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Evaluation of antinociceptive potential of new heterocyclic derivatives of 3-formyl-4-hydroxycoumarin in rats

KEYWORDS: 3-Formyl-4-hydroxycoumarin derivatives; Analgesic.

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ABSTRACT Coumarin is a fragrant organic chemical compound in the benzopyrone chemical class. It is a natural substance found in many plants. Coumarin has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks. It 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 analgesic activities of various 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. The nociceptive response were performed in adult male Wistar rats using hot plate and formalin tests. The test compound produced significant antinociceptive activity in the hotplate (central) as well as in formalin tests (central and peripheral) suggesting the involvement of both central and peripheral mechanisms in alleviating the pain response.

Introduction

[1] Benzopyran-2H-ones (Coumarins) reported to possess multiple biological activities (Aries, 1974) 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 (Soine, 1964). In recent times [1]benzopyran-2[H]-ones have been extensively used as laser materials (Drexhage and Reynold, 1974), photosensitizers (Czerney et al., 1981), brightner (Kaidbey and Kligman, 1981), as intermediates for dyes, pesticides and pharmaceuticals (Hagen and Kohler, 1981) as well as in perfume formulations (Pozdnev, 1987; Pozdnev, 1990) and in enzymology as biological probes (Tamura et al., 1982). Coumarins show activities such as antifungal (Sangwan et al., 1990), anticoagulant (Stahman et al., 1941), antibacterial (Honmantgad et al., 1985), analgesic, antipyretic, anti-inflammatory and anti-arthritic (Santaqati et al., 1993; Kontogiorgis and Hadjipavlou-Litina, 2005).

Drugs having analgesic property 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 (Kluger, 1991), 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.

Materials and methods

This research was conducted in the department of pharmacology, JN Medical College in collaboration with the department of chemistry, Aligarh Muslim University, Aligarh. Adult male Wistar Albino rats (weight 100–150g) (Tomazetti et al., 2005) used for the study were obtained from Laboratory Animal Breeding and Research Center, Jamia Hamdard, New Delhi. The animals were given a week time to get acclimatized with laboratory conditions and were housed in polypropylene cages (4 per cage) with sterilized paper cuttings as bedding material 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 hours before the experiment. All measures were taken between 10:00 to 16:00 hours (Yochim JM 1968). The study protocol was approved by the Institutional Animal Ethical Committee.

1.1 Chemicals and test compounds

Following heterocyclic compounds were synthesized in the research laboratory of the Department of Chemistry and studied for their physiochemical and spectral properties (Siddiqui and Asad, 2006). They were tested for analgesic activity in animal models.

1.3-Acetoacetyl pyrano [3,2-c] [1] benzopyran 2,5-dione (fig.1)

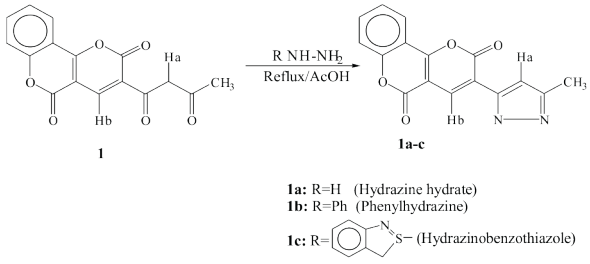

Fig 1: Structure of some novel new heterocyclic derivatives of 3-formyl-4-hydroxycoumarines synthesized and screened

It 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-

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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:
Baker's yeast (Britannia food products), Formalin (Merck, India), Paracetamol (IPCA) and Pentazocine (Ranbaxy)

1.2. Experimental Protocol
The following experimental models were used for test compounds.

***a.* Analgesic activity**
Adult male 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) orally 30 min before experiment.

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

Group III: Pentazocine (15 mg/kg) administered intraperitoneally 15 min prior to experiment.

Experimental design and drug treatment:
(i) *Hot-plate method* (Eddy and Leimbach, 1953; Woolfe and Mac Donald, 1994*)***
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±1C 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. Pentazocine 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 Pentazocine intraperitonealy, 20 µ lit 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 (Hunskaar *et al.*, 1985). The early phase represents neurogenic pain while the latter phase is of inflammatory pain.

b. 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:
Two 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 (Bruce. R-D 1985: Fundam Appl. Toxicol 5: 151-157). 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 LD50 was considered to more than 1000 mg/kg.

c. 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.

Results
***3.1.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 up to cut-off time with the test compounds, whereas reference drug Pentazocine a centrally acting analgesic, markedly increased pain latency at 1 h (2.5 fold) and achieving maximum effect (up to cut-off time) at 2 and 3 h (Table 1).

Table 1: Effects of test drugs and Pentazocine on hot plate reaction time in rat.

		Reaction time in second						
Compounds	**Dose/kg**	**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) *P value of < 0.05 was considered as significant in comparison to control.

3.2.b. Formalin test:
As shown in table-2 the pretreatment with test compounds caused a significant inhibition of the neurogenic (early phase) and inflammatory phases (late phase) of formalin induced licking in mice. The standard drug, Diclofenac sodium (5 mg/kg) also significantly inhibited formalin induced licking in mice but only in late phase (15-30 minute). In contrast, the reference antinociceptive drug Pentazocine (15 mg/kg) significantly reduced the licking activity against both phases of formalin-induced nociception.

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

Total time spent in Paw licking time (s)					
Compounds	Dose /kg	Phase (0-5 Min)	% Inhibition	Phase (15 -60 Min)	% 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

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The results are mean ± SEM from 6 animals *P<0.001 **P<0.0001, when compared to vehicle control (DMSO)

3.2. 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.

Discussion & Conclusion

Various coumarin-related derivatives are recognized as inhibitors of lipoxygenase and cyclooxygenase pathways of arachidonate metabolism (Neichi, et al., 1983) but also of neutrophile-dependent super oxide anion generation (Ozaki, et al., 1986). 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 (Paya, et al., 1992) 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 agent when tested on various animal models. Thermic painful stimuli (hot-plate test) are selectively centrally acting in nature. In the present study, the test compound produced a significant inhibitory effect on the nociceptive response at 2, 3 and 4 hours though less potent than that of the Pentazocine, a centrally acting analgesic drug, which significantly increased the reaction time in hot-plate test at 1, 2, 3, and 4 hours.

The formalin test is another pain model, which assesses the way an animal responds to moderate continuous pain generated by injured tissue (Tjolsen *et al*., 1992). 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. (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987). In the present study the test compounds significantly inhibited both the neurogenic pain (early phase) and inflammatory phase (later phase) except Ia that have no significant role in inhibiting neurogenigc pain. Pentazocine 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 possibly suggested the extract's potential to inhibit chemically and thermally induced noxious stimuli. Other than that, the ability to inhibit or reverse the former and latter tests could also be associated with the extract's potential to inhibit the inflammation-induced (Ballou LR et.al; 2000) and non-inflammation-related (Pini LA et al; 1997) nociception, respectively. Interestingly, according to Hunskaar et al (1986), the ability to inhibit both types of stimuli also indicates that the extract possesses a characteristic of a strong analgesic with centrally mediated activity (Chan TF et al; 1995), which is also supported by the observation that the extract inhibited both phases of the formalin test. Furthermore, the antinociceptive activity seen in the early phase of the formalin test reflects the extract's potential to produce an antinociceptive, non-anti-inflammatory effect (Hunskaar S et al; 1985) when given systemically.

In general, several mechanisms of action could be used to explain the observed antinociceptive activity of the test compounds. The ability to inhibit/reverse the centrally synthesized prostaglandins or cyclooxygenase (Uzcátegui B et al; 2004) could be one of the possible mechanisms that contribute to the central antinociceptive activity 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 by Chan et al. (1995) and Hosseinzadeh and Younesi (Hosseinzadeh H et al; 1985) 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 activity. Additions of different functional groups have varying effects. Significant increase in the analgesic effect of compound I was observed after addition of phenylhydrazine group.

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