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Research Article

EVALUATION OF ANTIPYRETIC AND ANTINOCICEPTIVE POTENTIAL OF NEW HETEROCYCLIC DERIVATIVES OF 3-FORMYL-4-HYDROXYCOUMARIN IN RATS

Razi Ahmad [a,*], Mohammad Asad [b], Zeba N Siddiqui [b], Anil kumar [a]
[a] Department of Pharmacology, J N Medical College, Aligarh Muslim University, Aligarh 202 002, India
[b] Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, India

Corresponding Author : **Razi Ahmad**, Email: rahmad50@gmail.com

Abstract: Coumarins have multiple biological activities; various coumarin-related derivatives are recognized as inhibitors of the lipoxygenase and cycloxygenase pathways of arachidonate metabolism. Several natural or synthetic coumarins with various hydroxyl and other substitutes were found to inhibit lipid peroxidation and to scavenge hydroxyl radical and superoxide anion and to influence processes involving free radical mediated injury. Thus, the objective of the present study was to evaluate the antipyretic and analgesic activities of heterocyclic derivatives of 3-formyl-4-hydoxycoumarin (synthesized by us) in animal models. All compounds synthesized were evaluated for the above activity and their effects were compared with the standard drugs. In the antipyretic test, a new model of pyrexia suggested by Tomazetti et al., 2005 was used where backer's yeast is used to induce pyrexia in juvenile male Wistar rats, which received Backer's yeast at 135 mg/kg intraperitonealy. The test compound or Paracetamol was given orally 1 h after injection of yeast (when average rise in rectal temp was about 1 ºC). Meanwhile, in the analgesic test the nociceptive response using hot plate and formalin tests were performed in adult male Wistar rats. The test compounds (heterocyclic derivatives of 3-formyl-4-hydoxycoumarin) showed significant antipyretic activity in rats. The test compound also produced significant antinociceptive activity in the hot plate (central) as well as in formalin tests (central and peripheral) nociceptive tests suggesting the involvement of both central and peripheral mechanisms in alleviating the pain response.

Keywords: 3-Formyl-4-hydroxycoumarin derivatives; Antipyretic; Analgesic, Backer yeast.

1. Introduction

[1] Benzopyran-2H-ones (Coumarins) reported to possess multiple biological activities[1] are used in the treatment of vitiligo, psoriasis and other dermal diseases. The physiological properties of natural and synthetic [1] benzopyran-2[H]-ones have been reviewed by various workers[2]. In recent times [1] benzopyran- 2[H]-ones have been extensively used as laser materials[3], photosensitizers[4], brightner[5], as intermediates for dyes, pesticides and pharmaceuticals[6] as well as in perfume formulations[7,8] and in enzymology as biological probes[9]. Coumarins show activities such as antifungal[10], anticoagulant[10], antibacterial[11], analgesic, antipyretic, anti-inflammatory and anti-arthritic[12,13].

Drugs having analgesic and antipyretic properties are one of the most widely used drugs for various medical and surgical conditions to the patients.

Although a significant progress for the understanding of the mechanisms of thermoregulation has been achieved in the past 30 years[14], the number of safe and effective antipyretics available in the clinics remained practically unaltered during this period. Keeping this in view, the present study has been undertaken to investigate the analgesic and antipyretic activities of the synthetic heterocyclic compounds in experimental animal models.

2. Materials and methods

2.1 Chemicals and test compounds

Following heterocyclic compounds were synthesized in the research laboratory of Department of Chemistry and studied for their physiochemical and spectral properties[15]. They were tested for antipyretic and analgesic activities in animal models.

1. 3-Acetoacetyl pyrano [3,2-c] [1] benzopyran 2,5-dione (Fig.1) was prepared from intramolecular translactonization of 4-hydroxycoumarins and triacetic acid lactone. The resulting compounds **1,** which possessed a 1,3-diketone unit in its structure were converted to pyrazoles by treatment with hydrazine, phenylhydrazine and hydrazinobenzothiazole to afford

1a. 3-(3-methyl pyrazol-5-yl)-pyrano[3,2-c][1] benzopyran 2,5-dione.

1b. 3-(3- methyl-1-phenyl pyrazol-5-yl)- pyrano [3,2-c] benzopyran-2, 5-dione and

1c. 3-(3-methyl-1-benzothiazolopyrazol-5-yl)-pyrano[3,2-c][1] benzopyran-2,5-dione.

The test compounds were dissolved in 2.5% DMSO (Dimethyl sulphoxide) prior to administration in different concentration so that animal received equal volume each time (5 ml/kg).

Dose selection of the test compound was based on preliminary trial carried out in our laboratory over a dose

range 5 mg/kg to 40 mg/kg in geometric increasing order and maximal effect was found at the dose of 20 mg/kg.

Drugs used: Bakers yeast (Britannia food products)**,** Formalin (Merck, India) , Paracetamol (IPCA), Pentazocin (Ranbaxy)

2.2. ***Experimental Animals***

For analgesic activity adult male Wistar Albino rats (weight 100–150 g) and for antipyretic activity young male Wistar Albino rats 28-30 days of age (weight 90-100 g) were used. They were obtained from Laboratory Animal Breeding and Research Center Jamia Hamdard University New Delhi, were used for the present study. The animals were given a week time to get acclimatized with laboratory conditions

The animals were housed in polypropylene cage (4 per cage) with sterilized paper cuttings as bedding material under laboratory conditions with control environment of temperature 22 ± 3 °C, humidity (60 % ± 10 %) an 12 h light/dark cycle. They were given free access to food with standard rodent pellet diet (from Lipton India) and drinking water. The animals were transferred to the experimental room 2 h before the experiment. All measures temperatures were taken between 10:00 to 16:00 h, when the rectal temperature reported to be stable [16].

The study protocol was approved by the institutional ethical committee.

2.3. Experimental Protocol

The following experimental models were used for test compounds.

a. Baker's yeast induced pyrexia

a. Effects of test compounds and Paracetamol on Baker's yeast induced pyrexia.

Rats were divided into four groups (n = 6). The animals were set in their cages individually throughout the experiment. Rectal temperature was measured with a lubricated thermister probe inserted 3 cm deep into the rectum. The probe was linked to telethermometer (range 31–41 °C with 0.1 ºC precision) for 5 h, Rectal temperature was measured every 15 min for each 5 h and recorded manually at specified intervals.

To minimize the stress response of the animals to the lightly restrained condition, we made a careful handling and performed two sets of acclimatizing training in the cage for 2 days before starting the experiments.

Fever was induced by intraperitoneal injection of baker yeast 135 mg/kg, which induced a sustained increase in rectal temperature for 5 h. Paracetamol and other novel antipyretics, reverted baker yeast-induced fever. The test compounds and Paracetamol was administered 1 h after injecting yeast when there was an average increase in temp of about 1 ºC.

Group 1: (Control) only yeast was injected and continuously temperature was monitored and recorded at specified interval for 5 h.

Group II: received 2.5 % DMSO (0.5 ml) was given orally 1 h after administering yeast.

Group III: Test compounds (20 mg/kg) dissolve in DMSO (0.5 ml) was administered orally 1 h after administering yeast.

Group IV: Paracetamol (150 mg/kg) was given orally 1 h after administering yeast.

b. Effect of test compounds and Paracetamol on basal rectal temperature.

Test compounds and Paracetamol were given orally and rectal temperature was measured every 15 min for each 5 h and recorded manually at specified intervals.

Group I: Received 2.5 % DMSO (0.5 ml) given orally.

Group II: Test compounds (20 mg/kg) dissolved in DMSO (0.5 ml) was administered orally.

Group III: Paracetamol (150 mg/kg) was given orally.

c. Analgesic activity

Adult rats weighing 100–150 g were divided into three groups (n = 6) and analgesic activity was tested by (i) Hot-plate method (ii) Formalin test.

Group I: received 2.5 % DMSO (0.5 ml) was given orally 30 min before experiment.

Group II: Test compounds (dissolved in 0.5 ml DMSO) were administered orally 30 min before experiment.

Group III: Pentazocin (15 mg/kg) was given intraperitoneal 15 min prior to experiment.

Experimental design and drug treatment:

(i) ***Hot-plate method*** **[17,18]**

Male rats, weighing 100 to 150 g were used. Rats were screened by placing them on the hot plate (Eddy's hot plate from Techno India) maintained at 55±1°C and reaction time in seconds for hind paw licking or jumping were recorded. Only rats, which reacted within 5 to 10 seconds, were used in the study. Those animals in which the reaction time is increased to at least twice the mean reaction time for control animals or control reading plus eleven seconds (control+11 seconds) were taken as showing significant analgesia. Pentazocin was used as standard drug.

(ii) ***Formalin test***

Thirty minutes after administration of the test compounds or Diclofenac sodium (5 mg/kg) and 15 minutes after pentazocin intraperitonealy, 20 µl of 2.5% formalin in saline was injected subcutaneously to a hind paw of the rat. The rat was observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection[26]. The early phase represents neurogenic pain while the latter phase is of inflammatory pain.

e. Toxicity study

The acute oral toxicity was carried out as per the guideline set by the organization for the economic co-operation and development (OECD) received from the committee for the purpose of control and supervision of experimental animals (CPCSEA).

Experimental design and drug treatment:

2 rats (one from either sex) were dosed at predetermined [250, 500 and 1000 mg/kg dissolved in fixed amount (1.5 ml) of DMSO] and administered by stomach feeding

cannula. They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality (Litchfield et al., 1949). If there was no mortality or if no more than one rat of either sex died at the highest level tested (1000 mg/kg) with the total of 10 rats (5/sex) dosed at 1000 mg/kg and monitored for 7 days period LD_{50} was considered to more than 1000 mg/kg.

f. Statistical analysis

All values are presented as mean ± S.E.M. of six rats and difference between means were assessed by one-way analysis of variance (ANOVA), followed by student's *t* test. Difference between means were considered to be significant at $P<0.05$ as compare to control.

3. Results

3.1. a. Effect of test compounds on Yeast-induced pyrexia

The experimental rats showed a mean increase of about 1 ºC in rectal temperature 1 h after Backer's yeast injection (135 mg/kg, i.p). The test compound (**1**, **1a** and **1c**) produced significant ($P<0.05$) antipyretic activity at 2, 3, 4 and 5 h, whereas test compound **1b** and the reference drug Paracetamol (150 mg/kg) showed significant antipyretic activity throughout the observation period up to 5 h (Fig 2).

3.1. b. Effect of test compounds and Paracetamol on basal rectal temperature

The result showed by the test compounds and paracetamol on normal body temperature in rats is presented in Fig. 3. The test compound **1** caused significant lowering of body temperature upto 2 h (0.5 ºC) following its administration. While the maximum lowing of the rectal temperature noticed with the test compound **1b** was 0.2 ºC upto 1 to 3 h and that of standard drug paracetamol was 0.1 ºC up to 1 to 2 h period.

3.2. a. Hot plate reaction time in Rat

The results of hot-plate test indicated a significant increase in reaction time at 2 h (2.5 fold) 3 h (3.0 fold) and 4 h maximum effect upto cut-off time) with the test compounds, whereas reference drug pentazocin a centrally acting analgesic drug, markedly increased pain latency at 1 h (2.5 fold) and achieving maximum effect (upto cut-off time) at 2 and 3 h (Table 3).

3.2. b. Formalin test:

As shown in Table 4 the pretreatment with test compounds caused a significant inhibition of the neurogenic (early phase) and inflammatory phases (late phase) of formalin induced licking in rats.

The standard drug, Diclofenac sodium (5 mg/kg) also significantly inhibited formalin induced licking in rats but only in late phase (15-30 minute)

In contrast, the reference antinociceptive drug Pentazocin (15 mg/kg) significantly reduced the licking activity against both phases of formalin-induced nociception.

3.3. Acute Toxicity study evaluation

In acute toxicity study the test compounds did not show any toxicity and mortality up to maximum dose of 1000 mg/kg body weight in rats. No gross change in behavior was observed at this dose. Weight of rats had a normal variation after 7 days of observations.

Table 1: Effects of test compounds and Pentazocin on hot plate reaction time in rat.

Compounds	Dose/ kg	Reaction time in second						
		0 min	15 min	0.5 h	1 h	2 h	3 h	4 h
DMSO	5ml	8.50 ± 0.2	8.50 ± 0.2	8.50 ± 0.2	8.50 ± 0.2	8.30 ± 0.2	8.10 ± 0.3	8.10 ± 0.2
1	20 mg	8.33 ± 0.21	11.66 ± 0.21	11.66 ± 0.21	15.66 ± 0.23*	25.00± 0.40*	29.33 ± 0.42*	>30*
1a	20 mg	9.00 ± 0.36	10.33 ± 0.21	13.33 ± 0.55	16.00 ± 0.36*	18.00 ± 0.40*	21.00 ± 0.63*	25.00 ± 0.36*
1b	20 mg	9.33 ± 0.55	9.66 ± 0.21	14.33 ± 0.55	19.66 ± 2.01*	25.33 ± 1.11*	28.00 ± 0.73*	>30*
1c	20 mg	9.00 ± 0.36	10.66 ± 0.21	15.00 ± 0.36	19.33 ± 0.76*	23.33 ± 0.55*	26.00 ± 0.36*	28.33 ± 0.21*
Pentazocin	15 mg	8.50 ± 0.30	12.00 ± 0.20	16.00 ± 0.20*	20.23 ± 0.10*	> 30*	> 30*	24.50 ± 0.40*

The results given are mean ± S.E.M; number of animals used (n=6)

Table 2: Anti-nociceptive activity of test compounds on formalin induced pain.

Test compounds	Dose/ kg	Total time spent in Paw licking time (s)			
		Phase (0-5)	% inhibition	Phase (15-60)	% inhibition
DMSO	5ml	62.2 ± 5.2	-	146.4±12.3	-
1	20 mg	40.1 ± 2.2*	35.53	88.3 ± 7.0*	39.68
1a	20 mg	46.2 ± 1.4	25.72	96.2 ± 4.4*	34.28
1b	20 mg	34.3 ± 2.0*	44.85	52.4 ± 6.2**	64.20
1c	20 mg	38.7 ± 6.3*	37.78	96.2 ± 8.4*	34.28
Penta-Zocin	15 mg	18.2 ± 3.2**	70.79	40.6 ± 8.7**	72.26
Diclofenac Sodium	5 mg	54.3 ± 2.4	12.70	64.2 ± 5.3**	56.14

The results are mean ± SEM from 6 animals *P<0.01 **P<0.001, when compared to vehicle control (DMSO)

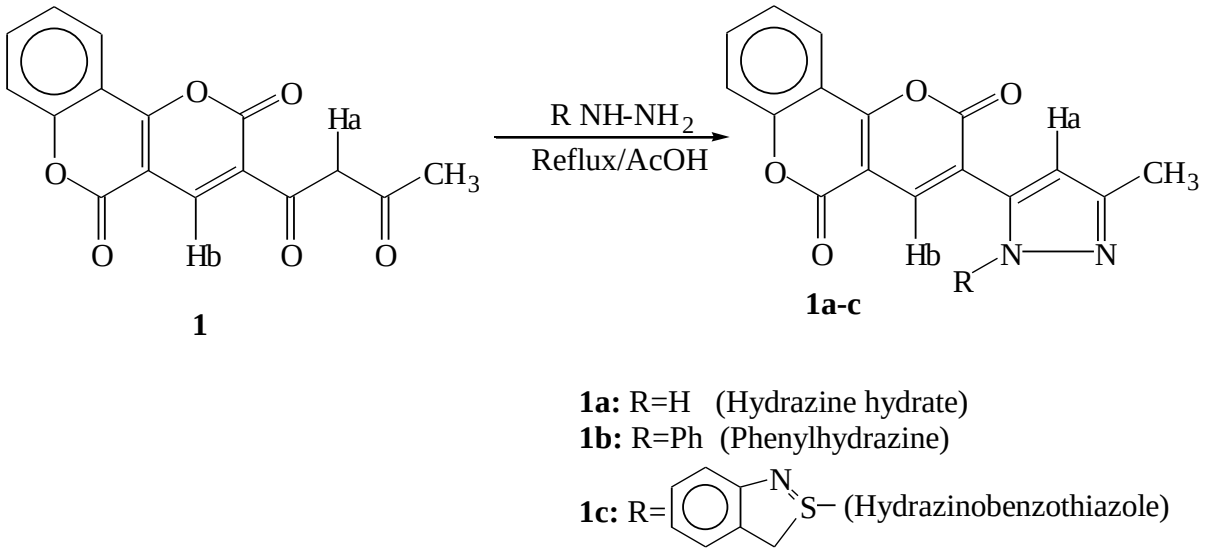

Fig 1: Structure of new heterocyclic derivatives of 3-formyl-4-hydroxycoumarines.

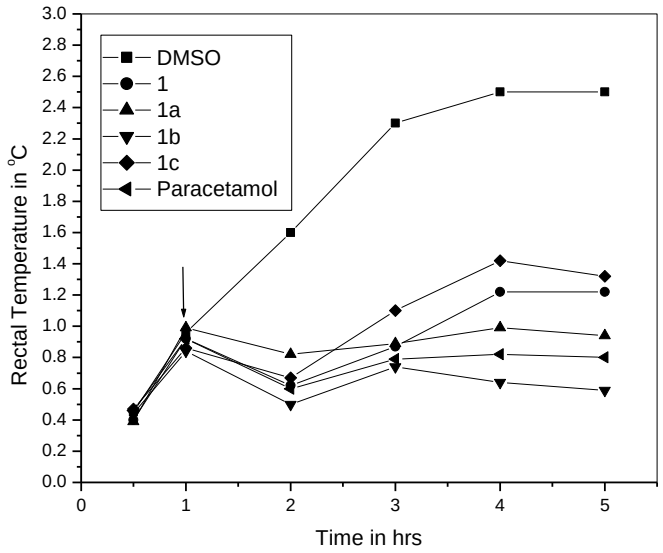

Fig 2: The Effect of test compounds and Paracetamol on yeast-induced pyrexia in rats

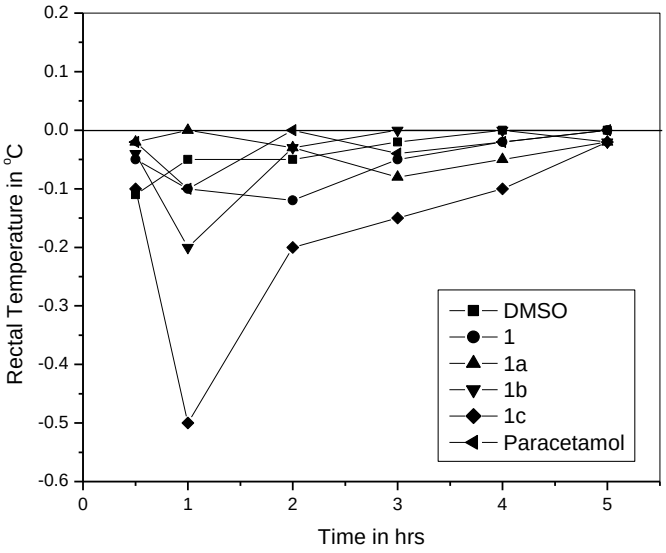

Fig 3: The Effect of test compounds on basal rectal temperature of rats

4. Discussion

Various coumarin-related derivatives are recognized as inhibitors of lipoxygenase and cycloxygenase pathways of arachidonate metabolism[19] but also of neutrophile-dependent super oxide anion generation[20]. Several natural or synthetic Coumarins with various hydroxyl and other substituents were found to inhibit lipid peroxidation and to scavenge hydroxyl radicals and superoxide anion[21] and to influence processes involving free radical-mediated injury, as can some plant phenolics and flavonoids.

The present study has demonstrated the pharmacological potential of the synthetic new heterocyclic derivatives of 3-formyl-4-hydroxycoumarin with addition of different groups as an antinociceptive and antipyretic agent when tested on various animal models.

Thermic painful stimuli (hot-plate test) are known to be selective to centrally, but not peripherally, acting analgesic drugs[22]. In the present study the test compound produced a significant inhibitory effect on the nociceptive response at 2, 3 and 4 h though less potent than that of the pentazocin a centrally acting analgesic drug, which significantly increased the reaction time in hot-plate test at 1, 2, 3, and 4 h.

The formalin test is another pain model, which assesses the way an animal responds to moderate, continuous pain generated by injured tissue[23]. Centrally acting drugs such as morphine inhibited both of the early and late phases equally while peripherally acting drugs such as aspirin only inhibited the second phase[24,25].

In the present study the test compounds significantly inhibited both the neurogenic pain (early phase) and inflammatory phase (later phase) except **1a** that have no significant role in inhibiting neurogenigc pain. Pentazocin significantly reduced the licking activity in both phases while Diclofenac decreased the licking activity only in the late phase.

The ability of test compounds to prolong the latency to discomfort in the respective formalin and hot plate tests plausibly suggested the compounds potential to inhibit chemically and thermally induced noxious stimuli. Other than that, the ability to inhibit/reverse the former and latter tests could also be associated with the compounds potential to inhibit the inflammation-induced[28] and non-inflammation-related[29] nociception, respectively. Interestingly, according to Hunskaar et al[29], the ability to inhibit both types of stimuli also indicates that the test compounds possesses a characteristic of a strong analgesic with centrally mediated activity[30], which is also supported by the observation that the compounds inhibited both phases of the formalin test. Furthermore, the antinociceptive activity seen in the early phase of the formalin test reflects the compounds potential to produce an antinociceptive, non-anti-inflammatory effect[31] when given systemically.

It is well known that most of the anti-inflammatory analgesic drugs possess antipyretic activity.

The test compounds (**1**, **1a** and **1c**) revealed weak antipyretic effect but **1b** produced marked antipyretic activity in Backer's yeast induced febrile rats its effect is comparable to that of the standard antipyretic drug paracetamol. In general, non-steroidal antiinflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus[31-32]. Various coumarin-related derivatives are recognized as inhibitors of lipoxygenase and cycloxygenase pathways of arachidonate metabolism, though there is no direct evidence of coumarin to interfere with prostaglandin synthesis in hypothalamus.

In general, several mechanisms of action could be used to explain the observed antinociceptive and antipyretic activity of the test compounds. The ability to inhibit/reverse the centrally synthesized prostaglandins or COX[34] could be one of the possible mechanisms that contribute to the central antinociceptive, as well as antipyretic activities of the test compounds seen in the present study. The involvement of the opioid system in the antinociceptive activity could also be suggested based on the claim[33-35.] that centrally acting drugs like Opioids affect both phases of the formalin and hot

plate tests, respectively. To conclude the synthetic new heterocyclic derivatives of 3-formyl-4-ydroxy Coumarins have potent analgesic and antipyretic activity. Additions of different functional groups have varying effects. Significant increase in analgesic and antipyretic effect of compound I was observed after addition of in phenylhydrazine.

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