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Design, Synthesis and Anticonvulsant Evaluation of N-[(Substituted 1*H*-pyrazol-3-yl)amino]-2-(4-methylphenyl)quinazolin-4(3*H*)-one Derivatives

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A series of quinazolinone derivatives (**7a-7e**) have been designed, synthesized and evaluated for the anticonvulsant activity using maximal electroshock seizure (MES) model. The compound 3-[(5-(4-chloro)phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)amino]-2-(4-methylphenyl)quinazolin-4(3*H*)-one (**7c**) was found to be most active compound showing 85.23 % protection and was found to be non-neurotoxic up to 4 h. This study indicates that the quinazolinone based derivatives could be further exploited for the discovery of newer and more effective anticonvulsant agents.

Keywords: Anticonvulsants, Quinazolinone, Maximal electroshock seizure (MES) model, Neurotoxicity.

INTRODUCTION

Epilepsy is the fourth most common neurological problem affecting human population after migrane, stroke and Alzheimer's disease (Epilepsy foundation). Epilepsy belongs to a group of neurological disorders showing neuronal hyper-excitability characterized by recurrent seizures in different parts of the brain [1]. According to WHO, it is estimated that the condition affects approximately 50 million people worldwide, 80 % of which resides in developing countries. Enormous AEDs belonging to different classes are used nowadays to treat epilepsy including phenytoin, carbamazepine, valproic acid, topiramate, gabapentin, felbamate, levetiracetam, *etc*. [2]. However, the current therapy using the presently available antiepileptic drugs shows limitations *viz.* insufficient, seizure control, unpredictability of effect and its loss, poor efficacy, inadequate information about the receptors involved, *etc*. Moreover AEDs do not prevent epilepsy in persons at risk of drug-resistance.

Further, long term therapy with these drugs pose an array of side effects which includes but is not limited to drowsiness, ataxia, gastrointestinal disturbances, megaloblasticanaemia and hirsutism [3,4]. Hence there exists the continuous need for the discovery and development of new antiepileptic agents with higher activity, minimal or negligible toxicity and side effects.

A number of heterocyclic derivatives possessing nitrogen has been reported to play an important role in pharmaceutical industry [5]. Amongst, quinazolinone moiety is one of the important scaffold embedded with a variety of biological activities including anticonvulsant [6], CNS depressant activities [6-8], antibacterial [9-12], anti-inflammatory [13-15], anticancer [16] *etc*. Methaqualone, a quinazolinone analogue, is reported to be a prime marker in the field of anti-convulsants drug development. Structure-activity relationship studies has revealed that the modifications at position 2 and 3 in quinazolinone nucleus might favour the anticonvulsant activity. Pandeya developed a 4-point pharmacophore model for anticonvulsants [17]. Accordingly, the molecule should possess four features *viz.* an electron donor group, a hydrogen bonding domain, a hydrophobic ring and a hydrophobic-hydrophilic ring.

Based on the discussions, in the present work, we designed and synthesized a series of quinazolinone derivatives, possessing the necessary four pharmacophoric features and evaluated the anticonvulsant activity of these derivatives using *viz.* maximal electroshock seizure (MES) model [18]. Further neurotoxicity of synthesized compounds was assessed by the rotarod apparatus [19]. The design approach for the synthesized quinazolinone derivatives is illustrated in Fig. 1.

EXPERIMENTAL

All the chemicals and reagents used were of commercial quality obtained from Sigma-Aldrich & Merck and were used without further purification.

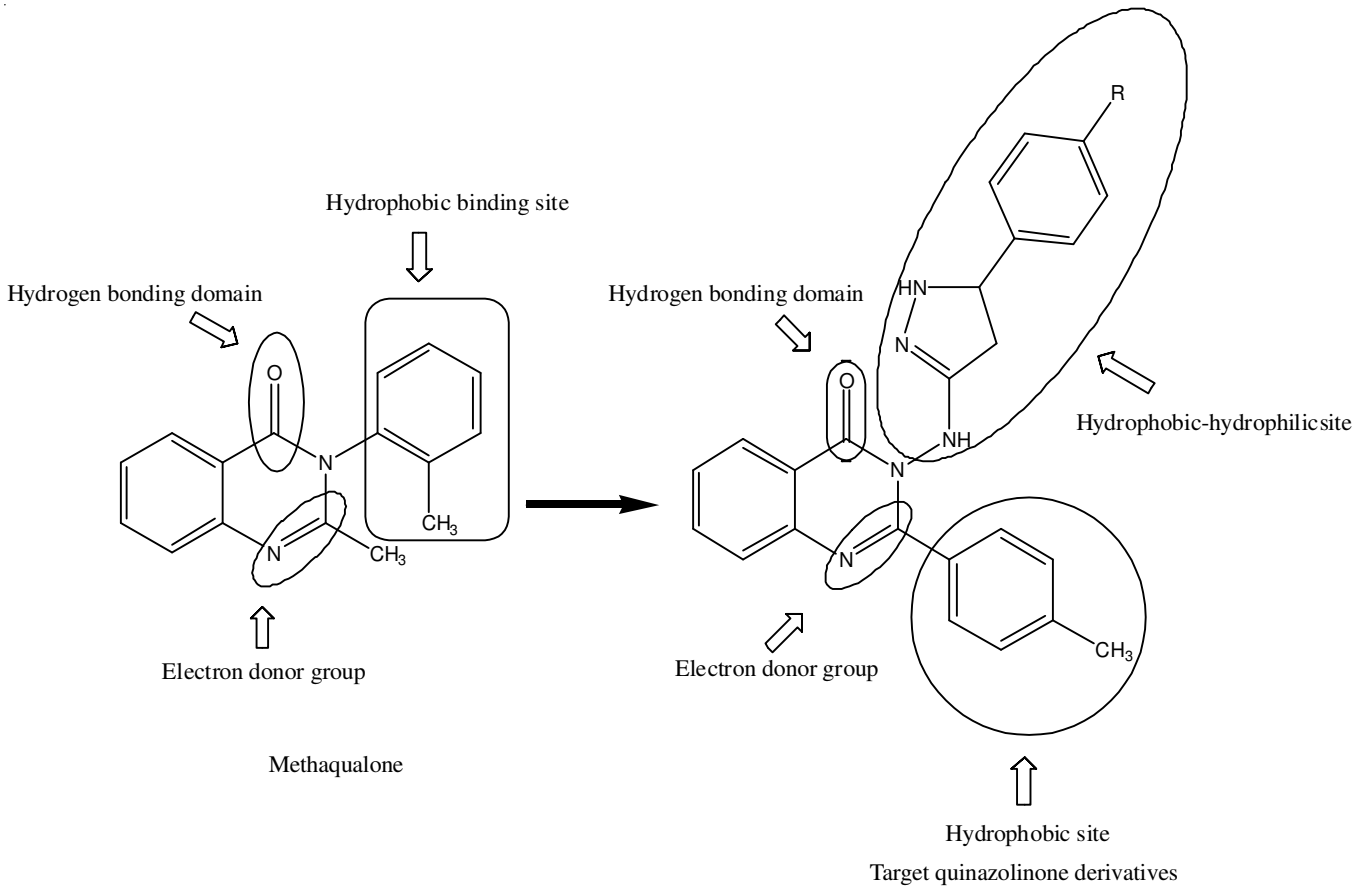
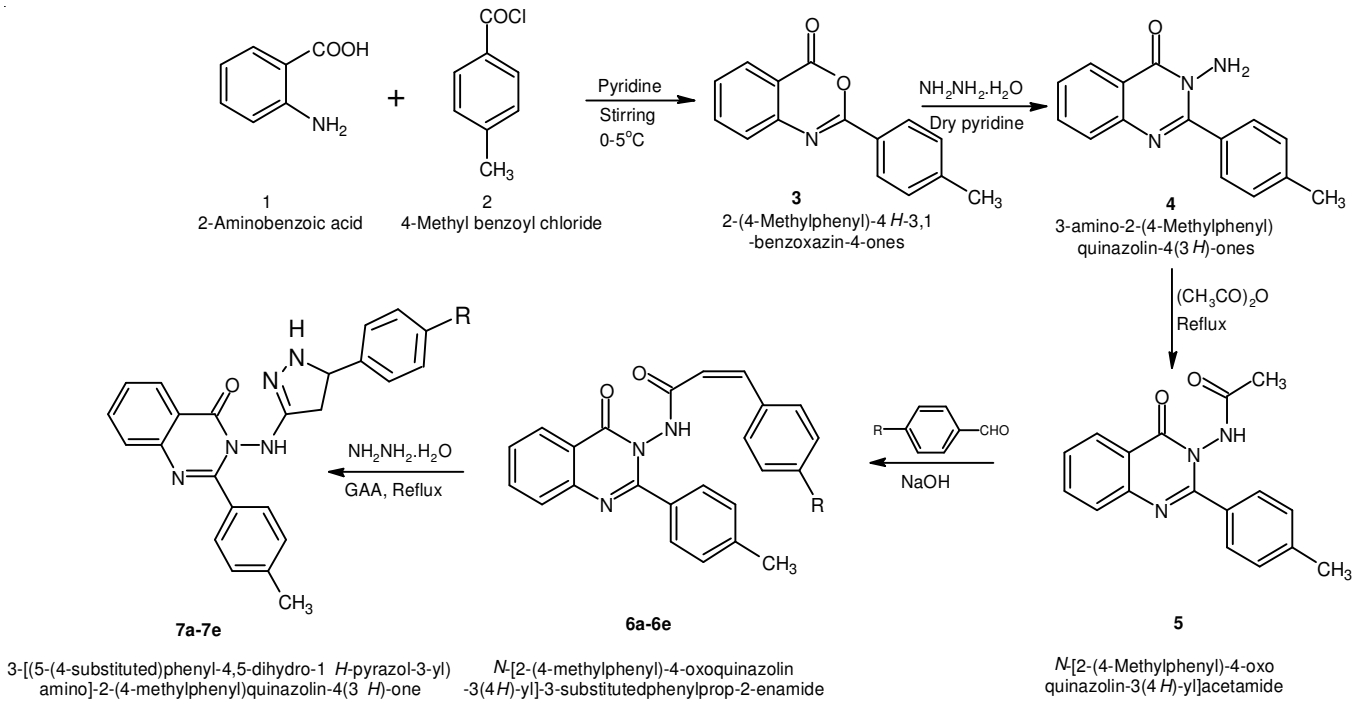


Fig. 1. Design approach for the target N-[(substituted-1*H*-pyrazol-3-yl) amino]-2-(4-methylphenyl) quinazolin-4(3*H*)-one derivatives incorporating the pharmacophoric features necessary for anticonvulsant activity

The synthesis of target compounds (**7a-7c**) was accomplished in accordance to **Scheme-I**. 2-Aminobenzoic acid (**1**, 0.1 mol) was reacted with 4-methylbenzoyl chloride (**2**, 0.2 mol) in presence of pyridine at 0-5 °C to produce the first intermediate 2-(4-methyl phenyl)-4*H*-3,1-benzoxazin-4-ones (**3**) which on further reaction with hydrazine hydrate (0.01 mol)



Scheme-I: Synthesis of target N-[(substituted-1*H*-pyrazol-3-yl) amino]-2-(4-methylphenyl) quinazolin-4(3*H*)-one derivatives

lead to the formation of next intermediate 3-amino-2-(4-methyl phenyl)quinazolin-4(3*H*)-one (**4**). Reaction of compound **4** (0.01 mol) with acetic anhydride (0.01 mol) in presence of hydrated sodium acetate and HCl resulted in the formation of the N-[2-(4-methylphenyl)-4-oxoquinazolin-3(4*H*)-yl]acetamide (**5**). Compound **5** was then reacted with substituted benzaldehydes to obtain the compounds **6a-6e** which on further cyclization with hydrazine hydrate (0.01 mol) yielded the corresponding final compounds (**7a-7e**).

Characterization of all the intermediates and final compounds were accomplished by various physico-chemical and spectral techniques. The melting point of all the final compounds was recorded on digital melting point apparatus (Kshitij Innovation) using one end open capillary tubes and are uncorrected. The progress of the reaction and its purity was confirmed by the thin layer chromatography on silica gel G plates. FTIR spectra was recorded on Shimadzu IR affinity spectrophotometer using KBr disc method. The ^{1}H NMR was recorded on a Varian AC 400 spectrometer using $CDCl_3$ as a solvent. TMS was taken as the internal standard. Mass spectra were on a Varian 1200L mass spectrometer. The data is expressed as $(M+1)^+$ values. Elemental analysis (C, H, N) was determined using a Carlo-Erba 1160 elemental analyzer. The physio-chemical and spectral characterization data of all the final compounds is presented in Tables 1 and 2, respectively.

Pharmacology: All the experimental procedures were carried out in accordance to CPCSEAs guidelines and approved by IAEC of the Institute (1205/c/08/CPCSEA).

Animals: Swiss albino mice weighing about 18-25 g were used in the entire study. The animals were kept in individual cages for one week to acclimatize them at the laboratory conditions. They were allowed free access to water and food.

TABLE-1
PHYSICO-CHEMICAL CHARACTERIZATION DATA OF FINAL COMPOUNDS **7a-7e**

Compd. code	R	m.f.	m.w.	m.p. (°C)	Yield (%)
7a	-OH	$C_{24}H_{21}N_5O_2$	411.47	210-215	68
7b	$-OCH_3$	$C_{25}H_{23}N_5O_2$	425.49	236-240	60
7c	-Cl	$C_{24}H_{20}N_5OCl$	429.91	234-239	65
7d	-Br	$C_{24}H_{20}N_5OBr$	474.36	295-300	70
7e	-F	$C_{24}H_{20}N_5OF$	413.46	231-235	57

Anticonvulsant activity: All the synthesized final compounds (**7a-7e**) were evaluated for the anticonvulsant activity using maximal electroshock seizure model. The animals were divided into three groups consisting of 6 animals each *viz.* test, control and standard. All the test compounds were dissolved in 1 % CMC solution and administrated intra-peritonially to the animals at three doses of 25, 75 and 150 mg/kg; 30 min before delivering the seizures. Phenytoin (25 mg/kg) was used as a reference drug. Control animals were given the 0.9 % saline solution. Seizures were induced in mice by delivering electro-shock of 50 mA for 0.2 s by means of electro-convulsiometer through a pair of ear clip electrodes. The results of the maximal electroshock seizure model for the synthesized compounds (**7a-7e**) is summarized in Table-3.

Neurotoxicity screening: The compounds (**7a-7e**) were treated for the neurotoxicity using rotarod apparatus. The animals were trained to balance on rotating rod (diameter: 3 cm) revolving at a speed of 6 rpm. The test compounds were considered to be neurotoxic at a particular dose if the trained animal is unable to balance itself on the rotating rod for at least 1 min. The animals were tested at four time intervals *viz.* 0.5, 1, 2 and 4 h for 1 min, after drug administration. The results are expressed as animals toxic/total number of animals used and is summarized in Table-4.

TABLE-2
SPECTRAL CHARACTERIZATION DATA OF THE FINAL COMPOUNDS **7a-7e**

Compd. code	IR (KBr, ν_{max}, cm^{-1})	^{1}H NMR ($CDCl_3$, δ in ppm)	Mass (*m/e*) $[M+1]^+$	Elemental analysis (%)	
				Calculated	Observed
7a	3550 (O-H str), 3410 (N-Hstr), 3100 (C-H str), 2920 (C-H str) 2917 (C-H asym str.), 2860 (C-H str), 1690 (C=O str), 1660 (C=C str), 1516 (C-C str), 1470 (C-H bend), 1250 (C-N)	7.4-7.9 (m, 4H, ArH), 7.0 (s, 1H, N-H), 7.13-7.55 (m, 4H, ArH), 2.3 (s, 1H, N-H), 6.5 (s, 1H, N-H), 6.68-6.95 (m, 4H, ArH), 5.0 (s, 1H, -OH), 2.35 (s, 3H, $-CH_3$).	412.47	C (70.06 %) H (5.14 %) N (17.02 %) O (7.78 %)	C (70.13 %) H (5.19 %) N (17.10 %) O (7.83 %)
7b	3415 (N-Hstr), 3050 (C-H str), 2916 (C-H str) 2929 (C-H asym str), 2872 (C-H str), 2835 (O-CH_3 str) 1681 (C=O str), 1664 (C=C str), 1510 (C-C str), 1472 (C-H bend), 1243 (C-N str)	7.2-7.7 (m, 4H, ArH), 7.6 (s, 1H, N-H), 7.01-7.45 (m, 4H, ArH), 2.8 (s, 1H, N-H), 7.0 (s, 1H, N-H), 6.72-7.01 (m, 4H, ArH), 3.74 (s, 1H, $-CH_3$), 2.39 (s, 3H, $-CH_3$)	426.49	C (70.57 %) H (5.45 %) N (16.46 %) O (7.52 %)	C (70.66 %) H (5.39 %) N (16.49 %) O (7.58 %)
7c	3425 (N-H str), 3054 (C-H str), 2935 (C-H str) 2912 (C-H asym str.), 2867 (C-H str), 1694 (C=Ostr), 1658 (C=C str), 1522 (C-C str), 1445 (C-H bend), 1255 (C-N str), 800 (-Cl)	7.8-7.13 (m, 4H, ArH), 9.0 (s, 1H, N-H), 7.09-7.50 (m, 4H, ArH), 3.0 (s, 1H, N-H), 7.4 (s, 1H, N-H), 7.08-7.24 (m, 4H, ArH), 2.4 (s, 3H, $-CH_3$)	430.91	C (67.05 %) H (4.69 %) Cl (8.25 %) N (16.29 %) O (3.72 %)	C (67.12 %) H (4.23 %) Cl (8.40 %) N (16.35 %) O (3.92 %)
7d	3422 (N-H str), 3049 (C-H str), 2954 (C-H str) 2920 (C-H asym str.), 2866 (C-H str), 1686 (C=O str), 1659 (C=C str), 1519 (C-C str), 1475 (C-H bend), 1243 (C-N str), 1100 (Br)	7.7-7.12 (m, 4H, ArH), 8.4 (s, 1H, N-H), 7.14-7.57 (m, 4H, ArH), 2.0 (s, 1H, N-H), 7.8 (s, 1H, N-H), 7.01-7.17 (m, 4H, ArH), 2.30 (s, 3H, $-CH_3$)	475.36	C (60.77 %) H (4.25 %) Br (16.84 %) N (14.76 %) O (3.37 %)	C (60.83 %) H (4.36 %) Br (16.75 %) N (12.62 %) O (3.43 %)
7e	3419 (N-H str), 3043 (C-H str), 2926 (C-H str)2921 (C-H asym str.), 2867 (C-H str), 1693 (C=O str), 1658 (C=C str), 1520 (C-C str), 1475 (C-H bend), 1248 (C-N str), 1150 (F)	7.1-7.6 (m, 4H, ArH), 8.1 (s, 1H, N-H), 7.0-7.41 (m, 4H, ArH), 2.2 (s, 1H, N-H), 8.0 (s, 1H, N-H), 7.11-7.27 (m, 4H, ArH), 2.40 (s, 3H, $-CH_3$)	414.46	C (69.72 %) H (4.88 %) F (4.59 %) N (16.94 %) O (3.87 %)	C (69.83 %) H (4.74 %) F (4.61 %) N (16.32 %) O (3.92 %)

TABLE-3
PRELIMINARY ANTICONVULSANT SCREENING DATA OF THE COMPOUNDS (**7a-7e**) IN MES MODEL

Compd. code	Dose (mg/kg)	Onset convulsion threshold (Mean ± SEM)	Protection (%)	ED_{50} value[a]	Potency (%)[b]
7a	25	5.34 ± 0.27	50	158.4	57.26
	75	7.33 ± 0.27	65.60		
	150	8.34 ± 0.31	83.30		
7b	25	8.3 ± 0.41	33.33	133.4	66.45
	75	9.04 ± 0.14	60.50		
	150	7.04 ± 0.50	79.40		
7c	25	6.01 ± 0.18	54.66	108.4	64.90
	75	8.35 ± 0.45	67.75		
	150	9.45 ± 0.20	85.23		
7d	25	7.54 ± 0.77	45.80	150.0	71.16
	75	9.23 ± 0.44	59.30		
	150	9.34 ± 0.32	80.00		
7e	25	8.65 ± 0.34	53.90	158.4	62.47
	75	7.02 ± 0.42	68.34		
	150	7.25 ± 0.32	81.80		
Phenytoin (Std.)	25	12.23 ± 0.33	100.00	–	–

[a]$ED_{50} = ED_{100}$-Σ(Difference between two successive doses x average number of survived animals)/n, n = 6
[b]Potency (%) of compound = (OCT of compound/OCT of the reference drug) × 100; *OCT = onset convulsion threshold
Results were analyzed by ANOVA, Dunnett's test.

TABLE-4
NEUROTOXICITY SCREENING DATA
OF THE COMPOUNDS (**7a-7e**)

Compd. code	0.5 h	1 h	2 h	4 h
7a	0/4	0/4	0/4	0/4
7b	0/4	0/4	1/4	2/4
7c	0/4	0/4	0/4	0/4
7d	0/4	0/4	0/4	0/4
7e	0/4	0/4	0/4	1/4
Phenytoin	0/4	0/4	0/4	0/4

The data indicates number of animals toxic/total number of animals used.

RESULTS AND DISCUSSION

The synthesis of title compounds **7a-7e** were accomplished according to the reaction sequence illustrated in **Scheme-I**. The compounds were analyzed by FTIR, ^{1}H NMR, mass spectrometry and elemental analysis. The structural data was found to be in accordance with the structures of the synthesized compounds.

Anticonvulsant activity

Maximum electroshock seizure (MES) model: The preliminary MES screening results expressed in the form of percentage protection is presented in Table-3. The MES model is the mechanism-independent animal seizure model that enables identification of compounds preventing seizure spread. This test uses an electrical stimulus to produce generalized tonic-clonic seizures and thus is thought to be an experimental model of tonic-clonic epilepsy and partial convulsions with or without secondary generalization in humans. The compounds **7a**, **7c** and **7e** showed protection in half or more of the animals tested at dose 25 mg/kg. All compounds except **7d** showed 60 to 70 % protectionat dose 75 mg/kg while compounds **7a**, **7c**, **7d** and **7e** showed 80 to 85 % protection at a dose of 150 mg/kg. Further percentage potency of all the compounds was calculated compound **7d** possessed maximum percentage protection of 71.16 % among all the compounds.

Neurotoxicity screening: The results of neurotoxicity screening are presented in Table-4. None of the compounds were neurotoxic upto 1 h. All the compounds were non-neurotoxic at 2 and 4 h except **7b** and **7e**. Compound **7b** was found to be mildly neurotoxic (25 % neurotoxic) at both the time intervals *i.e.* of 2-4 h while **7e** was found to be mildly neurotoxic (25 % neurotoxic) at 4 h.

Conclusion

In this study, we designed and synthesized a series of N-[(substituted-1*H*-pyrazol-3-yl) amino]-2-(4-methylphenyl)-quinazolin-4(3*H*)-one derivatives (**7a-7e**) and evaluated them for the anticonvulsant activity in maximum electroshock seizure (MES) model. Compound **7c** was found to be most active revealing about 85.23 % (Fig. 2) protection and was found to be non-neurotoxic upto 4 h in neurotoxicity screening.

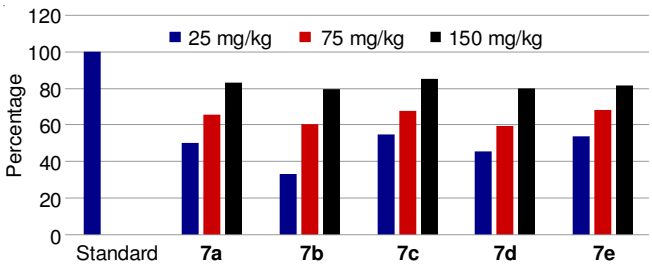


Fig. 2. Comparison of percentage protection of synthesized compounds (**7a-7e**) at different doses tested

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