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Anti-inflammatory and Antinociceptive Activities of 6-phenyl (3'-imino-benzylidene)-4-benzylidene-2,3,5-trihydro-3-(*2H*)-pyridazin-3-one Compounds

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Abstract

Currently used non steroidal anti-inflammatory drugs are one of the most commonly used medications for the treatment of pain and inflammation. Although routine and long-term uses limited due to their gastrointestinal and renal side effects. In order to minimize these side effects, there is increased focus on developing new non steroidal anti-inflammatory drugs. Stimulated by these above findings, our attention has been focused on the new 6-phenyl (3'-imino-benzylidene)-benzylidene-2,3,5-trihydropyridazin-3-one derivatives (**5a-5i**) were evaluated for antiinflammatory by right hind paw oedema and antinociceptive activity by hot plate and tail flick method respectively. All the compounds (**5a-5i**), exhibited significant antiinflammatory as well as antinociceptive activity as compared to standard drug indomethacin and aspirin against right hind paw oedema and tail flick, Eddy's hot plate method in albino rat and mice after intra-peritonially administration of 10 mg/kg and 50mg/Kg body weight dose. The compounds (**5a, 5b, 5e, 5g** and **5i**) showed lesser white spot or gastric ulcer and compounds (**5c, 5d, 5f** and **5h**) no white spot or gastric ulcer in the mucosal layer of the rat stomach as compared to the standard drug (indomethacin), which showed 100% ulceration.

Keywords: Antinociceptive, ulcer, inflammation, pyridazinone.

Introduction

During the past few decades increasing interest in the synthesis and properties of pyridazine derivatives has been observed. Pyridazine derivatives show wide spectrum of biological activities as described in the literature indicated that the a substantial number of substituted pyridazines have been reported to possess antimicrobial (Islam et al. 2008), analgesic, anti-inflammatory (Balkan et al. 2003, Banoglu et al. 2004, Doğruer et al. 2003, Gökçe et al. 2001), antifeedant, herbicidal (Cao et al. 2003), antihypertensive (Ogretir et al. 2002, Barbaro et al. 2001), antiplatelet activities (Coelho et al. 2004, Sotelo et al. 2002) anticancer effects (Malinka et al. 2004), intermediates for drugs and agrochemicals, antiphlogistics, antipyretics, cardiovascular, antitubercular, anticonsulvants (Rubat et al. 1990, Siddiqui et al. 2007) and

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other anticipated biological (Youssef et al. 2005) and pharmacological properties.

In particular, a large number of arylpyridazinone derivatives are well known as anti-inflammatory and analgesic agents (Asif et al. 2011, Gokce et al. 2001). So, many researchers have been interested in pyridazinone compounds for the development of new potential analgesic and anti-inflammatory agents. Among them, emorfazone (Figure 1) is marketed in Japan as an analgesic and anti-inflammatory, which was launched at the beginning of the last decade. After this, Dal Piaz et al. (1996) synthesized and evaluated the antinociceptive activities of the compounds having 2-substituted 4, 5-functionalized 6-phenyl-3(2H) - pyridazinone structures. They observed that some were more potent than Emorfazone (Piaz et al. 1996, Takaya et al. 1979).

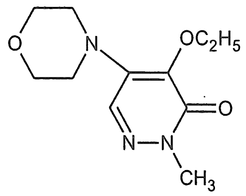


Figure 1. Emorfazone

In view of this fact, Nonsteroidal anti-inflammatory drugs are one of the most commonly used medications worldwide to inhibit cyclo-oxygenase (COX1 and COX2) activities for the treatment of pain and inflammation (Okcelik et al. 2003). Although they are effective in the treatment of pain and inflammation, their routine and long-term administrationis limited due to their gastrointestinal and renal side effects. In order to minimize these side effects, there is increased focus on developing non steroidal anti-inflammatory drugs. Stimulated by these above findings, our attention has been focused on the synthesis of a series of new 6–phenyl-4'- iminobenzylidene-4-benzylidene-3-(2H) pyridazin-3-one derivatives (**5a-5i**) which are expected to show antinociceptive and antiinflammatory activities. Syntheses of the designed compounds (**5a-5i**) were carried out per the scheme I and the structures of all the synthesized compounds were established based on elemental analysis, FT-IR and 1H-NMR spectral data.

Experiment

General synthetic procedure for Synthesis of benzoyl propionic acid (**1**)

A mixture of benzene (30 ml) and anhydrous aluminium chloride (0.15 mole) was refluxed under anhydrous condition, followed by addition of succinic anhydride (0.10 mole) in small quantity with continuous stirring. The stirring and heating were continued for 4 h. After 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 (Siddiqui et al. 2004, Furnish et al. 1989). The β-benzoyl propionic acid was re-crystallized from aqueous ethanol. 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 β-m-nitro benzoyl propionic acid (**2**)

To a mechanically stirred mixture of 12 ml concentrated nitric acid and 12 ml of concentrated sulphuric acid, 6 gm of β-benzoyl propionic acid was added in the portion while keeping the mixture at 0-10 ^0C by efficient cooling (30-40 min.) The temperature was further allowed to rise to 15^0C 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 (Furnish et al. 1989, Samantha et al. 2011). Lightly yellow color compound was obtained. IR Spectra: 3091 cm^{-1} (CH), 1705 cm^{-1} (C=O), 1353 cm^{-1} (NO_2), 1617 cm^{-1} (C=C). ^{1}HNMR (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).

Synthesis of β-m-Amino benzoyl propionic acid **(3)**

0.05mole β-m-nitro benzoyl propionic acid was taken in 50 ml of ethanol and was refluxed with 5.00 gm of tin and 10 ml of Conc. HCl for 30-40 min. (Furnish et al. 1989, Samantha et al. 2011).The reaction mixture was then poured into water treated with sodium carbonate and extracted with ether. The ethereal solution after washing with water, dried with sodium Sulphate, evaporated and crystallized from methanol. IR Spectra: 3400 cm^{-1} (NH_2), 1700cm^{-1} (C=O), 1617cm^{-1} (C=C), 3090cm^{-1} (CH). NMR Spectra: ^{1}HNMR ($CDCl_3$) ppm 2.8 (t,2H,CH_2), 3.20 (t,2H,CH_2), 7.40-8.0 (m,H,Ar-H), 8.8-9.0 (s,H,NH)(s,2H,NH_2).

Synthesis of 6-(3'-Amino phenyl)-2, 3, 4, 5-tetrahydro pyridazin-3-one **(4)**

The β-m-amino benzoyl propionic acid (0.01 mole) was refluxed for 6 h with hydrazine hydrate (0.01 mole) in methanol (10 ml.) containing sodium acetate (50 mg). The content was concentrated and then poured into ice cold water to get the compound (Siddiqui et al. 2004, Samantha et al. 2011). It was re-crystallized from ethanol. IR Spectra: 1685cm^{-1} (C=O), 1352cm^{-1} (NO_2), 3100cm^{-1} (CH), 3550cm^{-1} (NH). ^{1}HNMR ($CDCl_3$) ppm 2.8 (t,2H,CH_2), 3.2 (t,2H,CH_2), 7.40-8.0 (m,H,Ar-H), 8.8-9.0 (s,H,NH) (s,2H,NH_2).

Synthesis of-(3'-Iminophenyl-Benzylidene)-4-Benzylidene 2, 3, 5-trihydropyridazin-3-one derivatives: **(5a-5i)**, *Condensation of compound* **(4)** *with benzaldehyde*

A mixture of sample **(4)** (0.005 mole) and different aldehydes (0.005 mole) were taken in glacial acetic acid (20 ml) and sodium acetate (2 gm.) was added in it. The content was refluxed for 6-8 hours (monitored by TLC), cooled and then poured into ice (Samantha et al. 2011). The solid compound was obtained and then re-crystallized with ethanol. IR Spectra: 1360 cm^{-1} (NO_2), 1700cm^{-1} (C=O), 1580cm^{-1} (C=C) exo, 3450cm^{-1} (NH). ^{1}HNMR Spectra: ($CDCl_3$) ppm 3.10 (s,2H,CH_2), 7.2(s, H =CH), 7.40-8.0 (m, H, Ar-H) 8.8.0 (s,H,NH).

Pharmacology

Animals

Experimental animals were used for pharmacological study. 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 ethical committee of department of pharmacy in GRD (PG) IMT, Dehradun. Animals had free access to food and water, except during the experiment and housed at controlled room temperature with 12 h light 12 h dark cycle.

Anti-inflammatory Activity

Anti-inflammatory activity of all compounds was studied by the carrageenan–induced rat hind paw edema model as described by Winter, *et al.* Albino rats of the Wistar strain (150–200 g) of both sex were divided into different groups (control, test, and standard) containing six animals each (Winter et al. 1962). Animals were made to fast for 12 h before the experiment, and only water was allowed. The test samples and standard compound were suspended in 1% carboxymethylcellulose and administered orally to each animal by using a gastric gavage needle. The control group animals, however, received the same volume of vehicle. One hour after administration of the compounds, carrageenan was injected into the subplantar surface of the right hind paw of animals. In this study, the animals were administered a 50 mg/kg (body weight) dose of the test drugs and 10 mg/kg (body weight) dose of the standard drug Indomethacin. The paw volume was measured immediately using a plethysmometer (initial paw volume) and thereafter the paw volume was measured at 3 and 6 h after the administration of carrageenan. The results are summarized in Table 2.

Antinociceptive activity by Hot Plate Method

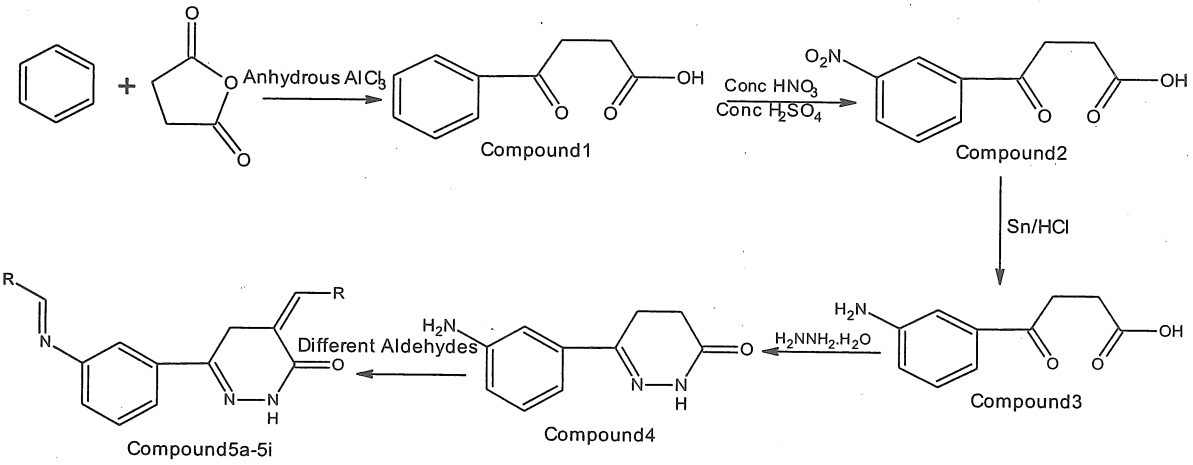
Antinociceptive activity was measured by Eddy and Leimbach's (1953) hot plate method, using a radiant heat type analgesiometer. Reaction time was measured as hind paw licking or jump response, whichever appears first in the animals. When placed on a hot plate maintained at constant temperature (55°C), normally animals show such response in 6–8 sec. A cut-off period of 15 sec is observed to avoid damage to the paws. For each animal, hind paw licking or jump response reaction time was obtained thrice before drug administration, and the mean was used as pre drug reaction time. Swiss albino mice (25–30 g) of both sexes were divided into different groups: control, test, and standard (containing six animals each). The test compounds and standard compound were suspended in 1% carboxymethylcellulose and administered intraperitoneally. The control group animals, however, received the same volume of vehicle. In this study, the animals were administered a 50 mg/kg (body weight) dose of the test drugs and 50 mg/kg (body weight) dose of the standard drug aspirin. After administration of the drug, the jumping or hind paw leaking times were measured at 30, 60, 120, and 180 min and are reported in Table 3.

Antinociceptive activity by Tail Flick Method

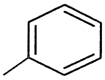
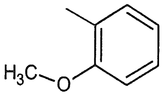
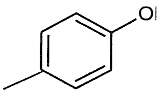
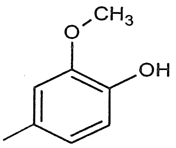
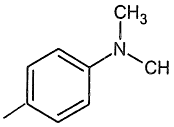
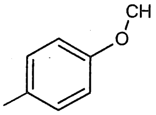
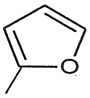
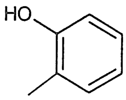
Antinociceptive activity was measured by the tail flick method using a radiant type analgesiometer. Reaction time to radiant heat was taken by placing the tip of the tail on the radiant heat source. Swiss albino mice (25 – 30 g) of either sex were divided into different groups (control, test, and standard) containing six animals each. For each animal, the tail flick reaction time was obtained thrice before drug administration and the mean was used as pre drug reaction time. After administration of the drug, the tail flick reaction times were measured at 30, 60, 90, 120, and 180 min. The test and standard drug were given intraperitoneally, while the control group received only vehicle. The animals were administered a 50 mg/kg (body weight) dose of the test drugs and 50 mg/kg (body weight) dose of the standard drug (aspirin). Increase in reaction time after administration of the test drugs and the standard drugs were calculated at 30, 60, 120, and 180 min and are reported in Table 4.

Gastro-ulcerogenic effect

Albino rats of the Wistar strain (150–200 g) of both sexes were divided into different groups: control, test, and standard (containing six animals each). The test group, control group, and standard group received the test drug, vehicle, and standard drug, respectively. The test compounds and standard compound were suspended in 1% carboxymethylcellulose and administered orally to each animal by using a gastric gavage needle. The control group animals, however, received the same volume of vehicle. In this study, the animals were administered a 50 mg/kg (body weight) dose of the test drugs and 10 mg/kg (body weight) dose of the standard drug (Indomethacin). The animals were treated once a day with the same dose of the test compounds (50 mg/kg) and the standard compound Indomethacin (10 mg/kg), and the control group received the same volume of vehicle up to a period of 5 days. During this period they have free access to food and water. On day 6 (24 hours after the last dosing), the animals were sacrificed and the stomachs and intestines were removed. A longitudinal incision along the lesser curvature was made. The stomach and intestine of the animals was rinsed in running water, and the presence or absence of ulcers was determined in the control group, the test group, and the standard group, and the results are reported in Table 5.



Scheme I

Compounds	R	Compounds	R	Compounds	R
5a	-CH_3	5d		5g	
5b		5e		5h	
5c		5f		5i	

Statistical analysis

Results were expressed as means ± 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.

Table 1. Characterization data of intermediate and pyridazinone derivatives

Synthesized Compounds	Yield (%)	M.P (°C)	Molecular Formula	Mass (g/mol)
Benzoyl propionic acid (1)	70	120	$C_{10}H_{10}O_3$	178.18
β-m-nitro benzoyl propionic acid (2)	50	108	$C_{10}H_9NO_5$	223.18
β-m-Amino benzoyl propionic acid (3)	52	95	$C_{10}H_{11}NO_3$	193.2
6-(3'-Amino phenyl)-2,3,4,5-tetrahydro pyridazin-3-one (4)	65	90	$C_{10}H_{11}N_3O$	189.21
6-(3'-Iminophenyl-methylidene)-4- methylidene 2,3,5-trihydro pyridazi-3-one (5a)	48	130	$C_{14}H_{15}N_3O$	241.29
6-(3'-Iminophenyl-4-hydroxy benzylidene)-4-(4-hydroxybenzyl- idene)-2,3,5-trihydro pyridazin-3-one (5b)	42	140	$C_{19}H_{24}N_3O_3$	342.41
6-(3'-Iminophenyl-4- methoxy benzylidene) - 4- (4- methoxy benzylidene) 2, 3, 5- trihydro pyridazin-3-one (5c)	55	127	$C_{26}H_{23}N_3O_3$	425.48
6-(3'-Iminophenyl-benzyl -idene) - 4- benzylidene 2, 3, 5- trihydro pyridazin-3-one (5d)	51	139	$C_{24}H_{19}N_3O$	365.43
6-(3'-Iminophenyl-3- methoxy -4-hydroxy benzylidene) - 4- (3- methoxy-4-hydroxy benzyl -idene) - 2, 3, 5 trihydro pyridazin-3-one (5e)	62	164	$C_{26}H_{25}N_3O_5$	459.5
6-(3'-Iminophenyl-furfuryl id ene) - 4- furfurylidene 2, 3, 5- trihydro pyridazin-3-one (5f)	53	158	$C_{20}H_{15}N_3O_3$	345.35
6-(3'-Iminophenyl-2-methoxy -benzylidene) -4- (2- methoxy benzylidene) 2, 3, 5- trihydro pyridazin-3-one (5g)	42	146	$C_{26}H_{23}N_3O_3$	425.48
6-(3'-Iminophenyl-4-dimethyl -aminobenzylidene) - 4- (4-dimethylamino benzylidene) 2, 3, 5- trihydro pyridazin-3-one (5h)	55	157	$C_{28}H_{29}N_5O$	451.57
6-(3'-Iminophenyl-2- hydroxy -benzylidene)-4- (2- hydroxy-benzylidene) 2, 3, 5- trihydro pyridazin-3-one (5i)	62	145	$C_{24}H_{19}N_3O_3$	397.43

Result and Discussion

In the initial step of the synthesis, corresponding 6-phenyl (3'-imino-benzylidene)-benzylidene 2,3,5-trihydro pyridazin-3-one derivatives **(5a-5i)** were synthesized from Friedal craft acylation of benzene with succinic anhydride in presence of anhydrous aluminium chloride yield β-benzoyl propionic acid **(1)** followed by nitration and reduction to yield β-m-nitrobenzoyl propionic acid **(2)** and β-m-aminobenzoyl propionic acid **(3)**. β-m-aminobenzoyl propionic acid were cyclised with hydrazine hydrate to form 6-phenyl(3'-imino-benzylidene)–4-benzylidene-2,3,5-trihydropyridazin-3-one derivatives **(4)**. 3'-aminophenyl)-2,3,4,5-tetrahydro pyridazin-3-one by reaction with substituted aldehydes (Scheme I).

All the synthesized compounds **(5a-5i)**, the IR and 1 H NMR spectra were consistent with the proposed chemical structures. 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 multiplet near δ=2.8 for CH_2 protons at 5-position, another multiplet is observed at about δ=3.0 for CH_2 at 4 position of compounds. Aromatic proton also observed in the aromatic region ranging from δ=7.0-8.0. Presence of other substitutes also authenticated in the ^{1}HNMR spectra at the assigned value and their physical characterization in Table 1. The test compounds **(5a-5i)** have anti-inflammatory activity as compare to the standard drug indomethacin after 3 and 6 h of carrageenan administration (Table 2). The carrageenan-induced hind paw edema assay suggested that compounds 5d, 5g and 5h having the phenyl, o-phenyl methoxy and p-dimethyl amino phenyl (electron donating) group in the R position showed comparable inhibition of inflammation (Table 2) after 6 h. Though the exact reason behind this abnormality is not known, the metabolic conversion of the different R group cannot be ignored as a possible reason.

Inflammation induced by carrageenan involved three distinct phases of release of mediator, including serotonin and histamine in the first phase (0–2 h) and kinins in the second phase (0–4 h). The results of this study indicate that compounds **(5a-5i)** significantly inhibit paw edema induced by carrageenan in the first and second phase, suggesting an inhibitory effect on the release of histamine, serotonin, kinins, and also on the release of prostaglandins. From the results it is apparent that compounds **(5a-5i)** showed a significant suppression of carrageenan induced paw edema in rats and demonstrated significant antinociceptive activity in tail flick, and hotplate induced pain. However, the analgesic activity of the test compounds was found to be significant in the both tests, tail flick and hot plate method, and thus it appears that the test compounds inhibit predominantly the peripheral pain mechanism.

Anti-inflammatory activity assessment

The anti-inflammatory activity of the synthesized compounds was studied using a carrageenan-induced hind paw edema model in mice at 50 mg/kg dose. The results are shown in Table 2. All pyridazinone derivatives showed remarkably potent anti-inflammatory activity, especially 180 min after the drug was administered, but all compounds had the less potent anti-inflammatory activity throughout the study. These compounds might have effect in both stages of inflammation.

Interestingly, most of the compounds in the present series had strong anti-inflammatory effects ranging from 50 to 75 % edema reduction, in comparison to around 86.36 % seen with the reference, indomethacin after 3 hr (Table 2). One compound caused only 50 % edema reduction. Compound6-(3'-Iminophenyl-2-methoxy-benzylidene)-4-(2- methoxy benzylidene) 2, 3, 5- trihydro pyridazin-3-one **(5g)**, showed maximum anti-inflammatory activity to that of other synthesized compounds. After 6hr Compound **5g** showed 46.93% Inhibition of paw edema, in comparison to around 60.20 % with the reference, indomethacin after 6 h. The ascending order of potency of % Inhibition of paw oedema **5e** < **5a** < **5b** < **5i** < **5h** < **5f** < **5c** < **5d** < **5g** were obtained. These findings indicate that 6-phenyl (3'-imino-benzylidene) and 4-benzylidene-3-(2H) pyridazinone derivatives could represent a highly promising group for the development of anti-inflammatory drugs.

Antinociceptive activity assessment

The antinociceptive activity of the compounds was studied using tail flick and hot plate model. As shown in Table 1, all compounds showed significant antinociceptive activity at 50 mg/kg dose level. All compounds except for **5c** and **5e** were more potent than aspirin in on tail flick response test in mice at 50 mg/kg dose. Remaining compounds (**5a, 5b, 5d, 5f, 5g, 5h** and **5i**) exhibited less antinociceptive activity than reference drug. Compounds 5c, 5e and Vh were more potent than aspirin in on hot plate test in mice at 50 mg/kg dose. Remaining compounds (**5a, 5b, 5d, 5f, 5g** and **5i**) exhibited less analgesic activity than reference drug. Among the compounds synthesized, compound **5e** was the most potent in terms of antinociceptive activity and had 33% ulcerogenic side effects. The degree of potency in ascending order for antinociceptive activity in tail flick method was **5a** <, **5d** <, **5i** <, **5g** <, **5b** <, **5f** <, **5h** <, **5c** <, **5e** and for hot plat method was **5a**<, **5b**<, **5d**<, **5f**<, **5g**<, **5i**<, **5h**<, **5c**< **5e**. All tested compounds exhibited antinociceptive activities (Table 3 & 4) that lasted for 120 minutes and the potency increased with time.

Anti-ulcerogenic effect assessment

On the other hand, compounds **5a**, **5b**, **5e**, **5g**, and **5i** resulted in an ulcerogenic effect Table 5. The synthesized compounds were devoid of gastrointestinal side effects (ulcerogenic effect), which is the most frequent untoward or adverse reactions associated with orally ingested anti-inflammatory or anti-arthritic agents. In the whole biological evaluation study (anti-inflammatory, as well as antinociceptive, and ulcerogenic effect) the results were encouraging. The above compounds deserve further clinical trials.

Table 2. Anti-inflammatory activities of substituted pyridazinones (**5a-5i**) against carrageenan-induced rat paw edema.

Compounds	Average change in paw volume after 3 h (mean ± SEM)	% Inhibition of paw edema after 3 h (mean)	Average change in paw volume after 6 h (mean ± SEM)	% Inhibition of paw edema after 6 h (mean)
Control	0.88 ± 0.02	–	0.98 ± 0.04	–
Indomethacin	0.24 ± 0.01	86.36	0.41 ± 0.03	60.2
5a	0.52 ± 0.03	54.54	0.69 ± 0.03	30.63
5b	0.45 ± 0.03	62.5	0.68 ± 0.01	36.36
5c	0.42 ± 0.03	65.9	0.66 ± 0.02	44.89
5d	0.38 ± 0.03	70.45	0.55 ± 0.02	45.91
5e	0.50 ± 0.03	56.81	0.73 ± 0.02	27.55
5f	0.56 ± 0.06	50	0.66 ± 0.02	44.89
5g	0.44 ± 0.06	75	0.54 ± 0.02	46.93
5h	0.43 ± 0.03	64.77	0.57 ± 0.04	43.87
5i	0.51 ± 0.03	55.68	0.61 ± 0.03	39.79

n=6 in each group. All the compounds showed significant result when compared with control group (***$p<0.001$)

Table 3. Antinociceptive activities of substituted pyridazinones (**5a-5i**) on tail flick response in mice.

Compounds		Post-drug reaction time, sec (mean ± SEM)			
		30 min (mean ± SEM)	60 min (mean ± SEM)	90 min (mean ± SEM)	180 min (mean ± SEM)
Control	4.26 ± 0.15	4.42 ± 0.19	4.39 ± 0.15	4.24 ± 0.08	4.44 ± 0.11
Aspirin	4.24 ± 0.07	6.78 ± 0.11	8.25 ± 0.15***	7.64 ± 0.15***	7.63 ± 0.14***
5a	4.39 ± 0.22	4.20 ± 0.11	4.56 ± 0.14	4.84 ± 0.12*	5.16 ± 0.01**
5b	4.0 ± 0.15	4.18 ± 0.02	4.58 ± 0.11	5.46 ± 0.11***	6.56 ± 0.12***
5c	4.26 ± 0.09	4.65 ± 0.13	5.74 ± 0.24***	6.61 ± 0.14***	8.10 ± 0.09***
5d	4.23 ± 0.10	4.22 ± 0.04	5.27 ± 0.09**	5.65 ± 0.14***	6.47 ± 0.03***
5e	4.16 ± 0.04	5.12 ± 0.12***	5.78 ± 0.14***	6.69 ± 0.07***	8.24 ± 0.13***
5f	4.22 ± 0.02	4.47 ± 0.08	5.42 ± 0.10***	6.86 ± 0.08***	6.96 ± 0.15***
5g	4.31 ± 0.08	4.52 ± 0.08	4.97 ± 0.13	5.44 ± 0.03***	6.54 ± 0.02***
5h	4.18 ± 0.16	5.03 ± 0.02**	5.57 ± 0.11***	6.63 ± 0.14***	7.62 ± 0.13***
5i	3.95 ± 0.10	4.52 ± 0.11	5.34 ± 0.02***	5.87 ± 0.08***	6.52 ± 0.21***

$n = 6$ in each group. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 4. Antinociceptive activities of substituted pyridazin-3-ones (**5a-5i**) by Eddy's hot plate method in mice.

Compounds		Post-drug reaction time, sec (mean ± SEM)			
		30 min (mean ± SEM)	60 min (mean ± SEM)	90 min (mean ± SEM)	180 min (mean ± SEM)
Control	4.86 ± 0.05	4.87 ± 0.05	4.87 ± 0.05	4.86 ± 0.06	4.88 ± 0.05
Aspirin	4.56 ± 0.01	6.55 ± 0.01^{a}	7.83 ± 0.03a	8.84 ± 0.06^{a}	8.40 ± 0.20^{a}
5a	4.70 ± 0.07	4.95 ± 0.06	5.39 ± 0.03^{c}	6.93 ± 0.02^{a}	8.78 ± 0.02^{a}
5b	4.72 ± 0.19	4.92 ± 0.18	4.99 ± 0.15	5.73 ± 0.07^{a}	6.42 ± 0.03^{b}
5c	4.29 ± 0.03	4.79 ± 0.09	5.64 ± 0.05^{a}	7.55 ± 0.14^{a}	8.72 ± 0.05^{a}
5d	4.78 ± 0.06	4.90 ± 0.06	5.76 ± 0.03^{a}	6.66 ± 0.02^{a}	7.13 ± 0.02^{a}
5e	4.73 ± 0.11	5.17 ± 0.06	6.64 ± 0.14^{a}	7.38 ± 0.07^{a}	8.78 ± 0.10^{a}
5f	4.11 ± 0.02	4.67 ± 0.13	5.85 ± 0.08^{a}	6.64 ± 0.16^{a}	7.45 ± 0.16^{a}
5g	4.33 ± 0.10	4.95 ± 0.09	5.87 ± 0.05^{a}	6.34 ± 0.02^{a}	7.84 ± 0.05^{a}
5h	4.69 ± 0.09	5.05 ± 0.03	6.52 ± 0.13^{a}	6.95 ± 0.18^{a}	8.69 ± 0.11^{a}
5i	4.68 ± 0.11	4.98 ± 0.01	5.76 ± 0.21^{a}	7.65 ± 0.03^{a}	8.20 ± 0.13^{a}

$n = 6$ in each group. *** $Pa < 0.001$, ** $Pb < 0.01$, * $Pc < 0.05$

Table 5. Ulcerogenic effect of substituted pyridazinone derivatives (**5a-5i**)

Compounds	Ulcer score	Ulceration (%)
Control	00/06	00.00
Aspirin	06/06	100.00
5a	01/06	16.66
5b	03/06	50.00
5c	00/06	00.00
5d	00/06	00.00
5e	02/06	33.33
5f	00/06	00.00
5g	01/06	16.66
5h	00/06	00.00
5i	02/06	33.33

Conclusion

In conclusion, for a series of antiinflammatory compounds, the substitution of 4-benzylidine and 6-phenyliminobenzylidine of 3-(*2H*)-pyridazinone derivatives or 4-methylidine and 6-phenyliminomethylidine of 3-(*2H*)-pyridazinone was synthesized, and the resulting antiinflammatory as well as antinociceptive activity was evaluated by an in vivo test. All the compounds, exhibited significant antiinflammatory as well as antinociceptive activity as compared to standard drug indomethacin and asprin against right hind paw oedema and tail flick, Eddy's hot plate method in albino rat and mice after intra-peritonially administration of 50mg/Kg body weight dose. Compounds (**5a-5i**) showed significant anti-inflammatory and as well as antinociceptive activity, showed lesser white spot or gastric ulcer in compounds (**5a, 5b, 5e, 5g** and **5i**) and no white spot or gastric ulcer in compounds(**5c, 5d, 5f** and **5h**) in the mucosal layer of the rat stomach as compared to the standard drug (indomethacin), which showed 100% ulceration.

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