

Synthesis and Evaluation of Some Novel N, N-Dialkylaminoalkoxy-2-Oxo-Indole-3-Ylidene Benzohydrazides as Anticonvulsant Agents

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Abstract: A series of N,N-dialkylaminoalkoxy-2-Oxo-indole-3-ylideneBenzohydrazides were synthesized by using the well designed synthetic route and the purity of the compounds were assessed by TLC, and characterized by FT-IR, NMR spectral data. The anti-convulsant activities of the synthesized compounds were evaluated against MES and PTZ induced seizure models in mice. All the test compounds were administered at doses of 50mg/kg body weight orally for MES method. The stimulus parameters in mice were 50mA in a pulse of 60Hz for 200ms. Intraperitoneally for a PTZ method from the series of compounds it was noted that compound N,N-dimethylaminoPropoxy-2-Oxo-5-methyl-3-ylidenebenzo hydrazides(VIIb) protected more than 80% of the mice against MES and PTZ induced seizures compound VIId, VIIa, VIIe were found to be next in the order of reducing the duration of convulsions in both MES and PTZ methods. Molecular docking studies were also carried out by using windows 2007 using Moe 2008.10 version. The 5HT_{2c} mouse receptor was retrieved from the protein data bank and used as target receptor was retrieved from the protein data bank.

Keywords: Anticonvulsant activity, Benzo hydrazides, Maximal electroshock, Pentylenetetrazole, 5-HT_{2c} receptors, Molecular docking studies

I. Introduction

Epilepsy is the most common neurological disorder after a migraine, stroke, Alzheimer's disease¹ affecting 50 million individual worldwide. The average incidence of epilepsy each year in the U.S is estimated as 150,000 or 48 for every 100,000 people^{2,3}. The word epilepsy is derived from the Greek word epilambanien meaning to attack or seize. Epilepsy is not a disease, but a syndrome of different cerebral disorders of the central nervous system. This syndrome is characterized by paroxysmal, excessive, and hypersynchronous discharges of large number of neurons these discharges disrupt normal brain function by creating fluctuations in electrochemical balances^{4,5} epilepsy treatment is still one of the major problems in medicine because of the uncontrolled seizures in some types of epilepsia, toxicity of medication, several side effects of currently used drugs, which may limit their maximal usefulness, obligation of administration of some anti-epileptics used symptomatically in the treatment of epilepsies for a long time even throughout one's life as they have to be used chronically, and also the risk of tolerance developing against the present drugs^{6,7}. Although there are a number of antiepileptic drugs available in the market, because of above mentioned reasons, development of new compounds having anticonvulsant activity is still necessary⁸⁻¹¹

Isatin (1H-indole-2,3-dione,) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids^{13,14}. Isatin (1H-indole-2, 3-dione) pharmacophore has attracted and has been attracting much attention from medicinal chemists because of great importance in their biological as well as synthetic approach of medicinal chemistry. The various derivatives of isatin are known to possess range of biological properties including antimicrobial^{15,16}, anticancer¹⁷, antiviral¹⁸, anticonvulsant^{19,20}, antidepressant²¹, anti-inflammatory and analgesic²². Surendranath pandeya^{23,24} et al, reported the synthesis and anti-convulsant activity of some novel n-methyl/acetyl, 5-unsubstituted isatin-3- semicarbazones²⁵ olesen and kanstrup reported synthesis and anti-convulsant activity of pyrido [2, 3-b] indoles²⁶ Evano et al synthesized and reported anti-convulsant activity of 1H-pyrido [3, 4-b] indole-4-carboxamide derivatives²⁷. In the last few years, new isatin derivatives have been discovered which show potential hypnotic²⁸, anti-bacterial²⁹ and MAO inhibitory³⁰ activity. Binedaline, amedalin, pindolol, siramesine and oxypertine etc are the marketed drugs bearing indole moiety used against different types of CNS disorders and which encouraged authors to further explore the pharmacological properties of the indole nucleus³⁰ Literature survey reveals that benzohydrazide exhibit wide variety of biological activities such as anti-microbial activity³¹, Hypnotic³² and anti-convulsant³³.

Keeping in view of biological importance of the two molecules, namely isatins with benzohydrazide has been felt worthwhile as depicted in scheme. It is evident from the literature survey that isatin derivatives, and benzo hydrazide derivatives showing more promising effects central nervous system. Keeping in view of these two molecular moieties into a single molecular frame as a model for molecular conjunction by appropriate synthetic routes and to screen them for anti-convulsant activity and neurotoxicity. The objective of the present study was to design a synthetic scheme to carry out molecular conjunction between isatin and benzohydrazide derivatives and to screen the resultant novel derivatives for anticonvulsant activity using *in vitro* docking and *in vivo* studies.

II. Materials And Methods

The compounds were mostly synthesized by conventional synthetic methods and as described in experimental section and also by the methods established in our laboratory.

2.1. Materials: Leptazole, Diazepam, Dialkylaminoalkylhalides Purchased From Sigma Aldrich Chemicals Private Limited, Hyderabad, India, Hydroxyl Amine Hydrochloride, Dry Methanol (HPLC Grade), Hydrazine Hydrate Were Purchased From Merck Chemicals Private Limited Hyderabad, India

2.2. Chemistry:

All the Solvents employed in the study were dried or distilled before use. Melting points were obtained on a thoshniwall melting point apparatus in open capillary tubes and were uncorrected. The purity of the compounds were ascertained by TLC on silicagel-G plates (merck) Infrared spectra (IR) were recorded with KBr pellet using Perkin-Elmer BX series, Infrared spectrophotometer, ¹H-NMR spectra were recorded on Bruker spectrometer 400MHz spectrophotometer in DMSO-d⁶

2.2.1. Synthesis of Indole-2,3-diones (Isatins, III)

Isatin was synthesised by using sandmeyer method and it consists reaction of aniline (I) with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulphate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulphuric acid, furnishes isatin in > 75% overall yield³⁴

Isonitrosoacetanilides (II): In a 5lit. RB flask, chloral hydrate (0.54M) and 1200mL of water were placed. To this solution, crystallized sodium sulphate (1300g) was then added followed by a solution of an appropriate aromatic aniline (I) in 300ml of water and concentrated hydrochloric acid (0.52M). Finally, a solution of hydroxylamine HCl (1.58mol) in 500mL of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried, and purified by recrystallization from Methanol(s).

Indole-2,3-diones (III): Sulphuric acid (600g, d, 1.84, 326mL) were warmed at 50°C in a one liter RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (II, 0.46M) was added at such a rate so as to maintain the temperature between 60°C to 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of iso nitroso compound the temperature of the solution was raised to 80°C and maintained the same for 10 minutes, to complete the reaction. Then the reaction mixture was cooled to room temperature and poured on to crushed ice (2.5kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water, and dried. Purification of the compound was affected by the recrystallization from methanol.

2.2.2. Preparation of 4-hydroxy benzohydrazides (V):

In 500ml RBF The mixture of methyl paraben (IV) (0.02 mol) and hydrazine hydrate (99%), (30 mL, 0.6 mol) was refluxed for 12 h. The excess solvent was removed under vacuum up to 10 mL. The reaction mixture was cooled at 4-5°C. The separated solid crystals (2) were filtered and washed with cold water. The crystals were dried and purified by recrystallization from ethanol (99.9%). Yield: 90% .M.P. 246-248°C.

2.2.3. Preparation of N,N-Dialkyl aminoalkoxy benzohydrazides (VI):

A mixture of (0.1 moles) 4-hydroxy benzohydrazide (V) (0.12mol) alkyl halide in 60ml alcoholic KOH refluxed for 24hr at room temperature. The reaction mixture was filtered and filtrate taken into benzene, washed with water and with Hydrochloric acid. combine aqueous acid extracts and were basify with ammonium hydroxide and extracted with chloroform the organic extracts are dried over calcium sulphate and the product recrystallized from ethyl acetate. % yield: 80, melting point: 230-232°C

2.2.4. Preparation of N,N-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazides (VII):

A mixture of an appropriate indole-2,3-dione (III, 0.01mol.) Dialkyl aminoalkoxy benzohydrazides (IV, 0.01mol.) In dry methanol (50ml) was heated under reflux for 30 min. mixture was filtered and dried and purified by methanol.

The physical data of the compounds were presented in table-1.

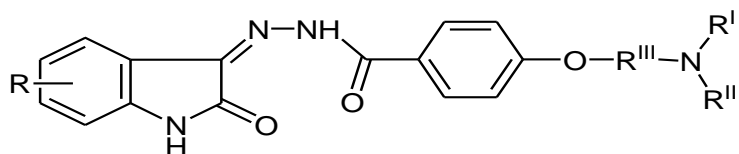
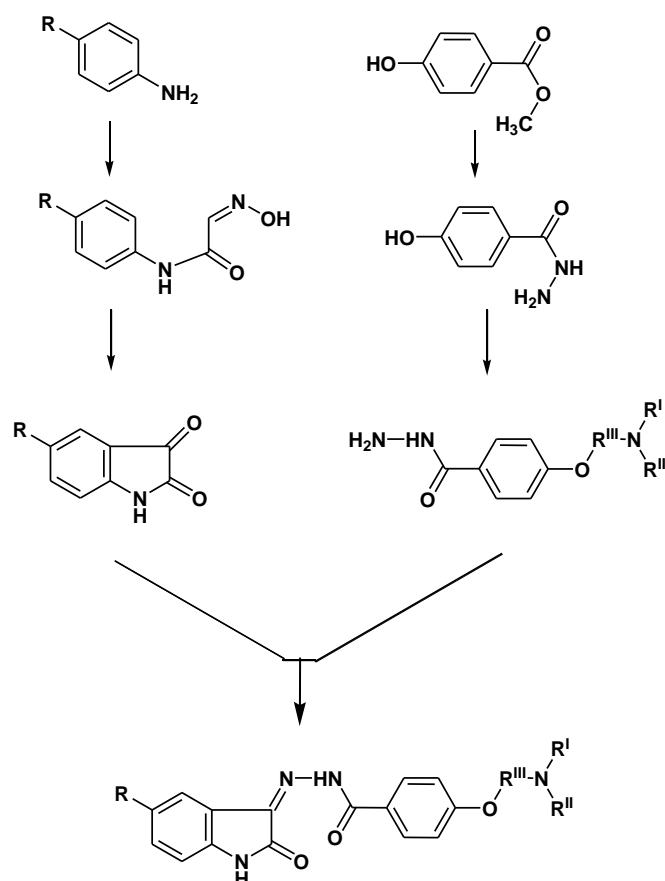


TABLE 1: Physical data of the *N,N*-dialkylaminoalkoxy-2-oxo-indole-3-ylidenebenzohydrazides

S.NO.	Compound	R	R'R''	R'''	Mol. Formula	Mol.Wt	M.p(°c)	%yield
1	VII	-H	-CH ₃	Ethyl	C ₁₉ H ₂₀ N ₄ O ₃	352.4	272-274	81
2	VIIa	-H	-CH ₃	Propyl	C ₂₀ H ₂₂ N ₄ O ₃	366.47	280-282	82
3	VIIb	-CH ₃	-CH ₃	Propyl	C ₂₁ H ₂₄ N ₄ O ₃	380.4403	284-286	81
4	VIIc	-Cl	-CH ₃	Propyl	C ₂₀ H ₂₁ ClN ₄ O ₃	400.8587	262-264	80
5	VIIId	-Br	-CH ₃	Propyl	C ₂₀ H ₂₁ BrN ₄ O ₃	430.27	236-238	85
6	VIIe	-F	-CH ₃	Propyl	C ₂₀ H ₂₁ FN ₄ O ₃	384.1	232-234	75
7	VIIIf	-H	-CH ₃	Isopropyl	C ₂₀ H ₂₂ N ₄ O ₃	366.47	280-282	80
8	VIIg	-CH ₃	-CH ₃	Isopropyl	C ₂₁ H ₂₄ N ₄ O ₃	380.44	252-254	83
9	VIIh	-Cl	-CH ₃	Isopropyl	C ₂₀ H ₂₁ ClN ₄ O ₃	400	242-246	82
10	VIIi	-Br	-CH ₃	Isopropyl	C ₂₀ H ₂₁ BrN ₄ O ₃	430	278-280	81
11	VIIj	-F	-CH ₃	Isopropyl	C ₂₀ H ₂₁ FN ₄ O ₃	384	236-238	81



Isatin Derivatives :

VIIa : R-H, R',R''-CH₃, R''' - Propyl

VIIb : R-CH₃, R',R''-CH₃, R''' - Propyl

VIIc : R-Cl, R',R''-CH₃, R''' - Propyl

VIIId : R-Br, R',R''-CH₃, R''' - Propyl

VIIe : R-F, R',R''-CH₃, R''' - Propyl

VIIIf : R-H, R',R''-CH₃, R''' - Isopropyl

VIIg : R-CH₃, R',R''-CH₃, R''' - Isopropyl

VIIh : R-Cl, R',R''-CH₃, R''' - Isopropyl

VIIi : R-Br, R',R''-CH₃, R''' - Isopropyl

VIIj : R-F, R',R''-CH₃, R''' - Isopropyl

Scheme 1: Schematic steps of *N,N*-dialkylaminoalkoxy-2-oxo-indole-3-ylidenebenzohydrazides

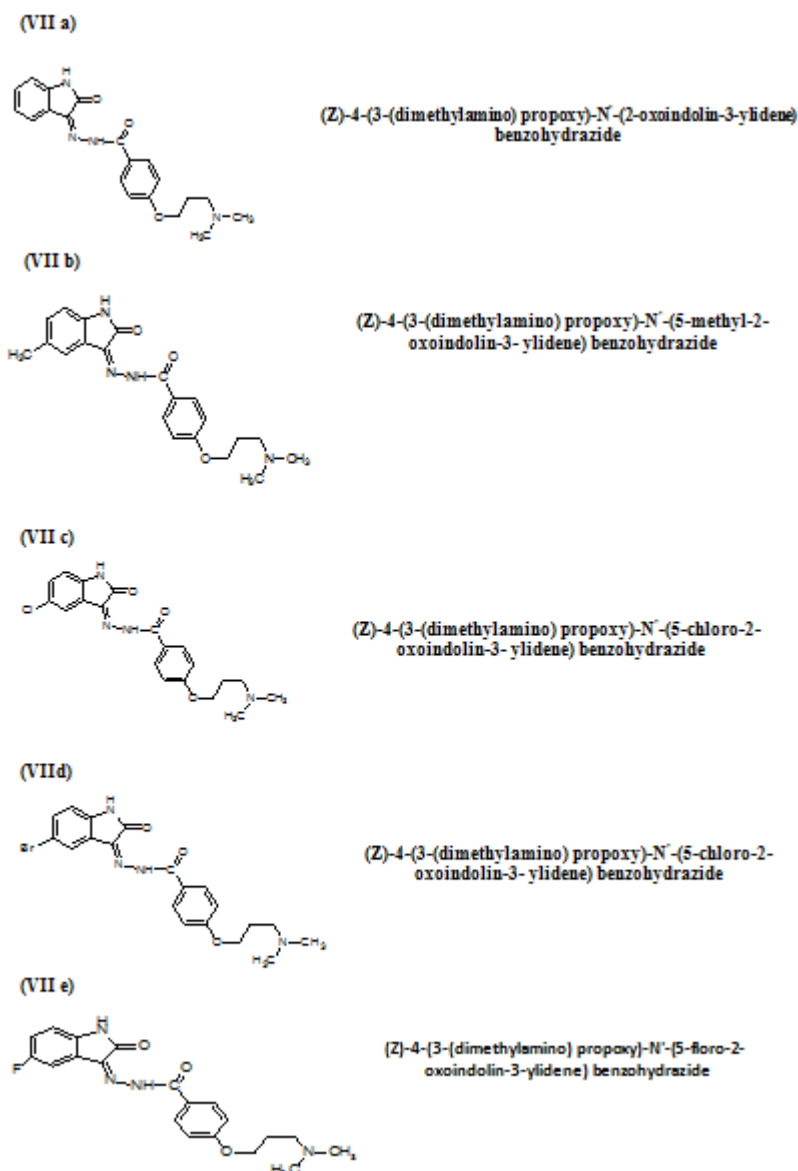


Figure1: structures of the substituted *N, N*-dialkylaminoalkoxy-2-oxo-indole-3-ylidenebenzohydrazide

2.3. Spectral data:

(Z)-4-(3-(dimethylamino) ethoxy)-*N'*-(5-chloro-2-oxoindolin-3-ylidene)benzohydrazide(VII)

¹H NMR(300 mHz, DMSO): δ [ppm];2.3(S,6H-C₂H₆),6.9-7.7(M,8H, Ar-H),10.5(S, 1H -CONH), and 13.8(S, 1H,N-H) IR(Kbr)cm⁻¹: 3189(N-H), 1676,1626(C=O),1608(C=C),1537(C=N),1466(C-H bending), 1268(C-O,Ar-O-C)

(Z)-4-(3-(dimethylamino)propoxy)-*N'*-(2-oxoindolin-3-ylidene)benzohydrazide (VIIa) :

IR(Kbr)cm⁻¹: 3189(N-H), 1676,1626(C=O),1608(C=C),1537(C=N),1466(C-H bending), 1268(C-O,Ar-O-C)

(Z)-4-(3-(dimethylamino)propoxy)-*N'*-(5-methyl-2-oxoindolin-3-ylidene)benzohydrazide (VIIb) :

IR(Kbr)cm⁻¹: 3186(N-H), 1676,1626(C=O),1603(C=C),1549(C=N),1484(C-H bending), 1262(C-O,Ar-O-C)

(Z)-4-(3-(dimethylamino)propoxy)-*N'*-(5-chloro-2-oxoindolin-3-ylidene)benzohydrazide (VIIc) :

IR(kbr)cm⁻¹: 3317, 3197(N-H),1618(C=O), 1538(C=N),1467(C-H bending), 1278(C-O,Ar-O-C)

(Z)-4-(3-(dimethylamino)propoxy)-*N'*-(5-bromo-2-oxoindolin-3-ylidene)benzohydrazide (VII d) :

IR(Kbr)cm⁻¹: 3214, 3197(N-H),1684(C=O), 1538(C=N),1472(C-H bending), 1262(C-O,Ar-O-C)

(Z)-4-(3-(dimethylamino)propoxy)-*N'*-(5-methyl-2-oxoindolin-3-ylidene)benzohydrazide (VIIe) :

IR(Kbr)cm⁻¹: 3425(N-H),1597(C=O), 1544(C=N),1422(C-H bending), 1253(C-O,Ar-O-C)

III. Anticonvulsant Activity

It is evident from the literature that isatins exhibits anti-convulsant, psychotropic and angiogenic, properties therefore, it has been worthwhile to screen the new isatin-benzohydrazide derivatives for CNS activity. All the synthesised isatins were subjected to the following screening tests

3.1. Acute toxicity:

Healthy and adult male albino mice weighing between 20-25g were used in the investigation. Animals were fasted for 24 hr and divided into groups of six animals each. The test compounds, dissolved in DMSO were administered, orally, at the dose of 5mg to 300mg per kg(b.w.) The controlled group of animals received only the vehicle(DMSO) the animals were observed for 48hr from the time of administration of the test compound to record the mortality.

3.2. Locomotor activity:

The locomotor activity was studied by using actophotometer, which operates on photoelectric cells, which are connected in circuit with a counter. When a beam of light falling on the photocells is cut of by animals, a count was recorded. Healthy mice weighing between 20-25gm were used. Animals were fasted for overnight and divided into groups of six animals each. The test compounds were dissolved in DMSO and administered at a dose of 50mg/kg body weight orally and the control group animals received only vehicle (DMSO) the response (counts) was recorded after 60 min. administration of test compound. The animals were placed in actophotometer for 10min .and scores were recorded(deflections)the results were compared with the control and presented in table-2:

All the test compounds were administered in a dose of 50 mg/kg (b.wt) i.p. and motor activity in an open field was determined data are expressed as the number of movements per minute(mean) percentage decrease in activity was calculated

Table 2: Readings of actophotometer:

Compounds	Before administration of compound (10 min.)	After administration of compound (10 min.)	% change in activity
VIIa	462.00	185.12	59.93
VIIb	455.62	127.29	72.00
VIIc	447.12	161.92	63.78
VIIId	470.32	147.65	68.60
VIIe	442.63	210.55	52.43

n= 6 in each group



Figure 2: Locomotor activity of isatin derivatives

3.3. In vivo Anti-Convulsant Activity:

3.3.1. Maximal electroshock induced convulsions:

The anti-convulsant activity was studied by using electroconvulsometer. Healthy male mice weighing between 20-25gm, were fasted for overnight and divided into six groups of six animals each . The test compounds suspended in 0.1% sodium CMC were administered at a dose of 50 mg/kg body weight orally. The control group animals received only vehicle (DMSO). The test started 30 min after oral administration.

Maximal seizures were induced by the application of electrical current to the brain via corneal electrodes. The stimulus parameters for mice were 50mA in a pulse of 60Hz for 200ms. Abolition of the hind limb tonic extensor spasm was recorded as a measure of anti convulsant activity^{37,38} results are presented in table-3

Table 3: Anti-convulsant activity of N,N-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazides against MES-induced convulsions:

Compounds	Substituent R	Duration of tonic hind limb extensor phase (in secs.).n=6	Animals protected in %
VIIa	H	37.2±12	68.7
VIIb	5-CH ₃	24.5±3	83.19
VIIc	5-Cl	29.7±8	73.40
VIIId	5-Br	26.2±6	76.6
VIIe	5-F	42.6±3	61.19
Control		93.3	0
Phenytoin ^c		0	100

n=six in each group.

All animals are recovered

The compounds were tested at a dose of 50mg/kg(oral)

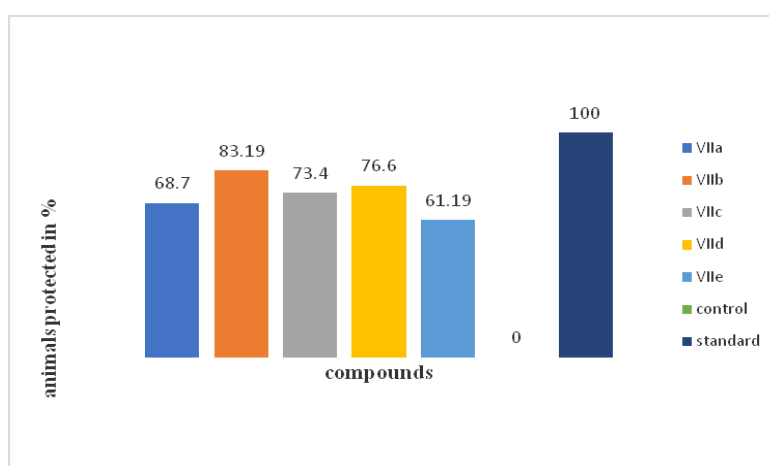


Figure 3: Anticonvulsant activity of N,N-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazides against MES-induced convulsions

3.3.2. Pentylentetrazole induced convulsions:

Animals were divided into three groups each comprising of 6 animals in each group one group was used for studying the effects of pentylentetrazole alone (control) and other groups for studying the protective effect of phenobarbitone(standard) as well as studying the anti-convulsant effect of test compounds. Injected pentylentetrazole to control and noted the onset of action (indicated by straub's tail, jerky movements of whole body and convulsions) and severity of convulsions due to the drug.

injected phenobarbitone to second group of animals and test compounds to third group of animals after 30 min. Injected pentylentetrazole to these animals which received phenobarbitone and test compounds.

Noted either delay or complete abolition of convulsions in mice treated with phenobarbitone and test compounds³⁹. And results were presented in table-4

Table-4 Anticonvulsant activity of N,N-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazides

Compounds	Substituent R	Duration of tonic hind limb extensor phase (in secs.). n=6	Animals protected in %
VIIa	H	8.5±2	70.8
VIIb	5-CH ₃	3.5±1.2	85.16
VIIc	5-Cl	6.6±2	72.8
VIIId	5-Br	5.7±3	78.7
VIIe	5-F	8.8±2.2	68.16
Control		21.23±5	0
Phenobarbitone ^c		0	100

All animals are recovered

The compounds were tested at a dose of 50mg/kg(oral)

n=six in each group.

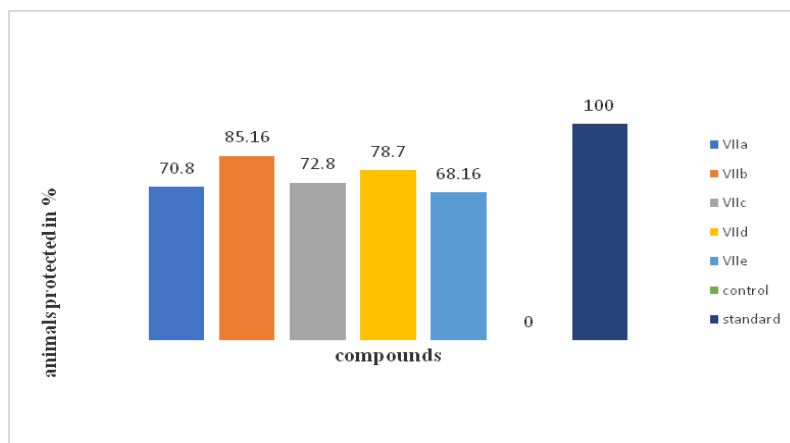


Figure 4: Anticonvulsant activity of *N,N*-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazides

3.4. *In vitro* studies: Molecular Docking:

Docking was performed on windows 2007 using MOE 2008.10 version. 5HT_{2C} mouse receptor was retrieved from the protein data bank (PDB code: 2MHO) and the enzyme was visualized using sequence option and further co-factors were deleted. The partial charge of the protein was adjusted with the help of force field method AMBER 99. All the synthesised compounds were docked into the 5-HT₂ receptor as it is evident from the literature that 5-HT₂ receptor is one of target for isatins. Docking study results were presented in the table-5. Compounds VIIb and VIId showed good docking scores with 5-HT₂ receptor. The compounds VIIc, VIIa, VIIe were shown least scores with 5-HT₂ receptor agents. Because of good binding scores 76.9, 27.2 for compound VIIb and 77.2 for compound VIId with Lys-392 receptor residue. Same compounds also showed good *invivo* activity. And hence compounds VIIb, VIId were considered as good anti-convulsant.

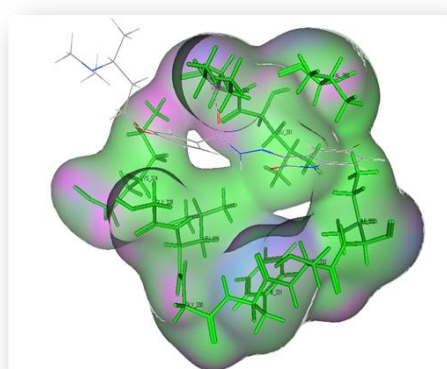
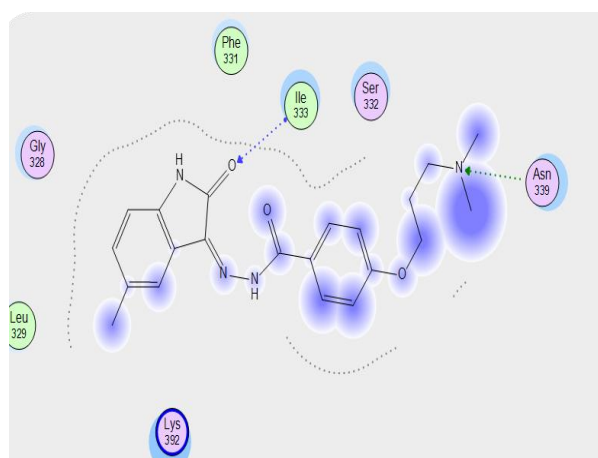


Figure 5: Docking studies of (Z)-4-(3-(dimethylamino)propoxy)-*N*'-(5-methyl-2-oxoindolin-3-ylidene) benzohydrazide(VIIb) on 5-HT_{2C} receptors

Table: 5 Docking Scores of Compounds With 5-HT_{2c}receptor

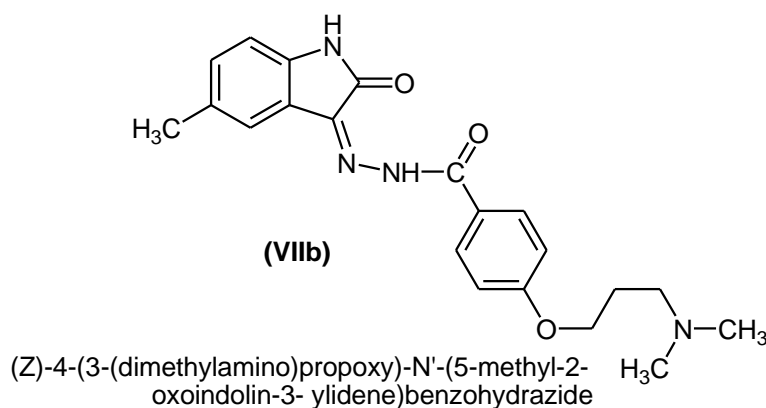
Compounds	Ligand	Receptor Residue	Chain Type	Score	Distance
VIIa	O 1429 NZ 1156	LYS 392	H-acc	71.3%	2.67
VIIb	H 1422 O 200	LYS 327	H-don	20.2%	1.98
	O 1428 NZ 1156	LYS 392	H-acc	27.4%	3.03
	O 1428 NZ 1156	LYS 392	H-acc	76.9%	2.44
VIIc	O 1428 NZ 1156	LYS 392	H-acc	48.1%	2.58
VIId	H1422 O 200	LYS327	H-don	19.3%	1.97
	N1424 NZ1156	LYS392	H-acc	26.6%	3.06
	O 1428 NZ1156	LYS392	H-acc	77.1%	2.44
VIIe	O 1423 NZ 1156	LYS392	H-acc	36.0%	2.50

IV. Results And Discussions

Novel isatin derivatives were synthesised by condensation of isatins with alkylated benzohydrazides in the presence of methanol. Details were given in scheme and Structures of all synthesised compounds were given in figure-1, Physical data were shown in table-1.

4.1. In-vivo anti-convulsant activity:

All synthesised compounds were evaluated for in-vivo anti-convulsant activity at the dose of 50 mg/kg by chemically induced convulsions and electrically induced by PTZ method and MES method. From the data it was revealed that all the tested compounds significantly reduced chemically induced convulsions and electrically induced convulsions. And the results were presented in table-3 and 4. Among the tested compounds, VIIb (R-CH₃) protected more than 80% of the mice against MES and PTZ induced convulsions. Compounds VIId, VIIc, VIIa, VIIe were found to be next in the order of reducing the duration of convulsions in both MES and PTZ methods. Interestingly, compounds with electron releasing substituents (R-CH₃) at C-5 of isatin nucleus produced 80% protection against MES and PTZ induced convulsions. Compounds VIIc(R-Cl), VIId(R-Br), VIId(R-F), VIIe(R-H) exhibited substantial activity with 78.7%, 72.8%, 68.9%, 70.8%. Compounds with electron releasing substituents (R-CH₃) at C-5 of isatin nucleus produced more anti-convulsant activity. Whereas compounds with electron withdrawing substituents such as (R-Cl, Br, F) at C-5 of isatin nucleus are next in the order of activity.



4.2. Docking-studies:

Docking was performed on windows 2007 using MOE 2008.10 version. 5HT_{2c} mouse receptor was retrieved from the protein data bank (PDB code: 2MHO) and the enzyme was visualized using sequence option and further co-factors were deleted. The partial charge of the protein was adjusted with the help of force field method AMBER 99. Later, the protein was subjected to 3D protonation at cut off 12.0, and further hydrogen was added according to standard geometry and the receptor was energy minimized using force field MMFF94x at 0.01 KJ mole gradients. After obtaining docking results, best pose was retained to understand molecular interaction out of 30 poses given for each chemical structure. The resultant best pose score values in the series

were used for analysis of docking and interaction. All the synthesised compounds were docked into the 5-HT_{2c} receptor and the results were presented in the table -5 Compounds VIIb and VIId showed good docking scores with 5-HT_{2c} receptor and these compounds also shown good iv-vivo anti-convulsant activity. The compounds VIIc, VIIa, VIIe were shown least scores with 5-HT_{2c} receptor. Compounds VIIb, VIId are considered as good anti-convulsant agents.

V. Conclusion

Present study described the synthesis and characterization of a novel NN-dialkylaminoalkoxy-2-oxo-indole-3-ylidene benzohydrazide derivatives. Among the tested compounds, VIIb (R-CH₃) has protected more than 80% of the mice against MES and PTZ induced convulsions. Compounds VIId, VIIc, VIIa and VIIe were found to be next in the order of reducing the duration of convulsions in both MES and PTZ methods. Compounds having electron donating groups at C-5 of isatin nucleus seemed to be necessary in providing higher anticonvulsant activity. Other substituents at various positions are next in the order of activity. It is evident from the anticonvulsant activity that electron donating groups seemed to be necessary moieties in showing higher anticonvulsant activity and *in vitro* docking studies have confirmed the same.

Acknowledgement

Authors are thankful to Dr Narendra Sharat Chandra for his guidance and kind support in carrying out docking studies.

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