29	25	32	27
4b	30	37	32	27	30
4c	27	24	35	29	27
4d	34	39	41	48	46
4e	32	31	26	22	26
4f	42	50	44	38	39
5a	29	35	31	31	29
5b	34	39	32	33	26
5c	26	29	20	28	30
5d	41	48	42	38	40
5e	30	27	31	30	28
5f	36	38	40	42	45
Ampicillin	39	48	40	---	---
Ketaconazole	---	----	---	42	40

ACKNOWLEDGEMENT

The authors are thankful to the DST Inspire Scholarship Program (SHE), New Delhi for providing financial support and also thankful to our Chairman, Dr Ch. V. Purushottam Reddy, Principal, Dr Veeravenkatiah for providing research facilities.

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