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Anticonvulsant Activity of 4-(Substituted Benzylidene)-6-(3-nitrophenyl)-4,5-dihydro Pyridazin-3(*2h*)-ones Against Maximal Electro Shock Induced Seizure

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Abstract: 4-(Benzylidene or substituted benzylidene)-6-(3-nitrophenyl)-4,5-dihydropyridazin- 3(2*H*)-ones (**4a-4c**) were synthesized from 6-(3-aminophenyl)-4,5-tetrahydro pyridazin-3(*2H*)-one (**3**) by condensation reaction with different benzaldehydes. The title compounds (**4a-4c**) were evaluated for anticonvulsant activity by maximal electro shock (MES) induced seizure method and these synthesized compounds exhibited significant anticonvulsant activity.

Key words: Anticonvulsant · Pyridazinone · Maximum Electroshock Method

INTRODUCTION

Many potent drugs derived from synthetic as well as natural sources, commonly in practice contain nitrogen in the heterocyclic ring system. The biological activities of various nitrogen hetero atoms containing compounds that are well established against various diseases or disorders like, natural compounds reserpine (antihypertensive and tranquilizer), morphine (CNS depressant and analgesic), etc and synthetic compounds, like Phenytoin, barbiturates, benzodiazepines, ethosuximide, carbamazepine etc. Pyridazinone [1,2]diazine derivatives have been reported to possess wide variety of biological activities such as antidiabetic, anticancer, anti-AIDs, antihypertensive, antimicrobial, fungicidal, herbicidal, antifeedant, antiplatelet, analgesic, anti-inflammatory and other anticipated biological activities [1-4]. On the other hand, a considerable number of pyridazin-3(*2H*)-ones endowed with anticonvulsant properties have been reported [5-16]. A series of 6-arylpyridazines derivatives exhibited appreciable anticonvulsant activity also. The discovery of biological activity in a series of pyridazine derivatives stimulated the vigorous growth of investigations in this area. Stimulated by these findings, our attention has been focused on the evaluation of three 6-nitrophenyl pyridazin-3(*2H*)-one (**4a-4c**) which are expected to show anticonvulsant activity.

Structural Feature of Aryl Pyridazinone Derivatives for Their Anticonvulsant Activity: Many investigations indicated that the presence of at least one aryl group, one or two electron donor atoms and/or an NH group in a special spatial arrangement is necessary for anticonvulsant activity [6- 9]. The pyridazinone ring system agrees with this salient feature. In order to explore the activity associated with the presence of an amide moiety, cyclic or not, is present in most anticonvulsants. The most common structural feature of clinically active drugs against epilepsy appeared to be a nitrogen hetero atomic system. The results revealed that, a number of aryl pyridazinones possessed greater protection in the maximal electro shock (MES) screening. It has been proposed that for activity in the MES test, a compound should have a large hydrophobic group in the close proximity to at least two electron donor atoms. The pyridazinones containing a hydrophobic moiety (aryl ring) as well as two electron donor atoms in the ring have been shown to possess activity in MES as well as subcutaneous pentylenetetrazole (scPTZ) screen. This study reported that higher is the hydrophobic parameter π (pi) of the substituent on phenyl ring, more potent anticonvulsant is the compound and also, only the compounds with an electron withdrawing substituent on the phenyl ring exhibited appreciable anticonvulsant activity [9-15]. Substituting the hydrogen (from –NH in pyridazine nucleus) with methyl and acetyl group enhanced

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the lipophilicity of the compounds. Lipophilic drugs must pass through blood brain barrier (BBB) and reach to its receptors in the central nervous system [17-19].

MATERIALS AND METHOD

Chemicals: Chemicals were procured from Central Drug House (P) Ltd., India of synthetic grade for synthesis of title compounds. All other chemicals & solutions were used were of analytical grade.

Experimental Section: Melting points of the title compounds were recorded in open capillary tube in liquid paraffin bath as well as in precision melting point apparatus and are uncorrected. Percentage yields were recorded accordingly (Table 1). Solvent system used throughout the experimental work for running TLC plates was toluene, ethyl acetate and formic acid (TEF) in the ratio of 5:4:1 and another solvent system also used were benzene and acetone in the ratio of 4:1. IR spectra were recorded by using KBr pellet technique on Perkin Elmer 337 IR spectrophotometer. 1HNMR spectra were recorded in deuterated chloroform using tetra methyl silane (TMS) as an internal reference standard on BRUKER AVANCE II 400 NMR spectrometer and elemental analysis also determined.

Synthesis of Benzoyl Propionic Acid (1): A mixture of benzene (30 ml) and anhydrous aluminium chloride (0.15 mol) was refluxed under anhydrous condition using calcium chloride guard tube, followed by slowly addition of succinic anhydride (0.10 mol) with continuous stirring. The stirring and heating were continued for 4 hrs. The mixture is leaving over night at room temperature [6,7-16]. The contents were poured into ice cold hydrochloric acid (2.5% v/v) followed by steam distillation. The aqueous solution was concentrated to small volume by evaporating on the water bath to obtain crude compound. It was purified by dissolving the 5% w/v of sodium bicarbonate solution followed by extraction with ether and chloroform. The aqueous layer on acidification with dilute hydrochloric acid gave benzoyl propionic acid and was recrystallized from aqueous ethanol. M.P- 120°C, Yield- 70%, Molecular formula- $C_{10}H_{10}O_3$, Molecular weight- 178.18. IR Spectra: 3250 cm-1 (OH), 1720 cm-1 (C=O).NMR Spectra: 1HNMR ($CDCL_3$) ppm 2.82 (2H, t, CH_2), 3.32 (2H, t, CH_2), 7.74 (CH_2, m, H-3, 5), 7.79 (2H, m, H-2, 6).

Synthesis of 3-Nitrobenzoyl Propionic Acid (2): To a mechanically stirred mixture of 12 ml conc. nitric acid and 12 ml of conc. Sulphuric acid, 6 g of benzoyl propionic acid **I** was added in the portion while keeping the mixture at 0-10°C by efficient cooling (30-40 min.). The temperature was further allowed to rise to 15°C in the course of 120 minutes and the solution was slowly stirred in ice-water [20]. The precipitated material was washed with cold water to free from acid and re-crystallized from methanol. Lightly yellow color compound was obtained. M.P-108°C, yield - 50%, molecular formula- $C_{10}H_9NO_5$, molecular weight- 223.18. IR Spectra: 3091 cm-1 (CH), 1705 cm-1 (C=O), 1353 cm-1 (NO_2), 1617 cm-1 (C=C).NMR Spectra: 1HNMR (DMSO) ppm 3.0 (t, 2H, CH_2), 3.36 ((t, 2H, CH_2),7.30- 8.2 (m, Ar-H), 8.82 (s, H, Ar-H).

Synthesisof6-(3-Nitrophenyl)-4,5-Tetrahydropyridazin-3(2*h*)-one (3): The 3-nitro benzoyl propionic acid (**II**) (0.01 mol) was refluxed for 6 hrs. with hydrazine hydrate (0.01 mol) in methanol (10 ml) containing sodium acetate (50 mg). The contents were concentrated and then poured into ice cold water (20) to get compound recrystrallized with ethanol. M. P- 90°C, yield- 65%, molecular formula- $C_{10}H_9N_3O_3$, molecular weight- 219.19. IR (cm-1)1685(C=O), 1352(NO_2), 3100(CH), 3550(NH), 1HNMR (ppm): 2.5 (s, 2H, NH_2), 7.17-8.05 (m, 7H, Ar-H), 8.15 (m, 1H, Ar-NH).

Synthesis of 4-(2-Hydroxybenzylidene) and (4-Methoxybenzylidene)-6-(3-Nitrophenyl)-4,5-Dihydro-Pyridazin-3(*2H*)-one (4a-4c)

Synthesis of 4-Benzylidene-6-(3-Nitrophenyl)-4,5-Dihydropyridazin-3(2H)-one (4a): Condensation of compound **(3)** with benzaldehyde, a mixture of compound **3** (0.005 mol) and benzaldehyde (0.005 mol) in glacial acetic acid (20 ml) and add sodium acetate (2 g) was refluxed for 6-8 hours (monitored by TLC) and cooled & poured on to ice. The solid compound was obtained and then recrystallized with ethanol. Melting point: 132°C, yield 65%, molecular formula $C_{17}H_{13}O_3N_3$, molecular weight 307.309 IR cm-1 -1360 cm-1 (NO_2), 1700cm-1 (C=O), 1580cm-1 (C=C) exo, 3450cm-1 (NH). 1HNMR ($CDCl_3$) ppm 2.3 (s, 2H, CH_2), 7.47-7.88 (m, H, Ar-H), 8.5 (d, H, Ar-H), 8.3 (d, H, Ar-H), 7.0 (s, H, NH), 8.9 (s, H, Ar-H).

Animals: Male Swiss albino mice weighing 20-30 g were maintained under controlled conditions of light (12 hr) and temperature 25±1°C in the animal house, Department of Pharmacy, GRD (PG) Institute of Management & Technology, 214, Rajpur Road, Dehradun, Uttaranchal, India, two weeks prior to the experiment for acclimatization. Animals had access to food and water *ad libitum*. All pharmacological activities were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals norms (Regn No: 1145/a/07/CPCSEA), after obtaining the approval from the Institutional Animal Ethics Committee of Department of Pharmacy, GRD (PG) Institute of Management & Technology, 214, Rajpur Road, Dehradun, Uttaranchal, India.

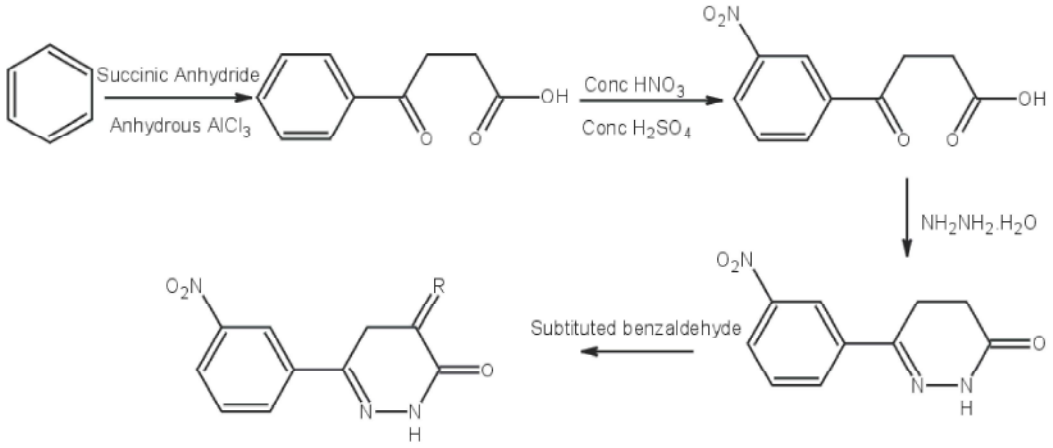
Preparation of Test Samples for Bioassay: Test samples 50 mg/kg. wt and phenytoin sodium50 mg/kg. wt were suspended in a mixture of distilled water and 0.5% sodium carboxyl methylcellulose (CMC), used as suspending agent and were given intraperitonealy to the experimental animals. The animals of the control group received the same experimental handling except that the drug treatment was replaced with appropriate volumes of the vehicle.

Anticonvulsant Activity: For the determination of the anticonvulsant effects on MES induced generalized or tonic clonic seizure, the electro shock model was employed. Male albino mice (25-35 g) were used to test the drugs (synthesized pyridazinone derivatives). Maximal Electro Shock (MES) method (21) was used for inducing seizures (Model**:** Techno Electro Convulsimeter). The MES induced convulsions in animal represent grand mal type of epilepsy. In MES, electro shock was applied through the ear pinna passing current of 60 mA for 0.2 sec. The MES convulsions were divided into five phases (i) Flexion (ii) Extensor (iii) Clonus (iv) stupor and (v) Recovery or Death. The female mice were excluded because of fact that oestrous cycle influence the seizure threshold. Reduction in the extension phase was calculated in comparison to diphenyl hydantoin sodium (Phenytoin Sodium) which was used as a standard drug (Table 2).

Statistical Analysis: Results were expressed as means±S.E.M. Statistical significance was analysed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where $p < 0.05$ was accepted to be a significant difference.

RESULTS AND DISCUSSION

All the three 6-(3-nitrophenyl)-4,5-dihydro pyridazin-3(2H)-ones (**4a-4c**) were synthesized (Table 1) and their structures were established based on spectroscopic data. IR spectrum showed the characteristics bond at 1700, 1352, 3450 and 1580 cm^{-1} authenticated the presence of C=O, NO_2, NH and C=C groups. The 1HNMR spectrum showed the signal in the form of triplet near δ=2.8 for CH_2 protons at 5-position, another triplet is observed at about δ=3.0 for CH_2 at 4 position of compounds [1-4]. Aromatic proton also observed in the aromatic region ranging from δ=7.0-8.0. Presence of other substitutes also authenticated



Scheme-I

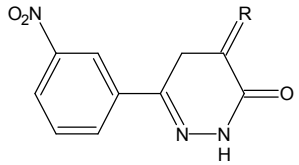


Table 1: Physical characteristic of the pyridazinone compounds

Compounds	R	Mol. formula	Mol. weight	M.P (°C)	Yield(%)
4-benzylidene-6-(3-nitro phenyl)-4,5-dihydropyridazin-3(2*H*)-one (4a)	C_6H_5	$C_{17}H_{13}N_3O_3$	307.30	130	48
4-(2-hydroxybenzylidene)-6-(3nitrophenyl) -4,5-dihydro pyridazin-3(2*H*)-one (4b)	*o*-C_6H_4OH	$C_{17}H13N_3O_4$	323.30	140	42
4-(4-methoxybenzylidene)-6-(3-nitrophenyl) -4,5-dihydro pyridazin-3(2*H*)-one (4c)	*p*-$C_6H_5OCH_3$	$C_{18}H_{15}N_3O_4$	337.32	127	55

Table 2: Anticonvulsant activity of synthesized pyridazinone compounds

Group (n= 5)	Treatment	Flexion (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	Recovery
I	Control	3.34±0.125	15.76±01.11	6.76±01.11	48.90±07.65	60%
II	Phenytoin (25mg/kg)	1.44±0.051^{a}	0.00±0.00^{a}	1.84±0.23	7.78±0.58^{a}	100%
III	4a(50mg/kg)	2.70±0.271$^{c\#\#\#}$	12.54±1.45$^{c\#\#\#}$	5.40±2.25$^{b\#}$	25.53±5.35$^{a\#\#\#}$	80%
IV	4b (50mg/kg)	2.62±0.278a$^{\#\#\#}$	10.83±1.64$^{a\#\#\#}$	6.50±2.54$^{\#\#}$	32.68±3.42a$^{\#\#\#}$	80%
V	4c (50mg/kg)	3.04±0.310$^{ns\#\#\#}$	9.80±1.85$^{a\#\#\#}$	5.72±1.035$^{\#\#}$	26.72±1.07$^{a\#\#\#}$	80%

Control: Distilled water; Standard drug: Phenytoin sodium (25mg/kg); Test drugs: (50 mg/kg); n= 5, no. of animals in each group; ^{a}P< 0.001, ^{b}P< 0.01, ^{c}P< 0.05, ns> 0.05 when compared to control Group and ###< 0.001, ##< 0.01, #<0.05, when compare with standard group.

in the ^{1}HNMR spectra at the assigned value. The present studies on the synthesized derivatives have shown significant protection against maximal electro shock (MES) induced seizure (Table 2) as compared to control. It is well known fact that the drugs which provide protection against seizure induced by maximal electro shock method are generally effective against toxic-clonic seizure. But the animals were observed for all the five phases as well as the duration. The maximum activity shown by the compounds were **4a** in extensor phase. The abolition of the extensor phase in drug treated group was taken as experimental criterion for anticonvulsant activity. Thereby, the compound **4a** may be assumed to bear most anticonvulsant activity among all three synthesized compounds. A number of substituents are aimed at studying for their contribution to pharmacological activity. In the view of above biological importance the new substituted pyridazinone derivatives will be developed as anticonvulsant roperties.

CONCLUSION

These 4-substituted bezaldehyde-6-(3-nitrophenyl) pyridazin(*2H*)-3-one derivatives of pyridazinone ring have shown significant anticonvulsant activity against MES induced seizure in albino mice after intra-peritonially administration of 50mg/Kg body weight dose. The potency order of the test compound on the extensor phase: **4a**> **4b**> **4c**. So, these compounds may be regarded as anticonvulsant because a substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES convulsions. The title compounds thus may have immense potential for contribution to human benefit. Scientific explorations of further studies of more derivatives as well as on more parameters are needed to elucidate the defined role of pyridazinone at the anticonvulsive levels.

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