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Novel drug designing approach for dual inhibitors as anti-inflammatory agents: implication of pyridine template

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Abstract

Compounds incorporating thiophene moiety, a pi excess five membered heterocycle, have attracted a great deal of research interest, owing to the therapeutic utility of the template as useful drug molecular scaffolding. We report the synthesis and pharmacological evaluation of thiophenes substituted with 4-methanesulfonyl benzoyl moiety at the fifth position of the ring, as possible anti-inflammatory lead candidates. The aryl sulfonyl methyl thiophene analogs AP29, AP82, and AP37, when screened for anti-inflammatory activity in carrageenin induced rat paw edema, an acute in vivo model, exhibited moderate to good activity at a dose level of 100mg/kg body weight P.o compared to Ibuprofen. In a five day formalin induced rat paw edema, a chronic in vivo anti-inflammatory model, candidates AP29, AP82, and AP37 inhibited the disease progression by 53%, 34%, and 65%, respectively on the fifth day, at a dose level of 100 mg/kg body weight P.o compared to Rofecoxib, Ibuprofen, and Dexamethasone at therapeutic doses which gave a protection of 53.8%, 81.5%, and 81.5%, respectively. The replacement of the 4-methanesulfonyl benzoyl moiety in AP82 with the pyridine template, 3,5-dimethyl-4-methoxy-2-pyridyl function, gave rise to AP84, which was less active in the acute model, but gave 54% and 75% protection both during the first day and fifth day, respectively, in the chronic model. A dual mechanism of action is proposed for AP84, a non-steroidal drug which has exhibited remarkable activity when compared to the steroid dexamethasone. These results open up new avenues in designing novel anti-inflammatory drugs as dual inhibitors with the incorporation of a pyridine template as part of the pharmacophore.

Keywords: Thiophene; Chronic model; Carrageenin

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain, fever, and inflammatory conditions including Osteoarthritis. Most of the classical NSAIDs exhibit their action by restricting the synthesis of prostaglandins, some of which are pro-inflammatory. This is essentially brought about by inhibiting the rate limiting cyclooxygenase (COX) enzyme involved in the inflammatory cascade [1]. The acid group present in the classical NSAIDs binds to arginine, the 120th amino acid residue in the cyclooxygenase, and causes the inactivation of the enzyme [2]. Thus NSAIDs act as preferential COX-1 inhibitors. Now it is well established that there are two isoforms of COX, namely COX-1 (constitutive) and COX-2 (inducible) [3]. Inhibition of the gastric constitutively expressed COX-1 is postulated to cause most of the side effects, especially intestinal disturbances in patients [2].

The hypothesis that selective inhibition of COX-2 will lead to potent anti-inflammatory drugs with higher gastrointestinal safety profile led to intense research efforts which culminated in the introduction of selective COX-2 inhibitors in the market [3–5]. A 1,2 disubstitution by two aryl groups on a central core, usually a five

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or six membered heterocycle, without a carboxylic acid group seemed to be a common structural feature of the majority of COX-2 inhibitors. In all these drug candidates the presence of methyl sulfonyl or sulfonyl amino substitution and the absence of the carboxylic acid group are presumed to be important in enhancing the biological activity and selectivity towards COX-2.

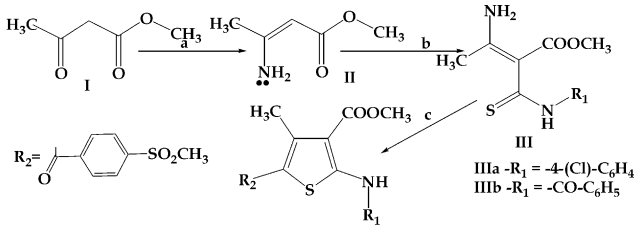
Among the five membered heterocycles, there are no reports on the study of thiophene analogs which had been designed and pharmacologically evaluated with different molecular scaffoldings which are involved in the inhibition of different rate limiting enzymes at different stages in the inflammatory cascade.

Our interest was to design some anti-inflammatory agents with (1) the incorporation of both COX-1 inhibitory (ester/acid as in mefenamic acid) and COX-2 inhibitory features (sulfonyl moiety) into a single experimental candidate as in AP29 and AP37, (2) with only the COX-2 inhibitory pharmacophore, (AP82), and (3) without the COX-1 or COX-2 inhibitory feature but, incorporating a pyridine template present in omeprazole [6], a proton pump inhibitor (AP84), which is implicated in the treatment of gastric disturbances related to NSAID therapy [6]. All the candidates were screened for their anti-inflammatory activity in both acute and chronic animal models.

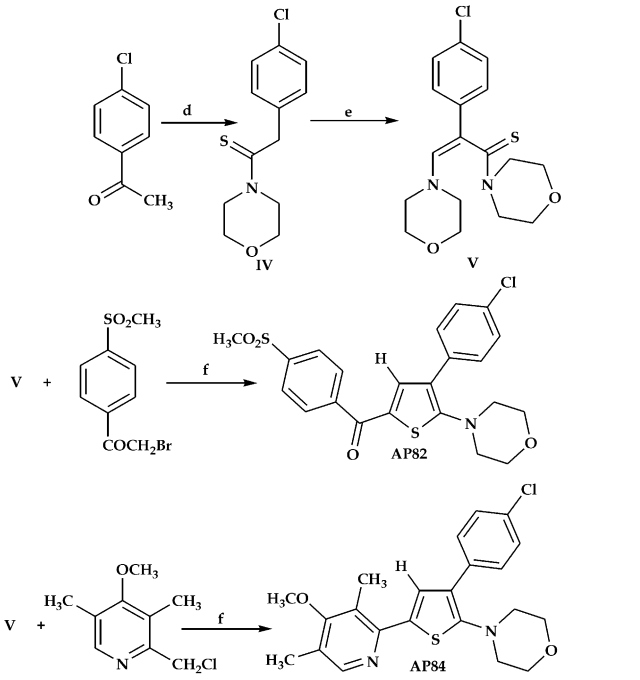
Materials and methods

Isothiocyanates were synthesized using the modified Kaluza method [7]. α-Haloketones were synthesized using reported procedure [8]. 2-Chloromethyl-3,5-dimethyl-4-methoxy-pyridine was a gift sample from Dishman Pharmaceuticals. Formalin and carrageenin were purchased from Qualigen, Mumbai. Rofecoxib was procured from Cadila Pharmaceuticals. Ibuprofen and Dexamethasone were gift samples from AVIK Pharmaceuticals, Mumbai.

Synthesis of tri and tetra substituted thiophenes. The synthetic sequence (Schemes 1 and 2), which we utilized [9] for preparing thiophene analogs, was analogs to the method reported in the literature [10–12]. The reaction was brought about by reacting equimolar amount of the enamine adduct (IIIa or IIIb) or thioacrylic acid morpholide intermediate (V) with α-halo carbonyl compound or halomethylene compound to yield tetra substituted or tri substituted thiophenes, respectively (Fig. 1).



Scheme 1. Synthesis of tetra substituted thiophenes. The adduct IIIa and IIIb was reacted with 4-(methane sulfonyl)-1-bromo acetophenone to yield the desired products.



Scheme 2. Synthesis of tetra substituted thiophenes. The adduct V was reacted with 4-(methane sulfonyl)-1-bromo acetophenone and 2-chloromethyl-3,5-dimethyl-4-methoxy-pyridine to yield the desired products AP82 and AP84, respectively.

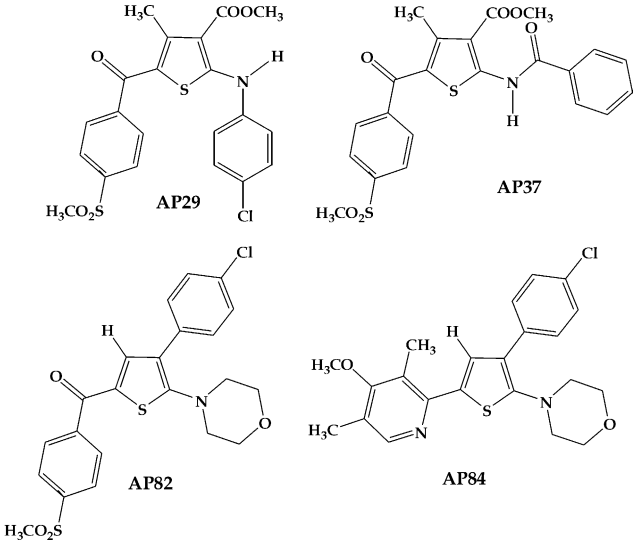


Fig. 1. Tetra substituted thiophenes synthesized and screened for anti-inflammatory activity: Compounds AP29 and AP37 had a methyl sulfonyl benzoyl substitution at the fifth position of the thiophene ring and an ester at the third position. Compound AP82 was devoid of the ester function. Compound AP84 did not have an ester or methyl sulfonyl benzoyl function. It had 3,5-dimethyl-4-methoxy-2-pyridyl-template at the fifth position.

Anti-inflammatory activity

Carrageenin induced 3 h rat paw edema model was chosen as an acute anti-inflammatory model [13]. Formalin induced five day rat paw edema model was chosen for chronic studies [14,15].

Carrageenin induced rat paw edema. Sprague–Dawley (Male/Female) rats weighing 150–250 g were used for the edema test. Animals were divided into 8 groups comprising six rats per group. Rats were put on fast for 18 h prior to the experiment. The standard drugs, Dexamethasone (5 mg/kg body weight), Rofecoxib (50 mg/kg body weight), and Ibuprofen (100 mg/kg body weight) and the test drugs AP29, AP37, AP82, and AP84 (100 mg/kg body weight) were given orally as a suspension, in 0.1% Sodium CMC as vehicle. One hour later, 0.1 ml of 1% carrageenin solution in saline was injected into the sub-plantar region of the right-hind paw of each rat. After 3 h of the carrageenin injection, the reduction in the paw volume compared to vehicle control was measured using plethysmometer. The experimental protocol was approved by the institutional *ethics committee, constituted by the Ministry of Social Justice and Empowerment, Government of India.*

Formalin induced rat paw edema. Animals were divided into 8 groups comprising six rats per group. The initial paw volumes were measured using plethysmometer and noted as zero hour reading (Z1). An accurately measured volume of 0.1 ml of 2% formalin was injected into the sub-plantar region of the right-hind paw of ether anesthetized animals. After 2 h of the insult paw volumes were measured. After 5 min the standard drugs, Dexamethasone (5 mg/kg body weight), Rofecoxib (50 mg/kg body weight), and Ibuprofen (100 mg/kg body weight) and the test drugs AP29, AP37, AP82, and AP84 (100 mg/kg body weight), were given orally as a suspension, in 0.1% Na CMC as vehicle. Final hour reading (Z2) was recorded 2.5 h after dosing. This exercise was continued for five consecutive days. Degree of inflammation was measured on day 1 and day 5.

Results and discussion

All the selected compounds (Fig. 1) had different structural features. Compounds AP29 and AP37 had an ester at the third position and a methyl sulfonyl benzoyl substitution at the fifth position of the thiophene ring. Compound AP82 was devoid of the ester function. Compound AP84 had 3,5-dimethyl-4-methoxy-2-pyridyl-template at the fifth position.

All the compounds were tested in both acute and chronic models for assessing their anti-inflammatory activity. In the acute model AP29 gave similar protection (60%) as that of Ibuprofen (Table 1). The selective COX-2 inhibitor Rofecoxib gave 40% protection, suggesting the induction of COX-2 within 3 h of the chemical insult. There are reports which show a substantial induction of COX-2 coinciding with enhanced TxB2 levels and local edema within 3 h of the carrageenin insult in carrageenin induced rat paw edema [16]. The very low activity exhibited by AP82, AP84, and AP37 in the acute model indicates that they are not acutely anti-inflammatory to a significant extent or a higher dose is required to elicit anti-inflammatory effect. The steroid dexamethasone gave 96.5% protection in the acute model.

The percent protection given by the experimental candidates and control drugs in chronic model on the first and the fifth days is shown in Table 2. The steroidal drug dexamethasone (PLA_2 inhibitor) showed a marginal activity (20.8%) on the first day and was giving (81.5%) protection on the fifth day. There are reports that both arthritis and induction of COX-2 are suppressed by glucocorticoids in adjuvant arthritic model, a more chronic arthritic model, where high levels of COX-2 protein develop rapidly [17,18]. The better activity exhibited by the steroid on the fifth day might be due to a synergetic effect of its PLA_2 inhibiting profile and suppression of induction of high levels of COX-2 developed, owing to the continuous chemical insult produced by formalin.

Rofecoxib which is a selective COX-2 inhibitor was inactive on the first day. One plausible reason for this could be the insufficient amount of COX-2 enzyme expressed with formalin at the time of measurement of paw volume. On the fifth day Rofecoxib gave 53.8% protection, explaining the fact that a continuous insult

Table 1
Acute anti-inflammatory activity data of carrageenin induced rat paw edema model

Group	Drug	Paw volume (% protection)
I	Control	2.25 ± 0.08
II	Dexamethasone	0.11 ± 0.006 (96.5)
III	Rofecoxib	1.35 ± 0.12 (40)
IV	Ibuprofen	0.89 ± 0.13 (60)
V	AP29	0.89 ± 0.19 (60)
VI	AP82	1.64 ± 0.11 (27)
VII	AP84	1.85 ± 0.14 (17)
VIII	AP37	1.64 ± 0.14 (27)

Table 2
The biological activity of the test drugs compared with the standard drugs, in formalin induced rat paw edema model

Group	Drug	Day 1 paw volume(A)* (Z2–Z1)	Day 5 paw volume(B)* (Z2–Z1)
I	Control	0.6 ± 0.15	0.65 ± 0.17
II	Dexamethasone	0.475 ± 0.04 (20.8)	0.12 ± 0.01 (81.5)
III	Rofecoxib	1.175 ± 0.12 (0)	0.32 ± 0.03 (53.8)
IV	Ibuprofen	1.1 ± 0.1 (0)	0.12 ± 0.01 (81.5)
V	AP29	1.05 ± 0.3 (0)	0.3 ± 0.04 (53)
VI	AP82	0.9 ± 0.18 (0)	0.4 ± 0.02 (34)
VII	AP84	0.275 ± 0.02 (54)	0.15 ± 0.01 (76.9)
VIII	AP37	0.82 ± 0.05 (0)	0.225 ± 0.01 (65.3)

A* and B* represent the% protection given by the standard and test drugs, during the first day and the fifth days, respectively.
The test drugs were dosed at 100 mg/kg body weight P.o.
Z1 is the initial paw volume recorded which corresponds to zero hour reading.
Z2 is the final paw volume recorded, 2.5 h after dosing.

can maintain the level of COX-2 and this could be the reason for its better activity.

Ibuprofen a preferential COX1 inhibitor did not give any protection on the first day. Reports suggest that the inflammation induced during formalin insult can be divided into two phases. In the first phase irritation is observed due to the action of formalin at the sensory fibers-C. In the second phase there is an inflammatory pain response. It is proved that NSAIDs inhibit only the second phase [19]. The above literature reports are supported by our finding that ibuprofen gave a reasonable protection (53.8%) to the inflamed tissue on fifth day. The test drugs AP29, AP82, and AP37 were giving 53, 34, and 65% inhibition, respectively, on the fifth day.

Among the series, AP84 turned out to be the best experimental candidate in which we incorporated the pyridyl template present in omeprazole, a proton pump inhibitor. This was the only experimental candidate which gave protection on both the first and fifth days, respectively. An interesting observation was that, being a non-steroidal drug, AP84 was showing comparable activity profile to dexamethasone which is steroidal in nature at the doses tested. Even though steroids are much better in suppressing chronic maladies the cumulative toxic effects on the skeleton, metabolism, and other organ systems limit the prolonged use of corticosteroids [20]. Our finding, that a NSAID with an omeprazole pyridyl template, which lacked the COX-1 or COX-2 inhibitory pharmacophore mimicked the activity profile of the steroid dexamethasone, gave us an impetus to explore this experimental candidate as an interesting non-steroidal anti-inflammatory lead candidate having similar efficacy to steroid. AP84 gave 54% protection in formalin test on the first day, probably mimicking to some extent the action of Dexamethasone. It gave 76.9% protection on fifth day. It could be possible that this candidate also inhibits both PLA_2 at an earlier stage and COX-1 or COX-2 at the later stage and thus maintains its anti-inflammatory activity profile for all the five days.

The most important effect attributed to the IL1 in inflammation is the stimulation of PLA_2, which in turn enhances PG production and stimulation of enzyme release by fibroblasts and chondrocytes which cause local tissue degradation [21]. Another possible explanation for the surprisingly potent activity exhibited by AP84 would be that it indirectly inhibits the stimulation of PLA_2, at an earlier stage by inhibiting IL1. If this were true then this candidate should have the capability to inhibit both PLA_2 and COX-1 at an earlier stage and IL1 and COX-2 at a later stage. The acute and chronic study data furnish clues in defining AP84 to be a probable dual inhibitor. If this were true, AP84 is worth considering to be a potent chemical lead, which could be exploited for further SAR studies to develop it into a drug like candidate with potency equivalent to steroids. Further studies to delineate the possible dual action of AP84 to be a PLA_2/IL1/COX-2 inhibitor are in progress and this will help us to identify a developable drug like candidate to treat chronic conditions like rheumatoid arthritis.

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References

[1] J.R. Vane, Nature 231 (1971) 232–235.
[2] C.J. Hawkey, The Lancet 353 (1999) 307–314.
[3] A. Garret, M.D. Fitzgerald, M.D. Carlo Patrono, N. Eng. J. Med. 345 (2001) 433–442.
[4] G.B. Zavioco, Drug Market Dev. 10 (1999) 337–345.
[5] J.J. Talley, S.R. Bertenshaw, D.L. Brown, J.F. Carter, M.J. Graneto, M.S. Kellogg, C.M. Koboldt, J. Yuan, Y.Y. Zhang, K. Seibert, J. Med. Chem. 43 (2000) 1661–1663.
[6] THE MERCK INDEX, 12th ed., Merck Research Laboratories Division of Merck & Co., Inc., 1996, p. 6977.
[7] J.E. Hodgkins, W.P. Reeves, J. Chem. 29 (1964) 3098–3099.
[8] W.A. Gregory, US Patent 2,763,692; CA-vol. 51-4429d, 1957.
[9] A.D. Pillai, P.D. Rathod, P.X. Franklin, H. Padh, K.V. Kamala, V. Sudarsanam, Indian J. Chem. (2002) (communicated).
[10] S. Rajappa, B.J. Advani, Indian J. Chem. 9 (1971) 759–760.
[11] A. Rolfs, J. Liebscher, Org. Syn. 74 (1973) 257–263.
[12] E.V. Brown, Synthesis (1975) 358–375.
[13] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[14] R.A. Greenwald, Methods Find. Clin. Pharmacol. 13 (1991) 75–83.
[15] A. Tjolsen, O.G. Berge, S. Hunskaar, J.H. Rosland, K. Hole, Pain 51 (1992) 5–17.
[16] K. Seibert, Y. Zhang, Y.K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee, P. Isakson, Proc. Natl. Acad. Sci. USA 91 (1994) 12013.
[17] H. Sano, T. Hla, J.A.M. Maier, L.J. Crofford, J.P. Case, T. Naciag, R.L. Wilder, J. Clin. Invest. 89 (1992) 97.
[18] G.D. Anderson, S.D. Hauser, K.L. McGarity, M.E. Bremer, P.C. Isakson, S.A. Gregory, J. Clin. Invest. 97 (1996) 2672.
[19] S. Husnkaar, K. Hole, Pain 30 (1987) 103–119.
[20] B.P. Schimmer, K.L. Parker, in: J.G. Hardman, L.E. Limbird (Eds.), Alfred Goodman Gilman (Consulting Ed.), The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill Medical Publishing Division, 2001, pp. 1649–1677.
[21] E.A. Urquhart, Agents Actions 32 (1991) 263.