ANTICOVULSANT ACTIVITIES OF SOME NOVEL 3-[5-SUBSTITUTED 1, 3, 4-THIADIAZOLE-YL]-2-STYRYL QUINAZOLINE-4(3H)-ONES

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Summary

Thiadiazole with styryl and quinazoline are reported to exhibit wide range of anticonvulsant, sedative, tranquilizer, analgesic, antimicrobial, anesthetic, anticancer, antihypertensive, anti-inflammatory, diuretic and muscle relaxant properties. With the intention to develop potent anticonvulsant agents we have designed, synthesized 3-[5-substituted 1, 3, 4-thiadiazol-yl]-2-styryl quinazolin-4(3H)-ones derivatives. The synthesized derivatives were tested in vivo for their anticonvulsant activity using MES, PTZ and Actophotometer model.

The synthesized compounds, showed significant anticonvulsant activity comparable to the standard phenytoin, diazepam and phenobarbital. The compounds BJ-1 (5.68Sec), BJ-7 (5.68Sec) and BJ-8 (6.3 Sec) were found to induce a significant anticonvulsant activity against MES model compared to standard drug Phenytoin 5.67 Sec and the compound BJ-8 (124 Sec), BJ-9 (122Sec), BJ-7 (122Sec) were most potent against PTZ model (standard drug diazepam). Around five compound were found to be significantly potent against Actophotometer model BJ-1 (2.59Sec), BJ-5 (264Sec), BJ-2 and BJ-9 (266Sec), BJ-4 (270Sec) and BJ-3 (272Sec) were found to exhibit significant anticonvulsant activity (decrease locomotor activity) compared to std drug phenobarbital (257Sec).

Thus, in conclusion, the compound BJ-1 was found to be most potent in MES whereas BJ-8 in actophotometer model exhibited significant activity against PTZ model indicating the critical role played by substituted on phenyl ring. This has complied us to get detailed insights of structure activity relationship studies of the synthesized derivatives. And it can be said that one electron rich [–N $(CH_3)_2$, –OH, OCH_3O] and another electron withdrawing group [-F,-NO2] are required for potent anticonvulsant activity of these type of compound.

Key Words: Diazepam, 3-[5-substituted 1, 3, 4-thiadiazole-yl]-2-styryl quinazoline-4(3H)-Ones, anticonvulsant MES, PTZ, Actophotometer.

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Introduction

The conditions grouped under the term epilepsy constitute an area of continuing medical need. It has been estimated that about 20% of the patients with epilepsy using the first generation of antiepileptic drugs (Phenobarbital, Phenytoin, Carbamazepine, Sodium valproate and Diazepam) were not able to acquire adequate control of seizure.

One of the most frequently encountered heterocycles in medicinal chemistry is 4(3H)-quinazolinone with wide applications including anticonvulsant, sedative, tranquilizer, analgesic, antimicrobial, anesthetic, anticancer, antihypertensive, anti-inflammatory, diuretic and muscle relaxant properties [1,2,3,4]. Literature survey revealed that the presence of substituted aromatic ring at position 3 and methyl group at position 2 are necessary requirement for the central nervous system (CNS) depression and anticonvulsant activities. In spite of the fact that literally hundreds of quinazolinones related derivatives have been synthesized and tested for central nervous system (CNS) depression and anticonvulsant activities, none of the drugs currently in use contain the 4(3H)-quinazolinone ring system. Among the few reports in the literature our attention was drawn to the earlier discovery by Boltze et al. [5] He discover the different substituents on the aromatic ring exert a significant influence on the biological activity by modulating the lipophilicity and thereby facilitating penetration across the blood-brain barrier. that modification of methyl group by some other chemical moiety yielded structural analogues with anticonvulsant activity.

Medicinal chemists over the years have substituted different heterocyclic rings at position 3 of the 4(3H)-quinazolinone to get potent CNS acting drugs. 1, 3, 4-Thiadiazoles nucleus itself exhibits anticonvulsant, sedative-hypnotic and CNS neurotoxicity activities [6]. In hope of getting synergistic response of 4(3H)-quinazolinone nucleus itself, substitution of 1, 3, 4-thiadiazoles nucleus at third position and chemically modifying second position of 4(3H)-quinazolinone, the present paper reports on the synthesis, anticonvulsant,CNS depressant activity of 3-[5-substituted 1, 3, 4-thiadiazole-yl]-2-styryl quinazoline-4(3H)-one derivatives. The compounds designed so, were found to posses much significant anticonvulsant activity with significant reduction in toxicity.

Material and Methods

Experimental Animals: [7,8,9]

Swiss albino mice of either sex weighing 20-25g obtained from National Centre for Cell Sciences, Pune, India .All the animals were housed under standard environmental conditions of temperature (24±2) and relative humidity of 30-70 %. A 12:12 h light: dark cycle was maintained. All the animals were allowed to have free access to water and standard palletized laboratory animal diet. All experimental procedure and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of college, Pune, constituted for the purpose of control and supervision of Experiment on Animals (CPCSEA), Government of India.

Chemicals:

All the chemicals used of analytical grade from procured from reported quality suppliers. Diazepam (Ranbaxy), PTZ (Ozone International, Mumbai), Phenobarbital (Anglo-French, Bangalore).All the chemicals used in the experimental study were of analytical grade.

Anticonvulsant Activity:

A freshly prepared suspension of Acacia (1.0%w/v, 0.1 ml) was triturated with the test and standard compound (10mg/kg) were subjected to anticonvulsant screening was carried out. In case of Maximal Electronic Shock (MES) the injection given orally, PTZ (Pentylene tetrazole) by Intraperotonial route in abdominal region and for screening using Actophotometer oral dose was given.

Data are expressed as % anticonvulsant activity ± S.E.M. and analyzed by one way ANOVA followed by Dunnett's test to determine the significant difference between the control group and test compounds. The difference in results were considered when $P<0.01$. All Statistical Calculation were carried out using Graph Pad Prism 3.0 (USA) software.

Acute oral toxicity Study:[10]

3-[5-substituted] 1, 3, 4-thiadiazol-2-yl]-2-styryl quinazolin-4(3H)-one derivatives (compound code: BJ1-BJ9) were subjected to acute toxicity study. Mice were then observed for incidences of mortality or any sign of toxicity up to 24 h after oral administration.

The dosing schedule followed was as per the OECD guideline 425 [9] as follows: Only one mouse received a dose at a particular time. First animal received a dose of 2000 mg/kg/p.o. Animal was observed for 3 hours after dosing for any toxicity signs, survival or death. If the first animal died or appeared moribund, the second animal received a lower dose. The dose progression or reduction factor was 3.2 times of the previous dose. If no mortality was observed in the first animal then the second animal received a 3.2 times higher dose. Dosing of the next animal was continued depending on the outcome of the previously dosed animal for a fixed time interval (3 hour). The test was stopped when one of the stopping criteria was met: i.e. i)5 reversals occur in any 6 consecutive animals tested. ii) 3 consecutive animals died at one dose level. Survived animals were observed for outcomes for a period of 24 hr. (AOT425 Guidelines)

PTZ studies: [11-13]

Animals were divided into three groups each comprising of 5 animals. One group was used for studying the effect of PTZ one group for control and third group to study effect for the protection of effect from Diazepam.

The PTZ at dose of 10 mg/Kg injected through intraperitonoal route to control group of animals and the onset of action in the form of jerky movement were measured Diazepam and test compound were administered through intraperitonoal route . After 30 min of PTZ administration the onset and severity of convulsion were noted. The delay on set of convulsion was referred to be anticonvulsant response .The results of test compound were compared with control and standard compounds.

The PTZ model was standardized using phenytoin as internal standard. The result are reported in the Table 1.

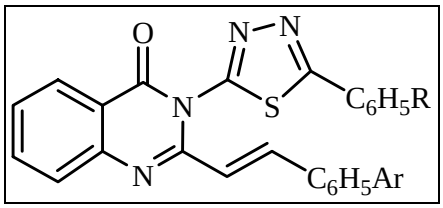
MES Studies:

Initially all the synthesized compounds were administrated i.p. in a volume of 0.01mg/g body weight for mice 0.04 ml/g body weight to result. At 10 mg/Kg body weight.

Animals were subjected to electroshock with 48Ma current for 0.2 sec .The animals were observed for 30 min convulsive responses. If any then all the animals were subjected to induce convulsion using MES model.

The MES model was standardized using phenytoin as internal standard. The results are reported in the Table 1.

Table 1: Chemical structure of 3-[5-substituted 1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones derivatives [17,18] and their anticonvulsant activity in mice by using PTZ, MES and Actophotometer model



Sr no	**Comp Code**	**-R**	**-Ar**	**(PTZ) Convulsions after test compound treatment (Sec)** Mean ± SEM	**(MES) Hind Limb Extension (Sec)** Mean ± SEM	**(Actophtometer) Locomotar activity (Sec)** Mean ± SEM
1	Control	-	-	63.66 ± 3.18	14.77 ± 1.35	425± 7.11
2	BJ1	$N(CH_3)_2$	3- NO_2	109.33 ± 2.6**	5.2 ± 0.6**	259.66± 6.79**
3	BJ2	p-F	$N(CH_3)_2$	107.66 ±1.45**	6.33± 1.15**	266.33 ± 2.472**
4	BJ3	3- NO_2	$N(CH_3)_2$	108.33 ± 0.88**	7.68 ± 1.28**	272.66 ± 8.36**
5	BJ4	4-OH	4-FC_6H_4	106.33 ± 0.88**	9.07 ± 0.92**	281 ± 4.612**
6	BJ5	3- NO_2	4-FC_6H_4	112.66 ±1.76**	7.58 ± 0.66**	264.66± 2.985**
7	BJ6	3- NO_2	3,4,5(CH_3O)	115 ±2.33**	6.11± 1.23**	274.66± 3.93**
8	BJ7	3- NO_2	4- Br	121± 4.58**	5.68± 0.81**	270.66 ±6.075**
9	BJ8	$N(CH_3)_2$	4-F	124.66 ±2.72**	6.3 ± 0.67**	274.66 ± 7.71**
10	BJ9	3- NO_2	4- OH	122 ± 5.03**	6.6 ±1.29**	266 .33 ±6.97**
11	Std	-	-	--	5.67 ±1.09**	275.33± 6.561**

Results are expressed as Mean ± SEM, Data was analyzed by one way ANOVA followed by Dunnett's ; (n =6),* P<0.05 **P<0.01 ***P< 0.001

Actophotometer:

Weigh animal and number them. Place individually each mice in the activity cage for 10 min. Note the basal activity score of all the animals .Inject (standard Phenobarbital) and measure the mean change of locomotor activity.

The Actophotometer model was standardized using phenytoin as internal standard. The results are reported in the Table 1.

Results and Conclusions

Oral administration of [5-substituted] 1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3h)-one[13-16] (comp BJ1-BJ9) did not produce any toxic effect in mice. The 3-[5-substituted] 1, 3, 4-thiadiazole-yl]-2-styryl quinazoline-4(3H)-one (compound code: BJ1-BJ9) and were found to be safe and no mortality was observed up to 2000 mg/kg. The data was analyzed with help of AOT425 Software.

Standard drug used for **PTZ** model was Diazepam (Fig. 1), **MES** model was Phenyltoin (Fig. 2), **Actophotometer** model was Phenobarbital (Fig. 3).

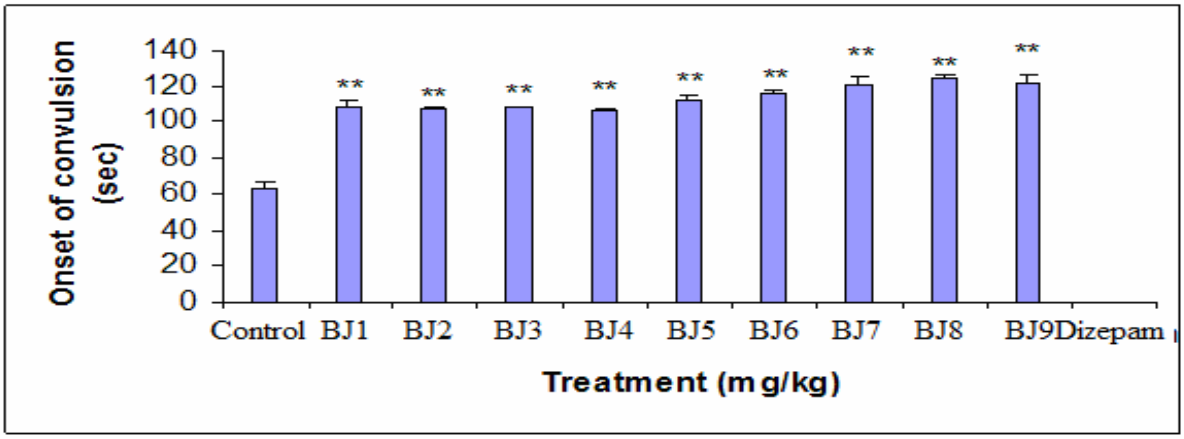


Fig. 1: Comparison of Anticonvulsant activity of test compound (PTZ)

Data was analyzed by one way ANOVA followed by Dunnett's test (n =6),* P<0.05 **P<0.01

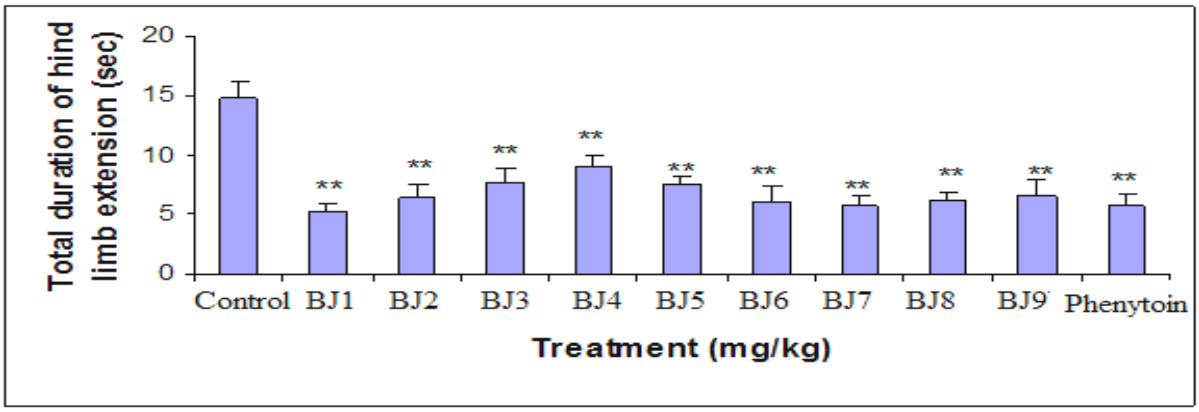


Fig. 2: Comparison of Anticonvulsant activity test compounds of MES model

Data was analyzed by one way ANOVA followed by Dunnett's test (n =6),* P<0.05 **P<0.01

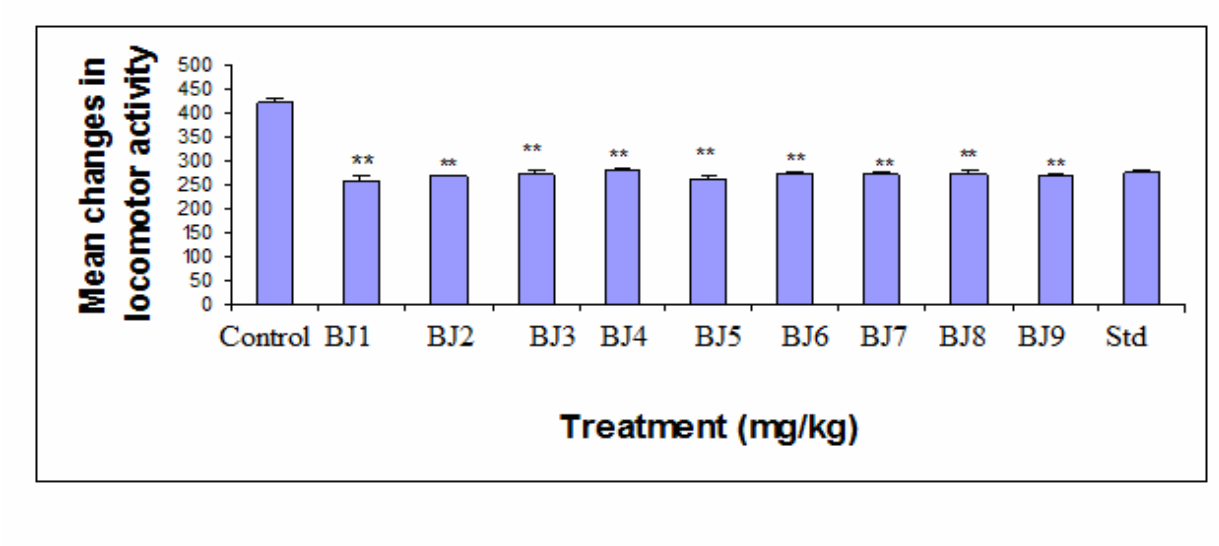


Fig. 3: Comparison of Behavioral studies of test compounds using Actophotometer model

Data was analyzed by one way ANOVA followed by Dunnett's test (n =6),* P<0.05 **P<0.01

The compounds were tested at equimolar doses equivalent to 10 mg/kg dose of standard drug i.e. Diazepam 28.47 mol Phenobarbital 25.23 mol and Phenytoin 23.24 mol. The tested compounds showed a potent anticonvulsant activity using different three animal model Viz.

1. PTZ Model: The compounds BJ1-BJ9 are significantly increase onset of convulsion when compared with control. The compound BJ8 was found to be more potent as model among all the synthesized compound (Fig. 1).

2. MES Model: The compounds BJ1-BJ9 are significantly decrease hind limb extension when compaied with control. The compound BJ-1 (5.2 Sec) BJ-7 (5.67Sec) were found to be significantly potent as compared with other compound (Fig. 2).

3. Actophotometer Model: The compounds BJ1-BJ9 significantly decreased locomotor activity when compared to the control. The compound BJ1 was found to be more potent as compared to the other compounds(Fig. 3).

Thus in conclusion the compound BJ-1 was found to be most potent in MES whereas BJ-8 in Actophotometer model exhibited significant activity against PTZ model indicating the critical role played by substituted on phenyl ring. This has complied us to get detailed insights of structure activity relationship studies of the synthesized derivatives. And it can be said that one electron rich [–N $(CH_3)_2$, –OH, OCH_3O] and another electron withdrawing group [-F,-NO2] are required for potent anticonvulsant activity of these type of compound.

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