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SYNTHESIS AND ANTICONVULSANT ACTIVITY OF SOME NOVEL SEMICARBAZONE DERIVATIVES CONTAINING QUINOXALINE MOIETY

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ABSTRACT

Various 1-(substituted benzylidene)-5-(3-hydroxyquinoxaline-2-yl) carbonohydrazide were synthesized starting from orthophenylenediamine. Structures of all the compounds were confirmed on the basis of spectral analyses. All the newly synthesized compounds were screened for anticonvulsant activity. Some of the compounds showed significant anticonvulsant activity with no neurotoxicity.

INTRODUCTION: The term epilepsy refers to a disorder of the brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsies are common and frequently devastating and affect around 1-2% of world population. Approximately 25% of epileptic convulsions are inadequately controlled by standard drug therapy [1, 2]. The search for antiepileptic compound with more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry.

Aryl semicarbazones represents one of the most active class of compounds possessing anticonvulsant activity [3-5]. According to the pharmacophoric model based on semicarbazone, essential features for anticonvulsant activity are presence of a hydrophobic aryl ring, a hydrogen bonding domain, an electron donor acceptor system and an another hydrophobic aryl ring determining pharmacokinetic properties [6].

Functionalized quinoxaline represents an important an important class of nitrogen-containing heterocyclic compounds. Quinoxaline derivatives have been shown to possess a diverse biological activity [7].

Recently quinoxaline moiety has been exploited for anticonvulsant activity. Some quinoxaline derivatives possess anticonvulsant activity [8]. In this paper quinoxaline has been selected as hydrophobic domain with the hope to potentiate biological activity.

MATERIAL AND METHODS: The chemicals and solvents used for the experimental work were commercially procured from E. Merck, CDH, S. D. Fine Chem. and Qualigens, all from India. Syntheses of the title compounds were accomplished according to scheme-1. Melting points were determined in an open capillary tube and are uncorrected. IR spectra (cm^{-1}) were recorded on a FTIR-8400s Shimadzu system. The proton magnetic resonance spectra (H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO-d_6 using tetramethylsilane as internal standard.

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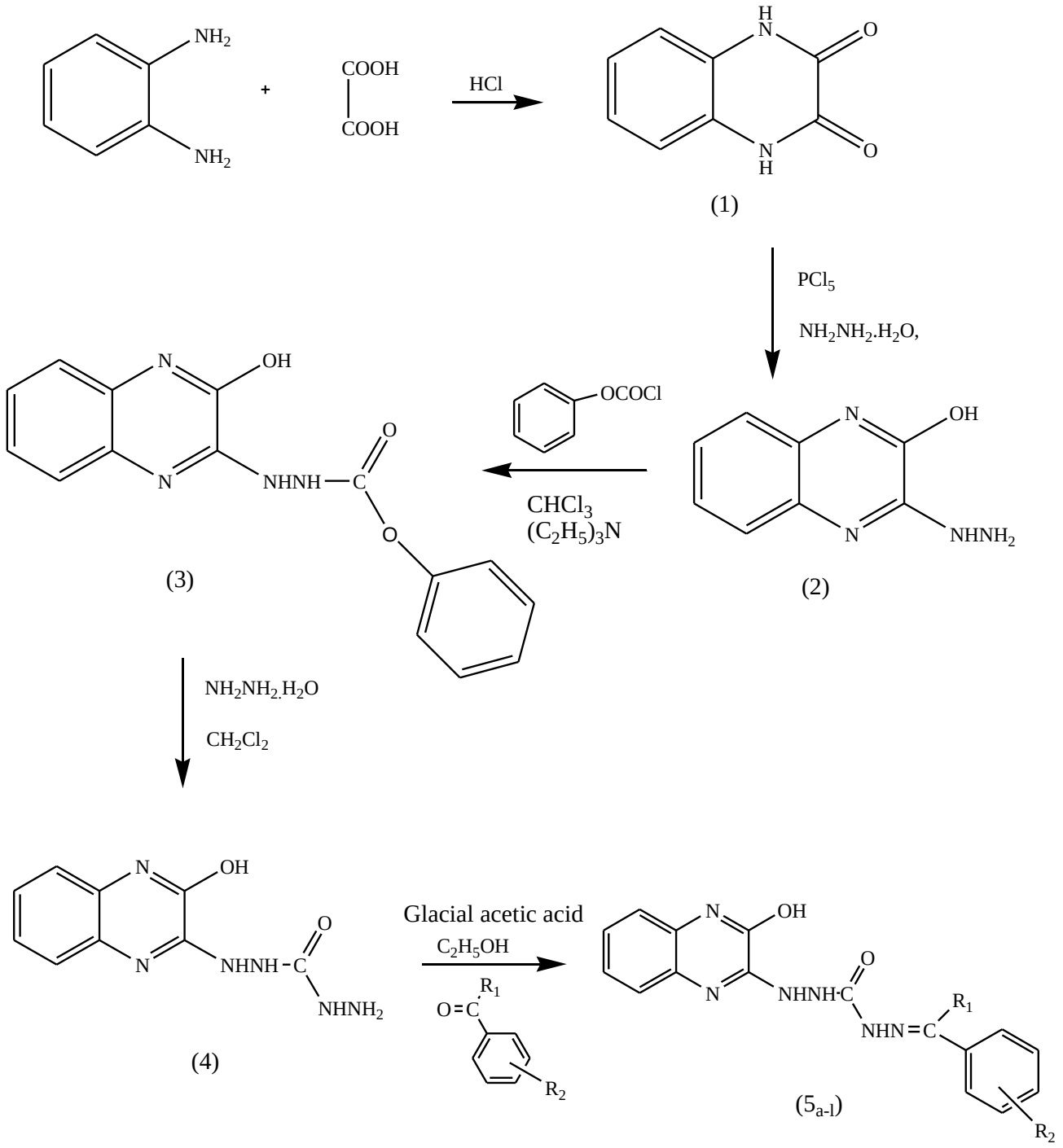
Chemical shifts (δ) were expressed in ppm. Progress of each step was observed by TLC. The *in vivo* experiment was approved by the institutional ethics committee and as per CPCSEA guidelines.



R_1= H, CH_3
R_2= H, 2-Cl, 3-Cl, 4-Cl, 2-NO_2, 3-NO_2,
2-OH, 4-OH, 4-Br, 4-OCH_3, 2,5-OCH_3, 4-N(CH_3)$_2$

Scheme - 1

Preparation of 2,3-Diketoquinoxaline (1): *O*-Phenylene diamine (0.25 mol), oxalic acid (0.36 mol) and 4N HCl were refluxed for 2h on oil bath. Grey colored crystals were obtained on cooling which were filtered and washed. Yield 85%; m.p. 295°C. IR (KBr, cm^{-1}) 3345, 2948, 1750, 1595, 1035.

Preparation of 2-hydrazino 3- hydroxy quinoxaline (2): Preparation of 2, 3-Diketoquinoxaline (0.01 mol) on treatment with phosphorous pentachloride yielded 2-chloro-3-hydroxy quinoxaline. Equimolar quantity of the chloro compound and hydrazine hydrate in ethanol (30 ml) was refluxed for 3h. The product separated was recrystalized from ethanol to yield 2-hydrazino 3-hydroxy quinoxaline. Yield 80%; m.p. 175°C. IR (KBr, cm^{-1}) 3285, 3170, 1625, 1190. ^{1}H NMR (DMSO-d_6): 2.52 (s, 3H), 4.23 (br, 2 H, NH_2 D_2O exchangeable) 6.2 (br, 1H, NH), 7.75 and 7.89 (d, 2H, quinoxaline ring protons).

Preparation of Phenyl 2-(3-hydroxyquinoxaline-2yl) hydrazine carboxylate (3): Phenyl chloroformate (0.1 mol) was dissolved in 40 ml of chloroform and equimolar quantity of 2-hydrazino 3- hydroxy quinoxaline and tri ethyl amine (0.1 mol) were added drop wise and stirred in room temperature for five hours. The reaction mixture was concentrated to one third volume and 100 ml of petroleum ether was added. The precipitate, which appeared was washed with water, filtered and dried to obtain product. Yield 77%; m.p. 95°C. IR (KBr, cm^{-1}) 1650 (C=N Str), 1191 (C-N Str), 1050 (N-N), 3300 (N-H), 1750 (C=O Str), 1250 (C-O Str). ^{1}H NMR (DMSO-d_6): 7.52-8.87 (m, 9H, ArH), 7.78 (s, 1H, OH), 4.65 and 8.91(s, 2H, NH, D_2O exchangeable).

Preparation of 1-(3-hydroxyquinoxaline-2yl) carbono hydrazide (4): Phenyl 2-(3-hydroxyquinoxaline-2yl) hydrazine carboxylate (0.05 mol) was dissolved in 100 ml of dichloromethane. To this solution 4.85 ml of hydrazine hydrate (0.1 mol) was added and refluxed with stirring for 24 hours. The precipitate was separated by vaccume filtration, washed with dichloromethane and dried to obtain product. Yield 56%; m.p. 105°C. IR (KBr, cm^{-1}) 1650 (C=N Str), 1195 (C-N Str), 1050 (N-N), 3350 (N-H), 1700 (C=O Str), 1255 (C-O Str). ^{1}H NMR (DMSO-d_6): 6.37-7.90 (m, 9H, ArH), 8.5 (s, 1H, OH), 5.25 (s, H, Ar-NH), 7.12 (s, 2H, 2×NH), 5.42 (s, 2H, NH_2 D_2O exchangeable).

Preparation of 1-(substituted benzylidene)-5-(3-hydroxyquinoxaline-2-yl) carbonohydrazide (5_{a-l}): To a solution of 1-(3-hydroxyquinoxaline-2yl) carbono-hydrazide (0.003 mol) in 25 ml of ethanol, an equimolar quantities of appropriate aldehyde or ketone in 5 ml of ethanol and glacial acetic acid (1-2 drops) were added. The mixture was stirred with heating for 1-4 hours until completion of the reaction and the resultant precipitate was filtered and dried. The solid was recrystalized from 95% ethanol to afford the titled compounds. Physical data and the spectral data are the following.

TABLE 1: PHYSICOCHEMICAL DATA OF SYNTHESIZED COMPOUNDS 5_{a-l}

Comp No.	R_1	R_2	Mol. formula	Mol. Weight [a]	Yield (%)	M. p. (°C)[b]	R_f[c]
5_a	H	2-Cl	$C_{16}H_{13}ClN_6O_2$	356.46	65	135	0.6
5_b	H	3-Cl	$C_{16}H_{13}ClN_6O_2$	356.46	70.2	130	0.51
5_c	H	4-Cl	$C_{16}H_{13}ClN_6O_2$	356.46	83.3	164	0.83
5_d	H	2-NO_2	$C_{16}H_{13}N_7O_4$	367.31	51	170	0.64
5_e	H	3-NO_2	$C_{16}H_{13}N_7O_4$	367.31	83	143	0.75
5_f	H	2-OH	$C_{16}H_{14}N_6O_3$	338.32	55	155	0.86
5_g	H	4-OH	$C_{16}H_{14}N_6O_3$	338.32	70	205	0.92
5_h	H	4-Br	$C_{17}H_{16}N_6O_3$	401.21	75	179	0.56
5_i	H	4-OCH_3	$C_{16}H_{13}BrN_6O_2$	352.34	46	135	0.85
5_j	H	2,5-OCH_3	$C_{18}H_{18}N_6O_4$	382.37	62	140	0.61
5_k	H	4-$N(CH_3)_2$	$C_{18}H_{19}N_7O_2$	462.50	65	154	0.75
5_l	CH_3	H	$C_{17}H_{16}N_6O_2$	336.34	50	208	0.46

[a] Solvent of crystallization: ethanol. [b] Melting point at decomposition. [c] Solvent system: chloroform: methanol (95:5)

Spectral data of Synthesized Compounds 5_{a-l}:

***N*''-(2-chlorobenzylidene)-*N*'''-(3-hydroxyquinoxaline-2-yl)carbonohydrazide (5_a):** IR (KBr, cm^{-1}) 1645 (C=N Str), 1182 (C-N Str), 1045 (N-N), 3355 (N-H), 1695 (C=O Str), 1267 (C-O Str), 879 (C-Cl). ^{1}H NMR (DMSO-d_6) δ (ppm):7.25-7.69 (m, 8H, ArH), 7.45 (s, 1H, OH), 4.65 (s, H, Ar-NH), 6.85 (s, 2H, 2×NH), 8.12 (s, 1H, imine H).

***N*''-(3-hydroxyquinoxaline-2-yl)-*N*'''-(2-nitrobenzyl idene) zcarbonohydrazide (5_d):** IR (KBr, cm^{-1}) 1650 (C=N Str), 1195 (C-N Str), 1050 (N-N), 3350 (N-H), 1700 (C=O Str), 1255 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.55-8.25 (m, 8H, ArH), 6.95 (s, 1H, OH), 5.15 (s, H, Ar-NH), 6.50 (s, 2H, 2×NH), 8.56 (s, 1H, imine H).

***N*''-(2-hydroxybenzylidene)-*N*'''-(3-hydroxy quinoxaline-2-yl)carbonohydrazide (5_f):** IR (KBr, cm^{-1}) 1754 (C=N Str), 1180 (C-N Str), 1065 (N-N), 3400 (N-H), 1765 (C=O Str), 1205 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.25-8.95 (m, 8H, ArH), 6.90 (s, 2H, 2×OH), 5.55 (s, H, Ar-NH), 6.15 (s, 2H, 2×NH), 8.15 (s, 1H, imine H).

***N*''-(4-hydroxybenzylidene)-*N*'''-(3-hydroxy quinoxaline-2-yl)carbonohydrazide (5_g):** IR (KBr, cm^{-1}) 1650 (C=N Str), 1195 (C-N Str), 1050 (N-N), 3350 (N-H), 1700 (C=O Str), 1255 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.12-8.52 (m, 8H, ArH), 6.94 (s, 2H, 2×OH), 5.42 (s, H, Ar-NH), 6.28 (s, 2H, 2×NH), 8.25 (s, 1H, imine H).

***N*''-(4-bromobenzylidene)-*N*'''-(3-hydroxyquinoxaline-2-yl)carbonohydrazide (5_h):** IR (KBr, cm^{-1}) 1750 (C=N Str), 1200 (C-N Str), 1125 (N-N), 3350 (N-H), 1698 (C=O Str), 1242 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 6.95-8.25 (m, 8H, ArH), 7.45 (s, 1H, OH), 4.62 (s, H, Ar-NH), 6.56 (s, 2H, 2×NH), 8.10 (s, 1H, imine H).

***N*''-(3-hydroxyquinoxaline-2-yl)-*N*'''-(4-methoxybenzyl idene)carbonohydrazide (5_i):** IR (KBr, cm^{-1}) 1632 (C=N Str), 1265 (C-N Str), 1132 (N-N), 3364 (N-H), 1663 (C=O Str), 1245 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.55-8.25 (m, 8H, ArH), 6.95 (s, 1H, OH), 5.15 (s, H, Ar-NH), 6.50 (s, 2H, 2×NH), 8.56 (s, 1H, imine H), 3.45 (s, 3H, Ar-OCH_3).

***N*''-(2,5-dimethoxybenzylidene)-*N*'''-(3-hydroxy quinoxaline-2-yl)carbonohydrazide (5_j):** IR (KBr, cm^{-1}) 1600 (C=N Str), 1263 (C-N Str), 1196 (N-N), 3345 (N-H), 1663 (C=O Str), 1266 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.62-8.01 (m, 7H, ArH), 6.23 (s, 1H, OH), 5.63 (s, H, Ar-NH), 6.50 (s, 2H, 2×NH), 8.36 (s, 1H, imine H), 3.45 (s, 6H, Ar-OCH_3).

***N*''-[4-(dimethylamino)benzylidene]-*N*'''-(3-hydroxy quinoxaline-2-yl)carbonohydrazide (5_k):** IR (KBr, cm^{-1}) 1623 (C=N Str), 1702 (C-N Str), 1165 (N-N), 3395 (N-H), 1663 (C=O Str), 1243 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.12-8.63 (m, 8H, ArH), 6.56 (s, 1H, OH), 5.56 (s, H, Ar-NH), 6.23 (s, 2H, 2×NH), 8.56 (s, 1H, imine H), 3.05 (s, 6H, $N(CH_3)_2$).

***N*''-(3-hydroxyquinoxaline-2-yl)-*N*'''-(1-phenylethyl idene)carbonohydrazide (5_l):** IR (KBr, cm^{-1}) 1785 (C=N Str), 1231 (C-N Str), 1112 (N-N), 3387 (N-H), 1668 (C=O Str), 1294 (C-O Str). ^{1}H NMR (DMSO-d_6) δ (ppm): 6.95-8.25 (m, 9H, ArH), 7.05 (s, 1H, OH), 4.62 (s, H, Ar-NH), 6.56 (s, 2H, 2×NH), 1.56(s, 3H, Carbamino-CH_3).

Anticonvulsant activity: Albino mice (18-25 g) were used as experimental animals. The synthesized compounds and standard drug were suspended in 0.5% methyl cellulose/water mixture. Each compound was administered as an i.p. injection at three 30 mg/kg dose levels and anticonvulsant effects were assessed at 30 min and 4 h intervals after administration. Anticonvulsant evaluation was undertaken using reported procedure [9, 10].

Maximal Electroshock Seizure Screen (MES): Maximal electroshock seizures were elicited with a 60 cycle alternating current of 50 mA intensity delivered for 0.25 s via ear clip electrodes. The maximal seizure typically consists of a short period of tonic extension of the hind limbs and a final clonic episode. Abolition of the hind limb tonic extensor component of the seizure is defined as protection.

Neurotoxicity Screen: The rotarod test was used to evaluate the neurotoxicity. The animal was placed on a 3.2 cm diameter knurled rod rotating at 6 rpm. Normal mice can remain on a rod rotating at this speed indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min. results are expressed as number of animals exhibiting toxicity/number of animals tested.

RESULTS: 1-(substituted benzylidene)-5-(3-hydroxy quinoxaline-2-yl) carbonohydrazide (5_{a-l}) obtained from reactional sequence were injected intraperitoneally to mice and evaluated for anticonvulsant activity by maximal electroshock seizure test and neurotoxicity screen by rotarod test at the dose of 30 mg/kg. Observations were carried out at two different time intervals of 0.5 and 4 h. Phenytoin was used as the standard drug for the comparison at dose level of 30 mg. The data obtained (**Table 2**) revealed that all the compounds showed anti-MES activity indicative of their ability to prevent seizure spread.

The compound that showed 100% protection at 30 mg/kg body mass was **5_c** and **5_h** both after 0.5 and 4 h. Compounds **5_e** and **5_i** showed 83% protection. Compounds **5_a**, **5_b**, **5_g**, **5_j**, **5_k** and**5_l** showed 66% protection, whereas compounds **5_d** and **5_f** were found to be only 50% protective against electroshock induced seizure. Selected compounds which showed more than 66% protection in the MES screen were further evaluated for neurotoxicity screen at the dose of 30 mg/kg. In this screen none of the compounds showed any sign of motor impairment (Table 2).

TABLE 2: ANTICONVULSANT AND NEUROTOXICITY OF SYNTHESIZED COMPOUNDS FOLLOWING INTRAPERITONEAL INJECTION TO MICE[a] 4_{a-l}

Compd. no	MES screen protection (%) 0.5hour	MES screen protection (%) 4hour	Neurotoxicity screen 0.5hour	Neurotoxicity screen 4hour
5_a	66	66	NT	NT
5_b	66	66	NT	NT
5_c	100	100	-	-
5_d	50	50	NT	NT
5_e	83	83	-	-
5_f	50	50	NT	NT
5_g	66	66	NT	NT
5_h	100	100	-	-
5_i	83	83	-	-
5_j	66	66	NT	NT
5_k	66	66	NT	NT
5_l	66	66	NT	NT
Control	-	-	-	-
Phenytoin	100	100	-	-

Number of animal in each group (n=6). [a] Dose of 30 mg/kg was administered i.p. Test and the standard compounds were suspended in 1% Tween 80. Animals were examined 0.5 and 4 h after administration. The (-) indicates absence of activity and NT denotes not tested.

DISCUSSION: Basic structure of the compounds fulfilled all the pharmacophoric structural requirements like presence of hydrophobic domain, hydrogen binding site, electron donor-acceptor moiety, and another distal hydrophobic domain. In general, the presence Cl and Br at para position of distal phenyl ring resulted in highly potent compound. Presence of Cl and NO_2 at distal phenyl ring resulted in compounds with significant activity. Substitution of Cl, Br and NO_2 with electron donating groups like OCH_3, $N(CH_3)_2$ at distal phenyl ring led to decrease in potency.

CONCLUSION: The present study revealed that semicarbazone derivatives containing quinoxaline moiety are active against tonic convulsion induced by electrical stimuli in mice with less neurotoxicity.

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