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SYNTHESIS OF NOVEL QUINAZOLINONE DERIVATIVES AS BROAD SPECTRUM ANTICONVUL-SANT AND ANTIMICROBIAL AGENT

ARCHANA

Medicinal Chemistry Laboratory, Department of Chemistry, Meerut College, Meerut (U.P.), India

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The present work was carried out to synthesize compounds with 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 quinazoline derivatives and their anticonvulsant, antimicrobial 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 analogue showed potent anticonvulsive activity. Some of the synthesized compounds were equipotent to the well known antiepileptic agent phenytoin. The synthesized compounds were found to potent anticonvul-sant with less neurotoxicity. The synthesized compounds were also studied for antimicrobial potency. The antibacterial activity of synthesized quinazoline semicarbazones were characterized by antimicrobial screening against several gram-positive, gram negative bacteria. Antimicrobial screening for all the compounds exhibits characteristic microbial inhibition. A detailed study is in progress to modify the structural activity, and toxicological barriers for the enhanced pharmacological efficiency of synthetic antibiotics.

KEYWORDS : Quinazolinone, anticonvulsant, Maximal electro shock, Neurotoxicity, antibacterial.

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INTRODUCTION

Epilepsy is all-pervading disease characterized by intermittent seizures and inflicts more than 60 million people worldwide according to epidemiological studies [1, 2, and 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, deprestients suffer from a lot of specific problems like neurotoxicity, deprestients 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, anti-bacterial, 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 a-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.

Since there are many quinazoline derivatives were reported as antimicrobial agents the current synthesized compounds were also investigated for antimicrobial activity by the disk diffusion method using four gram negative and four gram-positive nonpathogenic bacteria and one fungus.

MATERIALSAND 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 re-corded on a Bruker Advance spectrometer. The purity of the com-pounds 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 (0.01mole) was dissolved in dry pyridine (30 ml) by stirring slowly at room temperature. The solution was cooled to 0° and a solution of benzoylchloride (0.02 mole) 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 (50 ml) 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 di-luted ethanol (M.p. 125-130°C, yield 79%).

Step ii: Synthesis of quinazolinone urea

2-Aryl-3-amino-4(3H) quinazolinone (0.1 mole) was dissolved in 10 ml of glacial acetic acid and diluted to 100 ml with water . To this equimolar quantity of sodium cyanate in 50 ml of warm water was added with stirring and then allowed to stand for 30mts, cooled in ice for further 30mts. 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 200 ml of water equimolar quantity of hydrazine hydrate was added. The reaction mixture was made alkaline by 4gm of NaOH and added 20 ml 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 (0.01 mole) was dissolved in ethanol (20ml) and added slowly to an ethonolic solution of aromatic carbonyl compound (0.01 mole). The reaction mixture was catalyzed with 5 ml 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 scheme involved in the synthesize of titled compounds were shown in figure i. The physical data of the com-pounds are presented in Table i. The spectral data of the synthesized compounds are as follows:

3-N'- (1-Phenylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one (1) IR (KBr): 880, 1588, 1665, 1261, 3446 cm^{-1}; ^1H NMR dppm 6.68-7.67 (aromatic H), 7.92 (HC=N), 9.21 (NHCO).

3-N'-(4-Nitro benzylidene semicarbazone) - 2 –phenyl- 3H- quinazolin-4-one (2) IR (KBr): 890, 1560, 1658, 1258, 3444 cm^{-1}; ^1H NMR dppm 6.70-7.77 (aromatic H), 7.98 (HC=N), 9.10 (NHCO).

4-(1-Oxo-3-phenylisoquinolin-2(1H)-yl)-1-(1-phenylethylidene) semicarbazide (3) IR (KBr): 786, 1610,1665,1248,3446 cm^{-1}; ^1H NMR dppm 6.68-7.67 (aromatic H), 7.92 (HC=N), 9.21 (NHCO).

3-N'- (4-Methoxy benzylidene semicarbazone) - 2- phenyl- 3H-quinazolin-4-one (4) IR (KBr): 786, 1580, 1655, 1248, 3444 cm^{-1}; ^1H NMR dppm 3.7-3.8 (methoxy), 6.80-7.77 (aromatic H), 7.83 (HC=N), 9.32 (NHCO).

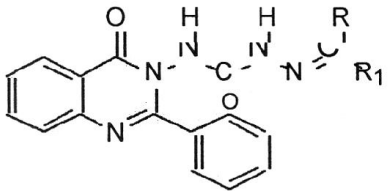
3-N'-(1-Styrenylidene semicarbazone) - 2- phenyl- 3H- quinazolin-4-one (5) IR (KBr): 706, 1632, 1666, 1265, 3418 cm^{-1}; ^1H NMR dppm 1.76 (OH) 6.80-7.78 (aromatic H), 7.91 (HC=N), 9.20 (NHCO).

Pharmacology

(a) Anticonvulsant screening:

The anticonvulsant activity of synthesized compounds was established using the MES, scSTY tests. The MES test was per-formed 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.

Table i : Physical data of compounds

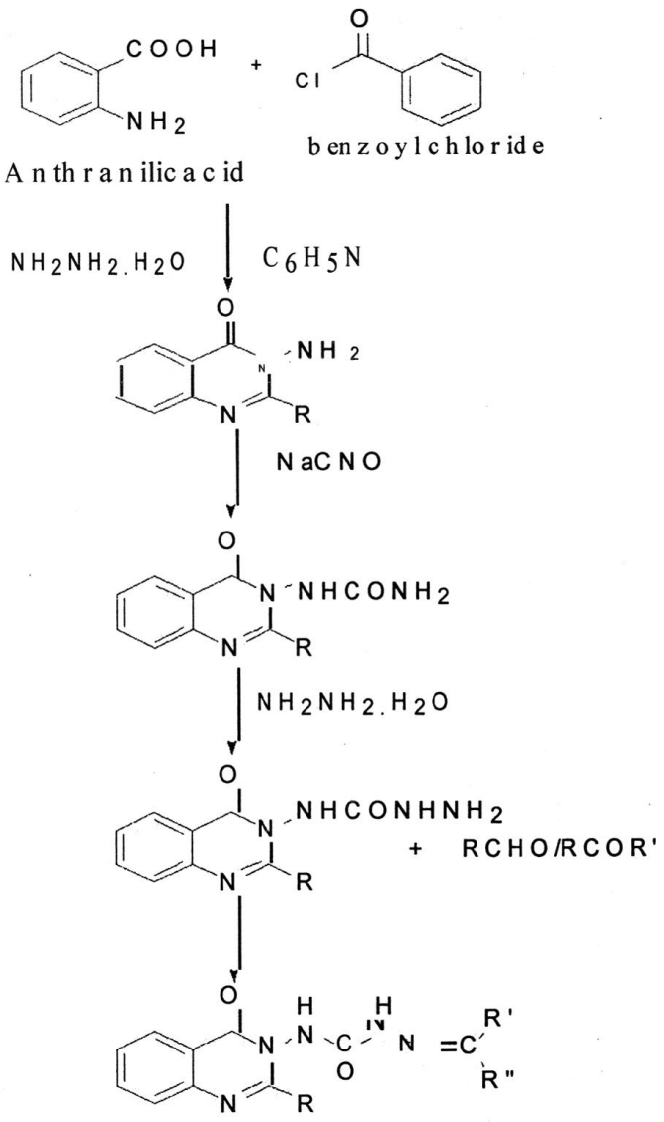


Cpd	Substituents		Yield (%)	Mp (°C)	Mol. For	Mol.wt	R_f	logP^*
	R	R_1						
01	H	C_6H_5-	96	238	$^C23^H18^N4^O2$	382	0.52	2.18
02	H	4-NO_2-Ph	80	225	$^C23^H17^N5^O4$	427	0.54	2.12
03	CH_3	H	94	228	$^C24^H20^N4^O2$	396	0.42	2.06
04	H	4-MeO-Ph	89	227	$^C24^H20^N4^O3$	412	0.50	2.52
05	H	C_6H_5-CH=CH	94	236	$^C25^H20^N4^O2$	408	0.48	2.64

Log P was calculated by partition coefficient determination using Octanol and buffer system; R_f. Solvent system- ethyl acetatebutanol : water (6:3:1) 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.

(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.



Quinazolinesemicarbazone

R = pheny

I; R′ = H

R″ = 2 – OH – Phenyl, 4 – OH – Phe

Nyl, R′ = R″ = furan, 1s is tin, phenyl

Fig. i : Synthetic scheme for the title compounds

(c) Antimicrobial screening:

The biological evaluation of synthesized compound was performed using the disk diffusion method. In the present study four gram-positive, four-gram negative, and one fungus were selected. The gram positive strains were *Bacillus lentus* (NCIM 2018), *Bacillus cereus* (NCIM 2018), *Micrococ-cus luteus* (NCIM 2155), *Staphylococcus albus* (NCIM 2178); gram negative strains were *Escherichia coli* (NCIM 2065), *Klebsiella aerogenes* (NCIM 2075), *Salmonella paratyphi* (NCIM 2075), *Proteus vulgaris* (NCIM 2239), and fungus *Candida albicans* (NCIM 0707). The strain was confirmed for its purity and identity by the gram-staining method and it was further character-ized by chemical reaction. The selected strains were preserved by periodical sub culturing on agar slant and storing them under frozen condition; for the study fresh 24 hours broth cultures were used. Each bacterial and fungal pure culture was transferred into 100 ml of Muller Hinton nutrient broth and Sabouraud's dextrose broth, respectively. The inoculated broths were incubated at 37°C for 24 hours and 27°C for 72 hours for bacteria and fungus respectively. After incubation, inocula were standardized to 108 colony-forming units (CFU)/ml for bacteria and 106 CFU/ml for fungus by colony forming unit method. Muller Hinton agar media was prepared by using Beef infusion 300 g, Casein acid hydrolysation 17.5 g, starch 1.5 g, and agar 17 g. Accurately weighed quantities of these ingredients were suspended in 1,000 ml of distilled water. They were boiled to dissolve completely. The pH was adjusted to 7.3 ± 0.2 at 25°C. It was then sterilized by autoclaving at 15 lbs. pressure (121°C for 15 minutes). The prepared Muller Hinton agar medium was transferred into sterile Petri plates; 200 µl of the standardized bacterial inoculums and fungus inoculum were spread on agar medium using sterile cotton swab. The synthesized product of quinazoline semicarbazone derivatives were dissolved in suitable chloroform solvent to a final concentration of 50 µl of drug solution, assuming that each disk absorbed approximately 10 µl of the drug. The drug was impregnated on disk and placed on the inoculated agar medium. Ciprofloxacin and clotrimidazole were used as a standard for the antibacterial and antifungal activity, respectively. All the bacterial Petri plates were kept in an incubator and the fungal Petri plate was kept at room temperature for approximately 18 hours. Then the zones of inhibition were measured.

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 character-istic 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. 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 neuro-toxicity 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 ii. 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 **compounds1,2,4** 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 **3**and **5** were active at 30mg/kg only at 0.5h, indicating that they have

rapid onset and shorter duration of action. The compounds were also screened in the scSTY pattern test. All the compounds showed protection against scSTY-induced sei-zure threshold test, indicative of their ability to prevent seizure.

Table ii: Anticonvulsant activity of phenytoin and newly synthesized compounds

	MES screen		scSTY screen		Neurotoxicity screen	
Compd	**0.5hr**	**4.0hr**	**0.5hr**	**4.0hr**	**0.5hr**	**4.0hr**
1	3 0	3 0	30	100	300	-
2	30	30	30	100	100	-
3	30	300	100	300	300	-
4	30	30	100	300	300	-
5	30	100	30	100	300	-
Phenytoin	30	30	30	30	-	-

Table iii: Diameter of zone of inhibition by individual compounds against gram-positive, gram-negative bacteria, and fungus

Zone of inhibition in mm	Compounds						
Organism	STD[a]	1	2	3	4	5	Solvent[b]
Gram +ve bacteria							
Escherichia coli	0 9	1 2	1 4	1 4	1 3	1 7	0 6
Micrococcus luteus	0 8	1 3	1 3	1 1	1 0	1 2	0 3
Bacillus cereus	0 9	1 0	1 2	1 1	1 1	1 3	0 3
Staphylococcus albus	0 8	1 1	1 0	0 9	0 7	1 0	04
Gram –ve bacteria							
Escherichia coli	16	18	17	16	18	18	04
Klebsiella aerogenes	10	15	15	14	14	15	05
Salmonella paratyphi	08	14	13	13	14	13	04
Proteus vulgaris	08	15	13	14	16	16	04
Fungus							
Candida albicans	14	16	16	15	17	18	03

[a] Standard ciprofloxacin for bacteria, clotrimazole for fungal

[b]Chloroform

The antimicrobial screening of all the compounds showed an excellent zone of inhibition against both gram-positive and gram-negative bacteria than standard ciprofloxacin. The data's are presented in table iii. Similarly, the zone of inhibition on the fungal strain showed a stronger activity than the standard clotrimidazole. New derivatives of quinazoline semicarbazone series exhibits stronger inhibition on gram-negative *Escherichia coli* compared with other bacterial strains. On the other hand, *Candida albicans* zone was highly inhibited by quinazoline semicarbazone derivatives, which proves the efficiency of antifungal activity than antibacterial activity. The zone of inhibition on *Staphylococcus albus* comparatively smaller than other bacterial species. Discussing the antimicrobial activity against individual organisms, it was clear that all the compounds have significant inhibitions. It was found that

the *Escherichia coli* and *Candida albicans* were highly susceptible to killing by the synthesized quinazoline semicarbazone.

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 toxico-logical barriers. Based on the literature review, the compound will be screened for other central nervous system activity, such as sedatives, hypnotics, and psychotics. The present study provides the broad-spectrum anticonvulsant activity of substituted quinazoline semicarbazones that are comparatively higher or equipotent to the current available drugs in the comparison tests. Overall, the synthesized compounds emerged as more active and less neuro-toxic derivatives. The antimicrobial efficiency of our new quinazoline series, indicating an introduction of newer synthetic antibiotics to the world.

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References

1. Loscher, W., New versions in the pharmacology of anticonvulsants, *European Journal of Pharmacology*, **342**, p. 1-13 (1998).
2. Leppik, I. E., Treatment of epilepsy in the elderly, *Current Treatment Options in Neurology*, **10(04)**, 239-245 (2008).
3. Chen, L., Sun, X.Y., Chai, K.Y., Synthesis and anticonvulsant evaluation of 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazoles as open-chain analogues of 7-alkoxyl-4,5-dihydro[1,2,4] triazolo[4,3-*a*] quinolones, *Bioorganic and Medicinal Chemistry*, **15**, p. 6775-6781 (2007).
4. Kubota, M., Sakakihora, Y., Zonisamide - induced urinary lithiasis in patients with intractable epilepsy, *Brain & Development*, **22**, p.230-233 (2000).
5. French, J.A., Practice advisory: the use of felbamate in the treatment of patients with intractable epilepsy, *Epilepsia*, **40**, p. 803-808 (1999).
6. Patil, V.M., Sinha, R., Masand, N., Synthesis and anticonvulsant activi-ties of small n-substituted2, 5-dimethyl pyrrole and bipyrrole, *Digest Journal of Nanomaterials and Biostructures*, **4**, No. **3**, p. 471-477 (2009).
7. Kashaw, S.K., Kashaw, V., Mishra, P., Jain, N.K., Design, synthesis and potential CNS activity some novel 1-(4-substituted-phenyl)-3-(4-oxo-2-prpyl-4H-quinazolin-3-yl)-urea, *ARKIVOC*, **(xiv),** p. 17-26 (2008).
8. Jatav, V., Mishra, P., Kashaw, S., Stables, J.P., Synthesis and CNS depres-sant activity of some novel 3-[5-substituted 1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3*H*)-ones, **43**, p. 135-141 (2008).
9. Wolfe, J.F., Sleevi, T.L., Campbell, J.A., Greenwood, T.D., Synthesis and anticonvulsant activity of some new 2-substituted 3-aryl-4(3H)-quinazolinones, *J. Med. Chem.*, **33**, p.161-166 (1990)**.**
10. Georgey, H., Gawad, N.A., Abbas, S., Synthesis and anticonvulsant activity of some quinazoline- 4-(3H)-one derivatives, *Molecules*, **13**, p 2557-2569 (2008).

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