Screening of semicarbazones as anticonvulsant agents

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Abstract

Objective: The principal objective of the present investigation was the preparation of several analogs to further evaluate the binding site hypothesis. Aryl semicarbazides have also been reported to display excellent anticonvulsant activity in mice and rats.

Method: In this project, the synthesis of semicarbazone derivatives was carried out. All molecules were synthesized using the common starting material –aniline. In all compounds, an intermediate was first formed by substituted phenyl urea using substituted aniline and potassium cyanate, and then it was hydrolyzed to get substituted phenyl semicarbazide, which was directly coupled with ketones. All the synthesized compounds were biologically screened for their anticonvulsant activity by MES method.

Result: Standard error mean was calculated with the respect of standard and control drug, Phenytoin sodium (25mg/kg.) and DMSO. The synthesized semicarbazone were characterized by using IR Spectroscopy. One another representative molecule compound was characterized using 1H NMR Spectroscopy.

Conclusion: It can be concluded that designed semicarbazones were synthesized and characterized successfully. After synthesis of designed semicarbazones compounds were evaluated for anticonvulsant activity. Finally, two compounds have shown better activity in comparisons to the other molecules.

Keywords

Semicarbazone • NMR Spectroscopy • Anticonvulsant activity • SEM

Introduction

Epilepsy is a central nervous system (CNS) malfunction that leads either to generalized hyper activity involving essentially all parts of the brain or to hyper activity of only a portion of the brain. Epilepsy is a collective term that includes over 40 different types of human seizures disorders. Approx 1% of world population at any one time (>50 million people worldwide) is afflicted with this serious neurological disorders. Although the current drug provide adequate seizure control in many patients, it is roughly estimated that up to 28-30% of patients are poorly treated with the available anti epileptic drugs (AED's). Moreover, many (AED's) have serious side effects and lifelong medication may be required. Conventional antiepileptic drugs (AED's).

Phenobarbital, primidone, phenytoin, carbamazepine, ethosuximide and benzodiazepines, are widely used but exhibit and unfavorable side effect profile and failure to adequately control seizures in therecentyearnewdrugs have been added to the list of therapeutic agents against epilepsy. However there is a significant group of patients (up to 30%) who are resistant to the available antiepileptic drugs. The long established AED's control seizures in 50% of people developing partial seizures and in 60-70% of those developing generalized seizures. In the 1960s, research in antiepileptic drug development was stimulated by the creation of the epilepsy Branch and the epilepsy Advisory Committee with in the United states national institute of Neurological and communicative disorders and stroke (NINCDS).These programs were established to collate and review the neuroscience literature pertinent to epilepsy and encouraged to careen large numbers of potentially new anticonvulsants. From this point, the development of new drugs concentrated on different molecular structure, resulting in marketing of carbamazepine clonazepam and valproate It has been estimated that adequate control of seizures could not be obtained in up to 20%of the patients with epilepsy using first generation of antiepileptic drugs A group of new drugs including felbamate, gabapentin, lamotrigene, oxacarbazepine, topiramate, milacemide, vigabatrine and zonisamide are in to clinical practice .The convulsions of approximately 25% of epileptics are inadequately controlled by

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current clinically available drugs. Current drug therapy is accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbance, gingival hyperplasia, hirsutism and megaloblastic anemia.

The long established AEDs control seizures in 50% of patients developing partial seizures and in 60-70% of those developing generalized seizures. Hence there is urgent need to develop new AEDs. The search f or antiepileptic compounds with a more selective activity and lower toxicity continue to be an area of investigation in medicinal chemistry. A rational drug design process of a new anticonvulsant could be achieved in several ways. The first strategy is the identification of new targets through better understanding of molecular mechanism of epilepsy. Another way is to modify already existing drugs and formulations. AED's belong to many different chemical classes of compounds, including; hydantoines, iminostillbene, barbiturates, benzodiazepines, valproate, imides, oxazolidine2, 3-diones, sulfonamides and miscellaneous agents. The efficacy of AED's is due to the main activities which include interaction with ion channels or neurotransmitter systems.

The new AED's and anticonvulsant agents have been reviewed last few years. The chemical diversity and various mechanism of action of anticonvulsants make it difficult to find a common way of identifying new drugs. Novel anticonvulsant is discovered through conventional screening and /or structure modification rather than a mechanism driven design. Therefore, drug identification is usually conducted via in-vivo screening tests, on the basic of seizure type rather than etiology.

These review present new anticonvulsant agents representing various structures for which the precise mechanism of action is still not known. The newer agents include heterocyclic compounds, sulfonamides, amino acids, amides, enaminones and others. The new structural classes of compounds can be useful for the design of future targets and development of new drugs.

Most recently, a number of new antiepileptic drugs have been approved or are at the moment awaiting approval in a number of countries and these drugs include vigabetrine, gabapentin felbamate, oxacarbazepine and zonisamide. In addition, a number of active compounds with novel structures have been identified and are at various stages of preclinical and clinical development and these drugs include tigabine, eterobarb, remacemide, stiripentol, flunarizine, topiramate, levetiracetam. However, none of the available drug is ideal as they can be as upon the individual factors like age, sex type of syndrome etc. also in current clinical practice combination therapy is prescribed in significant proportion of patient with epilepsy. Hence there is urgent need to develop new AEDs. The search for antiepileptic compound with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry.

Limitation of current antiepileptic drugs

a) Available compounds do not treat the disease, but rather control only the symptoms toxicity.

b) The majority of antiepileptic drugs fail, not because of efficacy but because of unforeseen toxicity.

c) Current therapy fails to adequately control 50% of patients with several complex partial seizures.

d) Few current drugs are available for pediatric use.

e) Therapy is lacking to prevent epileptogenesis. Thus a percentage of patients with head injury or infants with febrile seizures will subsequently develop the epileptic syndrome.

f) Choice of drug is highly limited with reference to treatment of absence seizures.

g) Control of life threatening status epileptics with current therapy is to some extent adequate.

h) Some highly effective drugs produce tolerance and other cause fatal malformations.

i) Side effect often results in poor patient compliance.

Approaches for new antiepileptic drugs:

The ideal new antiepileptic drug is one that which will be effective in all seizures disorders in all patients and have no adverse effects. It must be easy to monitor, allowing in frequent clinic visits by the patients, can be administered once or twice daily and is in expensive. In addition, water solubility, a long half life and negligible protein binding properties are preferable.

However, because of the heterogeneity of epilepsy and inter patient, variability in metabolic handling and receptor responses to drug , the possibility of achieving such a drug is at present negligible. Thus current approaches are based on the following target characteristic:

a) As effective as existing therapy

b) Improved therapeutic ratio (i.e. less toxic in proportion to its observed benefit)

c) Simple pharmacokinetics with a half life of 12-24 hours so that once or twice daily administration would be possible.

d) Should not induce or inhibit liver enzymes and consequently have the potential to cause drug interactions.

e) Should not cause tolerance or withdrawal problems.

There are at least three approaches which are currently used for the development of new antiepileptic drugs:

1. Random screening of chemicals of diverse structural categories for anticonvulsant activity,

2. Structural variation of known antiepileptic drugs.

3. Rational drug design.

The efficacy of various antiepileptic drugs (AED's) is due to their activities which include interaction with ion channel or neurotransmitter system. Amongst these classes semicarbazones are of considerable interest because of their better CNS activity especially as anticonvulsants [43-71].

The extensive SAR studies of semicarbazones postulate a specific binding site which include.

i) An aryl hydrophobic binding site with halo substituent preferably at the Para position

ii) A hydrogen bonding domain

iii) An electron donor group.

iv) Another hydrophobic –hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant.
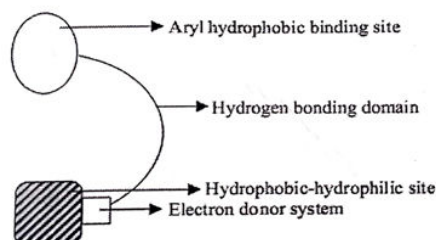

Figure 1. Pharmacophore of the designed Semicarbazone

Structural requirements for the semicarbazones displaying anticonvulsant activity Development of semicarbazones was initially based on the hypothesis Who correlated the MAO inhibiting activity of hydrazine derivatives with anticonvulsant activities specifically subcutaneous metrazol tests (ScMet).in the course of investigations aimed at developing structurally novel anticonvulsants ,a number of aryl semicarbazones, including series 1, were found to display significant activity.

These compounds were to interact at two locations on a putative binding site designated a hydrogen bonding area and an aryl binding site .However ,since the aryl group can be replaced by other hydrophobic moieties with retention of anticonvulsant activity the proportion of the binding site with which the aryl group of series.

The related compounds will be referred to as a hydrophobic bonding area rather than an aryl binding site. The principal objective of the present investigation was the preparation of a number of analogues of with a view to further evaluating the binding site hypothesis.

Aryl semicarbazides have also been reported to display excellent anticonvulsant activity in mice and rats. In terms of interaction at the binding site, as proposed previously the pharmacophoric elements were thought to be a lipophilic aryl ring and hydrogen bonding semicarbazone moiety. The attachment of a second aryl ring designated as the distal ring to the proximal aryl ring to increase the van der Waals bonding at the binding site and to increase potency have also been reported. Substitutions in the aryl ring by halogens have been found to increase potency in the MES screen.

The presence of electron-rich atom or group attached at the Para position of the aryl ring showed increased potency in the MES screen Due to importance of the hetero aryl semicarbazones, we have designed the novel molecules with nucleus of semicarbazones Based on pharmacophore model as cited in literature review, we design the novel derivative of semicarbazone or thiosemicarbazone. The designed scaffold structure is given in Figure-2.
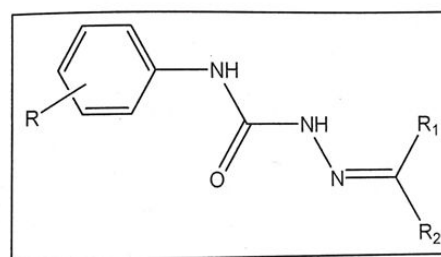
Figure 2. Designed scaffold

Where R=H, 4-Cl, Alkyl and R1=R2=H, alkyl, cyclic structure aromatic ring.

Materials and methods

The purity of the starting material was confirmed by melting point/ boiling point and thin layer chromatography. The purity and structures of the synthesized compounds were confirmed by melting point/ boiling point and thin layer chromatography, infrared spectroscopy and nuclear magnetic spectroscopy.

The melting point of the compound synthesized were uncorrected and recorded by open glass capillary method on 'Janki implex melting point apparatus" and compared with the reported melting point wherever applicable 1H-NMR spectra and 13CNMR spectra were reported on GEOLAR -300 shifts were expressed in parts per million (ppm). IR spectra were recorded using "BRUKER ALFA–E infrared spectrophotometer. Analytical thin layer chromatography (TLC) was carried out on pre-coated plates (silica gel G254). For anticonvulsant activity Electroconvulsometer was used.

The following are the chemicals used for biological testing:

1)   Dimethylsulfoxide (A.R.Grade, S.D.Fine Chem.Ltd. Mumbai).

Synthesis of compounds aryl semicarbazones (7a-j)

To a solution of substituted phenyl semicarbazide (5a) in ethanol was added an equimolar quantity of the appropriate ketone (6a-d).The pH of the reaction mixture was adjusted between 5 and 6 by adding glacial acetic acid. Reaction was monitored with TLC. The reaction mixture was refluxed for 1-2 h. The product obtained after cooling was filtered and recrystallized from 95% ethanol.

Animals

Male albino mice weight (25-35 gm) was used to test drug synthesized. Maximal Electro shock induced seizures.

Female animal were excluded because of fact that estrous cycle influences the seizure threshold. Animal were housed in propylene cage with dust free rice husk as bleeding material under laboratory condition with controlled environment of temperature 25 degree ± 2, humidity 60% and before subjecting them to experimentation the animal were given a week of time to acclimatize with laboratory condition . The animals were fasted over night before experiment.

Evolution of Anticonvulsant activity an experimental evaluation of Anticonvulsant activity of synthesized semicarbazone compounds done by Maximal Electroshock (MES) induced method.

The by Maximal Electroshock (MES) induced convulsion in animals represent grandma type of epilepsy.

The Maximal Electroshock (MES) convulsion divided in 5- phases.

1. Tonic flexion

2. Tonic Extensor

3. Clonic convulsion

4. Stupor

5. Recovery/ Death

Drug treatment

a)   Standard sample: the concentration of phenytoin sodium was prepared in DMSO solution and concentration of final solution was2.5mg/ml and to equate with t of compound synthesized.

b)   Test sample: Suspension of synthesized derivatives was prepared in DMSO having concentration of 2.5mg/ml.

Requirements- Test tube, mice, syringes, Needles, Electroconvulsometer, Volumetric flask, Oral feeding needle etc...

Study of Activity- Weighed and numbered the animals, divided in 12 groups, each group contain 5 animals. One group was used for study the effect of control. One group was used for the study effect of standard drug (Phenytoin Sod.) and another 10 group were used for the study of effect of synthesized final molecules.

MES induced seizures in albino mice

The albino mice were chosen for preliminary screening. Mice with showed extension of hind limb were induced in the study. The seizure was induced by MES in albino mice (weight25-35 gm) with the help of electroconvulsometer by passing current of 60MA for 0.2 sec. using electrode to the cornea of mice. The drug and DMSO were given one hour prior to induction of convulsion .The animal observed for the extensor phase as well its duration. The reduction and abolition of extensor phase (Tonic phase) in drug treated group was taken as criteria for anticonvulsant activity.

RESULT AND DISCUSSION

In this project the synthesis of semicarbazone derivatives was carried out. All molecules were synthesized using the common starting material –aniline. In all compounds, an intermediate was first formed by substituted phenyl urea using substituted aniline and potassium cyanate, and then it was hydrolyzed to get substituted phenyl semicarbazide which was directly coupled with ketones.

The general scheme utilized for the synthesis of aryl semicarbazone derivatives are outlined below in (Figure-3).
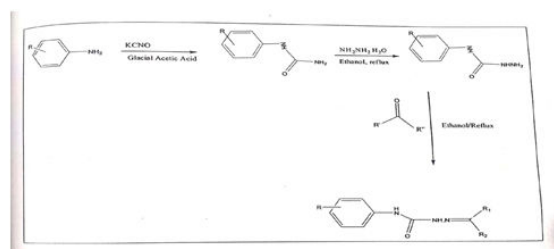

Figure -3. General synthetic scheme for aryl semicarbazone

Result of biological evaluation of synthesized molecules

All the synthesized compounds were screened for their anticonvulsant activity by MES method.

Group (n=3)	Dose (100mg/kg)	Body wt. (gm.)	Dose(ml)	Duration(sec) in various phases of convulsion				
				Flaxer	Extens or	Convul sion	Stupor	R/D
1	Control	30	0.3	4.59	15.68	68.29	78.69	D
		29	0.29	4.56	16.2	A	73	R
		26	0.26	4.15	14.42	29.28	74	D
2	Phenytoin	31	0.31	2.48	3.58	10.4	39.48	R
		23	0.23	2.39	3.39	11.59	35.85	R
		27	0.27	2.19	3.98	11.28	37.85	R
3	7a	31	0.31	4.47	10.01	23.1	23.3	R
		32	0.32	3.07	10.75	24.31	55.52	R
		30	0.3	2.99	10.78	20.99	56.47	D
4	7b	30	0.3	2.25	10.5	21.5	47.42	R
		31	0.31	2.77	9.98	22.8	39.85	R
		30	0.3	2.52	9.95	22.84	37.01	D
5	7c	29	0.29	3.57	10.09	24.52	60.59	D
		32	0.32	3.57	10.98	21.19	56	D
		33	0.33	2.69	10.14	22.28	47.1	D
6	7d	31	0.31	4.19	11.99	25.29	50.69	D
		32	0.32	4.4	12.49	24.59	48.48	R
		28	0.28	4.01	12.88	24.9	50.94	D
7	7e	31	0.31	4.93	11.99	22.09	32.31	D
		33	0.33	3.27	11.2	19.34	60.21	R
		27	0.27	3.3	10.5	22.28	48.49	R

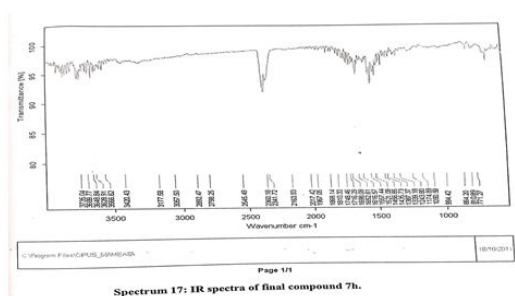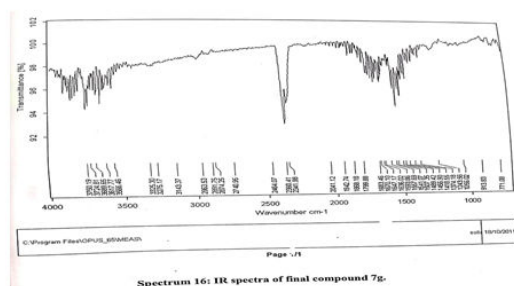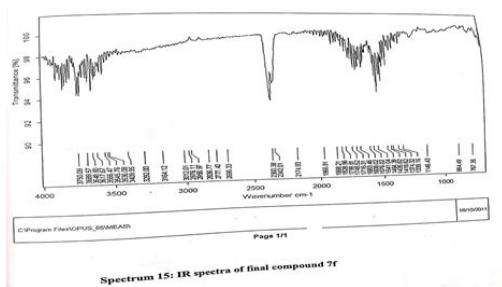|								R/D
8	7f	33	0.33	2.21	9.2	23.49	51.49	R
		32	0.32	1.98	10.01	20.89	27.98	R
		31	0.31	2.57	10.95	22.63	30.26	D
9	7g	31	0.31	2.54	9.8	23.01	44.1	R
		32	0.32	2.16	9.65	22.01	48.49	R
		32	0.32	2.67	9.98	22.86	55.2	D
10	7h	33	0.33	4.61	11.19	22.66	41	R
		31	0.31	4.85	10.99	22.97	55.1	D
11	7i	33	0.33	4.38	11.19	23.82	44.42	D
		33	0.33		4.59	11.99	22.89	41.85	R
		31	0.31		4.19	12.58	22.63	47.59	D
12	7j	28	0.28	3.98	11.37	11.28	33.79	R
		29	0.29	3.39	11.5	11.5	60.28	R
		30	0.3	3.58	10.99	11.22	A	D

Table -2. Anticonvulsant activities of synthesized compounds.

S. NO.	DOSE (25mg/kg)	Flaxer ±SEM	HLTE ±SEM	Convulsion ±SEM	STUPER ±SEM	R/D
1	Control	4.36±0.13	15.66±0.28	29.14±0.69	75.42±0.12	0.2
2	Phenytoin	2.31±0.04	3.61±0.04	11.19±0.19	38.20±075	1
3	7a	3.18±0.38	10.50±0.80	21.55±1.17	50.72±5.00	0.8
4	7b	2.49±0.05	10.01±0.29	19.79±2.19	41.80±2.20	0.6
5	7c	3.21±0.39	10.66±0.62	21.54±1.29	53.40±6.49	0.6
6	7d	4.20±0.20	12.50±0.53	25.54±0.99	50.70±5.53	0.4
7	7e	3.39±0.41	11.20±.068	20.55±1.49	49.10±6.10	0.6
8	7f	2.22±0.13	9.89±.0.15	21.16±0.71	35.89±6.89	0.8
9	7g	2.41±0.11	9.95±0.38	22.51±0.59	53.29±1.69	0.8
10	7h	3.65±0.25	11.30±0.38	23.41±0.78	53.26±3.11	0.6
11	7i	4.19±0.18	12.00±0.49	23.78±0.65	41.17±2.36	0.4
12	7j	3.64±0.16	11.25±0.48	23.73±1.20	51.71±5.81	0.6

Table 3. Anticonvulsant Biological Screening by MES method-

Spectrum 9: IR spectra of final compound 7a.

Spectrum 10: IR spectra of final compound 7b.

Spectrum 11: IR spectra of final compound 7c.

Spectrum 15: IR spectra of final compound 7f

Spectrum 16: IR spectra of final compound 7g.

Spectrum 17: IR spectra of final compound 7h.

CONCLUSION
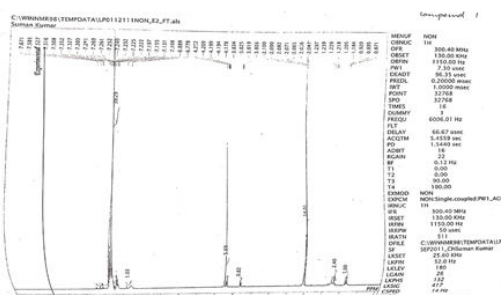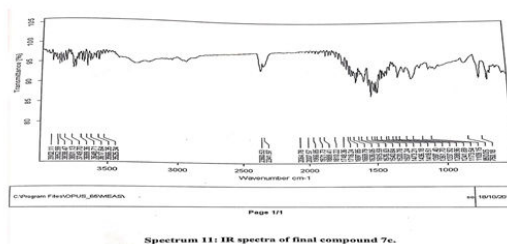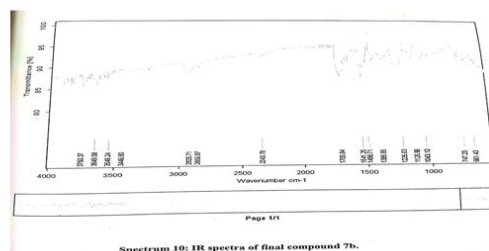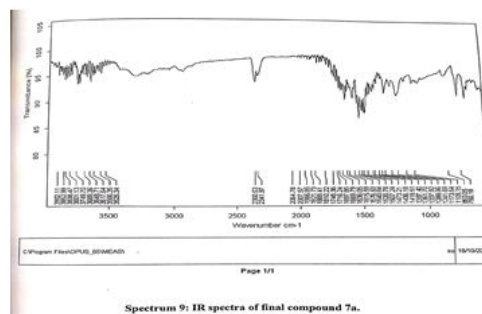
In summary, it can be concluded that designed semicarbazones were synthesized and characterized successfully. After synthesis of designed semicarbazones compounds were evaluated for anticonvulsant activity.

The electroshock assay in mice is used primarily as an indication for all compounds which are effective in Grand-mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by synthesized compounds. Finally, Compound 7f & 7g have shown better activity in comparisons to the other molecules.

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