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SYNTHESIS AND EVALUATION OF NOVEL QUINAZOLINONE DERIVATIVES AS BROAD SPECTRUM ANTICONVULSANTS

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The present work was carried out to synthesize a series of substituted quinazolinone semicarbazones at third position of the quinazoline nucleus and chemically modifying second position of quinazolinone to get the compounds with lesser side effects and more potent anticonvulsive agents. Although several new anticonvulsants are already in clinical use, some types of seizures are still not adequately treated with current therapy and have limitations, intolerable side effects. In response to these limitations, the development of new drugs to optimally manage seizures has been strongly advocated. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry. The present study describes the synthesis of newer quinazolinone derivatives and their anticonvulsant activities. The newly synthesized compounds were evaluated intraperitoneally into the mice in the maximal electro shock (MES), subcutaneous strychnine threshold test (scSTY), using doses 30, 100, 300 mg/kg, and neurotoxicity screens, observation was carried out at two different time intervals. Almost all the synthesized analogues were equipotent to phenytoin with very low neurotoxicity profile.

KEYWORDS : Quinazolinone, anticonvulsant, Maximal electric-shock method, Neurotoxicity.

INTRODUCTION

Epilepsy is ubiquitous disease characterized by recurrent seizures and inflicts more than 60 million people worldwide according to epidemiological studies [1-3]. Every year approximately 250000 new cases are added to this figure. It is roughly estimated that 28-30% of patients are resistant to the available medical therapies. Despite the development of several new anticonvulsants [4, 5], the treatment of epilepsy remains still inadequate, and the patients suffer from a lot of specific problems like neurotoxicity, depression and other CNS related diseases.

Although several new anticonvulsants are already in clinical use, some types of seizures are still not adequately treated with current therapy and have limitations, intolerable side effects. In response to these limitations, the development of new drugs to optimally manage seizures has been strongly advocated. Thus the search for new anticonvulsant drugs continues

to be an active area of investigation in medicinal chemistry [6]. The apprehension of such a possibility will impose a change in our current AED discovery approach. In the recent literature, various aryl semicarbazones with established pharmacophore requirements have been reported as a novel class of anticonvulsant agents with lesser central nervous system side effects.

In the recent years, the chemistry of quinazolinone and their derivatives has received considerable attention owing to their synthetic and effective biological importance. 4 (3*H*)-quinazolinone is one of the most frequently encountered heterocyclic compound in medicinal chemistry with wide applications including antiviral, antibacterial, antifungal, anti-inflammatory and anticonvulsant activities [7]. A literature survey revealed that the presence of aromatic or aliphatic group at position 2 and a substituted aromatic ring at position 3 are an essential requirement for CNS activities predominantly anticonvulsant properties. Various hypotheses were analyzed before the chemical synthesis of proposed compounds. First hypotheses was inspired from the 4 (3*H*)-quinazolinone nucleus containing well known CNS active Methaqualone (2-methyl-3-*o*-tolyl-4(3H)-quinazolinone). Modifications at the second and third position of this agent have lead to the generation of many CNS active agents. Second hypotheses explained, methyl group at the second position is not necessary for the CNS activity and other groups can also lead to potent CNS active agents [8, 9]. A literature survey exposed that replacement of the methyl group at the second position by some other functionality such as alkyloxy methyl or alkylthiomethyl groups reportedly yielded structural analogues which retained the anticonvulsant activity. The anticonvulsant activity of quinazolinone derivatives was attributed to its ability to bind the noncompetitive site of α-amino-hydroxy-methyl-4-isoxazolepropionic acid (AMPA) receptors [10]. Based upon the results of previous mentioned hypotheses the present work was carried out to synthesis compounds with substituted semicarbazones at third position and chemically modifying second position of quinazolinone to get the compounds with lesser side effects and more potent anticonvulsive agents and to synthesize compounds with low dose-related toxicity and without idiosyncratic side effects.[11].

MATERIALS AND METHODS

Melting points were determined by the open capillary tubes with electro thermal melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on JASCO 4100 FT-IR using KBr pellet disc technique.NMR spectra were recorded on a Bruker Advance spectrometer. Mass spectra were recorded on JOEL GC mate mass spectrometer at 70 eV. The elemental analysis was performed by using Perkin-Elmer model 240C analyzer. The purity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapor. Developing solvents used in TLC were ethyl acetate: n-butanol: water (6:3:1). The log *P* for all the synthesized compounds was calculated.

Synthesis of quinazoline derivatives

Step i: Synthesis of 2-aryl-3-amino-4(3H) quinazolinone

Anthranilic acid (0.01 mole) was dissolved in dry pyridine (30 ml) by stirring slowly at room temperature. The solution was cooled to 0° and a so lution of benzoylchloride (0.02mole) in dry pyridine (30 ml) was added slowly with constant stirring. After this addition the reaction mixture was further stirred for half an hour at room temperature and set aside for 1hr. The pasty mass obtained was diluted with water (50ml) and treated with aqueous sodium

bicarbonate solution. When the effervescence ceased the precipitate obtained was filtered off and washed with water, dried and recrystallized from diluted ethanol (M.p. 125-130°C, yield 79%).

Step ii: Synthesis of quinazolinone urea

2-Aryl-3-amino-4(3H) quinazolinone (0.1mole) was dissolved in 10ml of glacial acetic acid and diluted to 100ml with water . To this equimolar quantity of sodium cyanate in 50ml of warm water was added with stirring and then allowed to stand for 30mts, cooled in ice for further 30 mts. The precipitate obtained was filtered, washed with water and dried. The precipitate is recrystalized from boiling water and alcohol. (M.p. 190-195°C, yield 90%).

Step iii: Synthesis of quinazolinone semicarbazide

To a solution of quinazolinone urea (0.1 mole) in 200ml of water equimolar quantity of hydrazine hydrate was added. The reaction mixture was made alkaline by 4gm of NaOH and added 20ml of ethanol to get a clear solution. The reaction mixture was refluxed for 1.5hrs, cooled and filtered the precipitate. The precipitate was recrystallized from ethanol (M.p 160-165°C, yield 90%).

Step iv: General procedure for the synthesis of 2-Aryl-3-amino-4(3H)quinazolinone semicarbazone

2-Aryl-3-amino-4(3H) quinazolinone semicarbazide (0.01mole) was dissolved in ethanol (20ml) and added slowly to an ethonolic solution of aromatic carbonyl compound (0.01mole). The reaction mixture was catalyzed with 5ml of glacial acetic acid and refluxed for half an hour. The precipitate was collected and washed with the mixture of ether and water, dried. The product obtained was recrystallized from ethanol (yield 90%).The synthetic route for the titled compounds were shown in figure i. The physical data of the compounds were presented in Table i. The spectral and elemental analyses (Table ii) of some of the synthesized compounds were as follows:

3-N'- (4- Dimethyl amino benzylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one ***(1)***

IR (KBr): 780, 1580,1660,1261,3446 cm^{-1}; ^1H NMR δppm 1.8-2.1 (dimethyl amino), 6.68-7.67 (aromatic H), 7.92 (HC=N), 9.21 (NHCO).

3-N'-(3- Methoxy -4- hydroxyl benzylidene semicarbazone) - 2 –phenyl- 3H- quinazolin-4-one

IR (KBr): 870, 1550,1658,1252,3440 cm^{-1}; ^1H NMR δppm 1.70(OH), 3.7-3.8 (methoxy), 6.70-7.77 (aromatic H), 7.98 (HC=N), 9.10 (NHCO).

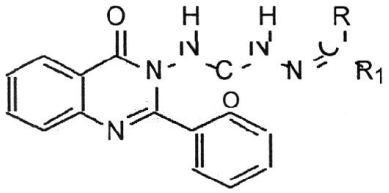
3-N'- (4- Chloro benzylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one **(3)** IR (KBr): 786, 1600,1665,1252,3444 cm^{-1}; ^1H NMR δppm 6.68-7.67 (aromatic H), 7.92 (HC=N), 9.21 (NHCO).

3-N'- (3-Nitro benzylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one **(4)** IR (KBr): 786, 1580,1655,1248,3444 cm^{-1}; ^1H NMR δppm 6.80-7.77 (aromatic H), 7.83 (HC=N), 9.32 (NHCO).

3-N'- (4- Hydroxyl benzylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one **(5)** IR (KBr): 706, 1629,1665,1255,3414 cm^{-1}; ^1H NMR δppm 1.76 (OH) 6.80-7.78 (aromatic H), 7.91 (HC=N), 9.20 (NHCO).

3-N'- (2- Hydroxyl benzylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one **(6)** IR (KBr): 700, 1633,1654,1265,3417 cm^{-1}; ^1H NMR δppm 1.81 (OH) 7.00-7.79 (aromatic H), 7.82 (HC=N), 9.27 (NHCO).

Table I: Physical data of compounds



Cpd	Substituents		Yield (%)	Mp (°C)	Molecular	Mole cular	R_f	logP^*
	R	R1			Formula	weight		
01	H	4-N $(CH_3)_2$	92	242	$C_{25}H_{23}N_5O_2$	425	0.62	2.19
02	H	3-MeO, 4-OH	88	231	$C_{24}H_{20}N_4O_4$	428	0.52	1.88
03	H	4-Cl	86	228	$C_{23}H_{17}ClN_4O_2$	416	0.40	2.68
04	H	3-NO_2	80	225	$C_{23}H_{17}N_5O_4$	427	0.54	2.12
05	H	4-OH	90	214	$C_{23}H_{18}N_4O_3$	398	0.46	2.07
06	H	2-OH	88	230	$C_{23}H_{18}N_4O_3$	398	0.60	2.10
07	CH_3	H	94	228	$C_{24}H_{20}N_4O_2$	396	0.42	2.06
08	H	Ar-Furan	92	209	$C_{21}H_{16}N_4O_3$	372	0.52	2.42
09	H	2-MeO	89	227	$C_{24}H_{20}N_4O_3$	412	0.50	2.52
10	Me	4-Cl	88	210	$C_{24}H_{19}ClN_4O_2$	430	0.42	2.60

*Log P was calculated by partition coefficient determination using Octanol and buffer system Rf- Solvent system- ethyl acetate-butanol:water (6:3:1)

Table II: Values obtained by elemental analysis for individual compounds

Compound	Carbon		Hydrogen		Nitrogen		Oxygen		Bromine		Exact mass
	A	B	A	B	A	B	A	B	A	B	
1.	70.57	70.48	5.44	4.80	16.46	14.56	7.52	7.49	-	-	425.19
2.	67.28	67.36	4.71	4.90	13.08	13.36	14.94	14.79	-	-	428.15
3.	66.27	67.36	4.11	4.08	13.44	13.36	7.68	7.70	-	-	416.10
4.	64.63	64.90	4.01	4.16	16.39	16.17	14.97	14.89	-	-	427.13
5.	69.34	69.46	4.55	4.67	14.06	14.18	12.05	13.18	-	-	398.14
6.	69.34	69.48	4.55	4.69	14.06	14.17	12.05	12.18	-	-	398.14

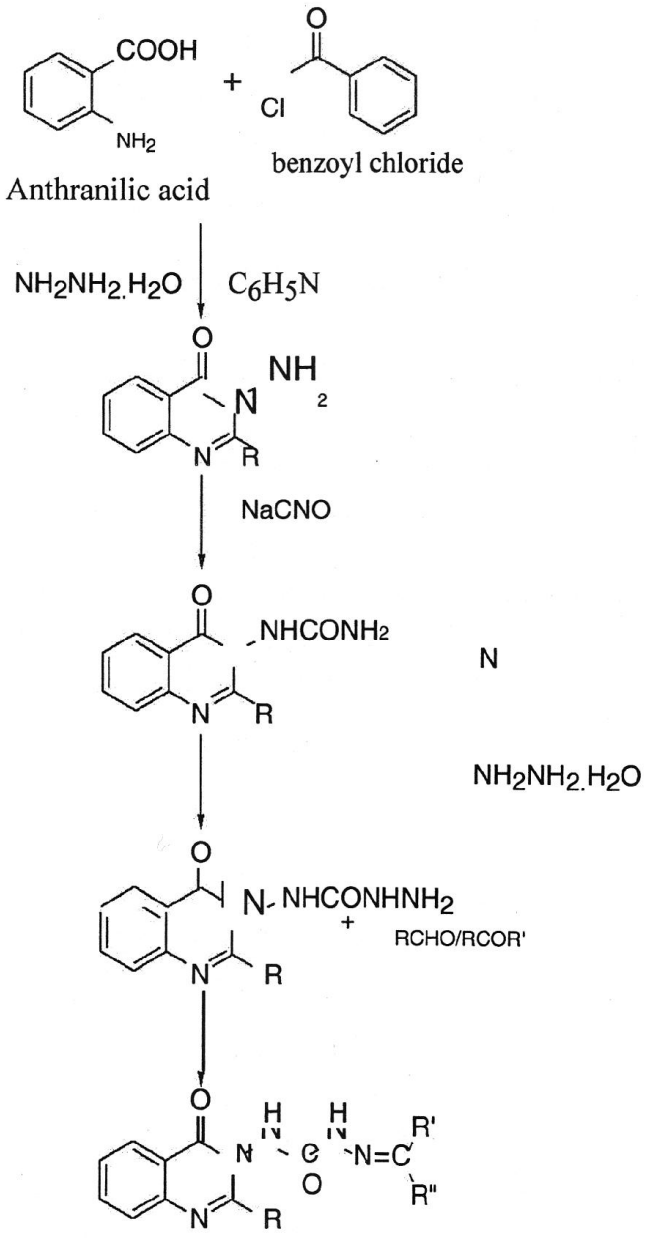
A-Calculated, B-Found

Pharmacology

a) Anticonvulsant screening:

The anticonvulsant activity of synthesized compounds was established using the MES, scSTY tests. The MES test was performed based on the protocols of National Institute of Neurological disorders and Stroke, NIH (USA).

Strychnine seizure pattern test (scSTY) was performed by using animals (mice) of either sex, weighing between 22 to25g, of the control group received polyethylene glycol vehicle (PEG). Drug solution was administered intraperitoneally to the other groups. After 1hr, the animals of both groups were injected with strychnine (2mg Kg^{-1} body mass) and observed for 45 minutes. The dose at which the hind leg tonic extensor component was abolished was noted.



Quinazoline semicarbazone

Figure I. Synthesis of the title compounds

b) Neurotoxicity screening

Minimal motor impairment was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod that rotates at 10 rpm. The rod diameter was 3.2cm.Trained animals were given ip injection of the test compounds in doses of 30, 100, 300 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 three trials.

RESULTS AND DISCUSSION

From the structural investigation, IR spectra showed the stretching frequency range between 1588 and 1629 cm^{-1}, which evinced the presence of imine linkage and also the absence of -NH_2 peak for the synthesized quinazoline semicarbazone derivatives. Dependant substitution of double-bonded nitrogen group of imine C=N could be the reason for the characteristic absorption close to the carbonyl C=O of amide (1630–1680 cm-1) or C=C of alkene (1600–1680 cm^{-1}) double bond stretching region. 1H-NMR spectra give a characteristic proton resonance shifts for all the synthesized quinazoline semicarbazone derivatives, which ensured the existence of aromatic, amine, amide, and imine protons. The wavelengths of maximum absorbance (kmax) for all the synthesized compounds were shown the specific absorptivity.

Table III: Anticonvulsant activity of phenytoin and newly synthesized compounds

	MES screen		scSTY screen		Neurotoxicity screen	
Compound	**0.5hr**	**4.0hr**	**0.5hr**	**4.0hr**	**0.5hr**	**4.0hr**
1	30	100	100	100	300	-
2	30	100	30	100	100	-
3	100	300	100	100	300	-
4	30	100	100	100	300	-
5	100	100	100	100	-	-
6	30	100	30	30	-	-
7	30	30	30	100	-	-
8	30	100	30	100	-	-
9	30	30	100	300	-	-
10	30	30	100	300	-	-
Phenytoin	30	30	30	30	-	-

The energetically most favorable p-p* excitation occurs from the highest energy bonding pi-electron to the lowest energy antibonding pi-electron systems. Conjugated pi-electron systems (unsaturated carbonyl and aromatic ring compounds) of synthesized compounds act as chromophores and absorbed the light, in the region of 296–390 nm. Estimated elemental compositions were within ±0.4% of the calculated values.

Almost all the synthesized analogue showed potent anticonvulsive activity. The newly synthesized compounds were injected intraperitoneally into the mice and evaluated in the maximal electro shock (MES), subcutaneous strychnine threshold test (scSTY), and neurotoxicity screens, using doses 30, 100, 300 mg/kg, and observation was carried out at two different time intervals. The data's are presented in table iii. All the compounds showed activity against MES screening method pinpointing their capability to prevent seizure spread. Most of the compounds showed activity both at 0.5h and 4.0h periods indicating that they have rapid onset and longer duration of action. The compounds **1, 2, 4** and **6 – 10** were active at 30mg/kg in the MES screen may prove to be useful in treating generalized tonic-clonic and complex partial seizures. The compounds **1,2,4,6** and **8** were active at 30mg/kg only at 0.5h, indicating that they have rapid onset and shorter duration of action. The compounds **7** and **9** showed protection at a dose of 30mg/kg, in half of the tested mice up to 4hrs, indicates that they have longer duration of activity. Compounds **9** and **10** showed protection up to 4hr at a dose of 30mg/kg indicating that, they have longer duration of action and equipotent to phenytoin in this study.

The compounds were also screened in the scSTY pattern test. All the compounds showed protection against scSTY-induced seizure threshold test, indicative of their ability to prevent seizure. The compounds **2,6,7,8** were showed protection at 30mg/kg in the scSTY pattern test. Compounds **1,3,4,5** were found to be active at a dose of 100mg/kg, after 0.5h and 4h, indicating longer duration of action.

Conclusion

In the present study, we have synthesized the biologically active condensed product of quinazoline semicarbazones. The semicarbazones was substituted at N-3 position of quinazoline, which again proves the novelty of biological efficiency of our new quinazoline series. In the future, the compounds will be modified further to reduce the molar mass and toxicological barriers. Based on the literature review, the compounds will be screened for other central nervous system activity, such as sedatives, hypnotics, and psychotics. Also the compounds will be modified further based on the literature, will be screened for antibacterial and antiviral activity [12].The present study provides the broad-spectrum anticonvulsant activity of substituted quinazoline semicarbazones that are comparatively higher or equipotent to the current available drugs. Overall, the synthesized compounds emerged as more active and less neurotoxic derivatives.

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