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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of some novel 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2-phenylquinazoline-4(3*H*)-ones as possible anticonvulsant agent

Anjali Gupta · Sushil K. Kashaw · Neha Jain · Harish Rajak · Abhishek Soni · J. P. Stables

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Abstract A novel series **7(a–f)** 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2 phenylquinazoline-4(3*H*)-ones have been synthesized and screened for its anticonvulsant and neurotoxic activity. After i.p. injection to mice at doses of 30, 100, and 300 mg/kg body weight 2,3-disubstituted-quinazolin-4(3*H*)-ones were examined in the maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (scPTZ) induced seizure models in mice. Spectroscopic data were consistent with the structure of newly synthesized compounds. The neurotoxicity was assessed using the rotorod method. In the prepared series, **7a** was found to be active in the MES screen at 0.5 h, whereas **7f** showed anticonvulsant activity at both 0.5 and 4 h.

Keywords 1,3,4-Oxadiazole · Semicarbazone · Anticonvulsant

Introduction

Epilepsy is a chronic brain disease of diverse etiology, characterized by recurrent paroxysmal episodes of uncontrolled excitation of brain neurons. There is currently a need for improved agents for the treatment of seizures, since currently available drugs are effective only in 60–80% of epileptic patients. Polytherapy with antiepileptic drugs (AEDs) is necessary in clinical practice because of the limited efficacy of monotherapy. In recent years, many new chemical entities (NCE) have been designed that were structurally dissimilar from many common anticonvulsant containing dicarboximide function (CONRCO), which contributes toxic side effects. 4(3*H*)-Quinazolinones and related quinazolines are classes of fused heterocyclic that are of considerable interest. Quinazoline and quinazolinone derivatives have diverse pharmacological activities like anti-tumor, anti-inflammatory, CNS depressant, stimulant, anthelmentic, muscle relaxant, antifertility, hypoglycemic, and anti-microbial activities (Raffa *et al.*, 2004a, b; Alagarsamy *et al.*, 2003; Mukerji *et al.*, 1980; Gupta *et al.*, 2008; Shukla and Saxena, 1979; Hussain and Gupta, 1982; Abdel-Aleem and Abdel-ghaffer, 1980). Literature survey revealed that presence of substituted aromatic ring at position 3 and methyl group at position 2 are necessary requirement for the central nervous system (CNS) depressant and anticonvulsant activities. Modification of methyl group by some other chemical moiety yielded structural analogues with anticonvulsant activity. The reported work is based on 4(3*H*)-quinazolinone nucleus containing well known sedative-hypnotic and anticonvulsant methaqualone (2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone) and already reported compounds by us in search of potential anticonvulsant compounds (Jatav *et al.*, 2008a, b; Kashaw *et al.*, 2008, 2009). The structural requirement as presented in the Fig. 1 prompted us to undertake the present work.

It was also expected to get synergistic response of 4(3*H*)-quinazolinone nucleus itself, placement of substituted 1,3,4-oxadiazole at third position and chemically modifying second position of 4(3*H*)-quinazolinone (substituting $-C_6H_5$).

A. Gupta · S. K. Kashaw (✉) · N. Jain · A. Soni
Pharmaceutical Chemistry Division, Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar 470003, MP, India
e-mail: sushilkashaw@gmail.com

H. Rajak
Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur, CG 495 009, India

J. P. Stables
Preclinical Pharmacology Section, Epilepsy Branch, National Institutes of Health, Bethesda, MD 20892-9020, USA

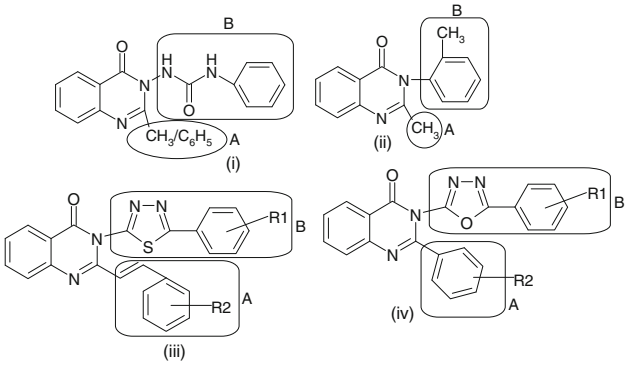


Fig. 1 Comparative pictorial representation of the proposed hypothesis (*iv*), methaqualone (*ii*) and previously reported compounds (*ii*) and (*iii*)

Chemistry

The synthetic route is outlined in Fig. 2. Substituted aryl semicarbazones **(2)** were prepared according to the method reported in the literature using semicarbazide hydrochloride and substituted aldehydes in alcohol (Furniss *et al.*, 2005a, b). 2-Amino-5-aryl-1,3,4-oxadiazoles **(3)** were prepared by cyclization of semicarbazones of the corresponding aromatic aldehydes in the presence of bromine in glacial acetic acid (Rajak *et al.*, 2007). *N*-benzoyl anthranilic acid **(5)** was prepared by reaction of anthranilic acid with benzoyl chloride. The reaction is followed by dehydrative cyclization of *N*-benzoyl anthranilic acid to form 2-phenyl benzoxazinone **(6)** (Raffa *et al.*, 2004a, b). Title compounds **7(a–f)** were synthesized by refluxing 2-phenyl benzoxazinone (**6**) with 2-amino-5-aryl-1,3,4-oxadiazole (**3**) in glacial acetic acid. All the synthesized compounds were characterized by spectral analysis (IR, H^1-NMR). Thin layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion.

Pharmacology

Initial anticonvulsant evaluation of the target compounds were undertaken by anticonvulsant drug development (ADD) program, NIH protocol. The profile of anticonvulsant activity was established after i.p. injections by one electrical and one chemical test. The electrical test

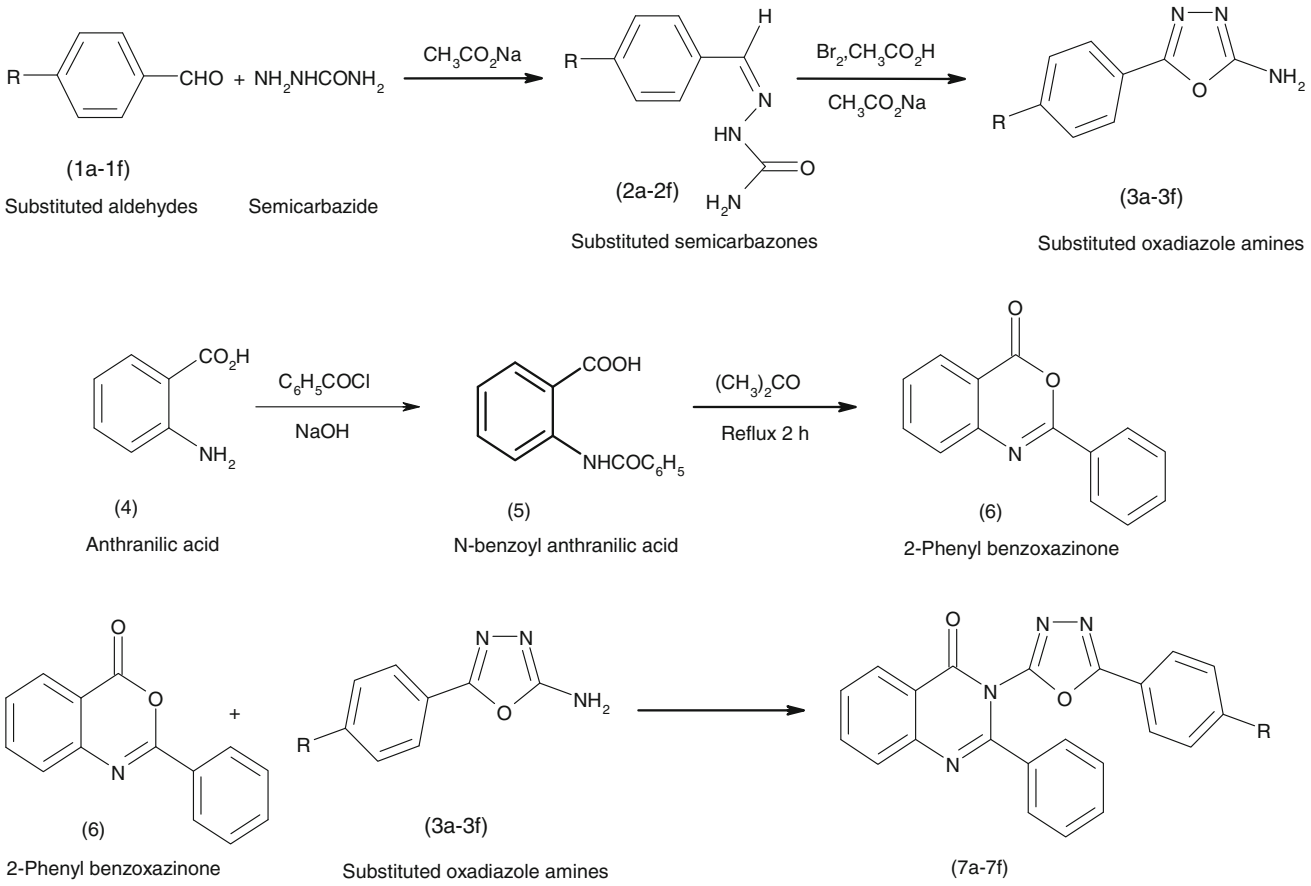


Fig. 2 Scheme for synthesis of target compounds

employed was the MES pattern test. The chemical test employed was the subcutaneous metrazole seizure threshold test. Minimal motor impairment was measured by the rotorod (neurotoxocity, NT) test.

Results and discussion

Structure of the prepared compounds was confirmed with the help of spectral analysis. Initial anticonvulsant activity and neurotoxicity data for the quinazolinone analogs are reported in Table 2. In the prepared series, **7a** and **7f** were found to be active at 300 mg/kg in the MES screen at 0.5 h, whereas **7f** showed anticonvulsant activity at 4 h. Out of six compounds only two compounds namely **7a** and **7f** exhibited activity at 300 mg/kg body weight. Three compounds out of six exhibited neurotoxicity at a maximum dose level of 300 mg/kg. None of the compounds showed neurotoxicity at a dose of 100 mg/kg. Compounds **7c**, **7d**, and **7e** showed extended level of neurotoxicity up to 4 h. It was observed that, as the electronegativity of substituted group increases, their anticonvulsant activity and duration of action also increases. In compound **7a**, R is –H which is less electronegative as compared to –F. Compound **7f** showed rapid onset of action. Compound **7a** which was active at 300 mg/kg in anticonvulsant screening showed no neurotoxicity, which suggested its selectivity.

Conclusion

Out of six synthesized compounds, **7a** and **7f** showed anticonvulsant activity while three compounds (**7c**–**7f**) exhibited the neurotoxicity (at 300 mg/kg body weight). Almost all the neurotoxic compounds also exhibited extended level of activity up to 4 h. Compound **7f** was active at 300 mg/kg body weight during 0.5 and 4 h time duration but unfortunately it was also toxic at the same dose. Compound with halogen substitution at position R was active but with neurotoxicity. Based on the experimental results, it may be concluded that the synthesized compounds showed more neurotoxic as compared to anticonvulsant.

Experimental

Chemistry

Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Perkin Elmer Spectrophotometer in KBr pellets, H^1-NMR spectra were recorded on Bruker Avance Spectrometer at 400 MHz using $CDCl_3$ as the solvent. Chemical shifts were reported in parts per million (ppm) using trimethylsilane (TMS) as an internal standard. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates and a solvent system of chloroform:methanol (8:2). The spots were developed in iodine chamber and visualized under ultra violet lamp.

Synthesis of 4-substitutedbenzaldehyde semicarbazone (**2a**–**2f**)

A solution of aromatic aldehyde (0.2 mol) in warm alcohol (250 ml) and a solution of semicarbazide (0.2 mol) and sodium acetate (0.4 mol) in 50–60 ml of warm water were mixed slowly with continuous stirring. The product separated immediately on cooling.

Synthesis of 2-amino-5-aryl-1,3,4-oxadiazoles (**3a**–**3f**)

Substituted semicarbazone (0.1 mol) and sodium acetate (0.2 mol) was dissolved in 300–350 ml of warm glacial acetic acid with continuous stirring. Bromine (7 ml in 50 ml of glacial acetic acid) was added slowly to it. Solution was stirred for an hour and then poured onto crushed ice. The separated solid was dried and recrystallized from 95% hot ethanol.

Synthesis of *N*-benzoyl anthranilic acid (**5a**–**5f**)

Anthranilic acid (4) (0.1 mol) was added in 360 ml of (5%) sodium hydroxide solution in a well-corked conical flask. Benzoyl chloride (36 ml) was added slowly with constant shaking until the odor of benzoyl chloride disappeared. The resulting solid was separated, dried and recrystallised from 95% ethanol.

Synthesis of 2-phenyl benzoxazinone (**6a**–**6f**)

The mixture of *N*-benzoyl anthranilic acid (**5**) (0.01mol) and acetic anhydride (0.1 mol) was refluxed under anhydrous condition for 2 h. The excess acetic anhydride was distilled off under reduced pressure. The product so obtained as a solid mass was used up immediately for next step. Physico-chemical data and m.p. of the **5** and **6** was in agreement with the data reported in the literature.

Synthesis of title compounds (**7a**–**7f**)

To the mixture of 2-phenyl benzoxazinone (0.1 mol), 2-amino-5-aryl-1,3,4-oxadiazole (0.1 mol) in 100 ml of glacial acetic acid was added and refluxed under anhydrous

condition for 4 h. After cooling, it was poured into crushed ice. The solid was filtered out, dried and recrystallized from hot ethanol (95%). The yields and melting point of synthesized compound are recorded in Table 1.

7a IR (cm^{-1}) 1683.1 (C=O str. in quinazolinone ring), 1608.7 (C=N str.), 1159.3 (C–O–C str. in oxadiazole ring), 849.3 (Ar-CH out of plane bending vibration), ^{1}HNMR (400 MH_z, δ) 7.22–7.9 (m, 14H, 3Ar-H)

7b IR (cm^{-1}) 1684.3 (C=O str. in quinazolinone ring), 1608.9 (C=N str.), 1180.6 (C–O–C str. in oxadiazole ring), 849.3 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.29–7.9 (m, 13H, 3Ar-H)

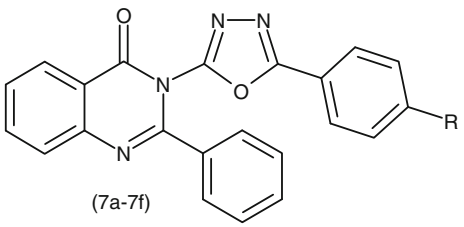
7c IR (cm^{-1}) 1684.4 (C=O str. in quinazolinone ring), 1609.4 (C=N str.), 1180.5 (C–O–C str. in oxadiazole ring), 1231.5 (Asym. C–O–C str. of aryl alkyl ether), 1025.0 (sym. C–O–C str. of aryl alkyl ether), 2983.4 (Alkane C–H str.) 848.3 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 6.83–7.9 (m, 13 H, 3Ar-H), 3.73 (s, 3H, OCH_3)

7d IR (cm^{-1}) 1660.9 (C=O str. in quinazolinone ring), 1583.8 (C=N str.), 1181.8 (C–O–C str. in oxadiazole ring), 2853.6 (Alkane C–H str.), 829.1 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.12–7.9 (m, 13H, 3Ar-H), 2.35 (s, 3H, CH_3)

7e IR (cm^{-1}) 1685.0 (C=O str. in quinazolinone ring), 1643.4 (C=N str.), 1158.3 (C–O–C str. in oxadiazole ring), 1538.5 (Asym. NO_2 str.), 1314.9 (Asym. NO_2 str.) 849.6 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.29–8.25 (m, 13H, 3Ar-H)

7f IR (cm^{-1}) 1684.8 (C=O str. in quinazolinone ring), 1645.6 (C=N str), 1157.9 (C–O–C str. in oxadiazole ring), 1039.2 (Aromatic C–F str.) 843.1(Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ), 7.03–7.9 (m, 13H, 3Ar-H)

Table 1 Physical data of 3-[5-(4-substituted)phenyl-1,3,4-oxadiazole-2yl]-2-phenylquinazoline-4(3*H*)-ones



(7a-7f)

S. no.	Code	Molecular formula	–R	M.P. (°c)	% Yield
1	**7a**	$C_{22}H_{14}N_4O_2$	–H	123–125	37.20
2	**7b**	$C_{22}H_{13}ClN_4O_2$	–Cl	113–116	42.56
3	**7c**	$C_{23}H_{16}N_4O_3$	$–OCH_3$	132–135	29.32
4	**7d**	$C_{23}H_{16}N_4O_2$	$–CH_3$	162–165	46.82
5	**7e**	$C_{22}H_{13}N_5O_4$	$–NO_2$	155–158	25.80
6	**7f**	$C_{22}H_{13}FN_4O_2$	–F	149–152	26.89

Pharmacology

The new derivatives obtained from the reaction sequence were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES), subcutaneous metrazole (ScMET), and neurotoxicity screens, using doses of 30, 100, and 300 mg/kg at two different time intervals. These data are presented in Table 2.

Anticonvulsant screening

Albino mice were used for this experiment. Food was withdrawn 12–15 h before the commencement of the experiments, while water was withdrawn immediately before the experiment. Maximum seizures were induced by applications of electrical current across the brain via corneal electrodes primed with normal saline (0.9% NaCl). The stimulus parameters were 50 mA AC in a pulse of 60 Hz for 0.2 s. After applying shock mice were observed for the type of convulsion produced and the hind limb extensor response was taken as the end point. Animals showing positive hind limb extensor response were used for testing drug substance. For ScMET test the test compounds were administrated i.p. to all animals in a group. Pentylenetetrazole (85 mg/kg) was injected subcutaneously, 30 min and 4 h after the administration of the compounds. The absence or presence of an episode of clonic convulsion was taken as the end point. Initially, all the compounds were administered i.p. in a volume of 0.01 ml/g body weight for mice and 0.004 ml/g body weight for rats at doses of 30, 100, and 300 mg/kg to one to

Table 2 Anticonvulsant activity and minimal motor impairment of 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2-phenylquinazoline-4(3*H*)-ones

Code no.	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicity screen	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
7a	300	–	–	–	–	–
7b	–	–	–	–	–	–
7c	–	–	–	–	300	300
7d	–	–	–	–	–	300
7e	–	–	–	–	300	300
7f	300	300	–	–	300	–
Phenytoin[b]	30	30	–	–	100	100
Carbamazepine[b]	30	100	100	300	100	300

[a] Doses of 30, 100, and 300 mg/kg were administered. The figures in the table indicates the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 h after injections. (–) indicates an absence of activity at maximum dose administered (300 mg/kg)

[b] Reference drug

four animals. Activity was established using the MES and ScMET test (White *et al.*, 1995a, b) and these data are presented in Table 2.

Neurotoxicity screen

The test is used to evaluate whether any drug is interfering with established anticonvulsant activity. Minimal motor impairment (Swinyard *et al.*, 1961) was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotated at six revolutions per minute. Only those animals showing ability to remain on the revolving rod for at least 1 min were selected for the test. These trained mice were divided into group of four animals each and were given test compounds intraperitoneally in doses of 30, 100, and 300 mg/kg. The rod diameter was 3.2 cm. 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.

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