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Synthesis of antimicrobial 2,9,10-trisubstituted-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines

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Abstract—A new series of 2,9,10-trisubstituted-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines was synthesized and submitted to antibacterial and antifungal activities. Result of the antimicrobial screening showed the compound **4j** being the most effective among the various treatments in antimicrobial screening. Compounds **4c**, **4d**, **4k**, and **4l** showed moderate activity against the microorganisms tested.

Nitrogen-containing heterocyclic compounds are indispensable structural units for both the chemists and the biochemists. Among the various classes of heterocyclic compounds, quinoxalines form an important component of pharmacologically active compounds. Quinoxaline ring is a part of various antibiotics such as hinomycin, levomycin, and actinoleutin[1,2] that are known to inhibit growth of Gram positive bacteria and are active against various transplantable tumors.[3] In addition quinoxaline derivatives are also associated with a wide spectrum of biological activities ranging from antihelmintic and anticancer to antimicrobial (as antifungal and antibacterial) antidepressant and anti-inflammatory agents.[4–8]

4-Hydroxycoumarin and its analogues are well known for their anticoagulant activity[9] along with antimicrobial activities. Also, 3-substituted 4-hydroxycoumarin generates considerable interest[10] in designing the synthesis of number of derivatives as probable HIV protease inhibitors with high therapeutic index.

Literature survey reveals that quinoxaline having coumarin constituent possesses antibacterial activity.[11] Recently some biologically active new heterocyclic compounds containing quinoxaline and coumarin moieties have been synthesized.[12]

In view of the vast biological importance of quinoxaline and coumarin derivatives, we tried to synthesize 2,9,10-trisubstituted-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quin-

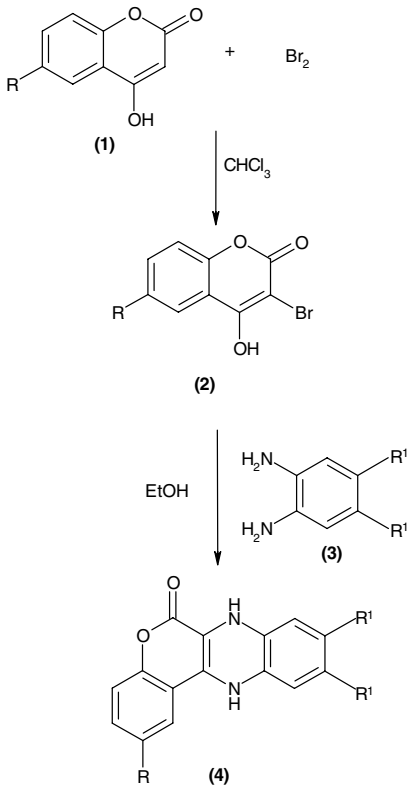

Figure 1.

Keywords: Quinoxallinones; Antimicrobial activity.
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Table 1. Experimental data of 2,9,10-trisubstituted-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4a**–**3n**)

Compound	R	R^1	Mp (°C)	Yield (%)	Molecular formula Molecular weight	Elemental analysis %		
						C Calcd Found	H Calcd Found	N Calcd Found
4a	H	H	185	75	$C_{15}H_{10}N_2O_2$	71.99	4.03	11.19
					250	71.90	4.00	11.13
4b	CH_3	H	245	78	$C_{16}H_{12}N_2O_2$	72.72	4.58	10.60
					264	72.69	4.52	10.55
4c	OCH_3	H	252–253	75	$C_{16}H_{12}N_2O_3$	68.57	4.32	9.99
					280	68.52	4.28	9.95
4d	Br	H	245	70	$C_{15}H_9BrN_2O_2$	54.74	2.76	8.51
					329	54.70	2.72	8.48
4e	Cl	H	240	73	$C_{15}H_9ClN_2O_2$	63.28	3.19	9.84
					284	63.25	3.15	9.80
4f	F	H	223–225	78	$C_{15}H_9FN_2O_2$	67.16	3.38	10.44
					268	67.25	3.46	10.51
4g	NO_2	H	201–202	71	$C_{15}H_9N_3O_4$	61.02	3.07	14.23
					295	61.10	3.12	14.30
4h	H	CH_3	243–246	75	$C_{17}H_{14}N_2O_2$	73.37	5.07	10.07
					278	73.32	5.02	10.02
4i	CH_3	CH_3	252–255	78	$C_{18}H_{16}N_2O_2$	73.96	5.52	9.58
					292	73.92	5.48	9.52
4j	OCH_3	CH_3	275	78	$C_{18}H_{16}N_2O_3$	70.12	5.23	9.09
					308	70.08	5.19	9.05
4k	Br	CH_3	275	80	$C_{17}H_{13}BrN_2O_2$	57.16	3.67	7.84
					357	57.12	3.62	7.80
4l	Cl	CH_3	272	80	$C_{17}H_{13}ClN_2O_2$	65.29	4.19	8.96
					312	65.25	4.16	8.90
4m	F	CH_3	268	78	$C_{17}H_{13}FN_2O_2$	68.91	4.42	9.45
					296	68.88	4.49	9.38
4n	NO_2	CH_3	252	71	$C_{17}H_{13}N_3O_4$	63.16	4.05	13.00
					323	63.10	4.01	13.08

Table 2. Antimicrobial activity[a] of synthesized compound

Compound	Zone of inhibition (mm)[b]					
	Bacteria				Fungi	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Fusarium oxysporum*
4a	2	1	2	3	2	3
4b	3	2	3	4	2	2
4c	8	5	6	7	5	6
4d	8	4	5	6	4	5
4e	6	3	4	5	3	4
4f	5	3	3	4	2	3
4g	5	4	4	4	4	3
4h	4	2	3	4	2	3
4i	5	3	4	5	2	4
4j	11	9	9	10	6	8
4k	9	7	6	7	4	5
4l	7	5	8	9	3	4
4m	6	5	7	8	3	4
4n	5	4	4	5	4	4
Standard antibiotic[c]	12	10	11	10	9	8

[a] These results are average results of four experiments.
[b] The compounds were used at the concentration of 100 μg/ml.
[c] Streptomycin for bacteria and Nystatin for fungi, were used at the concentration of 30 μg.

oxalines. Various substituted 4-hydroxycoumarin and 1,2-diphenylamine, were refluxed in ethanol to obtain the desired products[13] and to investigate their antimicrobial activity (Fig. 1).

The quinoxaline derivatives synthesized were tested to evaluate their antibacterial and antifungal activities at 100 μg/ml in DMSO. All the compounds (**4a**–**4n**) were found to have antimicrobial activities against different species of bacteria and fungi in our studies, (Table 2).[14] The obtained results mainly indicate that compound **4j** was the most effective, **4c**, **4d**, **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, and **4n** showed moderate activity, while **4a**, **4b**, **4h**, and **4i** exhibited scarce activity.

In spite of the scarce number of compounds tested this preliminary study evidences that the substituents on both quinoxaline and coumarin moieties exert significant influence on antimicrobial activities.

Furthermore, in comparison with antibiotics commonly used in therapy, our most potent compound (**4j**) shows comparable (although less potent) activities. Further studies are in progress to optimize these lead compounds and to characterize the mode of action.

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13. Experimental: Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. Chemical shifts δ (ppm) relative to TMS as an internal standard are reported. Electron spray ionization mass spectra (ESI-MS) were recorded on a Water-Micromass Quattro-II spectrometer. All of the reagents used were of AR grade and used without further purification. Column chromatography employed silica gel of 60–120 mesh.
General procedure: 3-Bromo-6-substituted-4-hydroxycoumarins (**2a**–**2g**) A mixture of 0.01 mol of 4-hydroxycoumarin in 50 ml of chloroform in a 250 ml flask. Cool it in an ice water bath. Add very slowly with constant stirring (about 15 min) from a dropping funnel 0.01 mol of bromine, stir the mixture by keeping the temperature below 10 °C, and keep it overnight. 3-Bromo-4-hydroxycoumarin commences as white needles. Filter and dry it. Washed with cold chloroform to give **2a**. All other compounds of this series were synthesized by following the above procedure.
2,9,10-Trisubstituted-6-oxo-7,12-dihydro-chromeno[3,4-*b*] quinoxalines (**4a**–**4n**): A solution of equimolar amount of (0.01 mol) of 3-bromo-4-hydroxycoumarin (**2a**–**2e**) and appropriate diamine in ethanol (25 ml) was refluxed for 4–6 h. The reaction was monitored by TLC. After completion of reaction, reaction mixture was cooled and poured into crushed ice. The crude compounds were purified by recrystallization with diethyl ether–hexane or by silica gel column chromatography to afford pure desired compounds. All other compounds of this series were synthesized by following the above procedure. The physical data of these compounds have been recorded in Table 1.
6-Oxo-7,12-dihydro-chromeno [3,4-*b*]quinoxalines (**4a**): ^{1}H NMR (DMSO-d_6) :δ = 4.02 (2H, br s, NH), 6.20 (2H, m, H-8,11), 6.34 (2H, m, H-9,10), 7.20 (1H, m, H-2), 7.25 (1H, m, H-4), 7.40 (H, m, H-3), 7.60 (1H, m, H-1). ^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ: 161, 149.2, 132.1, 131, 127.1, 126.2, 125.4, 124.1, 120.2, 118.2, 114.7, 102.1.
Mass (ES/MS) : *m*/*z* 249 (M-H).
2-Methyl-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines. (**4b**): ^{1}H NMR (DMSO-d_6) :δ = 2.29 (3H, s), 4.01 (2H, br s, NH), 6.20 (2H, m, H-8,11), 6.34 (2H, m, H-9,10), 7.01 (1H, d, H-4), 7.18 (1H, d, H-3), 7.35 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 147.1, 133.8, 133.2, 132.6, 127.6, 126.8, 118.6, 115.0, 102, 20.8.
Mass (ES/MS): *m*/*z* 263 (M-H).
2-Methoxy-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines. (**4c**): ^{1}H NMR (DMSO-d_6):δ = 4.01 (2H, br s, NH), 3.25 (3H, s), 6.20 (2H, m, H-8,11), 6.34 (2H, m, H-9,10), 6.88 (1H, d, H-3), 7.01 (1H, d, H-4), 7.10 (1H, d, H-1). ^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ: 161, 157.2, 142.1, 132.1, 131, 129.6, 121.1, 118.2, 118.1, 114.8, 112.6, 111, 101.1, 54.8.
Mass (ES/MS): *m*/*z* 279 (M-H).
2-Bromo-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4d**): ^{1}H NMR (DMSO-d_6): δ = 4.01 (2H, br s, NH), 6.19 (2H, m, H-8,10), 6.31 (2H, m, H-9,10), 7.01 (1H, d, H-4), 7.58 (1H, d, H-3), 7.72 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 148.9, 132.5, 132.4, 131.3,130.7, 129.07, 129.3, 122.8, 118.9, 118.5, 115, 102.8.
Mass (ES/MS): *m*/*z* 328 (M-H).
2-Chloro-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4e**): ^{1}H NMR (DMSO-d_6): δ = 4.01 (2H, br s, NH), 6.19 (2H, m, H-8,10), 6.31 (2H, m, H-9,10), 7.08 (1H, d, H-4), 7.40 (1H, d, H-3), 7.58 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 147.3, 132.2, 131.1, 129.6, 127.8, 126.8, 121.9, 118.6, 115, 102.8.
Mass (ES/MS): *m*/*z* 283 (M-H).
2-Fluoro-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4f**): ^{1}H NMR (DMSO-d_6): δ = 4.03 (2H, br s, NH), 6.19 (2H, m, H-8,10), 6.31 (2H, m, H-9,10), 7.08 (1H, d, H-4), 8.10 (1H, d, H-3), 8.32 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 155.1, 142.4, 132.2, 131.1, 129.6, 127.8, 126.8, 121.9, 118.6, 114.8, 102.8.
Mass (ES/MS): *m*/*z* 267 (M-H).
2-Nitro-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4g**): ^{1}H NMR (DMSO-d_6): δ = 4.03 (2H, br s, NH), 6.19 (2H, m, H-8,10), 6.31 (2H, m, H-9,10), 7.08 (1H, d, H-4), 8.10 (1H, d, H-3), 8.32 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 163.1, 158.4, 145.2, 131.1, 129.6, 127.8, 126.8, 121.9, 118.6, 114.8, 102.8.
Mass (ES/MS): *m*/*z* 294 (M-H).
9,10-Dimethyl-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4h**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (6H, S), 4.08

(2H, br s, NH), 6.85 (2H, d, H-8,11), 7.17 (1H, m, H-4), 7.19 (1H, m, H-2), 7.40 (1H, m, H-3), 7.58 (1H, m, H-1).
^{13}CNMR (50 MHz[$^{2}H_6$] DMSO) δ: 161, 149.2, 131, 129.1, 128.1, 127, 126.2, 125.2, 124.1, 120.1, 115.1, 101.1, 13.2.
Mass (ES/MS): *m*/*z* 277 (M-H).
2,9,10-Trimethyl-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4i**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (9H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-8-10), 7.03 (1H, d, H-4), 7.20 (1H, d, H-3), 7.39 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 146.2, 133.2, 131, 129.1, 128.1, 127.4, 126.4, 126.1, 120.1, 115.2, 102.1, 20.1, 13.9.
Mass (ES/MS): *m*/*z* 291 (M-H).
9,10-Dimethyl-2-methoxy-6-oxo-7,12-dihydro-chromeno-[3,4-*b*]quinoxalines (**4j**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (6H, s), 3.69 (3H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-8,11), 6.91 (1H, d, H-3), 7.01 (1H, d, H-4), 7.09 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ: 161, 157.2, 142.1, 131, 129.1, 128.1, 127.2, 121.1, 115.2, 112.2, 111.1, 102.1, 55, 14.2.
Mass (ES/MS): *m*/*z* 307 (M-H).
2-Bromo-9,10-dimethyl-6-oxo-7,12-dihydro-chromeno-[3,4-*b*]quinoxalines (**4k**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (6H, s), 3.69 (3H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-8,11), 6.91 (1H, d, H-3), 7.01 (1H, d, H-4), 7.09 (1H, d, H-1).
^{13}CNMR (50 MHz, [$^{2}H_6$] DMSO) δ = 161.5, 149, 131.1, 130.6, 129.4, 129, 128.7, 128.6, 122.6, 119, 116, 102.8, 13.8.
Mass (ES/MS): *m*/*z* 356 (M-H).
2-Chloro-9,10-dimethyl-6-oxo-7,12-dihydro-chromeno-[3,4-*b*]quinoxalines (**4l**)H^{1} NMR (DMSO-d_6): δ = 2.31 (6H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-8,11), 7.18 (1H, d, H-4), 7.38 (1H, d, H-3), 7.58 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 147.2, 131, 129.3, 129.1, 128.1, 127.2, 126.0, 121.3, 115.3, 102.1, 14.2, 14.1.
Mass (ES/MS): *m*/*z* 311 (M-H).
2-Fluoro-9,10-dimethyl-6-oxo-7,12-dihydro-chromeno-[3,4-*b*]quinoxalines (**4m**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (6H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-11), 7.18 (1H, d, H-4), 7.38 (1H, d, H-3), 7.58 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 147.2, 131, 129.3, 129.1, 128.1, 127.2, 126.0, 121.3, 115.3, 102.1, 14.2, 14.1.
Mass (ES/MS): *m*/*z* 295(M-H).
2-Nitro-9,10-dimethyl-6-oxo-7,12-dihydro-chromeno[3,4-*b*]quinoxalines (**4n**): ^{1}H NMR (DMSO-d_6): δ = 2.31 (6H, s), 4.08 (2H, br s, NH), 5.81 (2H, d, H-8,11), 7.18 (1H, d, H-4), 7.38 (1H, d, H-3), 7.58 (1H, d, H-1).
^{13}CNMR (50 MHz [$^{2}H_6$] DMSO) δ = 161, 147.2, 131, 129.3, 129.1, 128.1, 127.2, 126.0, 121.3, 115.3, 102.1, 14.2, 14.1.
Mass (ES/MS): *m*/*z* 322 (M-H).

14. Antimicrobial assay: The antimicrobial activity of the compounds was assayed by antimicrobial susceptibility test.[6] One hundred microliters of 24 h growth of each microorganism was spread on the surface of nutrient agar for bacteria (Mac Conkey's agar for *Escherichia coli)* and potato dextrose agar for fungi, in Petri plates. Fifty microliter compounds at the concentration of 100 μg/ml in DMSO saturated on discs of 6 mm diameter were kept on agar surface. The plates were refrigerated for 2 h to allow pre-diffusion of the compounds from the discs into the seeded agar layer and then incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for fungi. Zones of inhibition were measured in millimeter and size of the disc was subtracted from the zone size to measure final activity. DMSO saturated disc served as solvent control or negative control and Streptomycin saturated discs (30 μg) for bacteria and Nystatin (30 μg) for fungi as reference or positive control.