

Short Communication

SYNTHESIS AND ANALGESIC ACTIVITY OF 6-(M-NITROPHENYL)-4-SUSTITUTED BENZYLIDENE-4,5-DIHYDROPYRIDAZIN-3(2H)-ONE DERIVATIVES

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ABSTRACT

Many research groups have been interested in 3(2H)-pyridazinones for the development of potential analgesic and anti-inflammatory agents. Stimulated by these findings, three 6-(m-Nitrophenyl)-4-sustituted benzylidene-4,5-dihydropyridazin-3(2H)-ones (**IVa-IVc**) have been synthesized and evaluated for analgesic activity against hot plate method. Compounds **IVa-IVc** were prepared from 6-m-nitrophenyl-4,5-pyridazin-3(2H)-one by condensation with respective aldehydes. All compounds (**IVa-IVc**) having nitro phenyl and benzylidene groups at position 6 and 4 of the pyridazinone ring receptively. All compounds (**IVa-IVc**) showed significant analgesic activity when compare to control group and were found less potent than reference drug aspirin.

Key words: Pyridazinones, analgesic, benzylidene, nitrophenyl.

INTRODUCTION

The therapeutic uses of classical non-steroidal anti-inflammatory drugs (NSAIDs) are useful tools in the treatment of pain, inflammation, and fever. However, long-term clinical usage of NSAIDs is associated with several significant side effects including gastrointestinal lesions, bleeding, hepatotoxicity and nephrotoxicity (Mccarthy, 1998; Raskin, 1999). In view of this fact, research has been directed in recent years at designing compounds devoid of the typical side effects. Therefore, the discovery of new, potent and safer NSAIDs represents a challenging goal for such a research area. Because resistance to NSAIDs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent, and less toxic NSAIDs.

In recent years a substantial number of 6-aryl-3-(2H)-pyridazinones have been reported to possess antimicrobial, analgesic, anti-inflammatory, antifeedant, herbicidal, anti-hypertensive, antipyretic, antiplatelet, anti-cancer, anticonvulsant, antitubercular, cardiovascular activities and other anticipated biological properties (Samar, 2007; Siddiqui *et al.*, 2004; Islam *et al.*, 2008). On the other hand, a considerable number of 3(2H)-pyridazinone derivatives endowed with analgesic and anti-

inflammatory properties have been reported (Rubat C, *et al.* 1992, Pinna *et al.*, 1992; Flouzat *et al.*, 1993; Jing *et al.*, 2007). The results of the pharmacological screening indicated that some compounds possess good analgesic and anti-inflammatory activities associated with non-narcotic properties. Among them, emorfazone (Figure 1) is marketed in Japan as an analgesic and anti-inflammatory (Takaya *et al.* 1979; Heinisch *et al.*, 1990). Later, Dal Piaz *et al.* evaluated the analgesic activities of the compounds having 2-substituted 4,5-functionalized 6-phenyl-3(2H)-pyridazinones, some compounds were more potent than Emorfazone. In addition, 3-O-substituted benzyl pyridazinone derivatives have been found to exhibit *in vivo* potent anti-inflammatory activity through the mechanism involving selective COX-2 inhibition (Chintakunta *et al.*, 2002). Stimulated by these findings, our attention has been focused on the synthesis of some new 6-nitrophenyl-3(2H)-pyridazinone compounds (**IVa-IVc**) which are expected to show analgesic activity. We have recently synthesized 3(2H)-pyridazinones having nitro phenyl and benzylidene groups at position 6 and 4 of the pyridazinone ring evaluate their analgesic activity by using radiant heat-induced pain test.

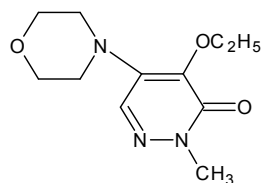


Figure 1. Emorfazone

METHODOLOGY

Preparation of test samples for bioassay

Test samples (100mg/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 (100mg/kg) for analgesic activity was used as reference drug.

Synthesis of benzoyl propionic acid (I)

A mixture of benzene (30mL) and anhydrous AlCl_3 (0.15mol) was taken in three neck flask and refluxed on a water bath under anhydrous condition using CaCl_2 guard tube at the top of condenser, followed by addition of succinic anhydride (0.10mol) in small quantity with continuous stirring (Siddiqui *et al.*, 2004). The stirring and heating were continued for 4 hrs. The reaction usually starts immediately. HCl gas is evolved and mixture becomes hot. Warm gently in the minimal and gentle reactions. After leaving over night at room temperature the contents were poured into ice cold HCl (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 Na_2CO_3 solution followed by extraction with ether or chloroform. The aqueous layer on acidification with dilute HCl gave β -benzoyl propionic acid and was re-crystallized from aqueous ethanol. Melting point: 120°C , yield 70% R_f value 0.77, molecular formula $\text{C}_{10}\text{H}_{10}\text{O}_3$, molecular weight 178.18. IR Spectra: 3250cm^{-1} (OH), 1720cm^{-1} (C=O). $^1\text{HNMR}$ Spectra: (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 β -m-nitro benzoyl propionic acid (II)

To a mechanically stirred mixture of 12mL conc. HNO_3 and 12mL of conc. H_2SO_4 , 6 gm of β -benzoyl propionic acid was added in the portion while keeping the mixture at $0-10^\circ\text{C}$ by efficient cooling (30-40min). 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. The precipitated material was washed with cold water to free from acid and re-crystallized from methanol. Lightly yellow color compound was obtained. Melting point: 108°C , yield 50 %, R_f value 0.70, molecular formula $\text{C}_{10}\text{H}_9\text{NO}_5$, molecular weight 223.18. IR Spectra: 3091cm^{-1} (CH), 1705cm^{-1} (C=O), 1353cm^{-1} (NO_2), 1617cm^{-1} (C=C). $^1\text{HNMR}$ (DMSO) ppm 3.0 (t, 2H, CH_2), 3.36 (t, 2H, CH_2), 7.30- 8.8 (m, Ar-H).

Synthesis of 6-(m-nitro phenyl)-4,5-dihydro pyridazin-3(2H)-one (III)

The β -m-nitro benzoyl propionic acid (0.01mol) was refluxed for 6 hr. with hydrazine hydrate (0.01mol) in methanol (10mL) containing sodium acetate (50 mg). (Islam *et al.* 2008). The contents were concentrated and then poured into ice cold water to get compound recrystallized with ethanol. Yield 52%, melting point. 95°C , R_f value 0.62, molecular formula $\text{C}_{10}\text{H}_9\text{NO}_5$, molecular weight 193.2. IR (cm^{-1}) 1685 (C=O), 1352 (NO_2), 3100 (CH), 3550 (NH). $^1\text{HNMR}$ (DMSO) ppm 3.0 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 7.2- 8.8 (m, Ar-H).

Synthesis of 6-(m-nitrophenyl)-4-sustituted benzylidene-4,5-dihydropyridazin-3(2H)-one (IVa-IVc)

Condensation of compounds (III) (0.005mol) with different substituted aromatic aldehydes (0.005mol) were taken in glacial acetic acid (20mL) and sodium acetate (2g) was added in it (Demirayak, *et al.*, 2004). The content was refluxed for 6-8 hours (monitored by TLC), cooled and then poured into ice. The solid compound was obtained and then re-crystallized with ethanol.

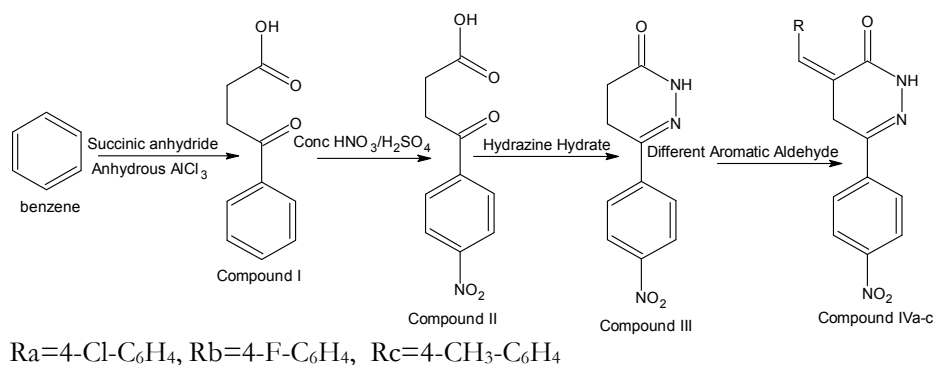


Figure 1. Analgesic activity of the 6-(*m*-Nitrophenyl)-4-sustituted benzylidene-4,5-dihydro pyridazin-3(2*H*)-one compounds

6-(*m*-nitrophenyl)-4-chlorobenzylidene-4,5-dihydropyridazin-3(2*H*)-one (IVa)

Melting point: 132°C, yield 65%. IR cm^{-1} 1360 cm^{-1} (NO_2), 1700 cm^{-1} ($\text{C}=\text{O}$), 1580 cm^{-1} ($\text{C}=\text{C}$) exo, 3450 cm^{-1} (NH). ^1H NMR (CDCl_3) ppm 2.3 (s, 2H, CH), 2.8 (t, 2H, CH_2), 3.0 (t, 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, ArH), 8.9 (s, H, N-H).

6-(*m*-nitrophenyl)-4-*p*-florobenzylidene-4,5-dihydro-pyridazin-3(2*H*)-one (IVb)

Melting point: 139°C, yield 55%. IR cm^{-1} 1685 cm^{-1} ($\text{C}=\text{O}$), 1350 cm^{-1} (NO_2), 3450 cm^{-1} (NH), 3600 cm^{-1} (OH), 1580 cm^{-1} ($\text{C}=\text{C}$). ^1H NMR (DMSO) ppm 2.0 (s, H, CH), 2.8 (t, 2H, CH_2), 3.0 (t, 2H, CH_2), 7.3 (s, H, CH), 6.9 (d, H, Ar-H), 7.78 (t, H, Ar-H), 6.8 (s, H, NH), 8.7 (s, H, N-H).

6-(*m*-nitrophenyl)-4-methl-benzylidene-4,5-dihydropyridazin-3(2*H*)-one (IVc)

Melting point: 146°C, yield 50%. IR cm^{-1} 1685 cm^{-1} ($\text{C}=\text{O}$), 1352 cm^{-1} (NO_2), 3450 cm^{-1} (NH), 1580 cm^{-1} ($\text{C}=\text{C}$), 1300 cm^{-1} ($\text{C}=\text{O}$). ^1H NMR (CDCl_3) ppm 2.2 (s, 2H, CH), 2.8 (t, 2H, CH_2), 3.0 (t, 2H, CH_2), 7.5 (s, H, NH), 7.4 – 8.2 (m, H, Ar-H), 8.6 (s, H, NH).

Experimental animals

Male albino mice (30-35g) 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 Commottee 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, 60 & 120 min interval on the hot plate after drug administration. A group of albino mice were treated intraperitonealy 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 I (Kulkarni, 1999).

Table I. 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
4a	4.53±0.02	5.73±0.01	6.68±0.02	9.42±0.01	11.50±0.01	12.14±0.01
4b	3.76±0.01	4.45±0.01	5.86±0.01	7.54±0.01	9.96±0.01	10.44±0.01
4c	3.84±0.01	4.68±0.01	5.94±0.01	6.79±0.01	8.92±0.01	9.65±0.01
Aspirin	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 $p < 0.001$.

Statistical analysis

Results were expressed as means \pm 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 6-(m-nitrophenyl)-4,5-dihydropyridazin-3(2H)-one derivatives (**IVa-IVc**) were prepared from 6-(m-nitrophenyl)-4,5-dihydro pyridazin-3-one (**III**) by reaction with substituted aromatic aldehydes. Friedal craft acylation of benzene in presence of anhydrous $AlCl_3$ yield β -benzoyl propionic acid (**I**) followed by nitration to yield β -m-nitrobenzoyl propionic acid (**II**). β -m-nitrobenzoyl propionic acid were cyclized with hydrazine hydrate to form 6-(3'-nitrophenyl)-2,3,5-trihydro pyridazin-3-one (**III**). Their structures were established based on spectroscopic analysis data (IR and 1H NMR). 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. 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. All tested compounds exhibited analgesic activities (Table I) that lasted for 120 minutes and the potency increased with time. The most potent compounds was (**IVc**), all the compound were less potent than reference drug aspirin. The degree of potency in ascending

order is **IVa** > **IVb** > **IVc**. On the basis of above finding, six membered heteroatom ring systems (pyridazine) is plays an important role in analgesic activity.

CONCLUSION

Synthesized 4-substituted-6-(m-nitrophenyl)-4,5-dihydropyridazin(2H)-3-one derivatives (**IVa-IVc**) have proven that these compounds showed analgesic activities. A number of substituents pyridazinones are aimed at studying for their contribution to various pharmacological activities.

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