

Anticonvulsant and Neurotoxicity Evaluation of Some Novel Cyclohexyl-[4-substituted benzylidene/2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazides

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A series of novel cyclohexyl-[4-substituted benzylidene/2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazides were synthesized and screened for anticonvulsant activity in maximal electroshock induced seizure (MES) and subcutaneous metrazol (scMET) induced seizure models in mice. The neurotoxicity was assessed using the rotorod method. The compounds cyclohexyl-[4-(4-chlorophenoxy)-benzylidene]thiosemicarbazide (**7d**) and cyclohexyl-[2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazide (**7i**) emerged as the most promising one with anti-MES activity in mice i.p. All the compounds exhibited no neurotoxicity in rotorod method.

Key Words: Cyclohexyl thiosemicarbazides, Anticonvulsant, Neurotoxicity.

INTRODUCTION

Epilepsy is a collective term for conditions characterized by recurrent episodes of abnormal events such as convulsive seizures resulting from paroxysmal aberration of brain functions. It is a leading neurological disorder in human, second only to stroke¹. Current drug therapy for epilepsy suffers from a number of disadvantages including the fact that the convulsions of approximately 25 % epilepsies are inadequately controlled by medication. Therefore, the need for more effective and less toxic antiepileptic drugs still exists².

Semicarbazones and thiosemicarbazones have documented consistent advances in the design of novel anticonvulsant agents³. Extensive structural activity relationship (SAR) studies have led to postulating a specific binding site of thiosemicarbazones/semicarbazones. The proposed pharmacophoric requirements in these molecules are: (1) aryl binding site with a hydrophobic group; (2) hydrogen bonding domain exemplified by the presence of the -NHCO-/-NHCS-grouping; (3) two electron donor systems; (4) hydrophobic binding site whose size determines the type of activity. Recently, [4-(6-chlorobenzothiazol-2-yl)-1-(3-isatinimino)thiosemicarbazone] has also shown strong activity in maximum electroshock induced seizures (MES) and scPTZ screens⁴. Knowing that isatin derivatives possess anticonvulsant properties^{5,6}, various compounds were designed as hybrid molecule incorporating a thiosemicarbazone fragment and an isatin molecule. The potency and spectrum of activity of these 6-chlorobenzothiazolyl thiosemi-

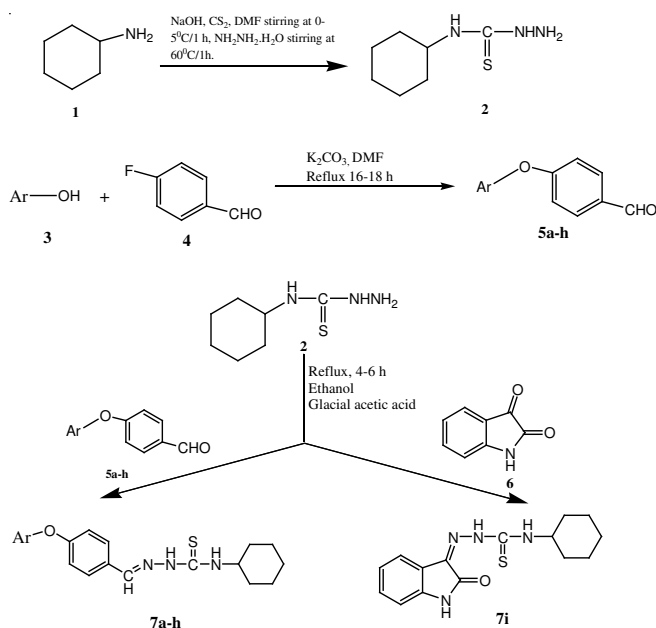
carbazones were comparable to those of standard drugs and represent a structurally novel class for subsequent molecular modifications. Bryans and Wustrow⁷ reported gabapentin and pregabalin, two γ -amino acids as having anticonvulsant, anxiolytic-like and analgesic actions. The presence of cyclohexyl moiety in gabapentin and analogs, prompt us to synthesize the cyclohexyl moiety based thiosemicarbazones and to evaluate their anticonvulsant activity.

EXPERIMENTAL

All the chemicals and solvents purchased from Merck (India), Spectrochem (India), Himedia (India) and S.D. Fine were used without further purification. The progress of reaction was monitored by thin layer chromatography, performed on a silica gel 60 F₂₅₄ coated aluminium sheet. The melting points were determined by using Thomas-Hoover melting point apparatus and are uncorrected. The FT-IR spectra were recorded on Perkin-Elmer Spectrum BX-II spectrophotometer. The ¹H NMR spectra were recorded on Bruker 300 MHz high resolution NMR spectrometer using TMS as an internal standard. Chemical shifts were reported in ppm (δ) and signals were described as singlet (s), doublet (d), triplet (t) and multiplet (m). All exchangeable protons were confirmed by addition of D₂O. The mass spectra were recorded on a Waters Micromass ZQ 2000 mass spectrometer. Elemental analysis (C, H, N) was undertaken with Perkin Elmer Model 240C analyzer.

The reaction sequence leading to the formation of the titled compounds, viz., cyclohexyl-[4-substituted benzylidene/

2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazides (**7a-7i**) is shown in **Scheme-I**.



Scheme-I: Synthesis of cyclohexyl-[4-substituted benzylidene/2-oxo-1,2-dihydroindol-3-ylidene]thiosemicarbazides (**7a-i**)

The cyclohexyl thiosemicarbazide (**2**) was obtained by reacting cyclohexyl amine (**1**) with carbon disulphide in presence of sodium hydroxide and then with hydrazine hydrate. Various 4-substituted benzaldehydes (**5a-h**) were prepared by refluxing various substituted phenol (**3**) with 4-fluoro benzaldehyde (**4**) in presence of potassium carbonate. The cyclohexyl thiosemicarbazide (**2**) was refluxed with various 4-substituted benzaldehyde (**5a-h**)/isatin (**6**) in the presence of catalytic amount of glacial acetic acid to yield the titled compounds (**7a-i**).

Synthesis of cyclohexyl thiosemicarbazide (2): To a solution of cyclohexyl amine (**1**) (0.01 mol) in DMF (15 mL) was added sodium hydroxide (0.011 mol) and carbon disulphide (0.01 mol). The mixture was stirred at 0-5 °C for 1 h, to the stirred mixture was added hydrazine hydrate (0.01 mol) and stirring continued at 60 °C for 1 h. On adding water a solid separated out which was recrystallized from 95 % ethanol afforded white crystalline solid.

Synthesis of 4-substituted benzaldehyde (5a-h): A mixture of substituted phenol (**3**) (37.4 mmol), 4-fluorobenzaldehyde (**4**) (37.4 mmol) and potassium carbonate (38.8 mmol) in N,N-dimethylformamide (30 mL) was refluxed for 16-18 h under nitrogen. After cooling, the product was extracted from the reaction mixture and purified by chromatography.

Synthesis of cyclohexyl-[4-substituted benzylidene/2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazides (7a-i): Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes (**5a-h**)/isatin (**6**) and cyclohexylthiosemicarbazide (**2**) were dissolved in warm ethanol containing 0.5 mL of glacial acetic acid. The reaction mixture was refluxed for 4-6 h and set aside. The resultant solid was washed with ethanol and recrystallized from 90 % ethanol. The physical data and elemental analysis data of all the thiosemicarbazones (**7a-i**) are presented in Table-1. The spectral data of thiosemicarbazones (**7a-i**) are given below.

Cyclohexyl-[4-phenoxybenzylidene]thiosemicarbazide (7a): IR (KBr, ν_{\max} , cm^{-1}): 3311 (-NH-), 2927, 2852 (CH_{str} of C_6H_{11}), 1595 (-N=CH-), 1247 (-O-), 1161 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.21-2.14 (a set of signals, 10H, - CH_2 of C_6H_{11}), 4.288 (m, 1H, -CH of C_6H_{11}), 7.041-7.841 (a set of signals, 9H, Ar-H), 7.331 (d, 1H, C-NH-CS, D_2O exchangeable), 7.875 (s, 1H, -CH=N-), 9.813 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 354.16 ($\text{M}^+ + 1$, 100).

Cyclohexyl-[4-(4-nitrophenoxy)benzylidene] thiosemicarbazide (7b): IR (KBr, ν_{\max} , cm^{-1}): 3313 (-NH-), 2928, 2851 (CH_{str} of C_6H_{11}), 1597 (-N=CH-), 1522 (N=O), 1249 (-O-), 1163 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.23-2.15 (a set of signals, 10H, - CH_2 of C_6H_{11}), 4.286 (m, 1H, -CH of C_6H_{11}), 7.043-7.840 (a set of signals, 8H, Ar-H), 7.33 (d, 1H, C-NH-CS, D_2O exchangeable), 7.871 (s, 1H, -CH=N-), 9.811 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 399.14 ($\text{M}^+ + 1$, 100).

Cyclohexyl-[4-(4-methylphenoxy)benzylidene] thiosemicarbazide (7c): IR (KBr, ν_{\max} , cm^{-1}): 3313 (-NH-), 2926, 2853 (CH_{str} of C_6H_{11}), 1595 (-N=CH-), 1246 (-O-), 1163 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.227-2.151 (a set of signals, 10H, - CH_2 of C_6H_{11}), 2.348 (s, 3H, - CH_3), 4.283 (m, 1H, -CH of C_6H_{11}), 7.041-7.843 (a set of signals, 8H, Ar-H), 7.327 (d, 1H, C-NH-CS, D_2O exchangeable), 7.875 (s, 1H, -CH=N-), 9.813 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 368.17 ($\text{M}^+ + 1$, 100).

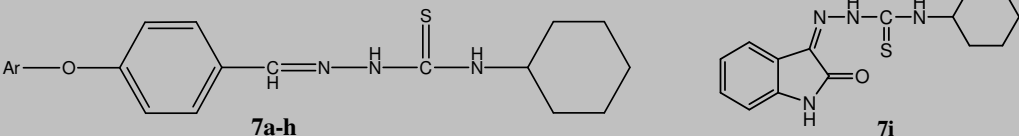
Cyclohexyl-[4-(4-chlorophenoxy)benzylidene] thiosemicarbazide (7d): IR (KBr, ν_{\max} , cm^{-1}): 3316 (-NH-), 2925, 2851 (CH_{str} of C_6H_{11}), 1598 (-N=CH-), 1249 (-O-), 1161 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.23-2.18 (a set of signals, 10H, - CH_2 of C_6H_{11}), 4.281 (m, 1H, -CH of C_6H_{11}), 7.043-7.841 (a set of signals, 8H, Ar-H), 7.328 (d, 1H, C-NH-CS, D_2O exchangeable), 7.871 (s, 1H, -CH=N-), 9.817 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 388.12 ($\text{M}^+ + 1$ for ^{35}Cl , 100.00), 390.10 ($\text{M}^+ + 1$ for ^{37}Cl , 35.1).

Cyclohexyl-[4-(4-bromophenoxy)benzylidene] thiosemicarbazide (7e): IR (KBr, ν_{\max} , cm^{-1}): 3317 (-NH-), 2921, 2853 (CH_{str} of C_6H_{11}), 1597 (-N=CH-), 1246 (-O-), 1163 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.228-2.178 (a set of signals, 10H, - CH_2 of C_6H_{11}), 4.283 (m, 1H, -CH of C_6H_{11}), 7.041-7.846 (a set of signals, 8H, Ar-H), 7.322 (d, 1H, C-NH-CS, D_2O exchangeable), 7.873 (s, 1H, -CH=N-), 9.815 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 434.10 ($\text{M}^+ + 1$ for ^{81}Br , 100.00), 432.07 ($\text{M}^+ + 1$ for ^{79}Br , 98.3).

Cyclohexyl-[4-(4-fluorophenoxy)benzylidene] thiosemicarbazide (7f): IR (KBr, ν_{\max} , cm^{-1}): 3317 (-NH-), 2924, 2852 (CH_{str} of C_6H_{11}), 1601 (-N=CH-), 1247 (-O-), 1163 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.228-2.181 (a set of signals, 10H, - CH_2 of C_6H_{11}), 4.283 (m, 1H, -CH of C_6H_{11}), 7.041-7.843 (a set of signals, 8H, Ar-H), 7.325 (d, 1H, C-NH-CS, D_2O exchangeable), 7.873 (s, 1H, -CH=N-), 9.815 (s, 1H, -NH=N-, D_2O exchangeable); MS (m/z , %): 372.16 ($\text{M}^+ + 1$, 100).

Cyclohexyl-[4-(3-methyl-4-chlorophenoxy)benzylidene]-thiosemicarbazide (7g): IR (KBr, ν_{\max} , cm^{-1}): 3311 (-NH-), 2927, 2857 (CH_{str} of C_6H_{11}), 1599 (-N=CH-), 1247 (-O-), 1163 (-C=S); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ in ppm: 1.229-2.153 (a set of signals, 10H, - CH_2 of C_6H_{11}), 2.359 (s, 3H, - CH_3), 4.281

TABLE-1
PHYSICAL AND ELEMENTAL ANALYSIS DATA OF CYCLOHEXYL-[4-SUBSTITUTED
BENZYLIDENE/2-OXO-1,2-DIHYDROINDOL-3-YLIDENE]THIOSEMICARBAZIDES



Compound	Ar	Yield (%)	m.p. (°C)	m.f.	m.w.	Elemental analysis % Found (calcd.)		
						C	H	N
7a		65	122	C ₂₀ H ₂₃ N ₃ OS	353.0	67.89 (67.96)	6.59 (6.6)	11.78 (11.8)
7b		68	150	C ₂₀ H ₂₂ N ₄ O ₃ S	398.49	60.29 (60.3)	5.61 (5.6)	13.98 (14.0)
7c		72	136	C ₂₁ H ₂₅ N ₃ OS	367.5	68.59 (68.6)	6.76 (6.8)	11.38 (11.4)
7d		70	228	C ₂₀ H ₂₂ ClN ₃ OS	387.9	61.87 (61.9)	5.69 (5.7)	10.77 (10.8)
7e		74	218	C ₂₀ H ₂₂ BrN ₃ OS	432.4	55.59 (55.6)	5.1 (5.1)	9.68 (9.7)
7f		75	157	C ₂₀ H ₂₂ FN ₃ OS	371.4	64.57 (64.6)	5.88 (5.9)	11.28 (11.3)
7g		72	215	C ₂₁ H ₂₄ ClN ₃ OS	401.9	62.67 (62.7)	6.01 (6.0)	10.38 (10.4)
7h		70	181	C ₂₄ H ₂₅ N ₃ OS	403.5	71.4 (71.4)	6.21 (6.2)	10.38 (10.4)
7i	-	77	208	C ₁₅ H ₁₇ N ₄ OS	301.4	59.66 (59.7)	5.68 (5.7)	18.59 (18.6)

(m, 1H, -CH of C₆H₁₁), 7.051-7.841 (a set of signals, 7H, Ar-H), 7.326 (d, 1H, C-NH-CS, D₂O exchangeable), 7.873 (s, 1H, -CH=N-), 9.811 (s, 1H, -NH=N-, D₂O exchangeable); MS (m/z, %): 402.12 (M⁺ + 1 for ³⁵Cl, 100.00), 404.15 (M⁺ + 1 for ³⁷Cl, 35.7).

Cyclohexyl-[4-(2-naphthoxy)benzylidene] thiosemicarbazide (7h): IR (KBr, ν_{max}, cm⁻¹): 3313 (-NH-), 2928, 2851 (CH_{str} of C₆H₁₁), 1597 (-N=CH-), 1249 (-O-), 1164 (-C=S), 836, 821 (β-naphthyl); ¹H NMR (CDCl₃, 300 MHz) δ in ppm: 1.208-2.139 (a set of signals, 10H, -CH₂ of C₆H₁₁), 4.298 (m, 1H, -CH of C₆H₁₁), 7.048-7.861 (a set of signals, 11H, Ar-H), 7.335 (d, 1H, C-NH-CS, D₂O exchangeable), 7.876 (s, 1H, -CH=N-), 9.815 (s, 1H, -NH=N-, D₂O exchangeable); MS (m/z, %): 404.33 (M⁺ + 1, 86).

Cyclohexyl-[1-(2-oxindolin-3-ylidene)] thiosemicarbazide (7i): IR (KBr, ν_{max}, cm⁻¹): 3311 (-NH-), 3147 (NH of isatin), 1687 (C=O), 1619 (C=N), 1167 (C=S); ¹H NMR (CDCl₃, 300 MHz) δ in ppm: 1.246-2.178 (a set of signals, 10H, -CH₂ of C₆H₁₁), 4.324 (m, 1H, -CH of C₆H₁₁), 6.927-7.609 (a set of signals, 4H, Ar-H), 7.67 (d, 1H, C-NH-CS, D₂O exchangeable), 8.118 (s, 1H, -NH=N-, D₂O exchangeable), 12.601 (s, 1H, NH of isatin, D₂O exchangeable); MS (m/z, %): 303.15 (M⁺ + 1, 100).

Anticonvulsant and neurotoxicity evaluation: Male albino mice (CF-1 strain, 18-25 g) were used as experimental

animal. The animals were housed in metabolic cages and allowed free access to food and water. The synthesized derivatives were suspended in 0.5 % methyl cellulose/water mixture or in polyethylene glycol (PEG 200). The test compound is usually manipulated with a mortar pestle to help preparation of suspension. In the preliminary screening each compound was administered as an i.p., injection at three dose levels (30, 100 and 300 mg/kg) and anticonvulsant and neurotoxic effects were assessed at 0.5 h and 4 h intervals after administration. Anticonvulsant efficacy was measured by MES and scMET tests and the data are presented in Table-2. The preliminary animal research study was carried out according to the protocols approved by the institutional animal ethical committee, S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar (Reg No. 876/AC/05/CPCSEA) and advanced anticonvulsant screening were carried out at Epilepsy Branch, National Institute of Neurological Disorder and Stroke, National Institute of Health, Bethesda, USA.

RESULTS AND DISCUSSION

In the present study, cyclohexyl-[4-substituted benzylidene/2-oxo-1,2-dihydro-indol-3-ylidene] thiosemicarbazides have been synthesized by refluxing cyclohexyl thiosemicarbazide with various 4-substituted benzaldehyde/isatin in the presence

TABLE-2
ANTICONVULSANT AND NEUROTOXIC ACTIVITY OF THE CYCLOHEXYL-[4-SUBSTITUTED
BENZYLIDENE/2-OXO-1,2-DIHYDROINDOL-3-YLIDENE]THIOSEMICARBAZIDES (**7a-7i**)

Compounds	Intraperitoneal injection in mice*					
	MES screen (h)		scMET screen (h)		Neurotoxicity screen (h)	
	0.5	4.0	0.5	4.0	0.5	4.0
7a	–	–	–	–	–	–
7b	–	–	–	–	–	–
7c	–	–	–	–	–	–
7d	–	100**	–	–	–	–
7e	–	–	–	–	–	–
7f	–	–	–	–	–	–
7g	–	–	–	–	–	–
7h	–	–	–	–	–	–
7i	–	300**	–	–	–	–
Phenytoin	30	30	–	–	100	100
Sodium valproate	–	–	300	–	–	–

*Dose of 30, 100 and 300 mg/kg were administered. The figure in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more mice. The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg). **In the MES screen compounds **7d** and **7i** showed protection (1/1, 4 h) at a dose of 100 and 300 mg/kg, respectively.

of catalytic amount of glacial acetic acid. All compounds gave satisfactory elemental analysis (Table-1). IR, ¹H NMR and mass spectra were consistent with the assigned structures.

All the thiosemicarbazones were evaluated for anti-convulsant activity in maximal electroshock induced seizure (MES) and subcutaneous metrazol (scMET) induced seizure models in mice using doses of 30, 100, 300 mg/kg. The observation was carried out at two different time intervals (0.5 and 4 h). The pharmacological test results were shown in Table-2. Compounds cyclohexyl-[4-(4-chlorophenoxy)-benzylidene]thiosemicarbazide (**7d**) and cyclohexyl-[2-oxo-1,2-dihydro-indol-3-ylidene]thiosemicarbazide (**7i**) showed 100 % protection (1/1, 4.0 h) at a dose of 100 and 300 mg/kg, respectively in MES test, indicating the compounds ability to prevent seizure spread. All the thiosemicarbazones exhibited no neurotoxicity at the highest administered dose (300 mg/kg) in rotorod method.

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