

## Analgesic Activity of Some 6-Phenyl-4-Substituted Benzylidene Tetrahydro Pyridazin-3(2H)-Ones

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**Abstract:** The synthesis of three 6-Phenyl-4-Substituted Benzylidene tetrahydro- pyridazin-3(2H)-one derivatives (III A-III C) were synthesized from 6-phenyl-4,5-dihydropyridazin-3(2H)-one (II). These compounds were characterized on the basis of IR and <sup>1</sup>H NMR spectral data. All three title compounds III A-III C exhibited significant (p<0.001) analgesic activities when compared with control group by using hot plate model and less active than Aspirin 100 mg/kg that was used as reference drug.

**Key words:** Pyridazinone • Analgesic activity • Aspirin • Spectral data

### INTRODUCTION

Pyridazine belong to an important group of heterocyclic compounds. A lot of research work on pyridazine derivatives has been done in the past. This heterocyclic nucleus or moiety possess almost all types of pharmacological activities. Recently, a substantial number of pyridazine derivatives have been reported to possess increasing interest in the synthesis and biological properties, such as analgesic, anti-inflammatory, antisecretory, antiulcer, antidepressants, anxiolytics, sedative-hypnotics, anticonvulsants, antiplatelet, antithrombotics, antitumor, cardiotonics, vasodilators, antiarrhythmics and antidiabetic, antitubercular agents etc [1-5]. The small and simple pyridazine nucleus is present in compounds involved in the research aimed at evaluating new product that possess interesting biological activities. They are also used as intermediates for drugs, agrochemicals and other anticipated properties [6-11].

It is well known that classical non-steroidal anti-inflammatory drugs (NSAIDs) are useful tools in the treatment of inflammation, pain and fever. In view of this fact, research has been directed in recent years at designing new compounds with more effective as well as less or without adverse effects as compare to currently used NSAIDs [12-14]. Therefore, the discovery of new, potent and safer NSAIDs represented a challenging goal

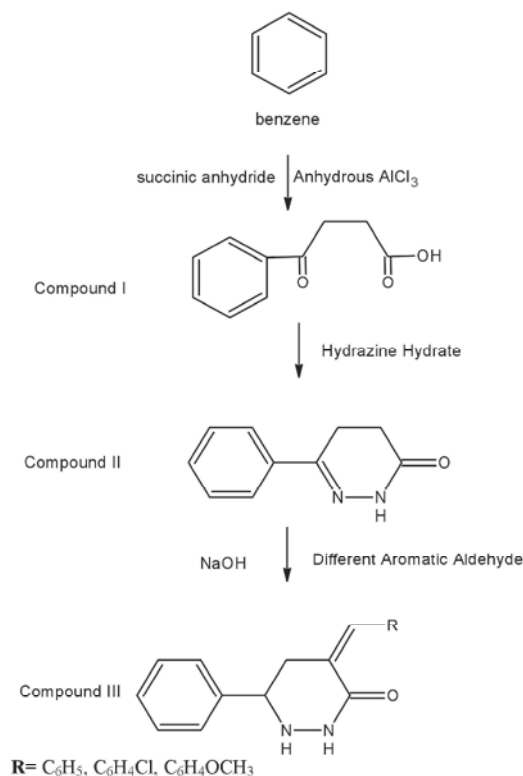
for such research area. A considerable number of substituted pyridazinones endowed with analgesic properties have been reported. The discovery of biological activity in a series of pyridazine derivatives stimulated the vigorous growth of investigations in this area [15-22].

Stimulated by these findings, our attention has been focused on the synthesis of some 6-phenyl-3(2H)-pyridazinone derivatives (III A-III C) for analgesic activity. These pyridazinones having phenyl and benzylidene groups at position 6 and 4 of the pyridazinone ring. These pyridazinone derivatives are evaluated for analgesic activity by using radiant heat-induced pain model.

### MATERIALS AND METHODS

**Chemicals:** Chemicals and solvents were procured from Central Drug House (P) Ltd., India of laboratory grade for synthesis of title compounds as well as some other supporting works.

**Experimental Section:** Melting points of all the synthesized 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). All the reactions were monitored by TLC using Toluene: Ethyl Acetate:



Scheme 1:

Table 1: Analgesic activity of the synthesized compounds

Compd. No.	Time (min)					
	10	20	30	60	90	120
Control	3.42±0.03	3.48±0.02	3.64±0.04	3.50±0.03	3.76±0.04	3.64±0.05
IIIA	3.51±0.02	4.73±0.01	6.62±0.02	9.24±0.01	10.56±0.01	11.92±0.01
IIIB	4.10±0.01	5.22±0.01	7.00±0.01	9.62±0.01	10.96±0.01	12.26±0.01
IIIC	3.84±0.01	4.65±0.01	5.88±0.01	8.24±0.01	9.98±0.01	10.84±0.01
Asprin	8.24±0.01	10.54±0.01	11.37±0.01	11.61±0.01	12.53±0.01	12.88±0.01

All results are significantly different from control at  $p < 0.001$

Formic Acid (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. <sup>1</sup>HNMR 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.

**Preparation of Test Samples for Bioassay:** Test samples (100 mg/kg) were suspended in a mixture of distilled water and 0.5% sodium carboxyl methylcellulose (CMC) and were given intraperitoneally to the test 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. Aspirin in 0.5% CMC (100 mg/kg) for analgesic activity was used as reference drug.

**Synthesis of Benzoyl Propionic Acid (I):** A mixture of benzene (30 ml) and anhydrous aluminium chloride (0.15 mol) was taken in three neck flask and refluxed on a water bath under anhydrous condition using calcium chloride guard tube at the top of condenser, followed by addition of succinic anhydride (0.10 mol) in small quantity with continuous stirring [1, 2, 23]. The stirring and heating were continued for 4 hrs. The reaction usually starts immediately HCl gas is evolved.

After this, the mixture is leaving over night at room temperature 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 re-crystallized from aqueous ethanol. Mol. formula  $C_{10}H_{10}O_3$ , mol. weight 178.18, m. p. 120°C, yield 70%  $R_f$  value 0.77. IR Spectra:  $3250\text{ cm}^{-1}$  (OH),  $1720\text{ cm}^{-1}$  (C=O). NMR Spectra:  $^1\text{H-NMR}$ ( $\text{CDCl}_3$ ) ppm 2.82 (2H, t,  $\text{CH}_2$ ), 3.32 (2H, t,  $\text{CH}_2$ ), 7.74 ( $\text{CH}_2$ , m, H-3, 5), 7.79 (2H, m, H-2, 6).

**Synthesis of 6-phenyl-4,5-dihydro pyridazin-3(2H)-one (II):** The benzoyl propionic acid (0.01 mol) was refluxed for 6 hr. 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 to get compound recrystallized with ethanol [1, 2]. Mol. formula  $C_{10}H_9NO$ , mol. weight 193.2, yield 50%, m.p. 251°C,  $R_f$  value 0.62. IR ( $\text{cm}^{-1}$ ) 1685(C=O), 1352( $\text{NO}_2$ ), 3100(CH), 3550(NH).  $^1\text{H-NMR}$  (ppm): 2.45 (t, 2H,  $\text{CH}_2$ ), 2.93 (t, 2H,  $\text{CH}_2$ ), 7.41-7.74 (ArH, m, CH), 10.94 (s, 1H, CONH).

**Synthesis of 6-phenyl-4(benzylidene)-tetrahydropyridazin-3(H)-one (IIIA):** A mixture of compound II (0.05 mol) and benzaldehyde (0.05 mol) in ethanol (20 ml) and an ethanolic sodium ethoxide solution prepared from sodium and dry ethanol was added and the reaction mixture was left overnight at room temperature, diluted with water and rendered just acidic with concentrated HCl [24]. The Contents were poured into ice-cold water, filtered and recrystallized with ethanol. Mol. formula  $C_{17}H_{16}N_2O$ , mol weight 264.32. IR (KBr) in  $\text{cm}^{-1}$ : 3400 (Ar-H), 3350 (NH), 2862 (C-H), 1603.52 (C=C), 1258.32 (C-N).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ,  $\delta$  in ppm): 12.09 (1H, s, NH), 10.04 (1H, s, CH), 7.33-7.89 (ArH, m, CH), 4.032 (2H, d,  $\text{CH}_2$ ), 1.749 (1H, s, NH).

**Synthesis of 6-phenyl-4-(p-methoxybenzylidene)-tetrahydropyridazin-3(2H)-one (IIIB):** The compound II (1g) was refluxed for 10 hours with anisaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water [24], filtered and recrystallized to get the crystals. Mol. formula-  $C_{18}H_{18}N_2O_2$ , mol. weight- 294.34. IR (Kbr) in  $\text{cm}^{-1}$ : 3400 (Ar-H), 3444 (NH), 2857 (C-H), 1602.56 (C=C),

1484.92 (C-Cl) 1292.07 (C-N).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ,  $\delta$  in ppm): 10.209 (1H, s, NH), 8.621 (1H, s, CH), 7.27-7.782 (ArH, d, CH), 4.383 (2H, d,  $\text{CH}_2$ ), 1.768 (1H, s, NH).

**Synthesis of 6-phenyl-4-(p-chlorobenzylidene)-tetrahydropyridazin-3(2H)-one (IIIC):** The compound II (1g) was refluxed for 10 hours with p-chlorobenzaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water [24], filtered and recrystallized to get the crystals. Mol. formula-  $C_{17}H_{15}ClN_2O$ , mol. weight-294.34. IR (KBr) in  $\text{cm}^{-1}$ : 3400 (Ar-H), 3435 (NH), 2831 (C-H), 1602.56 (C=C), 1509.99 (C-O- $\text{CH}_3$ ), 1255.32 (C-N), 1165.76 ( $\text{OCH}_3$ ).  $^1\text{H-NMR}$  (DMSO,  $\delta$  in ppm): 9.782 (1H, s, NH), 8.496 (1H, s, CH), 6.93- 7.77 (ArH, d, CH), 6.770 (1H, t, CH), 4.448 (2H, d,  $\text{CH}_2$ ), 4.435 (3H, s,  $\text{OCH}_3$ ), 2.498 (1H, s, NH).

**Experimental Animals:** Male albino mice (30- 35 g) were used for analgesic activity. All of the animals were left for 2 days in the laboratory for acclimatization before the day of experiment and on the last day they were given water only. Minimum of 5 animals were used in each group. All pharmacological activities were carried out as per CPCSEA (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.

#### Analgesic Activity

**Eddy's Hot Plate Method:** Heat is used as a source of pain. Animals were individually placed on the hot plate maintain at constant temperature (55°C) and the reaction of animals, such as paw licking or jump response was taken as the end response. Analgesic drugs/compounds increases the reaction time. The method was first described by Eddy & Leimbach (A cut off period of 15 sec is observed to avoid damage to the paw). Administration of the control, standard and test compounds to animals by i.p route and note the reaction of time of animals at 10, 20, 30, 40 & 50 min interval on the hot plate after drug administration. A group of albino mice were treated intraperitoneally with a dose of 100 mg/kg body weight with aqueous suspension in 0.5% CMC Na of the synthesized compounds. The method of Eddy and Leimbach using techno heated plat analgesic apparatus was used. The standard drug aspirin (50mg/kg) was used reference drug for comparison. The result was tabulated in Table 1 [25].

**Statistical Analysis:** Results were expressed as means  $\pm$  S.E.M. Statistical significance was analyzed 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

All the 6-phenyl-4-substituted benzylidene tetrahydropyridazin-3(2H)-one derivatives (IIIA-IIIC) were synthesized from 6-phenyl-tetrahydropyridazin-3(2H)-one (II) by reaction with different substituted benzaldehyde. These title compounds initially prepared from benzene to benzoyl propionic acid (I) followed by cyclization with hydrazine hydrate to form II. Their structures were established based on IR and NMR spectroscopic data. IR spectrum showed the characteristics bond at 1700, 3450 and 1580  $\text{cm}^{-1}$  authenticated the presence of C=O, NH and C=C groups. The  $^1\text{H}$ NMR spectrum showed the signal in the form of triplet near  $\delta=2.8$  for  $\text{CH}_2$  protons at 5-position, another triplet is observed at about  $\delta=3.0$  for  $\text{CH}_2$  at 4 position of compounds. Aromatic proton also observed in the aromatic region ranging from  $\delta=7.0-8.0$ . Presence of other substitutes also authenticated in the  $^1\text{H}$ NMR spectra at the assigned value. All tested compounds exhibited analgesic activities (Table 1) that lasted for 120 minutes and the potency increased with time. All these title compounds were exhibited significant analgesic activity when compare to control group. The most potent compound was (IIIB), all the compound were less potent than reference drug aspirin, but only compound (IIIC) was less potent than all compounds. The degree of potency in ascending order is IIIB>, IIIA>, IIIC. The result favours and proved that different substituted pyridazinone compounds are plays an important role in an analgesic activity.

## DISCUSSION

A number of substituted pyridazines are used for their contribution in analgesic as well as various pharmacological activities. Various pyridazinone derivatives are more active than reference drug like 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (emorfazone) is currently being marketed in Japan as an analgesic and anti-inflammatory drug [26]. Moreover, it has been reported that 4-amino-2-methyl-6-phenyl-5-vinyl-3(2H)-pyridazinone was seven-fold more potent than emorfazone [27] in bringing about analgesic and anti-inflammatory response. The synthesized different 6-phenyl-4-substituted benzylidene tetrahydropyridazin-

3(2H)-ones have proven that substituted pyridazinones showed analgesic activities but are less active as reference drug or some previous synthesized substituted pyridazinone derivatives with different substituted atoms or groups. The currently used analgesic compounds inhibit the synthesis of non selective or selective cyclooxygenase (COX1 & COX2) or prostaglandin, histamine and bradykinin. It has been suggested that prostaglandins and bradykinins play a major role in the analgesia or pain. So it may be predicted that the title compounds may be act by inhibiting the synthesis of these pain as well as inflammation inducing chemical mediators such as histamine, bradykinin and prostaglandin [12-14].

In conclusion, literature revealed that pyridazinone has diverse biological potential. By the present scenario it can be concluded that pyridazinone have a great potential which remain to be disclosed till date. The results of the pharmacological screening indicated that pyridazine compounds possess significant analgesic activity associated with NSAIDs properties. Pyridazinones further drew our attention because of their easy functionalization at various ring positions, which makes them attractive synthetic building blocks for designing and development of novel pyridazine as analgesic agents.

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