Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 6 June 2019

Research Article

Analgesic Activity of 6-(p-Chlorophenyl)-4-Substituted-Benzylidene tetrahydropyridazin-3(2H)-One

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Received: March 22, 2019; **Published:** May 07, 2019

DOI: 10.31080/ASPS.2019.03.0275

Abstract

Several studies have been focused on pyridazinone derivatives for design and developing potent and safer non-steroidal anti-inflammatory drugs (NSAIDs). So, our interest has been focused on the synthesis and biological evaluation of some 6-(p-chlorophenyl)-4-substitutedpyridazin-3(2H)-one compounds (IIIA-IIIC) as an analgesic agents. These three title compounds IIIA-IIIC were synthesized from 6-(p-chlorophenyl)-pyridazin-3(2H)-one (II). These title compounds IIIA-IIIC were characterized on the basis of IR and ^{1}HNMR spectral data analysis. The title compounds IIIA-IIIC were exhibited significant ($p<0.001$) analgesic activity compare to control group by using radiant heat-induced pain test model and acetyl salicylic acid (Aspirin, 100 mg/kg) was used as reference drug.

Keywords: Pyridazinone; Analgesic; Aspirin; Spectral Data

Introduction

The well recognized therapeutic utilization of non-steroidal anti-inflammatory drugs (NSAIDs) is valuable in the cure of pain, inflammation and fever. However, long lasting usages of NSAIDs are causes numerous undesirable effects together with gastrointestinal, kidney and hepato-toxicities. Therefore, the innovation of novel, potent and safer NSAIDs is a demanding object for researchers.

Lots of potent drugs are derived from synthetic as well as natural sources; usually in practice have nitrogen atom in the heterocyclic ring system. In recent years various 6-substituted pyridazinones have been reported to possess antimicrobial, analgesic, anti-inflammatory, antipyretic, antiplatelet, antifeedant, herbicidal, anticancer, anticonvulsant, antihypertensive and other anticipated properties [1-5]. On the other hand, the results of the pharmacological test indicated that some considerable figure of substituted pyridazinone compounds possess good non-narcotic analgesic properties [6-20]. Stimulated by these above findings, our concentration have been focused on the synthesis of some 6-(p-chlorophenyl)-4-substitutedpyridazin-3(2H)-one compounds (IIIA-IIIC) which are anticipated to show potent analgesic activities by using radiant heat-induced pain test model.

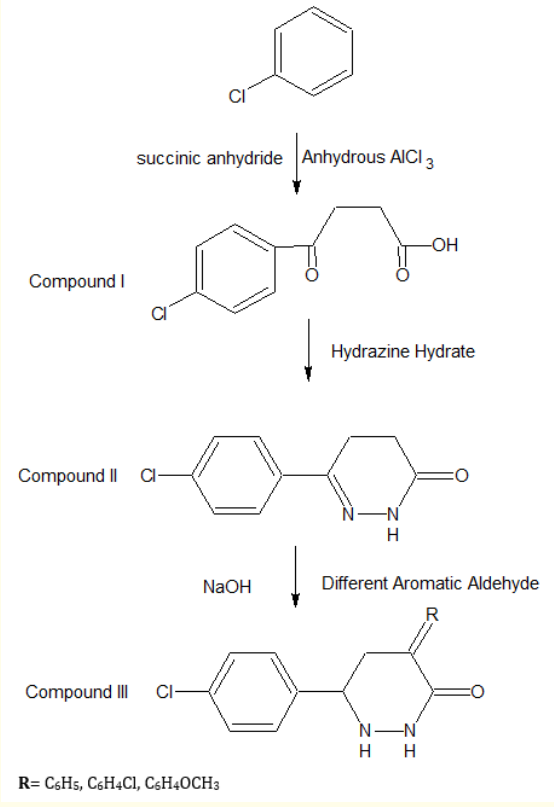
Materials and Method

Chemistry

All chemicals were procured from Merk and Central Drug House (P) Ltd., India of laboratory grade for synthesis of title compounds. Melting points (M.P.) of the synthesized compounds were recorded in open capillary tube in liquid paraffin bath as well as in melting point apparatus and are uncorrected. Percentage yields were recorded accordingly (Table 1). Solvent system used during the experimental work for running thin layer chromatography (TLC) plates was toluene, ethyl acetate and formic acid (TEF) in the ratio of 5:4:1 and another solvent system also used was benzene and acetone in the ratio of 4:1. IR spectra were recorded by using KBr pellet technique on Perkin Elmer 337 IR spectrophotometer. ^{1}HNMR spectra were recorded in deuterated chloroform using tetra methyl silane (TMS) as an internal reference standard on BRUKER AVANCE II 400 NMR spectrometer.

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	10.30 ± 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
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

Table 1: Analgesic activity of the synthesized pyridazinone compounds (IIIA-IIIC).
All results are significantly different from control at $a\ p < 0.001$.



Scheme I

Synthesis of 4-Chloro benzoyl propanoic acid (I)

A mixture of chlorobenzene (30 ml) and anhydrous aluminium chloride (0.15 mol) was taken in flask and refluxed under anhydrous condition, followed by addition of succinic anhydride in small quantity (0.10 mol) with continuous stirring [12,21]. The stirring and heating were continued for 5 hrs. The reaction starts immediately HCl gas is evolved. After this the reaction mixture is leaving over night 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 to obtain crude compound and purified by dissolving the 5% w/v of sodium bicarbonate solution followed by extraction with chloroform. The aqueous layer on acidification with dilute hydrochloric acid gave β-chlorobenzoyl propionic acid and re-crystallized with aqueous ethanol. Melting point: 1200C, yield 70% R_f value 0.77, molecular formula $C_{10}H_{10}O_3$, molecular weight178.18. IR Spectra: 3250 cm^{-1} (OH),1720 cm^{-1} (C=O).NMR Spectra: ^{1}HNMR($CDCL_3$) 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 6-chlorophenyl-4,5-dihydro pyridazin-3(2H)-one (II)

The β-p-chlorobenzoyl propionic acid (I) (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 was concentrated and poured into ice cold water to get 6-chlorophenyl-4,5-dihydro pyridazin-3(2H)-one and recrystrallized with ethanol [1,4,12]. Yield 52 %, melting point. 140^{0}C, R_f value 0.65, molecular formula $C_{10}H_8NOCl$, molecular weight 193.2. IR (cm^{-1}) 1685(C=O), 3100(CH), 3550(NH).

General synthesis of 6–(p-chlorophenyl)-4-benzylidine/substituted benzylidine tetrahydro pyridazin-3(2H)-ones (IIIA-IIIB)

A mixture of compound II (0.005 mol) and different aldehyde (0.005 mol) in ethanol (20 ml) and ethanolic sodium ethoxide solution was added and the reaction mixture was left overnight, diluted with water and rendered just acidic with hydrochloric acid [22]. The solid 6–(p-chlorophenyl)-4-benzylidine/substituted benzylidine tetrahydro pyridazin-3(2H)-ones (IIIA-IIIB) thus obtained, filtered and recrystallized with ethanol.

Synthesis of 6-(p-chlorophenyl)-4-(benzylidene)-tetrahydro pyridazin-3-one (IIIA)

IR (KBr) in cm^{-1}: 3402 (Ar-H), 3435 (NH), 2831 (C-H), 1602.56 (-C=C), 1255.3 (C-N). 1H-NMR (CDCl3, δ in ppm): ppm 8.688 (1H, s, NH), 5.25 (1H, s, CH), 7.25-7.47 (CH, m, ArH), 3.924 (1H, t, CH), 1.26 (2H, d, CH_2), 8.86 (1H, s, NH).

Synthesis of 6-(p-chlorophenyl)-4(p-methoxybenzylidene)-tetrahydropyridazin-3(2H)-one (IIIB)

IR (KBr) in cm^{-1}: 3402 (Ar-H), 3435 (NH), 2831(C-H), 1602.56 (C=C), 1509.99 (C-O-CH_3), 1255.32 (C-N), 1166.72 (OCH_3). 1H-NMR ($CDCl_3$, δ in ppm): 9.779 (1H, s, NH), 8.491 (1H, s, CH), 6.886-7.7.67 (CH, m, ArH), 3.809 (1H, t, CH), 2.495 (2H, d, CH_2), 3.051 (3H, s, OCH_3), 2.064 (1H, s, NH).

Synthesis of 6-(p-chlorophenyl)-4-(4-cholorobenzylidene)-tetrahydropyridazin-3-one (IIIC)

IR (KBr) in cm^{-1}: 3400 (Ar-H), 3444 (NH), 2857 (C-H), 1602.56 (C=C), 1489.79 (C-Cl), 1292.07 (C-N). 1H-NMR ($CDCl_3$, δ in ppm): 4.689 (1H, t, CH), 8.625 (1H, s, NH), 7.803 (1H, s, CH), 7.455-7.783 (CH, m, ArH), 2.048-2.002 (2H, d, CH_2), 1.441 (1H, s, NH).

Preparation of test samples for bioassay

Test compounds (100 mg/kg) were suspended in distilled water and 0.5% sodium carboxyl methylcellulose (CMC) and were given intraperitonealy (i.p) to the test animals. The animals of the control group received the same experimental handling except that the drug treatment was substituted with suitable quantity of the vehicle. Aspirin in 0.5% CMC (100 mg/kg) for analgesic activity was used as reference drug.

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 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 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)IMT, Dehradun, India.

Analgesic activity by Eddy's hot plate method

In this method, heat is used as a source of pain. Animals were alone placed on the hot plate maintain at stable temperature (55^0C) and the reaction of animals, such as paw licking or jump reaction was taken as the end response. Analgesic 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, 90, 120 min interval on the hot plate after drug administration. A group of albino mice were treated i.p with a dose of 100 mg/kg body weight with aqueous suspension in 0.5% CMC Na of the synthesized compounds. In this method, techno heated plat analgesic apparatus was used. The standard drug Aspirin (50 mg/kg) was used reference drug for comparison. The result was tabulated in table 1 [23].

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 6-(p-chlorophenyl)-4substituted-4,5-dihydropyridazin-3(2H)-one derivatives (IIIA-IIIC) were synthesized from 6-(p-chlorophenyl)-4,5-dihydro pyridazin-3-one (II) by reaction with substituted aldehydes. Friedal craft acylation of benzene yield β-chlorobenzoyl propionic acid (I). Compound I was cyclized with hydrazine hydrate to form compound (II). All the compounds were characterized on the basis of IR and ^{1}HNMR spectral 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 ^{1}HNMR 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. Aromatic proton also observed in the aromatic region ranging from δ=7.0-8.0. Presence of other substitutes also authenticated in the IR and 1HNMR 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 three compounds are showed significant ($p<0.001$) analgesic activity when compare to control group. The most potent compound was (IIIA), all the three compounds were less potent than reference drug aspirin, but compound IIIC was less potent among all compounds. The degree of potency in ascending order is IIIA>, IIIB>, IIIC.

Analgesic activity is commonly possessed by inhibiting the synthesis of cyclooxygenase (COX) or prostaglandin (PGs) [19]. These PGs cause or responsible for stimulus of pain receptors and sensitize the skin to painful stimuli possibly because they sensitize

pain receptors to mechanical and chemical stimulus like the pain producing effect of mediators (e.g. histamine, kinins) which are released in tissue injury and inflammation. Inhibition of PGs synthesis may account for the analgesic activity of the title compounds. Pain and inflammation is linked with numerous patho- physiologies of various clinical conditions like arthritis, cancer, tissue injury and vascular diseases. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception and the analgesic effect of the NSAIDs has also been credited to effects at peripheral or central neurons [24-26].

In the conclusion, synthesized 4-substituted-6-(p-chlorophenyl)-4,5-dihydropyridazin(2H)-3-one (IIIA-IIIB) derivatives have proven that analgesic activities. These compounds were less effective than standard drug Aspirin. Various different substituted pyridazinone compounds are aimed at studying for their contribution to analgesic and other pharmacological activities.

Acknowledgement

The authors are thankful to GRD (PG) IMT, Dehradun, India, Department of Pharmaceutical chemistry, Himalayan Institute of Pharmacy Research, Dehradun, India, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia as well as to SAIF, Punjab University, Chandigarh, India for providing financial as well as technical support and facilities to carry out this work.

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Volume 3 Issue 6 June 2019