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Synthesis and biological activity of 8-chloro-[1,2,4]triazolo [4,3-a]quinoxalines

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Abstract

8-Chloro-1-substituted-[1,2,4]triazolo[4,3-a]quinoxaline**s 6** underwent facile condensation with various hydrocarbon derivatives in the presence of TEBAC in acetonitrile was refluxed and afforded corresponding 8-chloro-1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxaline derivatives **7a-j.** Their chemical structures were characterized using IR, H^1 NMR and Mass spectral studies. All the above compounds were screened for anti-microbial activity, anti-oxidant activity and their bioassay showed them to possess significant antimicrobial activity and anti-oxidant activity.

Keywords: 8-Chloro-1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxaline**s**, spectral analysis, anti-microbial activity, anti oxidant.

Introduction

The recent literatures are enriched with progressive findings about the synthesis and pharmacological action of fused heterocyclic. The structural diversity and biological importance of nitrogen containing heterocyclic have made them attractive synthetic targets over many years and they are found in various natural products [1]. Quinoxalines are an important class of nitrogen containing heterocycles with a variety of biological activities. In particular quinoxaline scaffolds were found as a core unit in a number of biologically active compounds. These include anticancer [2,3], antibacterial [4], antiviral [5], anti-inflammatory [6], anti HIV [7-8] and antihelmintic activities [9]. Quinoxaline derivatives are also used in the development of novel organic dyes and organic semiconductors.

Experimental Section

The IR spectra were recorded in KBr discs (υ_{max} in cm^{-1}) on Perkin-Elmer FT-IR spectrophotometer. The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument. Mass spectra under electron impact conditions (EI) were recorded at 70 ev ionizing voltage with a VG Prospec instrument and the presented as m/z (% rel int.). Elemental analyses (C,N,H) results were found to be in good agreement with the calculated values. Melting points were determined with Capillaries Thomas Hoover melting point apparatus and are uncorrected. TLC monitored all reactions and purity of the synthesized compounds.

General Procedure

The general synthetic pathway is depicted in Scheme 1. Infact, **2** was obtained by the reaction of 1 equivalents of **1** with 1.2 equivalents of oxalic acid in 4N HCl under reflux [10-11]. Treatment of **2** with $POCl_3$, catalytic amount of DMF gave **3** [12-14]. The compound **3** with one equivalent of hydrazine hydrate in dioxane at RT in the presence of triethylamine as a base [15], lead to the replacement of only one chlorine yielding **4**. Further condensation of **4** with aliphatic/aromatic aldehydes in DMF [16-17] at RT gave a clean crystalline product which has been assigned **5**. The reaction of **5** with chloranil in refluxing with 1, 2 dichloroethane to give **6** [18-19]. It was considered worth while to carryout the dehydrohalogenation of **6** to **7** using for the purpose, TEBAC (triethylbenzylammonium chloride) and acetonitrile was considered to be a good choice, in such reaction, easy availability in the lab and low cost.

A mixture of **6** (10mM), hydrocarbon derivatives (15mM) and TEBAC (2.5mM) in acetonitrile (10 Vol) was refluxed for 6-8 hr. At the end of the period, the reaction mixture was cooled to RT. The acetonitrile was evaporated under reduced pressure to dryness and the residue was treated with water (20mL). The resulting mixture was filtered, washed with water and dried to obtained **7a-j**.

4, 8-Dichloro-1-(2-chloro-6-methoxyquinolin-3-yl)-[1,2,4]triazolo[4,3-a]quinoxaline ***7a*****:** Brown solid: Yield was found to be 76%, mp 265-266°C. IR (KBr) cm^{-1}: 1610 (C=N), 1291 (C=C), 1158 (N-N). ^{1}H NMR (DMSO-d_6), δ, ppm: 4.0 (s, 3H), 7.0-8.9 (m, 7H). Mass spectrum, m/z: 414 $(M+1)^+$. *Anal.* Calculated. For $C_{19}H_{10}Cl_3N_5$: C, 55.03; H, 2.43; N, 16.89; Cl, 25.65. Found, %: C, 55.10; H, 2.45; N, 16.85; Cl, 25.58.

*2-(8-Chloro-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxalin-4-ylthio)acetic acid 7****b*****:** Yellow solid: Yield was found to be 86%, mp 236-238°C. IR (KBr) cm^{-1}: 1651 (C=N), 1302 (C=C), 1156 (N-N). ^{1}H NMR (DMSO-d_6), δ, ppm: 4.21 (s, 2H), 7-7.9 (m, 8H), 12.2 (s, 1H). Mass spectrum, m/z: 371 $(M+1)^+$. *Anal.* Calculated. For $C_{17}H_{11}ClN_4O_2S$: C, 55.06; H, 2.99; N, 15.11; Cl, 9.56. Found, %: C, 55.10; H, 2.97; N, 15.08; Cl, 9.52.

8-Chloro-1-(4-fluorophenyl)-4-methoxy-[1,2,4]triazolo[4,3-a]quinoxaline ***7c****:* Yellow solid: Yield was found to be 87%, mp 304-305°C. IR (KBr) cm^{-1}: 1613 (C=N), 1298 (C=C), 1162 (N-N), C-O-C (1241, 1021). ^{1}H NMR (DMSO-d_6), δ, ppm: 2.57 (s, 3H), 7.2 (s, 3H), 7.5-7.61 (m, 2H), 8-8.3 (m, 2H). Mass spectrum, m/z: 329 $(M+1)^+$. *Anal.* Calculated. For $C_{16}H_{10}ClFN_4O$: C, 58.46; H, 3.07; N, 17.04; Cl, 10.78. Found, %: C, 58.49; H, 3.05; N, 17.08; Cl, 10.81

2-(8-Chloro-1-(furan-2-yl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-ylthio)aceticacid ***7d*****:** White solid: Yield was found to be 79%, mp 225-226°C. IR (KBr) cm^{-1}: 1611 (C=N), 1303 (C=C), 1145 (N-N). ^1H NMR ($CDCl_3$), δ, ppm: 4.2 (s, 2H), 7.2-8 (m, 6H), 12.1 (s, 1H). Mass spectrum, m/z: 361 $(M+1)^+$. *Anal*. Calculated. For $C_{15}H_9ClN_4O_3S$: C, 49.94; H, 2.51; N, 15.53; Cl, 9.83. Found, %: C, 49.90; H, 2.53; N, 15.51; Cl, 9.88.

4,8-Dichloro-1-(4-chlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline ***7e*****:** Light Yellow solid: Yield was found to be 81%, mp 286-287°C. IR (KBr) cm^{-1}: 1603 (C=N), 1297 (C=C), 1151 (N-N). ^1H NMR (DMSO-d_6), δ, ppm: 7.5-8.1 (m, 7H). Mass spectrum, m/z: 349 $(M+1)^+$. *Anal*. Calculated. For $C_{15}H_7Cl_3N_4$: C, 51.53; H, 2.02; N, 16.03; Cl, 30.42. Found, %: C, 51.64; H, 2.05; N, 16.08; Cl, 30.38.

(E)-8-Chloro-N,N-diethyl-1-(prop-1-enyl)-[1,2,4]triazolo[4,3-a]quinoxaline-4-amine ***7f*****:** Brown solid: Yield was found to be 85%, mp 165-166°C. IR (KBr) cm^{-1}: 1608 (C=N), 1295 (C=C), 1148 (N-N). ^1H NMR ($CDCl_3$), δ, ppm: 1.25 (t, 6H), 2.9 (q, 4H), 7.1 (s, 2H), 7.4-8.2 (m, 8H). Mass spectrum, m/z: 378 $(M+1)^+$. *Anal.* Calculated. For $C_{21}H_{20}ClN_5$: C, 66.75; H, 5.33; N, 18.53; Cl, 9.38. Found, %: C, 66.70; H, 5.31; N, 18.55; Cl, 9.35.

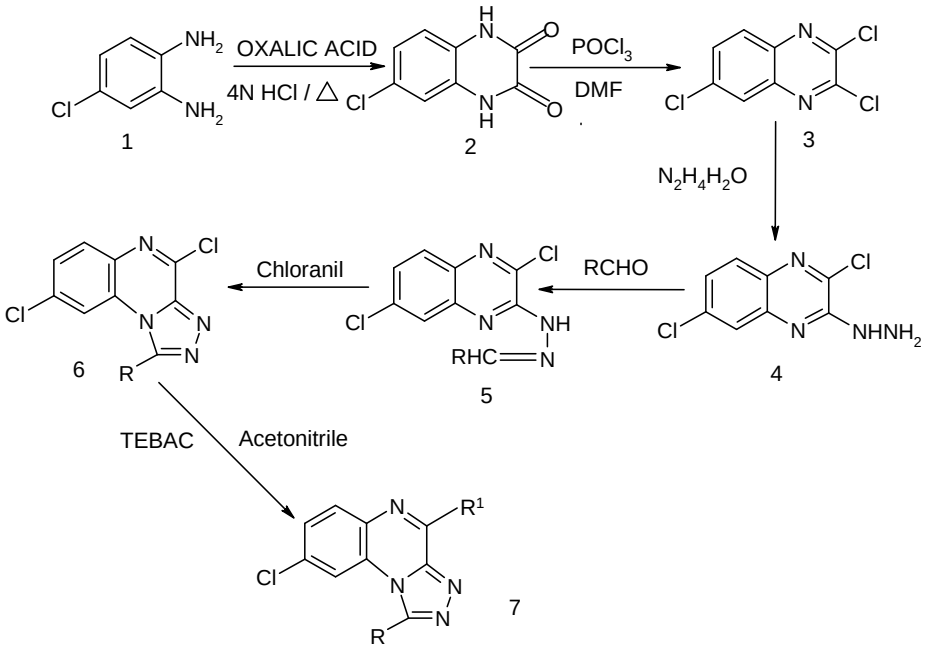
8-Chloro-4-(4-methylpiperazin-1-yl)-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxaline ***7g*****:** Light Yellow solid: Yield was found to be 75%, mp 164-165°C. IR (KBr) cm^{-1}: 1603 (C=N), 1292 (C=C), 1139 (N-N), 2782 (N-CH_3). ^1H NMR (DMSO-d_6), δ, ppm: 2.21 (s, 3H), 2.4 (t, 4H), 2.68 (t, 4H), 7.15-7.7 (m, 9H). Mass spectrum, m/z: 379 $(M+1)^+$. *Anal.* Calculated. For $C_{20}H_{19}ClN_6$: C, 63.41; H, 5.05; N, 22.18; Cl, 9.36. Found, %: C, 63.39; H, 5.09; N, 22.16; Cl, 9.38.

8-Chloro-4-(4-methylpiperazin-1-yl)-1-propyl-[1,2,4]triazolo[4,3-a]quinoxaline ***7h*****:** Yellow solid: Yield was found to be 78%, mp 144-145°C. IR (KBr) cm^{-1}: 1605 (C=N), 1292 (C=C), 1143 (N-N), 2792 (N-CH_3). ^1H NMR ($CDCl_3$), δ, ppm: 1.19-1.22 (t, 3H), 1.40(m, 2H), 1.65(t, 2H), 1.9 (s, 3H), 2.45-2.70 (t, 8H), 7.19-7.40 (m, 3H). Mass spectrum, m/z: 345 $(M+1)^+$. *Anal*. Calculated. For $C_{17}H_{21}ClN_6$: C, 59.21; H, 6.14; N, 24.37; Cl, 10.28. Found, %: C, 59.33; H, 6.10; N, 24.38; Cl, 10.30.

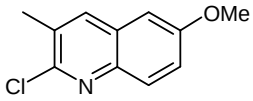
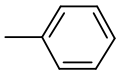
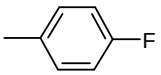
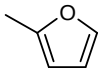
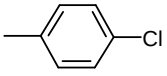
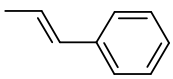
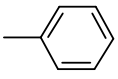
2-(8-Chloro-1-(thiophen-2-yl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-ylthio)aceticacid ***7i*****:** White solid: Yield was found to be 77%, mp 202-203°C. IR (KBr) cm^{-1}: 1611 (C=N), 1303 (C=C), 1145 (N-N), 659 (C-C). ^1H NMR ($CDCl_3$), δ, ppm: 3.9 (s, 2H), 7.3-8.0 (m, 6H), 12.01 (s, 1H). Mass spectrum, m/z: 375 $(M+1)^+$. *Anal.* Calculated. For $C_{16}H_{10}ClN_3O_2S_2$: C, 51.13; H, 2.68; N, 11.18; Cl, 9.43. Found, %: C, 51.16; H, 2.65; N, 11.15; Cl, 9.45.

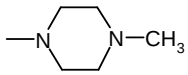
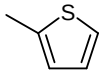
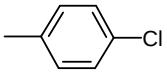
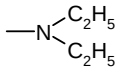
8-Chloro-1-(4-chlorophenyl)-N,N-diethyl-[1,2,4]triazolo[4,3-a]quinoxaline-4-amine ***7j*****:** Yellow solid: Yield was found to be 80%, mp 175-176°C. IR (KBr) cm^{-1}: 1608 (C=N), 1295 (C=C), 1148 (N-N). ^1H NMR ($CDCl_3$), δ, ppm: 1.23 (t, 10H), 2.8 (q, 4H), 7.1-8.2 (m, 6H). Mass spectrum, m/z: 384 $(M+1)^+$. *Anal.* Calculated. For $C_{20}H_{18}Cl_2N_4$: C, 62.35; H, 4.71; N, 14.54; Cl, 18.40. Found, %: C, 62.33; H, 4.69; N, 14.55; Cl, 18.38.

Scheme



Synthetic Scheme for 7a-j

Compound	R	R^1
7a		—Cl
7b		—SCH_2COOH
7c		—OMe
7d		—SCH_2COOH
7e		—Cl
7f		—$N(C_2H_5)_2$
7g		—N⟨⟩N—CH_3

7h	—C_3H_7	
7i		—SCH_2COOH
7j		

Antimicrobial Testing

The compound 7**a-j** was tested for in vitro antimicrobial activity at two different concentrations 100 and 200µg per disc. The antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris, Klebsiella pneumoniae* (Gram-nagative bacteria) on nutrient agar plates at 37 ^{0}C for 24 hrs using chloramphenicol as reference during. The compounds were also evaluated for their antifungal activity against *Aspergillus niger* and *Pencillium chrysogenium* using fluconazole as standard drug. Fungi cultures were grown on potato dextrose agar (PDA) medium at 25 ^{0}C. The spore suspension was adjusted to 10^6 pores ml^{-1} at an mg ml^{-1} concentration by the Vincent and Vincent method.

Table 1. Antibacterial activity of the target compounds 7a-j

Compound	Concentration	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
	(µg)	*S.aureus*	*B.subtilis*	*P.vulgaris*	*K.pneumoniae*
7a	100	20	22	17	19
	200	27	25	22	23
7b	100	30	31	31	35
	200	35	38	40	39
7c	100	25	23	20	23
	200	30	25	23	28
7d	100	12	11	14	13
	200	15	13	17	16
7e	100	32	27	24	26
	200	35	30	28	27
7f	100	26	26	20	21
	200	32	30	24	24
7g	100	12	13	12	14
	200	14	12	15	17
7h	100	11	12	11	11
	200	14	16	14	13
7i	100	28	31	22	21

	200	33	35	26	25
7j	100	25	24	20	21
	200	31	28	23	24
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

* c = 100 μg / ml.
* c = 200 μg / ml.

Table 2. Antifungal activity of the target compounds 7a-j

Compound	Concentration	Zone of Inhibition (mm)	
	(μg/ml)	*A.niger*	*P.chrysogenium*
7a	100	24	20
	200	27	26
7b	100	14	14
	200	19	17
7c	100	26	26
	200	30	29
7d	100	31	18
	200	38	19
7e	100	15	25
	200	18	28
7f	100	25	30
	200	27	32
7g	100	30	26
	200	33	28
7h	100	17	16
	200	22	18
7i	100	31	28
	200	37	34
7j	100	33	34
	200	35	36
Fluconazole	100	38	41
	200	42	44

* c = 100 μg / ml.
* c = 200 μg / ml.

Antioxidant Testing

The compounds 7**a-j** is tested for antioxidant property by nitric oxide and DPPH methods.

Assay for Nitric Oxide (NO) Scavenging Activity Sodium nitroprusside (5µM) in phosphate buffer pH 7.4 was incubated with 100 µM concentration of test compounds dissolved in a suitable solvent (methanol) and tubes were incubated at 25^0C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals 0.5 ml of incubation solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at 546 nm.

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH Method) The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100 µM) were added to DPPH (100 µM) in ethanol. The tubes were kept at an ambient temperature for 25 minutes and the absorbance was measured at 517 nm. The difference between the test and the control experiments was taken and expressed as the percentage scavenging of the DPPH radical.

Table 3: Antioxidant activity of the target compounds 7a-j

Compound	% Inhibition at 100 µM	
	Nitric oxide method	DPPH method
7a	82.25	84.74
7b	34.33	38.12
7c	91.18	93.65
7d	29.21	27.75
7e	70.23	72.25
7f	79.1	76.8
7g	25.1	35.22
7h	24.78	26.15
7i	72.14	75.25
7j	96.18	94.38

* c = 100 µM.

Results and Discussion

The results of the compounds of preliminary antimicrobial testing are shown in Tables-1 and 2. The results revealed that the inhibitory activity against Gram-positive bacteria was higher than Gram-negative bacteria. The imidazole derivatives **7d, 7g,** and **7h** were displayed least activity.

The compounds 7**a, 7b, 7c, 7e, 7f, 7i**, and 7**j** showed excellent activity against Gram-positive bacteria (inhibitory zone>25mm) and good activity against Gram negative bacteria (inhibitory zone>20mm). All the test compounds 7**a, 7c, 7d, 7f, 7g, 7i** and **7j** excellent activity and compounds **7b, 7e** and **7h** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Aspergillus niger*. Compounds **7a, 7c, 7e, 7f, 7g, 7i and 7j** excellent activity and compounds **7b, 7d** and **7h** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Pencillium chrysogenium* (Table 1 & 2). The compounds 7**a, 7c, 7e, 7f, 7i,** and **7j** exhibited high antioxidant property in both **Nitric Oxide** and **DPPH** methods at 100 µM concentration (Table 3).

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