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Tetra substituted thiophenes as anti-inflammatory agents: Exploitation of analogue-based drug design

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Abstract—A series of 17 novel tetra substituted thiophenes was designed, synthesized, and screened for anti-inflammatory activity in carrageenin induced rat paw edema model, an acute in vivo model. The lead molecule selected was Tenidap, a dual COX/LOX inhibitor. Compounds **I** (43%), **III** (60%), **IV** (60%), and **VIII** (64%) showed moderate to good anti-inflammatory activity. The best candidate among the whole series was **VIII**, which gave 64% protection to the inflamed paw. The side chain of candidate **VIII** had resemblance to that of Romazarit, a DMARD, which was withdrawn due to its toxicity profile. A probable reason for the metabolic stability of the proposed side chain not having the possibility of generating peroxy type radicals or acrylic acid moieties, unlike Romazarit, is discussed. The biological activity exhibited by the three designed series was in the order of methyl amino series > ethyl amino series > carbethoxy amino series. The –(C=O)–CH_2–COOR side chain at the fifth position as in candidate **VIII**, the methyl amino group at the second position, and the ester at the third position of the thiophene can be considered as a three-point pharmacophore for designing better anti-inflammatory agents. The present study is a classical example of the exploitation of an analogue based drug design, which culminated in the development of good anti-inflammatory agents that have the potential of becoming dual inhibitors.

1. Introduction

The analysis of known drugs or a drug-like candidate can provide valuable information on drug discovery and such candidates are invaluable probes to explore living systems and their emergent properties. By drawing lessons from their structures, we may both gain insight into drug discovery projects long since brought into completion and to provide guidelines for new drug discovery programs.[1,2] The common basis by which the various NSAIDs achieve their therapeutic effects is through their blockade of prostaglandin synthesis by their inhibition of cyclo-oxygenase enzyme. By interacting with appropriate targets, they transmit messages aimed to rectify the disease state. However, several structurally and functionally unrelated compounds used in the symptomatic treatment of inflammation show considerable side effects. Moreover, the discovery of isoforms of the COX enzyme, followed by the advent of COX-2 inhibitors, which was once thought to be a panacea for inflammatory disease treatment has recently met with a measure of disrepute due to the emergence of serious side effects in long-term clinical practice.[3]

In view of the polygenic nature of such diseases with a number of pathways in series and in parallel— and with a number of rate limiting steps, it seemed appropriate to look for candidates acting on more than one pathway.[4] In comparison with simple COX inhibitors, NSAIDs with dual inhibitory activities against COX and 5-LO, such as KME-4, E-5110, BF-389, and CI–1004, are hypothesized to be superior and are studied as potential

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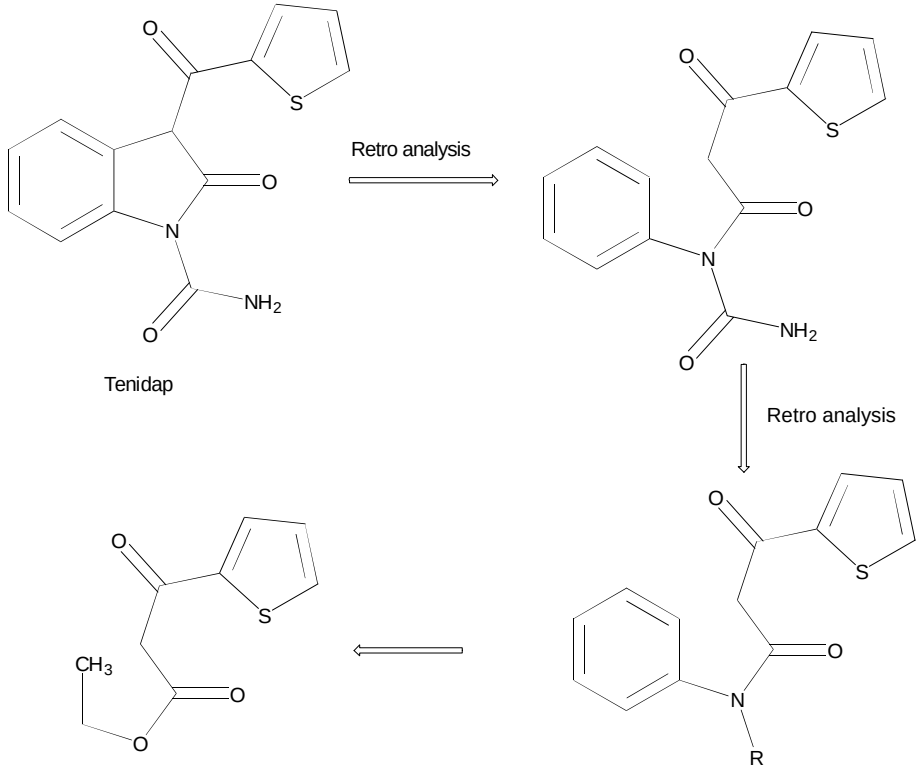
anti-inflammatory agents with an improved safety profile.[5]

With thiophene as a molecular scaffold, Tenidap (CP-66, 248, -2) was a dual cyclo-oxygenase/lipo-oxygenase inhibitor that also reduced the IL-1 levels in synovial fluids and inhibited monocyte migration, proving its potential as a cell migration inhibitor. Tenidap also turned out to be a collagenase inhibitor that inhibited (5-HPETE)-5-hydroperoxy eicosatetraenoic acid, which can be converted further into leukotrienes.[6] However, US FDA declined marketing permission indicating that Pfizer's clinical data had not sufficiently established its utility.

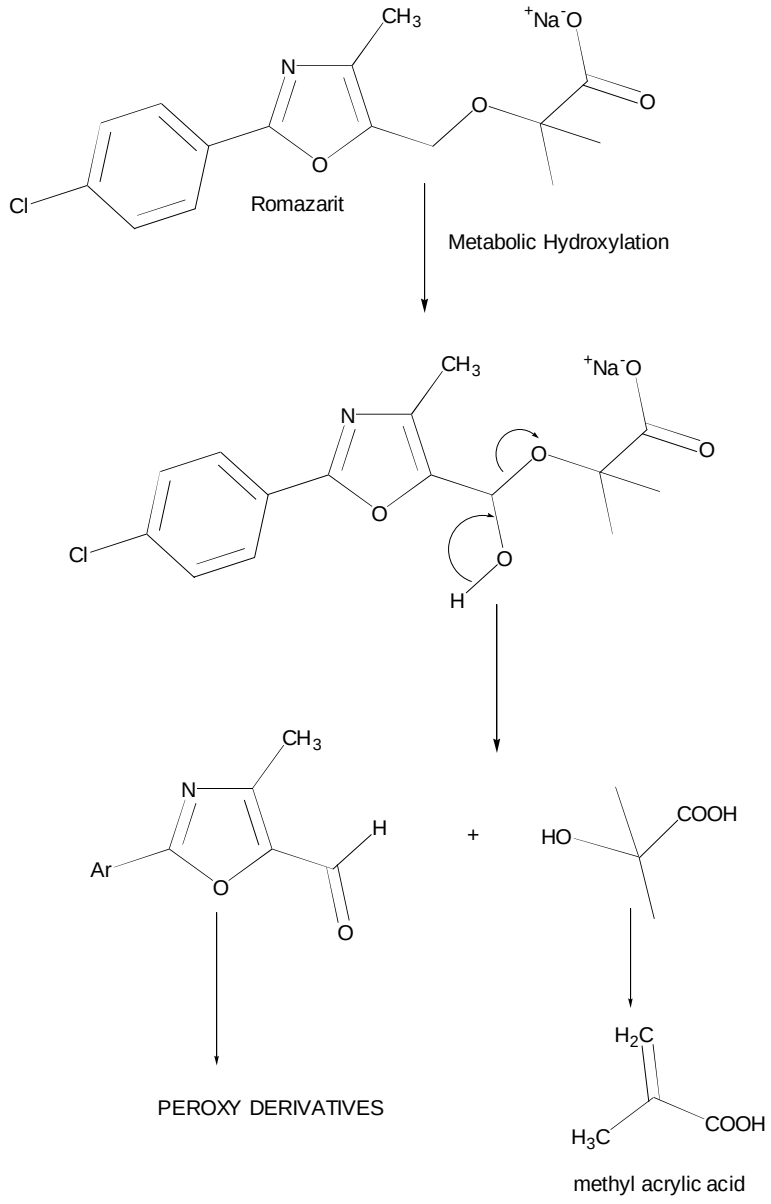
Considering the interesting pharmacological profile of Tenidap, a retro analysis of the structure was appropriate for us to arrive at a probable pharmacophore, as outlined in Scheme 1. In our previous studies on a derivatives of thiophene, a π excessive ring as molecular scaffold in designing better anti-inflammatory agents, we observed that 'the aliphatic oxime esters,' attached via a ketone bridge to the fifth position of the thiophene, were superior in enhancing the anti-inflammatory activity compared to the aryl oxime counterparts.[7] We anticipated that through careful modification of the pharmacophore obtained as a result of the retro analysis of Tenidap, by incorporating some aliphatic oximes or aromatic ketones that were established anti-inflammatory pharmacophores, as from our previous studies,[7,8] some novel potential anti-inflammatory agents could be designed and synthesized.

Our designed side chains, as well as overall structures, had some structural similarity to that of the Roche candidate (Ro-31-3948). The Roche candidate (Ro-31-3948), Romazarit, (2-[{2-(4-chlorophenyl)-4-methyl-5-oxazolyl} methoxy]-2-methyl propionic acid sodium salt, exhibited interesting anti-inflammatory activity. Though Romazarit had a desirable activity profile in various biological test systems and animal models, indicating its efficacy in arresting more than one deleterious pathway in the inflammatory cascade, it was withdrawn from the phase II clinical trials due to the adverse effects (bladder tumor) that emerged from chronic toxicity studies.[9–13] The toxicity produced by Romazarit could be due to metabolic hydroxylation of the CH_2 at fifth position (Scheme 2), which could give rise to an aldehyde and α-methyl acrylic acid. The aldehyde we presume could have led to peroxy type of compounds in vivo, and α-methyl acrylic acid being the probable cause of bladder tumor formation.

The designed thiophene analogues, taking Tenidap as the lead, are unlikely to produce the peroxy derivatives and methyl acrylic acid as was seen with the Roche candidate (Ro-31-3948), Romazarit. The present paper deals with the design, synthesis, and pharmacological evaluation of some thiophene derivatives with aliphatic oximes and some aromatic ketones as the side chains, exemplifying the importance of lead-based drug design leading to the synthesis of potential anti-inflammatory agents.



Scheme 1. Retro analysis of Tenidap, a dual COX–LOX inhibitor. Pharmacophore generated from retro analysis of Tenidap. The pharmacphore generated had side chain as that of Romaxarit, but hadn't had the likelihood of generating peroxy type radicals or methyl acrylic acid moieties probably responsible for bladder tumour formation.



Scheme 2. Probable mechanism of metabolic toxicity exhibited by Romazarit. Methyl acryic acid might be the moiety responsible for the baldder tumour.

2. Results and discussion

All the 17 compounds synthesized were evaluated for anti-inflammatory activity in carrageenin induced rat paw edema model. In the methyl amino series, compounds **I** (43%), **III** (60%), **IV** (60%), and **V** (32%) gave reasonable protection to the inflamed paw, eliciting good or moderate anti-inflammatory activity. The biological activity exhibited by candidate **I** was in consonance with the previous studies that aliphatic oximino esters acted as good anti-inflammatory pharmacophores.[7] In most of the selective COX-2 inhibitors, 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 toward COX-2.[14] The better activity exhibited by candidates **IV** and **III** might be due to the methyl sulfonyl group, which is an established pharmacophore in COX-2 inhibitors, such as Rofecoxib, and the fact that methylmercapto can rapidly undergo in vivo oxidation to sulfone, respectively.

In the ethyl amino series, the only candidate that was exhibiting a good biological activity was **VIII** (64%). Even though **VIII** was devoid of an oxime moiety at the fifth position of thiophene scaffold, the side chain –(C=O)–CH_2–COOR seemed to have good potential as envisaged in the retro analysis of Tenidap and **VIII** emerged as the best candidate among the whole series. Unlike the aliphatic oximino esters or methyl sulfonyl scaffolds, candidate **VIII** had –(C=O)–CH_2–COOR at the fifth position of the thiophene. The side chain also has resemblance to the side chain of Romazarit, but was unlikely to produce the peroxy derivatives and

methyl acrylic acid, as was envisaged with the Roche candidate (Ro-31-3948), Romazarit.

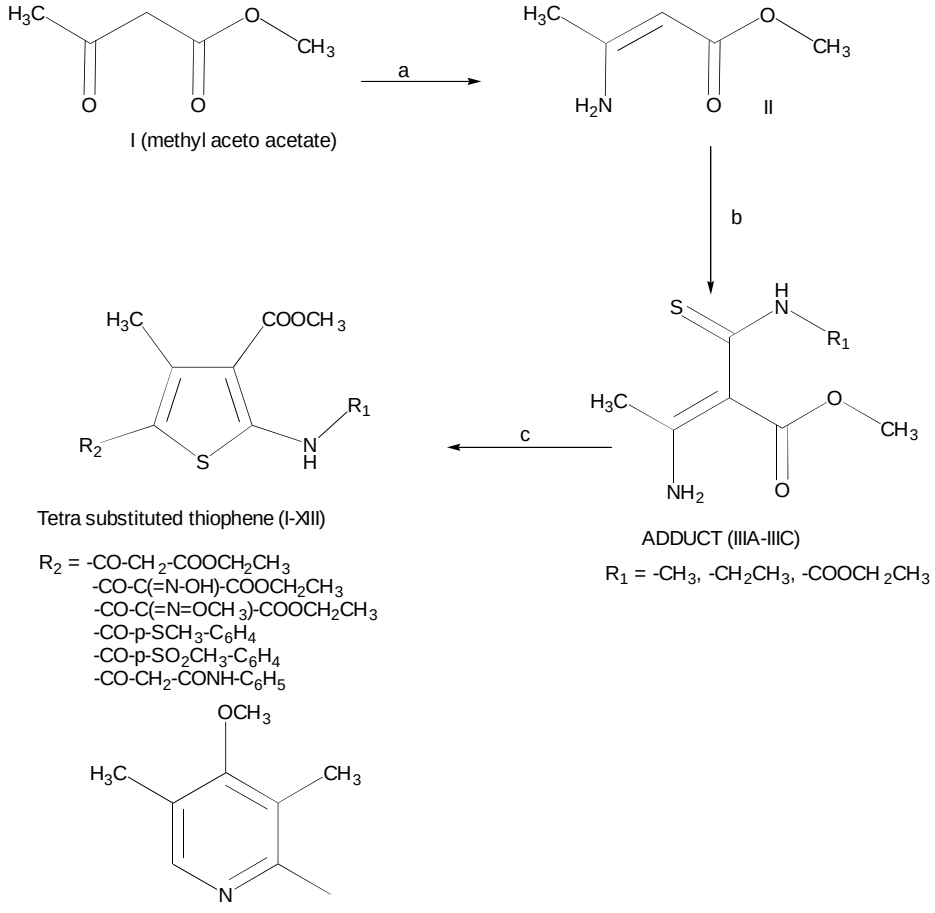
None of the candidates in the carbethoxy amino series exhibited good anti-inflammatory activity, indicating that a carbamate moiety is not preferred at the second position of the thiophene. An important outcome of this study is the optimization of the substitution at the second position of the thiophene in eliciting better biological activity. This is understood when we scrutinize and compare the activity profiles of

1. Candidates **I** (43%), **VI** (31%), and **XII** (27%)
2. Candidates **III** (60%), **IX** (19%), and **XV** (22%)
3. Candidates **IV** (60%), **X** (38%), and **XVI** (0%)
4. Candidates **V** (32%), **XI** (28%), and **XVII** (0%).

The biological activity exhibited by the three designed series were in the order of methyl amino series > ethyl amino series > carbethoxy amino series. The –(C═O)–CH_2–COOR side chain at the fifth position as in candidate **VIII**, the methyl amino group at the second position, and the ester at the third position of the thiophene can be considered as a three-point pharmacophore for designing better anti-inflammatory agents. By utilizing the pharmacophore generated by retro analysis of Tenidap, which is a dual inhibitor as the lead molecule and incorporating the aliphatic oximino esters or aromatic ketones, which were already established anti-inflammatory pharmacophores,[7,8] these results open up new avenues in designing candidates acting on more than one rate-limiting step along the inflammatory cascade.

3. Conclusion

A series of 17 tetra substituted thiophenes was designed based on the pharmacophore generated by the retro analysis of the structure of a dual inhibitor Tenidap. The aliphatic oximino esters and aromatic ketones, which were already established pharmacophores from our previous studies, were incorporated in the pharmacophore. The side chains had similarity to that of Romazarit, a DMARD, which was withdrawn due to its toxicity profile. A probable mechanism of the toxicity profile of Romazarit is described and a logical reason for the metabolic stability of the designed side chains not having the likelihood of generating peroxy type radicals or acrylic acid moieties, unlike Romazarit, is discussed. The present study discloses a novel three-point pharmacophore for designing better anti-inflammatory agents that is essentially generated from a lead-based drug design methodology and is an example of the exploitation of an analogue-based drug design.



Scheme 3. Synthesis of tetra substituted thiophenes. HVII was the only halomethylene compound used. Reagents: (a) ammonia, diethyl ether; (b) R_1-NCS, petroleum ether; (c) haloketones or halomethylene compound of the formula X-CH_2-R_2, acetonitrile.

Table 1. Structures and IUPAC nomenclature of haloketones (HI-HVI)

H	IUPAC nomenclature	Structure
HI	(*Z*)-γ-Chloro-β-oxo-α-hydroxyimino butyric acid, ethyl ester	Cl–CH_2–CO–C(=N–OH)–COO–CH_2–CH_3
HII	(*Z*)-γ-Bromo-β-oxo-α-methoxyimino butyric acid, ethyl ester	Br–CH_2–CO–C(=N–OCH_3)–COO–CH_2–CH_3
HIII	γ-Bromo-β-oxo-butyric acid, ethyl ester	Br–CH_2–CO–CH_2–$COOCH_2CH_3$
HIV	4-(α-Bromo acetyl)1-phenylmethylsulfone	Br–CH_2–CO–Ph–*p*-SO_2CH_3
HV	4-(α-Bromoacetyl)-thioanisole	Br–CH_2–CO–Ph–*p*–SCH_3
HVI	γ-Bromo-acetoacetanilide	Br–CH_2–CO–CH_2–CO–NH–Ph
HVII	(3,5-Dimethyl-4-methoxy)-2-chloromethyl pyridine	See Scheme 1

Halomethylene compound HVII used for the synthesis of novel designed thiophenes.

4. Experimental

4.1. Materials and methods

Methyl isothiocyanate was a gift sample from Dr. Reddy's Research Laboratories, Hyderabad. Carrageenan was purchased from Qualigen, Mumbai. Ibuprofen was a gift sample from AVIK Pharmaceuticals, Mumbai. 2-Chloromethyl-3,5-dimethyl-4-methoxy pyridine was kindly provided by Dishman pharma, Ahmedabad. The designed compounds were synthesized by the previously reported protocol, by Rajappa et al.[15–18] (Scheme 3). Isothiocyanates were synthesized using the modified Kaluza method.[19] α-Haloketones (Table 1) were synthesized using a reported procedure.[17]. The adduct of general formula III (IIIA-IIIC) was reacted with α-haloketones, α-haloketoximes, and one halomethylene compound of the general formula X–CH_2–R_2 to yield the tetra substituted thiophenes.

4.2. Experimental part

The melting point of the compounds was taken in open capillaries and is uncorrected. The infrared spectrum was recorded using KBr as the medium, utilizing Buck Scientific M-500 Infrared spectrophotometer. The mass of all the compounds was recorded on a Perkin Elmer Sciex Atmospheric pressure ionization liquid chromatography mass instrument. ^{1}H NMR were recorded at 200 MHz from CDRI Lucknow. Elemental analysis was taken on Heraeus Carlo Erba 1108 elemental analyzer at CDRI Lucknow. All the reactions were monitored using thin layer chromatography (TLC) using a glass plate coated with Silica Gel G or GF_{254}. TLC plates were developed using iodine.

4.2.1. Synthesis of thiophenes. The general procedure for the synthesis of tetra substituted thiophenes is as follows.[20] To a stirred solution of 0.001 mol of III in 25 parts of acetonitrile at room temperature was added 0.001 mol of the respective halo carbonyl compound (X–CH_2–R_2) or the halomethylene compound (HVII, Table 1), and the solution was stirred until the solid separated from the reaction mixture or the TLC showed the absence of the starting material. The solid that separated was filtered, washed with chilled acetonitrile, and dried, yielding a yellow colored product, which corresponded to the tetra substituted thiophene (I-XVII) characterized as per the analytical data. In case where the solid did not separate from the reaction mixture, the solvent was evaporated and chilled water was added and triturated when the solid separated.

4.2.2. Characteristics of synthesized compounds

4.2.2.1. (*Z*)-2-Methylamino-5-(2-ethoxycarbonyl-2-hydroxyimino-acetyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP58). Molecular formula: $C_{13}H_{16}N_2O_6S$; TLC: toluene/methanol (7:3); R_f: 0.7. Yield: 62%; mp: 150–152 °C, IR (KBR, cm^{-1}) = 3370 (NH stretching at second position), 1732 (C=O stretching of ester), 1692 (C=O stretching), 1580 (NH bending of alkyl amine), Mass: M+1 at *m*/*z* 329. ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.32–1.39 (t, 3H), 2.77 (s, 3H), 3.85 (s, 3H), 4.11 (s, 3H), 4.32–4.43 (q, 2H), 7.27 (s, 1H), 8.4 (s, 1H).

4.2.2.2. (*Z*)-2-Methylamino-5-(2-ethoxycarbonyl-2-methoxyimino-acetyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP56). Molecular formula: $C_{14}H_{18}N_2O_6S$; TLC: toluene/acetonitrile (7:3); R_f: 0.91. Yield: 29%, mp: 139–40 °C, IR (KBr, cm^{-1}) = 3400 (NH– at second position), 1732 (C=O stretching of ester), 1690 (C=O stretching of ester), Mass: M+1 at *m*/*z*: 343. ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.15–1.23 (t, 3H), 2.78 (s, 3H), 3.06 (s, 3H), 3.80 (s, 3H), 3.90 (s, 3H), 4.32–4.43 (q, 2H), 8.4 (s, 1H).

4.2.2.3. Synthesis of 2-methylamino-5-(*p*-methyl sulfonyl benzoyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP57). Molecular formula: $C_{16}H_{17}NO_5S_2$; TLC: toluene/methanol (7:3); R_f: 0.55. Yield: 45%, mp: 174–75 °C, IR (KBr, cm^{-1}) = 3290 (NH stretching), 1660 (C=O stretching of ester), 1612 (C=O stretching of ketone), 1352 (S(=O) asymmetrical stretching), 1128 (S=(O), symmetrical stretching), Mass: M+1 at *m*/*z* 368. ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.45 (s, 3H), 3.04 (s, 3H), 3.28 (s, 3H), 3.88 (s, 3H), 7.8–7.83 (d, 2H), 8.01–8.04 (d, 2H), 8.5 (s, 1H).

4.2.2.4. 2-Methylamino-5-(4-methylmercapto-benzoyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP156). Molecular formula: $C_{16}H_{17}NO_3S_2$; TLC: toluene/methanol (9:1); R_f: 0.42. Yield: 50%; mp: 165–66 °C, IR (KBr, cm^{-1}) 3320 (alkyl NH stretching), 1678 (C=O ester stretching), 1632 (C=O stretching of ketone), Mass: M+1 at *m*/*z* 336.

4.2.2.5. 2-Methylamino-5-(3,5-dimethyl-4-methoxy-2-pyridyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP53). Molecular formula: $C_{16}H_{20}N_2O_3S$; TLC: toluene/acetonitrile (7:3); R_f: 0.60. Yield: 45%; mp: 280–281 °C, IR (KBr, cm^{-1}) 3320 (aryl NH stretching),

1660 (C=O stretching of ester), Mass: M+1 at *m/z* 321, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.55 (s, 3H), 1.65 (s, 3H), 1.75 (s, 3H), 2.1 (s, 3H), 3.8 (s, 3H), 7.25 (s, 1H).

4.2.2.6. (*Z*)-2-Ethylamino-5-(2-ethoxycarbonyl-2-hydroxyimino-acetyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP61). Molecular formula: $C_{14}H_{18}N_2O_6S$; TLC: toluene/methanol (7:3); R_f: 0.23. Yield: 14%; mp: 150–152 °C, IR (KBr, cm^{-1}) 3360 (NH stretching), 1718 (C=O stretching of ester), 1672 (C=O stretching), Mass: M+1 = 343.

4.2.2.7. (*Z*)-2-Ethylamino-5-(2-ethoxycarbonyl-2-methoxyimino-acetyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP62). Molecular formula: $C_{15}H_{20}N_2O_6S$; TLC: toluene/acetonitrile (8:2); R_f: 0.91. Yield: 29%; mp 130–132 °C; IR (KBr, cm^{-1}) 3380 (NH stretching), 1736 (C=O stretching of ester), 1696 (C=O stretching), Mass: M+1 at *m/z* 357, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.25–1.38 (m, 6H), 2.77 (s, 3H), 3.30–3.39 (q, 2H), 3.85 (s, 3H), 4.11 (s, 3H), 4.34–4.4 (q, 2H), 8.43 (s, 1H).

4.2.2.8. 2-Ethylamino-5-(2-ethoxycarbonyl-2-acetyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP59). Molecular formula: $C_{14}H_{19}NO_5S$; TLC: toluene/methanol (8:2); R_f: 0.85. Yield: 52%; mp: 79–82 °C; IR (KBr, cm^{-1}) 3320 (alkyl NH stretching), 1724 (C=O stretching of ester), 1694 (C=O stretching), 1628 (C=O stretching of β-keto ester), Mass: (M+1 at *m/z* 314, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.2 (m, 6H), 2.354 (s, 3H), 3.20–3.27 (q, 2H), 3.68 (s, 2H), 3.78 (s, 3H), 4.1–4.23 (q, 2H), 8.32 (s, 1H).

4.2.2.9. 2-Ethylamino-5-(4-methylmercapto-benzoyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP155). Molecular formula: $C_{17}H_{19}NO_3S_2$; TLC: toluene/methanol (7:3); R_f: 0.33. Yield 78%; mp: 147–148 °C, IR (KBr, cm^{-1}) 3320 (alkyl NH stretching), 1676 (C=O stretching), Mass: M+1 at *m/z* 350, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.39 (t, 3H), 2.4 (s, 3H, SCH_3), 2.56 (s, 3H), 3.27–3.34 (q, 2H), 3.8 (s, 3H), 7.25–7.28 (d, 2H), 7.59–7.62 (d, 2 H), 8.4 (s, 1H).

4.2.2.10. 2-Ethylamino-5-(4-methylsulfonylbenzoyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP60). Molecular formula: $C_{17}H_{19}NO_5S_2$; TLC: toluene:acetonitrile (7:3); R_f: 0.70. Yield: 99%; mp: 115–116 °C, IR (KBr, cm^{-1}) 3250 (alkyl NH stretching), 1680 (C=O stretching of ester), 1612 (C=O stretching of ketone), 1346 (asymmetric S=O stretching), 1148 (symmetric S=O stretching), Mass: M + 1 at *m/z* 382, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.32–1.39 (t, 3H), 2.43 (s, 3H), 3.24 (s, 3H), 3.30–3.38 (q, 2H), 3.84 (s, 3 H), 7.78–7.82 (d, 2H), 8.007–8.047 (d, 2H), 8.44 (s, 1H).

4.2.2.11. 2-Ethylamino-5-(3,5-dimethyl-4-methoxy-2-pyridyl)-4-methyl-thiophene-3-carboxylic acid methyl ester (AP63). Molecular formula: $C_{17}H_{22}N_2O_3S$; TLC: toluene/methanol (7:3); R_f: 0.25. Yield: 31%; mp: 256 °C, IR (KBr, cm^{-1}) 3320 (aryl NH stretching), 1650 (C=O stretching of ester), Mass: M+1 at *m/z* 335, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.21–1.25 (t, 3H), 1.76 (s, 3H), 1.78 (s, 3H), 1.90 (s, 3H), 2.50 (s, 3H), 3.76 (s, 3 H), 7.7 (s, 1H, pyridine), 7.8 (s, 1H).

4.2.2.12. (*Z*)-2-carbethoxyamino-5-(2-ethoxycarbonyl-2-hydroxyimino-acetyl)-4- methyl-thiophene-3-carboxylic acid methyl ester (AP11). Molecular formula: $C_{15}H_{18}N_2O_8S$; TLC: toluene:acetonitrile (7:3); R_f: 0.32. Yield: 10.36%; mp: 140–141 °C, IR (KBr, cm^{-1}) = 3370 (NH stretching at second position), 1712 (C=O stretching of ester), 1614 (C=O stretching of ester), Mass: M+1 at *m/z* 387.

4.2.2.13. (*Z*)-2-carbethoxyamino-5-(2-ethoxycarbonyl-2-methoxyimino-acetyl)-4- methyl-thiophene-3-carboxylic acid methyl ester (AP10). Molecular formula: $C_{16}H_{20}N_2O_8S$, TLC: toluene:acetonitrile (7:3); R_f: 0.50. Yield: 22%; mp: 250–251 °C, IR (KBr, cm^{-1}) 3120 (NH at second position), 1746 (C=O stretching of ester), 1716 (C=O stretching), Mass; M+1 at *m/z* 401. ^{1}H NMR ($CDCl_3$, δ ppm) = 1.22–1.31 (m, 6H), 2.65 (s, 3H), 3.85 (s, 3H), 4.1 (s, 3H), 4.26–4.32 (m, 4H), 7.27 (s, 1H).

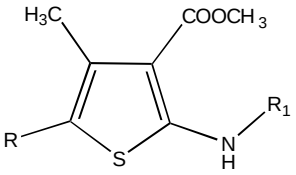
4.2.2.14. 2-Carbethoxyamino-5-(2-carboxanilido-acetyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP121). Molecular formula: $C_{19}H_{20}N_2O_6S$, TLC: toluene/acetonitrile (7:3); R_f: 0.22. Yield: 34%; mp: 170–171 °C, IR (KBr, cm^{-1}) 3270 (aryl NH stretching at fifth position), 3150 (alkyl NH stretching at second position), 1724 (C=O stretching of ester), 1676 (C=O stretching), Mass: M+1 at *m/z* 405, ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.4–1.5 (m, 6H), 3.07 (s, 3H), 3.7–3.78 (q, 2H), 7.23–7.31 (m, 4H), 7.51 (s, 1H), 7.76 (s, 1H).

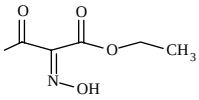
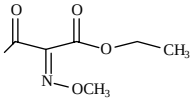
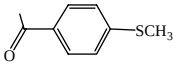
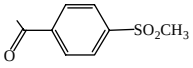
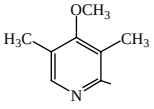
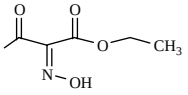
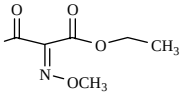
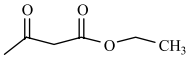
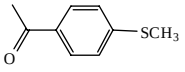
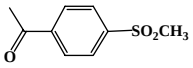
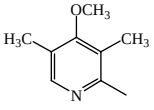
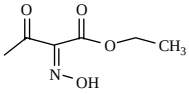
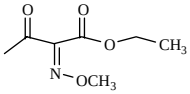
4.2.2.15. 2-Carbethoxyamino-5-(4-methylmercapto-benzoyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP157). Molecular formula: $C_{18}H_{19}NO_5S_2$; TLC: toluene/methanol (9:1); R_f: 0.90. Yield: 18.9%; mp: 122 °C, IR (KBr, cm^{-1}) 3080 (alkyl NH stretching), 1732 (C=O stretching of ester), 1664 (C=O stretching of ketone), Mass: M+1 at *m/z* 394. ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.29–1.34 (t, 3H), 2.38 (s, 3H), 2.52 (s, 3H), 3.91 (s, 3H), 4.2–4.26 (q, 2H), 7.54 (s, 1H), 7.83–7.87 (m, 4H).

4.2.2.16. 2-Carbethoxyamino-5-(4-methylsulfonylbenzoyl)-4-methyl-thiophene-3- carboxylic acid methyl ester (AP46). Molecular formula: $C_{18}H_{19}NO_7S_2$; TLC: toluene/methanol (7:3); R_f: 0.72. Yield: 17%; mp: 176 °C, IR (KBr, cm^{-1}) 3110 (alkyl NH stretching), 1720 (C=O stretching of ester), 1652 (C=O stretching of ketone), 1340 (asymmetric S=O stretching), 1150 (symmetric S=O stretching), Mass: M+1 at *m/z* 426. ^{1}H NMR ($CDCl_3$, δ, ppm) = 1.40–1.45 (t, 3H), 2.35 (s, 3H), 3.08 (s, 3H), 3.94 (s, 3 H), 4.24–4.33 (q, 2H), 7.2–8.0 (m, 4H), 10.82 (s, 1H).

4.2.2.17. 2-Carbethoxyamino-5-(3,5-dimethyl-4-methoxy-2-pyridyl)-4-methyl- thiophene-3-carboxylic acid methyl ester (AP45). Molecular formula: $C_{18}H_{22}N_2O_5S$; TLC: toluene/acetonitrile (7:3); R_f: 0.77. Yield: 23%; mp: 174–175 °C, IR (KBr, cm^{-1}) 3320 (alkyl NH stretching), 1710 (C=O stretching of ester), 1650 (C=O stretching of ester), Mass: M+1 at *m/z* 379, ^{1}H NMR ($CDCl_3$, δ, ppm) 1.25–1.56 (m, 6H), 2.25 (s, 3H), 2.53 (s, 3H), 2.7 (s, 3H), 3.78 (s, 3 H), 4.25–4.36 (q, 2H), 7.26 (s, 1H), 7.96 (s, 1H).

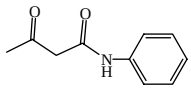
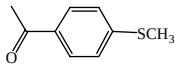
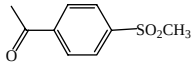
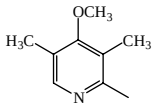
Table 2. The biological activity exhibited by designed thiophene analogues in a carrageenin induced rat paw edema model



Compound	Candidate code	R	R_1	*P*
I	**AP58**		CH_3	43
II	**AP56**		CH_3	0
III	**AP156**		CH_3	60
IV	**AP57**		CH_3	60
V	**AP53**		CH_3	32
VI	**AP61**		CH_2CH_3	31
VII	**AP62**		CH_2CH_3	0
VIII	**AP59**		CH_2CH_3	64
IX	**AP155**		CH_2CH_3	19
X	**AP60**		CH_2CH_3	38
XI	**AP63**		CH_2CH_3	28
XII	**AP11**		$COOC_2H_5$	27
XIII	**AP10**		$COOC_2H_5$	22

(*continued on next page*)

Table 2 (*continued*)

Compound	Candidate code	R	R_1	*P*
XIV	**AP121**		$COOC_2H_5$	5
XV	**AP157**		$COOC_2H_5$	22
XVI	**AP46**		$COOC_2H_5$	0
XVII	**AP45**		$COOC_2H_5$	0
Standard	Ibuprofen			60

P = % protection to inflammation given to the rat paw, in a carrageenin induced rat paw edema model.
The test drugs were dosed at 100 mg/kg body weight po. Ibuprofen was dosed at 100 mg/kg body weight po.
P represents the protection given by the experimental candidates to the inflamed rat paw. The test and standard drug were dosed at 100 mg/kg body weight po.

4.3. Pharmacological activity

Carrageenin induced rat paw edema:[21] Sprague–Dawley (male/female) rats weighing 150–250 g were used for the edema test. Animals were divided into 19 groups comprising six rats per group. Rats were put on fast for 18 h prior to the experiment. The standard drug, Ibuprofen (100 mg/kg body weight), and the test drugs (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 in the subplantar region of the right hind paw of each rat. After 3 h of the carrageenin injection, the reduction in paw volume compared to vehicle control was measured using plethysmometer. The percentage protection given to the inflamed paw was calculated (Table 2). The Institutional Ethics Committee, constituted by the Ministry of Social Justice and Empowerment, Government of India, approved the experimental protocol.

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