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Original Article

ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC EVALUATIONS OF NEW ISOQUINOLINE DERIVATIVES

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ABSTRACT

Objective: To evaluate the isoquinoline (N-substituted (E)-4-arylidene-isoquinoline-1,3-dione) derivatives (5a-h) for their anti-inflammatory, analgesic and antipyretic activity potentials in animal models using indomethacin and diclofenac sodium as the standard.

Methods: Peripheral and central analgesic activities were determined by induced writhing and tail immersion tests. Carrageenan stimulated rat paw edema model was used to evaluate the anti-inflammatory activities by examining the increase in paw volume and percentage inhibition of paw volume was calculated with plethysmometer at different time periods. Brewer's yeast induced pyresis model was used to assessing the anti-pyretic activity by measuring the decreased rectal temperature.

Results: Compounds 5g>5d>5h showed anti-inflammatory, analgesic and antipyretic activities and they were significant with $p<0.001$ and comparable with the control group. The results coincided with our previous report which suggests that the compounds 5g>5d>5h may take into further druggability evaluations.

Conclusion: New isoquinoline derivatives produced significant anti-inflammatory, analgesic, and antipyretic activities and this suggests that these derivatives need further drug development evaluations especially for the compounds 5g>5d>5h.

Keywords: N-substituted (E)-4-arylidene-isoquinoline-1,3-dione, Carrageenan, Anti-inflammatory, Analgesic, Diclofenac sodium, Antipyretic, Indomethacin

INTRODUCTION

Inflammation is a common and serious predicament in cases of hypersensitivity, autoimmune diseases, and negative response of transplanted organs [1]. Healing alternatives for inflammatory diseases generate unfavourable effects and being evidence being deficient in effectiveness. Non-steroidal anti-inflammatory drugs (NSAIDs) are precious in the healing of severe and persistent inflammation [2], fever [3], and pain [4] but long-term use of NSAIDs is related through the gastrointestinal wound, hemorrhage, and nephrotoxicity [5]. A number of isoquinoline alkaloids were reported for anti-inflammatory activity [6, 7]. We already have evaluated the *in vitro* anti-inflammatory [8], anticancer [9] properties of the proposed compounds. In this report, the *in vivo* anti-inflammatory, analgesic, and antipyretic potentials were evaluated and reported as mentioned in our previous report [8].

MATERIALS AND METHODS

Chemicals

λ-Carrageenan was procured from Sigma (St Louis, MO), Diclofenac Sodium was a legacy sample from Blessing Pharmaceuticals India (Maharashtra, India), Indomethacin and Brewer's yeast from Sigma-Aldrich Chemicals, USA and all other chemicals & reagents were used analytical grade. The test compounds (E)-4-arylidene isoquinoline-1,3-diones derivatives 5(a–h) were synthesized and reported previously [8] was taken for animal studies.

Chemical compounds for animal studies

A mixture of homophthalic acid (1) and substituted anilines (2a-c) (1:1 ratio) in toluene and 5 mol % ZnO were amended to the suspension and refluxed. The obtained pale yellow solid compounds N-substituted homopthalimides (3a-c) were purified and characterized by FT-IR, GC-MS, ^{1}H NMR and [13]C NMR. Condensation of aromatic aldehydes (4a-c) with 3a-c in ethanol using oxalic acid as a catalyst to obtain the (E)-4-arylidene isoquinoline-1,3-diones derivatives 5(a–h).

Animals

Wistar albino rats of either sex, 50-100g, were procured from the animal house, Annamalai University, India. They were in a controlled room with a 12 h dark-light cycle and fed with commercial pellet feed from Hindustan Lever Ltd. (Mumbai, India); water was freely available. The animal model study was approved (Vide No.1038, 2013) by the Institutional Animal ethics committee of Annamalai University, India and was conducted in accordance with the "Guidelines for care and Use of Laboratory Animals", Government of India.

Test of peripheral analgesic activity by acetic acid-induced writhing response

The acetic acid-induced writhing test was carried out in accordance with Collier *et al.* [10]. The rats were intraperitoneally injected with 0.6 % acetic acid (10 ml/kg b. wt) to elicit a writhing response. Immediately after the administration of acetic acid, the animals were placed in glass cages, and the number of writhes was recorded for the following 30 min. A considerable reduction in the number of writhes by drug treatment as compared to control animals was considered as a positive analgesic response. Test compounds (50 and 100 mg/kg) and diclofenac sodium (50 mg/kg b. wt) were administered intraperitoneally 30 min before the acetic acid injection. Mean writhing and average protection values were calculated.

Test of central analgesic activity of *p*-CA (*p*-Coumaric acid) by tail-immersion test

The tail-immersion test was carried out as described by Janssen *et al.* 1963, was immersed in a water bath thermostatically maintained at 55±1 °C [11]. The time in seconds for the tail withdrawal from the water was taken as the tail withdrawal latency or reaction time, with a cut-off time of immersion set at 10 seconds. The reaction time was measured before drug treatment at 15, 30, 45 and 60 min after the drugs were administered. Test compounds (50 and 100 mg/kg) and diclofenac sodium (50 mg/kg b. wt) were administered at a lower 5-cm portion of the tail. Writhing values and average protection percentage values were calculated and tabulated.

Acute toxicity studies

According to OECD guidelines, the acute toxicity was carried out [12]. The wistar rats (100±10 gm) either sex were used for this study. The animals were divided into four groups in which each group with three animals (n=3). The animals fasted overnight and test compounds were given orally to rats at a dose of 15, 30 and 45 mg/kg body weight. There was continued observation for the behavioral changes for first three hours and for mortality at the end of 24 h. The animals were examined daily up to 8-10th day for the behavioral change or mortality.

***In vivo* anti-inflammatory activity–carrageenan-induced rat paw edema method**

Wistar rats (100±10 gm) of either sex were used. Animals were weighed and randomized in 5 groups (n=6). Before treatment, the volume of the right paw of each animal was determined using a plethysmometer (UGO Basile, 7140). All the animals were starved for 12 h. To ensure, uniform hydration, the rats received 5 ml of water by stomach tube. Group I served as control (Vc) and do not receive any drug. Group II received the standard Indomethacin (10 mg/kg, p. o), and Group III to VI was received test compounds (5a-h) in different doses (50 and 100 mg/kg). 30 min later, the rats were challenged by a subcutaneous injection of 0.1 ml of 1% w/v freshly prepared a solution of λ-carrageenan in saline into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in the water reservoir of digital Plethysmometer up to that mark to measure the paw volume. The paw volume (Vt) was measured at 2, 4, 6, 12 and 24 h immediately after carrageenan injection in control, compound 5a-h treated and indomethacin-treated groups [13]. The % of inhibition of each group was determined using the following formulae:

$$\% \text{ Inhibition} = (Vc - Vt / Vc) X\ 100$$

Where, Vc stands for mean variation of edema for the control group; Vt equals mean variation of edema.

Antipyretic activity

Wistar strain rats of either sex weighing 100±10 gm were taken, and animals were fasted overnight and divided into different groups (n=6). Fever was induced by injecting 20 ml/kg (*s. c*) of 20% suspension of Brewer's yeast in normal saline below the nape of the neck, and the initial rectal temperature was recorded.

After 18 h, animals that showed an increase of 0.3-0.5 °C in rectal temperature were selected for the experiment. The test compounds (50 and 100 mg/kg), standard indomethacin (10 mg/kg) and control 0.1% sodium CMC was administered orally. The rectal temperature was measured every 30 min up to 120 min after compounds administration [14].

Statistical analysis

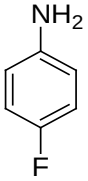
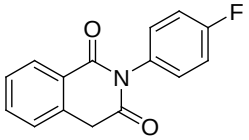
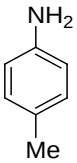
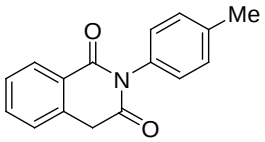
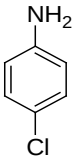
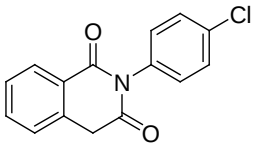
All biological *in vitro* and *in silico* experiments results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA with Dunnett's post test was performed. GraphPad Prism version 6.07 for Windows, GraphPad Software, San Diego California USA, www. graphpad. Com was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$.

RESULTS

Synthesis of (E)-4-arylidene isoquinoline-1,3-diones derivatives

All synthesized derivatives appeared as yellow solid and the chemical yield of the new compounds N-aryl homo phthalimide derivatives **(**3a-c) and (E)-4-arylidene isoquinoline-1,3-dione derivatives **(**5a-h) was calculated. The spectral confirmations of compounds 5a-h obtained were reported along with the *in vitro* evaluations in our previous report [8]. The purified compounds were taken for animal studies which were prepared in various doses 50–200 mg/kg. The product yield and time is taken for completions of reaction along with starting ingredients were depicted in table 1 and 2.

Table 1: Synthesis of N-aryl homo phthalimide derivatives

S. No.	Aniline (2a-c)	Product (3a-c)	Time (h)	Yield (%)
1			36 h	82%
2			36 h	78%
3			36 h	79%

Result of peripheral analgesic activity by acetic acid-induced writhing response

In the acetic acid-induced writhing test, the standard diclofenac sodium showed significant peripheral analgesic activity in a dose-dependent manner, and compound 5g and 5h showed equal activities to diclofenac sodium. Intraperitoneal injection of acetic acid in rats significantly increased the writhing. Among all the rest compounds, 5d showed a moderate activity when to compare with the diclofenac sodium, 5g and 5h (table 3).

Result of central analgesic activity of *p*-CA (*p*-Coumaric acid) by tail-immersion test

Table 4 below depicts the central analgesic activity of diclofenac and the test compounds as measured by the tail-immersion test administered with *p*-CA (*p*-Coumaric acid). Diclofenac (50/100 mg/kg b. wt) exhibited significant analgesic activity in a dose-dependent manner by delaying the tail withdrawal latency or reaction time, compared to the control group. Diclofenac treatment was found to have a better effect compared to all

tested compounds. Among all the compounds, 5d and 5g showed almost equal activity when to compare with the diclofenac sodium and 5h showed a moderate. The least activity was showed by 5b (table 4).

Table 2: Synthesis of (E)-4-arylidene isoquinoline-1, 3-dione derivatives

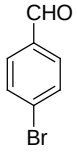
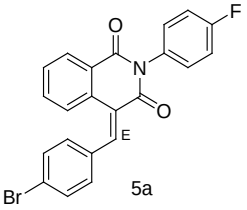
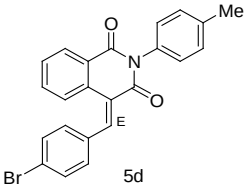
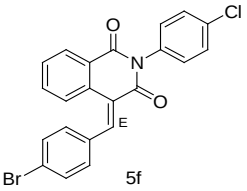
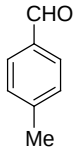
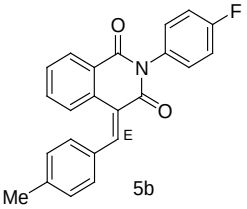
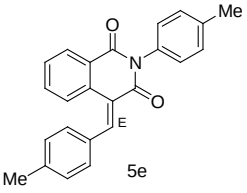
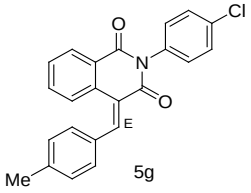
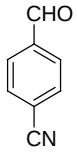
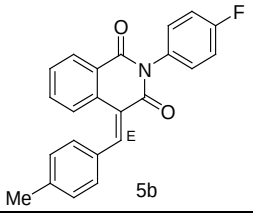
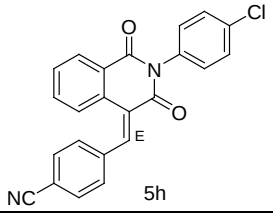
S. No.	Aldehyde (4a-c)	Product (5a-h)	Time (h)	Yield (%)
1		 5a  5d  5f	4-5 h	92, 89 90
2		 5b  5e  5g	4-5 h	90 91, 90
3		 5b  5h	4-5 h	88, 86

Table 3: Effect of diclofenac and compounds 5a-h on writhing response test in rats

Test samples	Dose	Mean writhing (X±SE)	Average protection (%)
Control	-	28.0±1.55	–
Diclofenac sodium	50 mg/kg	3.00±2.44**	**76.00**±6.54
5a	50 mg/kg	6.20±1.55**	61.33±5.54
	100 mg/kg	5.00±1.44**	
5b	50 mg/kg	5.40±1.32**	67.12±6.84
	100 mg/kg	4.70±1.24**	
5c	50 mg/kg	5.60±2.41**	62.33±7.42
	100 mg/kg	4.80±1.04**	
5d	50 mg/kg	4.00±1.12**	74.88±6.64
	100 mg/kg	3.20±1.74**	
5e	50 mg/kg	6.40±1.46**	68.54±7.22
	100 mg/kg	5.20±1.24**	
5f	50 mg/kg	6.20±2.44**	70.85±6.48
	100 mg/kg	4.20±1.12**	
5g	50 mg/kg	4.60±1.26**	75.92±6.54
	100 mg/kg	3.00±1.66**	
5h	50 mg/kg	4.80±1.84**	74.02±6.22
	100 mg/kg	3.10±1.54**	

Data represent mean values±SE n=6, compared with the control group. **statistical significance when $p<0.05$

Table 4: Effect of diclofenac and compounds 5a-h on tail-immersion test

Test samples	Dose	Mean latency time (X±SE)	Average protection (%)
Control	-	28.0±1.55	–
Diclofenac sodium	50 mg/kg	3.40±2.44**	**78.00**±4.98
5a	50 mg/kg	5.20±1.22**	61.33±4.62
	100 mg/kg	4.60±1.55**	
5b	50 mg/kg	6.20±1.22**	57.12±4.54
	100 mg/kg	5.00±1.22**	
5c	50 mg/kg	5.40±1.22**	62.33±5.44
	100 mg/kg	4.80±2.41**	
5d	50 mg/kg	4.40±1.22**	77.08±6.62
	100 mg/kg	3.20±1.12**	
5e	50 mg/kg	6.40±1.22**	58.54±4.48
	100 mg/kg	5.40±1.46**	
5f	50 mg/kg	6.20±2.44**	60.85±4.98
	100 mg/kg	5.20±1.22**	
5g	50 mg/kg	4.00±1.22**	77.92±5.64
	100 mg/kg	3.60±1.26**	
5h	50 mg/kg	4.20±1.22**	76.02±5.88
	100 mg/kg	3.80±1.84**	

Data represent mean values±SE n=6, compared with the control group. ** Statistical significance when $p<0.05$

Acute toxicity study results

Results of acute toxicity study showed that there was no mortality or any significant change in the behavior of the mice recorded up to the dose of 200 mg/kg of the tested compounds (5a-h). Based on the results of the preliminary toxicity testing, the doses of the compounds for further studies were decided to be 10, 50 and 100 mg/kg body weight of the rats.

Carrageenan-induced paw edema

Anti-inflammatory effect of Isoquinoline (N-substituted (E)-4-arylidene-isoquinoline-1,3-dione) derivatives (5a-h) were shown in table 2. From the observations, compounds 5d, 5g, and 5h have significant (P<0.05) anti-inflammatory activity when compared with the control. The average percentage inhibition in paw edema after 2 h was recorded as 78±4.24 for indomethacin and 70±3.88, 76±2.88, and 74±4.24 for 5d, 5g and 5h respectively. The least activity was found for 5a, 5b, and 5f with 38±4.64, 32±4.24 and 34±3.54 respectively. A moderate activity found for 5e with 64±5.44 which shows the close enough potency to compete with the top ranked compounds here (table 5).

Brewer's yeast induced pyrexia

The tested rats exhibited a mean raise of about 1 °C in rectal temperature 1 h after Backer's yeast injection (135 mg/kg, i. p). The test compounds (5d and 5h) produced significantly ($p<0.05$) antipyretic activity at 2, 3, 4 and 5 h, whereas test compound 5g and the reference drug Paracetamol (150 mg/kg) showed significant antipyretic activity throughout the observation period up to 5 h (fig. 1).

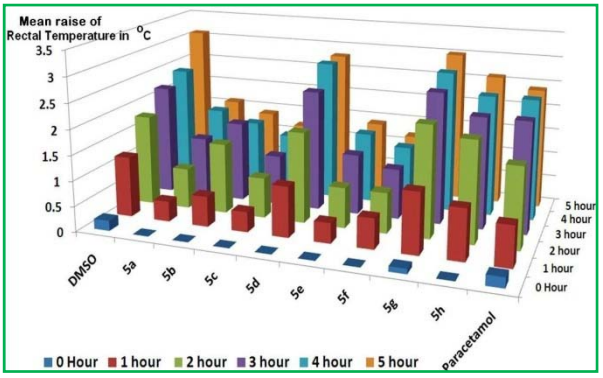


Fig. 1: Antipyretic activity results for compounds 5a-h, paracetamol as ref. drug

Table 5: Effect of Indomethacin (standard) and compounds 5a-h on carrageenan-induced rat paw edema

Group	Dose	Edema volume in ml				
		½ hr	1 hr	1½ hr	2 hr	mean±SD % reduction
Control	5 ml/kg	0.38±0.01	0.37±0.02	0.40±0.01	0.38±0.01	-
Standard	10 mg/kg	0.22±0.02	0.16±0.01*	0.15±0.01*	0.13±0.01*	78±4.24*
5a	50 mg/kg	0.54±0.01	0.48±0.01*	0.40±0.01*	0.38±0.01*	38±4.64**
	100 mg/kg	0.47±0.02	0.39±0.01*	0.36±0.01*	0.35±0.01*	
5b	50 mg/kg	0.48±0.01	0.45±0.01*	0.43±0.01*	0.46±0.01*	34±3.54**
	100 mg/kg	0.47±0.02	0.41±0.01*	0.40±0.01*	0.44±0.01*	
5c	50 mg/kg	0.38±0.01	0.35±0.01*	0.33±0.01*	0.44±0.01*	42±3.86**
	100 mg/kg	0.37±0.02	0.31±0.01*	0.27±0.01*	0.42±0.01*	
5d	50 mg/kg	0.28±0.01	0.22±0.01*	0.20±0.01*	0.17±0.01*	70±3.88*
	100 mg/kg	0.27±0.02	0.18±0.01*	0.17±0.01*	0.16±0.01*	
5e	50 mg/kg	0.38±0.01	0.25±0.01*	0.23±0.01*	0.17±0.01*	64±5.44*
	100 mg/kg	0.37±0.02	0.21±0.01*	0.17±0.01*	0.16±0.01*	
5f	50 mg/kg	0.58±0.01	0.50±0.01*	0.48±0.01*	0.42±0.01*	32±4.24**
	100 mg/kg	0.50±0.02	0.48±0.01*	0.42±0.01*	0.40±0.01*	
5g	50 mg/kg	0.22±0.01	0.20±0.01*	0.18±0.01*	0.14±0.01*	76±2.88*
	100 mg/kg	0.20±0.02	0.17±0.01*	0.14±0.01*	0.12±0.01*	
5h	50 mg/kg	0.26±0.01	0.22±0.01*	0.18±0.01*	0.16±0.01*	74±4.24*
	100 mg/kg	0.24±0.02	0.20±0.01*	.17±0.01*	.14±0.01*	

mean±SEM n=6, (one-way ANOVA), statistically significant from control *p<0.001, **not significant

DISCUSSION

Isoquinoline (N-substituted (E)-4-arylidene-isoquinoline-1, 3-dione) derivatives (5a-h) were evaluated for anti-inflammatory, analgesic and antipyretic activity by using standard experimental models. In the present investigation carrageenan-induced paw edema acute inflammatory model, isoquinoline derivatives inhibited inflammation efficiently by cyclooxygenase inhibition. COX (Cyclooxygenase) was alleged to be expressed constitutively with constant levels in individual tissues [15]. Prostaglandin synthesis was thought to increase in inflammation because of increased release of precursor [16]. COX activity increases in inflammation, and this increase can be prohibited by corticosteroids [17]. This is caused by the compounds may show anti-corticosteroid activity. Both the COX inhibition results and *in vitro* anti-inflammatory results coincided together for the compounds 5d, 5g and 5h. Acute inflammation is supposed to be biphasic, the first phase (1hr) involves the release of serotonin and histamine while the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase products and continuity between two phases is proved by kinins [18-20]. Among all the derivatives 5d, 5g and 5h at 100 mg/kg dose showed significant paw edema inhibition and this activity of isoquinoline derivatives. In the assessment of analgesic activity, the mechanical stimulations can suggest the pain by raising the synthesis of pain mediators (PG's, histamine, kinins, etc. [21] through pain receptors and the reaction time is noted as a therapeutic end point. This suggests that the compounds have proved inhibitory actions on cyclooxygenase reconciled pathway. In Brewer's yeast induced pyrexia model, evaluation in the body temperature is due to the fever mediators such as IL-Ib, IL-6, IFN-α and prostaglandins (PGE2), especially in the brain regions. The isoquinoline derivatives showed bradykinin and peptide synthesis inhibition [22]. As compared with other derivatives 5d, 5g, and 5h were shown a reduction of body temperature at 100 mg/kg when compared with control.

CONCLUSION

In conclusion, the new isoquinoline derivatives produced significant anti-inflammatory, analgesic, and antipyretic activities and this suggests that these derivatives have keen inhibitory actions on cyclooxygenase enzymes also the work may be extended to further drug development evaluations especially for the compounds 5g>5d>5h.

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CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. Huerre MR, Gounon P. Inflammation: patterns and new concepts. Res Immunol 1996;147:417–34.
2. Sng BL, Schug SA. The role of opioids in managing chronic non-cancer pain. Ann Acad Med Singap 2009;38:960–6.
3. Eccles R. Efficacy and safety of over-the-counter analgesics in the treatment of common cold and flu. J Clin Pharm Ther 2006;31:309–19.
4. Zahradnik HP, Hanjalic-Beck A, Groth K. Non-steroidal anti-inflammatory drugs and hormonal contraceptives for pain relief from dysmenorrhea: a review. Contraception 2010;81:185–96.
5. Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA 2001;286:954–9.
6. Wong CW, Seow WK, O'callaghan JW, Thong YH. Interrelation of chemical structure and pharmacological activity. Farmatsiya 1992;25:33-8.
7. Yasukawa K, Takido M, Ikekawa T, Shimada F, Takeuchi M, Nakagawa S. The relative inhibitory activity of berberine-type alkaloids against 12-o-tetradecanoylphorbol-13-acetate-induced inflammation in mice. Chem Pharm Bull 1991; 39:1462-5.
8. Manikandan A, Sathiyanarayanan KI, Sivakumar A**.** Molecular docking, design, synthesis, *in vitro* antioxidant and anti-inflammatory evaluations of new isoquinoline derivatives. Int J Pharm Pharm Sci 2015;7 Suppl 12:200-8.
9. Manikandan A, Sivakumar A. N-substituted (E)-4-arylidene isoquinoline-1,3-dione derivatives as potent anticancer agents. Der Pharm Chem 2015;7 Suppl 12;210-18.
10. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother 1968;32:295–310.
11. Janssen PA, Niemegeers CJ, Dony JG. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 1963;13:502–7.
12. OECD Publishing., Acute oral toxicity-Acute toxic class method. In: OECD Guideline for the testing of chemicals, Section 4: Health effects; 2001;423:1-14.
13. Jimenez-Estrada M, Chilpa RR, Apan TR, Lledias F, Hansberg W, Arrieta D. Anti-inflammatory activity of cacalol and cacalone sesquiterpenes isolated from *Psacalium decompositum*. J Ethnopharmacol 2006;105:34-8.
14. Teotino U, Friz LP, Gandini A, Bella DD. Thio derivatives of 2, 3-dihydro-4H-1, 3-benzoxazin-4-one. Synthesis and pharmacological properties. J Med Chem 1963;6 Suppl 3:248-50.
15. Tricarico C, Pinzani P, Bianchi S, Paglieriani M, Distante V, Pazzagli M, *et al.* Quantitative real-time reverse transcription polymerase chain reaction: normalization to rRNA or single housekeeping genes is inappropriate for human tissue biopsies. Anal Biochem 2002;309:293-300.
16. Hawkey C. COX-2 inhibitors. Lancet 1999;353:307-14.
17. Perianayagam JB, Sharma S, Pillai K. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethnopharmacol 2006;104 Suppl 3:410-14.
18. Asongalem E, Foyet H, Ekobo S, Dimo T, Kamtchouing P. Antiinflammatory, lack of analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. J Ethnopharmacol 2004;95 Suppl 1:63-8.
19. Silva GN, Martins FR, Matheus ME, Leitão SG, Fernandes PD. Investigation of anti-inflammatory and antinociceptive activities of Lantana trifolia. J Ethnopharmacol 2005;100 Suppl 3:254-9.
20. Vane JR, Mitchell JA, Appleton I, Tomlinson A, Baiely DB, Croxtall J, *et al.* Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc Natl Acad Sci U S A 1994;9:2046-50.
21. Roberts JL, Morrow JD. Analgesic–antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Gilman AG, Hardman JG, Limbird LE. Eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw Hill Co; 2001. p. 687–731.
22. Supriya M, Nilanjan P, Neeraj K. Synthesis and characterization of novel thiazole-isoxazole fused isatin as an analgesic and anti-inflammatory agent. Pharm Res 2010;3:51-9.