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ORIGINAL RESEARCH ARTICLE

Synthesis and Evalution of Some Novel Semicarbazone Derivatives for Anticonvulsant Activity and Antibacterial Activity

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ABSTRACT

The purpose of research involving the synthesis of a series of novel semicarbazone derivatives for their anticonvulsant and antibacterial activity . The anticonvulsant activity of the synthesized compounds was established after intraperitoneal administration in mice which include maximal electroshock seizure, while antibacterial activity was investigated using cup plate agar diffusion method, while all the compounds were evaluted for antibaterial against gram (+) bacterial: staphylococus aureus and gram (-) bacteria :Escherichia coli .The chemical structure of the compounds were proved by spectral analysis IR analysis and ^{1}H- NMR study . The most active compounds were L3 ,L7 , L8 , L12 while compound with lesser activity were L1,L5 ,L6 .Compounds L2 , L5 , L10, L12 exhibited considerable neurotoioxicity while compounds L1 , L9, showed lesser neurotoxicity at the administered dose of 50 mg /kg . Most active compound L8 was most potent among all the synthesized semicarbazone with lesser neurotoxicity .Synthesized compounds were tested for invitro antibacterial activity by the agar dilution method the MIC values of the synthesized compounds against pathogenic bacteria are presented in Table 4 which included is the activity of refrence compound Ampicillin It has been observed that all the compounds tested showed mild to moderate activity against the tasted bacteria .The most active compound against bacteria is L 10

Key words: Anticonvulsant activity,Antibacterial activity ,MES method ,Neurotoxicity Evalution ,semicarbazone

INTRODUCTION

Epilepsy, one of the most frequent neurological disorders, is a major public health issue, affecting about 4% of individuals over their lifetime Epilepsy is characterized by unprovoked seizures, and affects at least 50 million people worldwide. There is a continuing demand for new anticonvulsant agents as it has not been possible to control every kind of seizure with the currently available antiepileptic drugs. There is currently a need for improved agents for the treatment of seizure disorders, since available drugs are effective in only 60–80% of epileptic patients. During the past decade, several new drugs have been approved (Rufinamide, Retigabine, Pregabaline,Remacemide, etc.). Despite advances in the drug treatment of epilepsy, a number of limitations of antiepileptic drug therapy continue to exist. In recent years, aryl and heteroaryl semicarbazones and thiosemicarbazones have emerged as structurally novel anticonvulsants. The present work focuses on synthesis, anticonvulsant and antibacterial evaluation of 1-(3 Chloro – 2-methyl phenyl) –[(3-hydroxyzinoloxy carbonyl)] semicarbazone.

MATERIALS AND METHODS

Chemicals:

The chemicals and reagents used in the present project were of AR grade synthesis grade ,purchased from sigma aldrich ,S.D Fine chem. Ltd ,loba chem bombay . 3-chloro 2-methyl aniline ,sodium cynate ,water ,Triethylamine, Hydrazine hydrateDichloromethane,Glacial acetic acid , Ethanol, Phenylchloroformate ,phenytoin ,carbamazepine .polyethylene glycol(PEG)

Method for the determination of melting point

Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus and are uncor rected.

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Method for the estimation of synthesized compound: Infrared (IR) and proton nuclear magnetic resonance (1H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

Method for the determination of purity:

The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silicagel-G (Merck) coated aluminium plates, visualized by iodine vapor. Developing solvents were chloroform–methanol (9:1).

Biological evalution of the synthesized compound

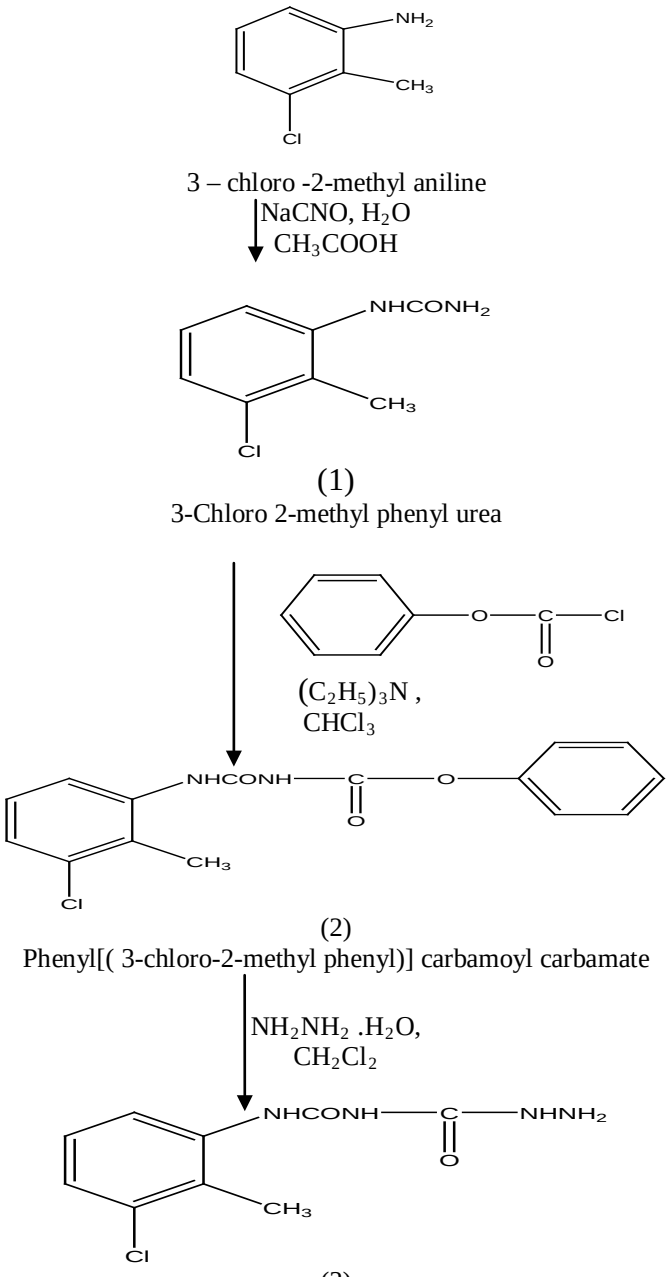
The anticonvulsant evaluations were undertaken using reported procedures. Male albino mice (CF-1 strain or Swiss, 18–25 g) and rats (Sprague–Dawley or Wistar, 100–150 g) were used as experimental animals. The tested compounds were suspended in 0.5% polyethylene glycol (PEG).

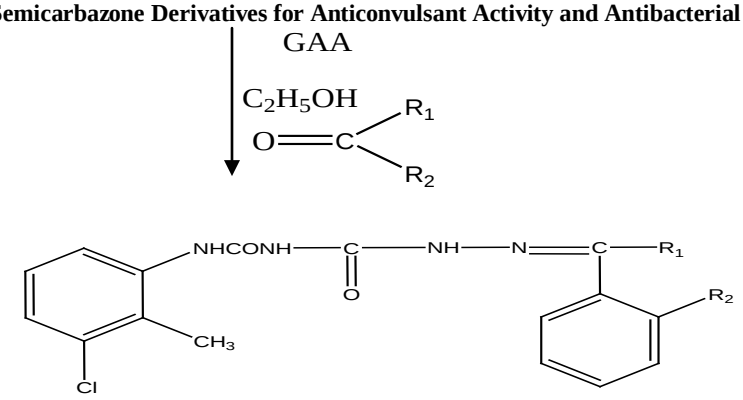
EXPERIMENTAL PROTOCOL

The synthesis of the semicarbazones is based on following reaction scheme

Preparation of scheme

Scheme - 1





(4)

1-(3 Chloro – 2-methyl phenyl) –[(3- hydroxyzinoloxy carbonyl)] semicarbazone (L1-L12)

Synthesis of 3-chloro-2-methyl phenyl urea (1)

3-Chloro-2-methyl aniline (0.1 mol, 14.1 g, 11.8 ml) was dissolved in 20 ml of glacial acetic acid and 10 ml of water. To this, 0.1 mol of sodium cyanate (6.5 g) in 80 ml of warm water was added with stirring. Allowed to stand for 30 min, then cooled in ice and filtered with suction, and dried. Recrystallized from boiling water to yield (1) with m.p. 201 °C, IR (KBr) mmax 3450, 1650, 840 cm–1, 1H-NMR (DMSO-d6) d 2.4 (s, 3H, CH3), 7.2–7.4 (m, 3H, ArH) 8.28 (s, 1H, ArNH, D2O exchangeable), 9.33 (s, 2H, CONH2, D2O exchangeable).

Synthesis of Phenyl [(3-chloro-2-methyl phenyl)] carbamoyl carbamate (2)

The urea derivative (**1**) treated with phenylchlorophoroformate (0.1 mol ,12.6 ml) was dissolved in 40 ml of chloroform and 3- chloro 2-methyl phenyl urea and trimethylamine (0.1mol,13.9 ml) were added dropwise and stirrered in the room temperature for 5 hours .the reaction mixture was concentrated to one third volume ad 100 ml of petroleum ether was was added .the precipitate was washed with water and filtered and dried yield found phenyl [(3- chloro 2-methyl)carbamayl] carbamate (2)IR (KBr) mmax 3450,3100 -3250, 1650, 840 cm–1; 1H-NMR (CDCl3) d 2.31 (s, 3H, CH3), 6.10(s, 2H, NH, D2O exchangeable), 7.18–7.19 (m, 3H,ArH), 8.10 (s, 1H,ArNH,D2O exchangeable)

Synthesis of 1-(3-chloro-2-methyl phenyl)-3- [(hydroxyzinoloxy carbonyl] semicarbazide (3)

To the solution of 2(0.05 mol) was dissolved in 100 ml of dichloromethane .To this solution 4.85 ml of hydrazine hydrate (0.1mol) was added and refluxed with stirring for 24 hous.The precipitate was separated by vaccum filtration and washed with dichloromethan and dried .IR (KBr) mmax 3450, 3269, 1640, 840 cm–1; 1H-NMR (CDCl3) d 2.38 (s, 3H, CH3), 6.16 (s, 2H, NH2, D2O exchangeable), 7.12–7.14 (m, 3H,ArH), 8.04 (s, 1H,ArNH,D2O exchangeable), 9.60 (bs, 1H, NHNH2, D2O exchangeable).

Synthesis of 1-(3 Chloro – 2-methyl phenyl) – [(3- hydroxyzinoloxy carbonyl)] semicarbazone (L1-L12)

The title compounds were synthesized following procedures reported earlier To a solution of 3 (0.003 mol), in 25 ml of ethanol,an equimolar quantity of appropriate aldehyde or ketone in 5ml ethanol and glacial acetic acid (1-2 drop) was added .The mixture was stirred with heating for 1-4 hours until the completion of the reaction and the resultant precipitate was filtered and dried .The product was recrystallized from 95% ethanol. The IR spectra of the semicarbazone derivatives were identical in the following aspects; 3450, 3300–3250, 1650, 1595, 840 cm–1. 1H-NMR (300 MHz, d) spectra of some representative compounds are as follows:

Characteriazation data of new semicarbazones

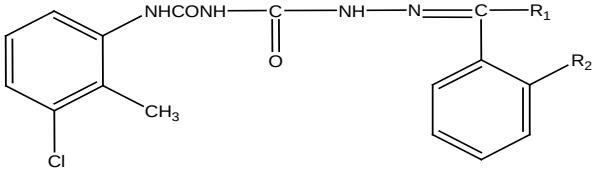


Table 1: Characterization of data of new semicarbazones

S No	Code	R_1	R_2	Molecular formula	M.P.(^{0}C)	Yield (%)	R_f
1	L-1	H	H	$C_{16}H_{15}O_2N_4Cl$	203	61	0.89
2	L-2	H	4-N(CH_3)	$C_{18}H_{21}O_2N_5Cl$	189	57	0.83
3	L-3	H	4-OCH_3	$C_{17}H_{18}O_3N_4Cl$	174	60	0.78
4	L-4	H	4-OH	$C_{16}H_{16}O2N_4Cl$	201	59	0.86
5	L-5	CH_3	CH_3	$C_{18}H_{21}O_2N_4Cl$	217	52	0.80
6	L-6	C_6H_5	H	$C_{22}H_{21}O_2N_4Cl$	198	74	0.90
7	L-7	H	P- Cl	$C_{16}H_{16}O_2N_4Cl_2$	185	80	0.83

8	L-8	H	2-Cl	$C_{16}H_{16}O_2N_4Cl_2$	200	67	0.91
9	L-9	H	3-NO_2	$C_{16}H_{16}O_4N_5Cl$	177	80	0.79
10	L-10	C_6H_5	4-OH	$C_{22}H_{21}O_2N_4Cl$	172	68	0.88
11	L-11	H	4- CH_3	$C_{17}H_{19}O_2N_4Cl$	180	65	0.85
12	L-12	C_6H_5	P- No_2	$C_{22}H_{20}O_4N_5Cl$	193	59	0.75

RESULTS

Anticonvulsant activity : Animals used in this study were Male albino mice (Swiss, 18–25 g) and rats (Wistar, 100–150 g) were used as experimental animal .The animals were housed in metabolic cages ,and allowed free access to food and water .The synthesizes compounds (L- l to L-12) were suspended in 0.5% in polyethylene glycol (PEG 200) .The anticonvulsant evolution were undertaken by the pinnacle Biomedical Research Institute ,Bholpal ,India using their reported procedures Initially all compounds were administeredthe i/p at dose of 50 mg /kg to one to four mice .Activity was stablished using the MES test .

Table 2: Anticonvulsant evaluation of some novel semicarbazones

Treatment	Dose	Duration Of Tonic Hind Limb Extension (In seconds) n=6Mean ± SD	Recovery
Control (saline)	2 ml / kg	19.23± 1.52	Yes
Phenytoin	25mg/kg	6.18± 1.06	Yes
L1	50mg/kg	13.14±1.64	Yes
L2	50mg/kg	8.46±2.03	Yes
L3	50mg/kg	8.34±2.23	Yes
L4	50mg/kg	9.17±1.13	Yes
L5	50mg/kg	10.54±1.27	Yes
L6	50mg/kg	10.11±1.54	Yes
L7	50mg/kg	7.43±1.32*****	Yes
L8	50mg/kg	6.11±1.67*****	Yes
L9	50mg/kg	6.32±1.59	Yes
L10	50mg/kg	7.29±1.22	Yes
L11	50mg/kg	6.19±1.19	Yes
L12	50mg/kg	7.26±1.06 *****	Yes

- ***** $p<0.01$
- One Way ANOVA followed by Dunnet's t-test.

Table 3: Neurotoxicity evaluation of synthesized semicarbazones

Treatment	Dose	Mean of time (s) spent on the rod ± SD
Control	2ml/kg	146.21± 22.28*****
L1	50mg/k	94.12±20.70
L2	50mg/k	88.11±28.17*****
L3	50mg/k	76.35±22.11
L4	50mg/k	99.25±19.23
L5	50mg/k	70.21±16.89
L6	50mg/k	75.13±14.41
L7	50mg/k	87.81±19.15
L8	50mg/k	82.38±11.47*****
L9	50mg/k	103.45±14.26*****
L10	50mg/k	74.43±10.23*****
L11	50mg/k	90.13±15.29*****
L12	50mg/k	77.13±18.19*****

Neurotoxicity screening

Minimal motor impairment was measured in mice by the rota rod test .The mice trained to say on an accelerating rota rod that rotates at 10 rpm .The rod diameter was 3.2 cm .Trained animals were given ip injection of the test compound in dose of 50 mg /kg .Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials .The result of anticonvulsant and neurotoxicity evolution are presented in (**Table 2 & 3**).

Antimicrobial activity

Antimicrobial activity was carried out by cup – plate agar diffusion method using nutrient agar .The compound were tested *in–vitro* for their antibacterial activity against two microorganisms *Escherichia coli* and *Staphylococcus aureus* which are pathogenic to human beings .The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface .The plates were dried at 37 ^{0}c before inoculation .The sterile disc contained test drug ,standard and blank were placed on the previously inoculated surface of the agar plate and it was kept in the refrigerator for 1 hour to facilitate uniform diffusion of the drug .plates were prepared were in triplicate and they were then incubated for 18 – 24 hours .Observations were made for zones of inhibition around the drug and compared with standard Standard drugs ampicillin were used .The concentration was 100 µg/ml compound were evaluated for their in vitro antibacterial activity against pathogenic bacteria procedure from shri Rawatpura Sarkar Institute of pharmacy.

Table 4: Antibacterial activities of the synthesized compounds

compound	*E.coli* (mm)	*S.aureus*(mm)
L-1	17	15
L-2	21	20
L-3	18	19
L-4	21	17
L-5	24	22
L-6	15	16
L-7	17	18
L-8	16	18
L-9	20	23
L-10	9	13
L-11	23	22
L-12	19	18
Ampicillin	25	25

DISCUSSION

The synthesis of 1-(3 Chloro – 2-methyl phenyl) –[(3- hydroxyzinoloxy carbonyl)] semicarbazone (L 1 to L12) was accomplished as presented in Scheme 1. 3-Chloro-2-methyl aniline was treated with sodium cyanate in the presence of glacial acetic acid according to the known urea preparation method, to yield 3-chloro-2-methyl phenyl urea (1). The urea derivative(1)treated with phenylchlorophoroformate (0.1 mol ,12.6 ml) was dissolved in 40 ml of chloroform and 3- chloro 2-methyl phenyl urea gave the phenyl [(3- chloro 2-methyl)carbamayl] carbamate (2).was dissolved in dichloromethane.To this solution hydrazine hydrate added and formed 1(3-chloro-2-methyl phenyl)-3-[hydrazinyloxy carbonyl]urea.(3). In the last step (3) is react with the glacial acetic acid and ethanol and equimoler quantity of appropriate aldehyde and ketone . and formed 1-(3 Chloro – 2-methyl phenyl) –[(3- hydroxyzinoloxy carbonyl)] semicarbazone(L1 to L12) .The semicarbazone derivatives were prepared by reaction of the appropriate aryl/alkyl alde- hyde or ketone or isatin derivatives with (3). Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. All the synthesized compounds were screened for their anticonvulsant potential through MES model .The majority of the compound showed anticonvulsant activity , The most active compounds were L3 ,L7 , L8 , L12 while compound with lesser activity were L1,L5 ,L6 .Compounds L2 , L5 , L10, L12 exhibited considerable neurotoioxicity while compounds L1 , L9, showed lesser neurotoxicity at the administered dose of 50 mg /kg . Most active compound L8 was most potent among all the synthesized semicarbazone with lesser neurotoxicity .Synthesized compounds were tasted for invitro antibacterial activity by the agar dilution method the MIC values of the synthesized compounds against pathogenic bacteria are presented in Table 4 which included is the activity of refrence compound Ampicillin It has been observed that all the compounds tasted showed mild to moderate activity against the tasted bacteria .The most active compound against bacteria is L 10.

CONCLUSION

If we relate structure of the compound with activity, it is found that compounds containing carbonyl system, aromatic system and more than one nitrogen atom are required for anticonvulsant activity.semicarbazone system possesses the above essential features required for the anticonvulsant activity and by incorporating NH-CO-NH group the activity is increases. From the structure of the compound it was found that by the presence of Chloro, Nitro group in the synthesized compound anticonvulsant activity increased as compared to the unsubstituted compound and methoxy or hydroxy substituted compound. In the semicarbazide substituted derivatives L8 shows maximum activity due to the presence of Chloro at the paraThe structures of synthesized compounds were confirmed by IR, ^{1}H-NMR analysis All the synthesized compounds were evaluated for anticonvulsant activity by Maximal electroshock seizure (MES) method. Male albino rats were taken as experimental animals. Measurements of the activities of the synthesized compounds were compared with phenytoin (standard) at dose of 50mg/Kg body weight. All the compounds were found to possess anticonvulsant activity position .

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