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# Synthesis and anti-inflammatory activity of some selected aminothiophene analogs

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**Abstract**
Some 2-aminothiophene analogs **1**–**6** were synthesized and characterized. Among the tested compounds, compound **1** ($IC_{50}$ 121.47 μM) exhibited highest while the compound **5** showed least anti-inflammatory potential ($IC_{50}$ 422 μM).

**Keywords:** *2-Aminothiophene, anti-inflammatory activity, inflammation, leukocyte migration*

## Introduction

Inflammation occurs as a defensive response, which induces physiological adaptations to limit tissue damage and remove the pathogenic infections. Such mechanisms involve a complex series of events including venules and capillaries with increased vascular permeability and exudation of fluids including plasma proteins and leukocyte migration into the inflammatory area followed by the loss of tissue function.

Diseases caused by inflammation are an important factor of morbidity and mortality in humans. In certain conditions, there is the appearance of lack of resolution of the acute signs of inflammation and the chronic state develops, that may lead to loss of life of the individual. Inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis.

Other characteristics, such as erythema and fever can also be observed during inflammatory events. The latter feature occurs when activated macrophages release cytokine (IL-1, TNF-α) which leads to vessel dilation resulting from smooth muscle relaxation followed by an increase in local blood flow (hypothermia) [1].

Acute inflammation is relatively ephemeral (days) and characterized by neutrophils as the primary cellular infiltrate. In contrast, chronic inflammation can continue up to months or longer, and is characterized by infiltration of macrophages and lymphocytes. Chronic inflammation may lead to immune granuloma formation and more serious immunological consequences such as autoimmune disease, *i.e.* immune response to the body's own constituents with chronic, debilitating and sometimes life threatening tissue and organ injuries.

As a part of our drug discovery programme we screened a focused library of compounds and found 2-aminothiophene and its analogs to have potent anti-inflammatory activity.

Thiophene and its analogs represent an important class of compounds with diverse biological activities.

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Its derivatives provide intermediates for many products in the fine chemical industries such as pharmaceutics, agrochemicals and dyestuffs.

Substituted thiophenes are also present in natural products. Thienopyrimidinone derivatives have potential radio-protective character [2], analgesic [3], hypocholesterolemic, antitussive [4], and CNS depressant [5], anticonvulsants [6], anti-inflammatory [7], bactericidal [8], fungicidal [9] activities and antifertility [10], sedative effects [11,12], antimalarial [13–15] and post-coital antifertility activities in rats [10]. Compounds containing fused pyrimidine rings have attracted great attention in recent years. Many potent drugs in the field of cancer and virus research have been developed [16–20]. Herein we report 2-aminothiophene and its analogs as a new class of anti-inflammatory compounds.

## Methods

### *Chemistry*

*2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid ethyl ester (**1**)*. A mixture of ethyl cyanoacetate (3.1 mL, 29.0 mmol), cyclohexanone (3 ml, 29.0 mmol), sulphur (0.93 g, 29 mmol), morpholine (2.52 mL, 29 mmol) and ethanol (26 mL) was stirred at room temperature. During this exothermic reaction, the reaction mixture was stirred on an ice bath. After 1 h of continuous stirring the reaction was completed and the reaction mixture was evaporated and extracted with ether. The ether extract was dried over anhydrous $Na_2SO_4$, evaporated to some extent and kept in the refrigerator to crystallize [21].

Yield = 3.53 g (54%); m.p. = 107.8°C; $R_f$ = 0.5 (9:1, Hexane/EtOAc); UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 209 (IR (KBr): 3408, 2918, 1732, 1608, 1474, 1355, 1145 $cm^{-1}$; $^1$H-NMR (500 MHz, $CDCl_3$): δ 5.91 (br. s, 2H, $NH_2$), 4.24 (q, 2H, $OCH_2$), 2.70–2.67 (m, 2H, H-2), 2.49–2.46 (m, 2H, H-7), 1.77–1.70 (m, 4H, H-5 and H-6), 1.31 (t, 3H, $CH_3$); EI MS: *m/z* (rel. abund. %) 225 ($M^+$, 67), 179 (100), 151 (61), 123 (30), 91 (41), 65 (40), 51 (22); Anal. Calcd. for $C_{11}H_{15}NO_2S$: C, 58.64; H,6.71, N, 6.22. Found: 58.69; H, 6.66; N, 6.22%.

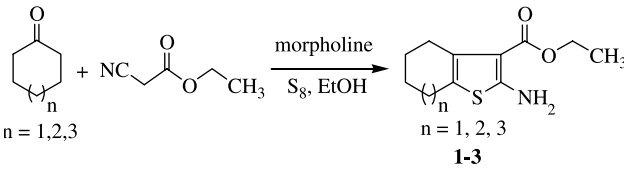
*2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylic acid ethyl ester (**2**)*. Yield = 2.24 g (55%); m.p. = 79°C; $R_f$ = 0.5 (9:1, Hexane/EtOAc); UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 211 (I.R. (KBr): 3429, 2973, 1726, 1601, 1470, 1365, 1160 $cm^{-1}$; $H^1$-NMR: (400 MHz, $CDCl_3$): δ 5.76 (br. s, 1H, $NH_2$), 4.27 (q, 2H, $OCH_2$), 2.97–2.94 (m, 2H, H-4), 2.57–2.54 (m, 2H, H-8), 1.81–1.76 (m, 2H, H-5), 1.65–1.56 (m, 4H, H-6 and H-7), 1.33 (t, 3H, $\mathcal{J}$ = 7.12, $CH_3$); EI MS: *m/z* (rel. abund. %) 239 ($M^+$, 77), 193 (100), 165 (38), 150 (21), 125 (8), 91 (10); Anal. Calcd for $C_{12}H_{17}NO_2S$: C, 60.22; H, 6.16; N, 5.85;. Found: C, 60.27; H, 6.11; N, 5.87%.

*2-Amino-4,5,6,1,8-hexahydrocycloocta[b]thiophene-3-carboxylic acid ethyl ester (**3**)*. Yield = 3.37 g (84%); $R_f$ = 0.6 (9:1, Hexane/EtOAc); UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 207 (IR (KBr): 3421, 2930, 1700, 1601, 1452, 1360, 1109 $cm^{-1}$; $H^1$-NMR (400 MHz, $CDCl_3$): δ 5.09 (br. s, 1H, NH), 4.23 (q, $\mathcal{J}$ = 7.12, 2H, $OCH_2$), 2.95–2.90 (m, 2H, H-4), 2.73–2.69 (m, 2H, H-9), 2.40–2.35 (m, 2H, H-8), 1.87–1.80 (m, 2H, H-7), 1.33 (t, $\mathcal{J}$ = 6.7, 2H, $CH_3$); EI MS: *m/z* (rel. abund. %) 253 ($M^+$, 67), 208 (13), 179 (29), 139 (28), 107 (11), 83 (100), 55 (87); Anal. Calcd for $C_{13}H_{19}NO_2S$: C, 61.63; H, 7.56; N, 5.53; Found: C, 61.69; H, 6.05; N, 5.94%.

*2-[(2-Chloroacetyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylic acid ethyl ester (**4**)*. 2-Amino-5,6,7-tetrahydro-1-benzothiophene-3-carboxylic acid ester (0.50 g, 2.2 mmol) **(1)** was treated with chloroacetylchloride (0.42 mL, 4.4 mmol) in dry chloroform (25 mL). The reaction was stirred at room temperature and was then refluxed for 3 h. When reaction was completed (TLC analysis), it was then fractionally distilled under reduced pressure to afford **4** as an oily product.

Yield = 84%; UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 226 (IR (KBr): 3454 (–NH), 2931 (–$CH_2$–), 1729 (–COO–), 1663 (amide), 1603 and 1472 (C=C aromatic), 764 (C–Cl), 712, 678 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$): δ 10.91 (br. s, 1H, NH), 4.28 (q, 2H, $OCH_2$), 4.23 (s, 2H, –$CH_2$Cl), 2.75–2.67 (m, 2H, H-2), 2.47–2.43 (m, 2H, H-7), 1.79–1.73 (m, 4H, H-5 and H-6), 1.31 (t, 3H. $CH_3$); EI MS: *m/z* (rel. abund. %), 303 ($M^+$2), 301 ($M^+$, 10), 266 (29), 252 (34), 224 (61), 179 (100), 151 (64), 123 (15), 91 (100), 65 (41), 51 (23); Anal. Calcd for $C_{13}H_{16}NO_3$-SCl: C, 51.74; H, 5.34; N, 4.64. Found: C, 51.81; H, 5.27; N, 4.68%.

*2-[(2-Chloroacetyl)amino]-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylic acid ethyl ester (**5**)*. Yield = 55%; UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 224 (IR (KBr): 3449 (NH), 2927 (–$CH_2$), 1738 (–COO–), 1666 (amide), 1604 and 1471 (C=C, arom.), 771 (C–Cl), 718, 667; $^1$H-NMR (400 MHz, $CDCl_3$): δ 6.23 (br. s, 1H, NH), 4.49–4.39 (m, 2H, –$CH_2$), 4.38 (s, 2H, –$CH_2$Cl), 4.24 (q, 2H, $OCH_2$), 3.21–3.13 (m, 2H, –$CH_2$), 1.93 – 1.79 (m, 4H, –2$CH_2$), 1.51 (t, 3H, $CH_3$), 1.32–1.30 (m, 2H, –$CH_2$); EI MS *m/z* (rel. abund. %): 317 ($M^+$2, 4), 315 (13), 279 (21), 265 (48), 237 (24), 192 (69), 164 (100), 150 (61), 118 (18), 93 (31), 69 (23); Anal. Calcd for




Scheme 1. Synthesis of compounds **1**–**3**

$C_{14}H_{18}ClNO_3S$: C, 53.24; H, 5.74; N, 4.44. Found: C, 53.29; H, 5.69; N, 4.38%.

*2-[(2,2-Dichloroacetyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylic acid ethyl ester (**6**).* 2-Amino-5,6,7-tetrahydro-1-benzothiophene-3-carboxylic acid ester (0.50 g, 2.2 mmol) **(1)** was treated with dichloroacetylchloride (0.41 g, 4.4 mmol) in dry chloroform (25 mL). The reaction was stirred at room temperature initially and then refluxed for 2 h. When reaction was completed (TLC analysis), it was then fractionally distilled under reduced pressure to afford **6** as an oily product.

Yield = 71%; UV (methanol) $\lambda_{max}$ (log$\varepsilon$) 224 (IR (KBr): 3463 (–NH), 2926 (–$CH_2$), 1736 (–COO–), 1667 (amide), 1605 and 1476 (C=C arom.), 773 (CCl), 719, 663; $^1$H-NMR (400 MHz, $CDCl_3$): $\delta$ 11.01 (br. s, 1H, NH), 4.27 (q, 2H, $OCH_2$), 2.76–2.71 (m, 2H, H-2), 2.48–2.44 (m, 2H, H-7), 1.81–1.75 (m, 4H, H-5/H-6), 1.32 (t, 3H, $CH_3$), 5.49 (s, 2H, –$CHCl_2$); EI MS: *m/z* (rel. abund. %) 340 ($M^+$4, 4), 338 ($M^+$2, 13), 336 ($M^+$, 21), 252 (37), 224 (53), 179 (69), 151 (38), 136 (21), 91 (100), 65 (43), 51 (28); Anal. Calcd for $C_{13}H_{15}NO_3SCl_2$: C, 46.44; H, 4.50; N, 4.17. Found: C, 46.49; H, 4.45; N, 4.11%.

### *Anti-inflammatory assay protocol*

*Isolation of human neutrophils*. Heparinized fresh venous blood was drawn from healthy volunteers and neutrophils were isolated by the method of Siddiqui *et al.* [22,23]. Briefly, whole blood was mixed with Ficoll or Dextran 6% in a ratio of 1:3 and allowed to settle down. Buffy coat was collected, layered on the bed of Ficoll (3 mL) and centrifuged at 1500 rpm for 30 min. The pallets were collected and washed with phosphate buffered saline (PBS) buffer (pH 7.4). The RBCs were lysed with ammonium chloride solution and then centrifuged. The pallets were washed with PBS and the cells were resuspended with the same buffer at a concentration of $1 \times 10^6$ Cell/mL.

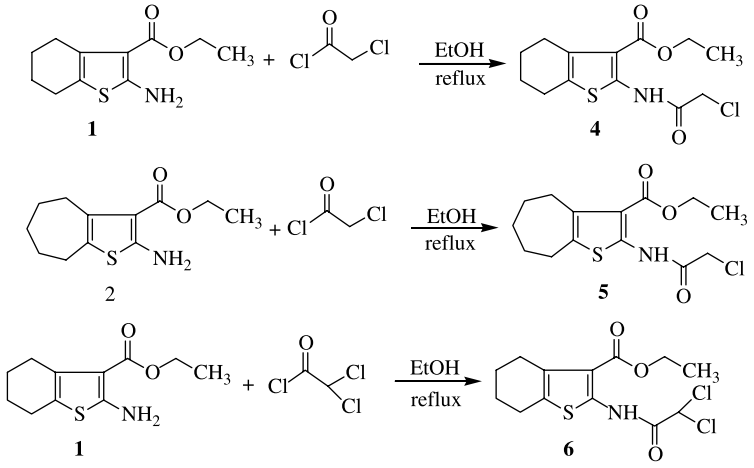
*Anti-inflammatory assay.* To determine the anti-inflammatory activity of the compounds the modified assay of Berridge *et al.* was used [17–20]. This *in vitro* assay is based on the reduction of highly water-soluble tetrazolium salt (WST-1) in the presence of activated neutrophils. Anti-inflammatory activity was determined in a total volume of 200 $\mu$L PBS (pH 7.4) containing $0.5–10^4$ neutrophils/mL, 750 $\mu$L WST-1 in various concentrations of test compounds. Control only contains buffer, neutrophils and WST-1. All compounds were equilibrated at 37°C for 10 min and reaction was initiated by adding Zymosan activated serum (ZAS).

Indomethacin was used as a positive control and the absorbance was measured at 450 nm using a spectra MAX 340 microplate reader kinetically for 30 min. Each value was the mean of reactions in six wells for a single compound in a 96-well plate. The $IC_{50}$ was calculated by comparing with DMSO as blank and expressed as % inhibition of superoxide produced.

## Results and discussion

### *Chemistry*

A number of 2-aminothiophenes were synthesised by treating different ketones with ethyl cyanoacetate




Scheme 2. Synthesis of compounds **4**–**6**

Table I. $IC_{50}$ μM values of compounds related to 2-aminothiophene.

| Compound | % Inhibition | $IC_{50}$ (μM) ± SEM |
|---|---|---|
| **1** | 61 | 121 ± 3.09 |
| **2** | 94 | 412 ± 1.86 |
| **3** | 30 | 323 ± 10.37 |
| **4** | 75 | 348 ± 4.08 |
| **5** | 71 | 422 ± 2.97 |
| **6** | 81 | 396 ± 1.89 |

Reaction mixture of 200 μL contains PBS buffer, neutrophils ($0.5 - 1 \times 10^5$ Cell/mL) and WST-1 (0.75 μM). Neutrophils were incubated with test compounds (10 min) and Zymosan was then added. **Standard drug:** Indomethacin.

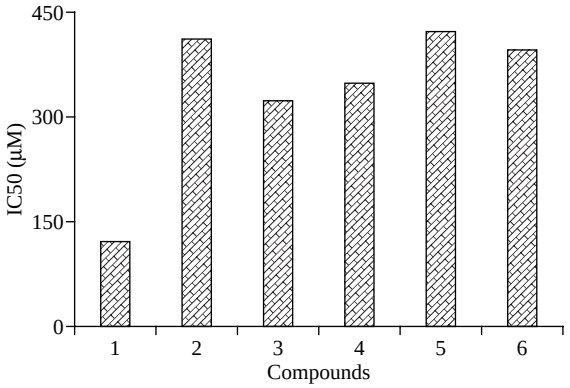



Figure 1. Bar-Graph representing anti-inflammatory activity ($IC_{50}$ μM).

and sulphur in the presence of morpholine (Scheme 1) and some of their derivatives were prepared (Scheme 2) [21].

*Biological evaluation*

*Anti-inflammatory activity.* Anti-inflammatory activity was done according to the literature protocol of Siddiqui et al. [22,23]. Compound **1** exhibited 61% anti-inflammatory activity with $IC_{50}$ 121 μM, compound **2** showed 94% activity with an $IC_{50}$ value of 412 μM and compound **3** indicated 30% activity with an $IC_{50}$ value of 323 μM. Among other thiophene analogs **4**, **5**, and **6** exhibited 75, 71 and 81% anti-inflammatory activity with $IC_{50}$ values of 348, 422 and 396 μM, respectively (Table I and Figure 1).

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