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Short communication

Synthesis and pharmacological evaluation of 2-substituted benzo[*b*]thiophenes as anti-inflammatory and analgesic agents

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Abstract

An efficient method for trapping isocyanate **4**, generated from the Curtius rearrangement, with ethyl alcohol to afford the carbamate **5** is reported. 5-Nitrobenzo[*b*]thiophene-2-carboxylic acid **1** is converted to the corresponding hydrazide **2** by the reaction with hydrazine hydrate and then to the azide **3** with nitrous acid, followed by thermal rearrangement, cooling, and trapping in one pot reaction. The carbamate **5** is treated with hydrazine hydrate to afford the desired, Zileuton analogue, 4-(5-nitrobenzo[*b*]thiophene-2-yl)semicarbazide **6**. Also the reactivity of hydrazide **2** towards some carboxyaldehydes and phenylisothiocyanate afforded the corresponding carbohydrazides **7**, **8** and phenylthiosemicarbazide **9**, respectively. Compounds **9**, **2** and **6**, respectively, were more potent as anti-inflammatory and anti-nociceptive agents.

Keywords: Zileuton; Benzo[*b*]thiophene; Anti-inflammatory and analgesic

1. Introduction

Inhibition of leukotriene synthesis has been an area of intense pharmaceutical interest as a means of targeting inflammatory and vascular diseases [1]. The 5-lipoxygenase enzyme catalyzes the formation of leukotriene-A4 ultimately from arachidonic acid [2]. The synthesis of 2-substituted benzo[*b*]-thiophenes is important as such compounds have a range of useful pharmaceutical properties. Zileuton (Zyflo) [*N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea] **I**, for example (Fig. 1), is a potent and selective inhibitor of 5-lipoxygenase [3]. Zileuton was discovered at Abbott Laboratories [4], as the first selective 5-lipoxygenase inhibitor that receives approval by the FDA. While many 2-substituted benzothiophenes are selective estrogen receptor modulators and one such compound, raloxifene **II** (Fig. 1), is used to treat osteoporosis [5]. Some inhibit serine proteases, such as thrombin [6,7] and factor Xa [8–10], and so have potential as anticoagulants, or inhibit the cysteine protease cathepsin K providing a potential alternative route for the treatment of osteoporosis [11].

Optimistic by the above observations and considering the interesting pharmacological profile of Zileuton, we turn our concentration to synthesize new 2-substituted benzo[*b*]-thiophenes. In the present study, the biological activities and structure–activity relationship (SAR) of the newly synthesized compounds were evaluated in comparison with classic NSAID piroxicam drug and were found to possess potent anti-inflammatory and analgesic activities.

2. Results and discussion

2.1. Chemistry

The Curtius rearrangement has proven itself to be a versatile and important chemical transformation. An important practical feature of this reaction is that a diverse assortment of carboxylic acids can be converted into their corresponding acyl

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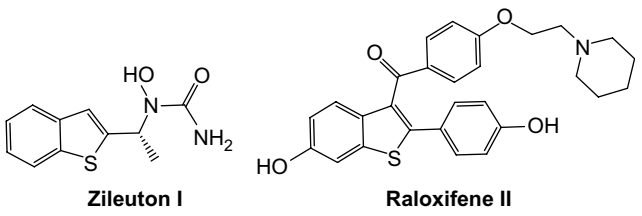


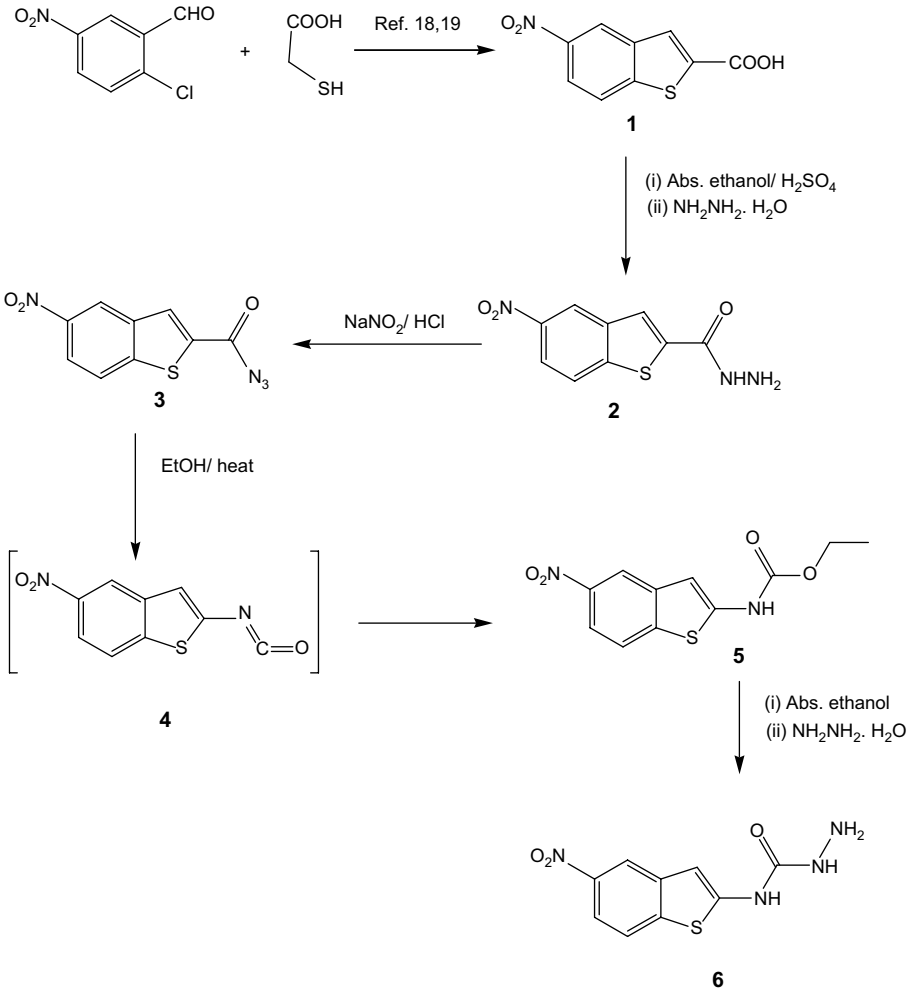
Fig. 1. Examples of 2-substituted benzo[*b*]thiophene drugs.

azides which can undergo thermal rearrangement to isocyanates in one pot reaction. Isocyanates considered an important intermediate in the synthesis of a variety of heterocyclic scaffolds, such as hydantoins [12] and quinazoline-2,4-diones [13]. Recently, the generation of isocyanates by the Curtius rearrangement and their succeeding trapping with resin-bound alcohols has been demonstrated [14].

In our continuing efforts towards the synthesis and pharmacological study of non-steroidal anti-inflammatory and analgesic agents [15] and also towards the synthesis of bioactive indole and benzo[*b*]thiophene compounds [16,17], we report here synthesis of some new 2-substituted benzo[*b*]thiophenes as promising anti-inflammatory and analgesic agents.

One pot reaction of 5-nitrobenzo[*b*]thiophene-2-carboxylic acid **1** [18,19], in absolute ethanol in the presence of concentrated H_2SO_4 and then the reaction mixture was gently heated for 10 h to give the intermediate ester, subsequently, hydrazine hydrate was added to the reaction mixture which was further refluxed for 3 h to afford the corresponding 5-nitrobenzo[*b*]thiophene-2-carbohydrazide **2**. Then, the carbohydrazide **2** was treated with nitrous acid to produce 5-nitrobenzo[*b*]thiophene-2-carboazide **3**. Next, the carboazide **3** underwent a thermal Curtius rearrangement to the isocyanate intermediate **4** which was trapped with absolute ethanol as nucleophilic agent to provide the corresponding ethyl (5-nitrobenzo[*b*]thiophene-2-yl) carbamate **5**. And then, refluxing the ethyl carbamate **5** with hydrazine hydrate in absolute ethanol afforded the desired, Zileuton analogue, 4-(5-nitrobenzo[*b*]thiophene-2-yl)semicarbazide **6** (Scheme 1). The structures of the synthesized compounds were assigned on the basis of its elemental analysis and spectral data (cf. Section 4).

Furthermore, condensation of carbohydrazide **2** with some heterocyclic carboxyaldehydes, such as indole-3-carboxyaldehyde and 5-methylfuran-2-carboxyaldehyde in refluxing



Scheme 1.

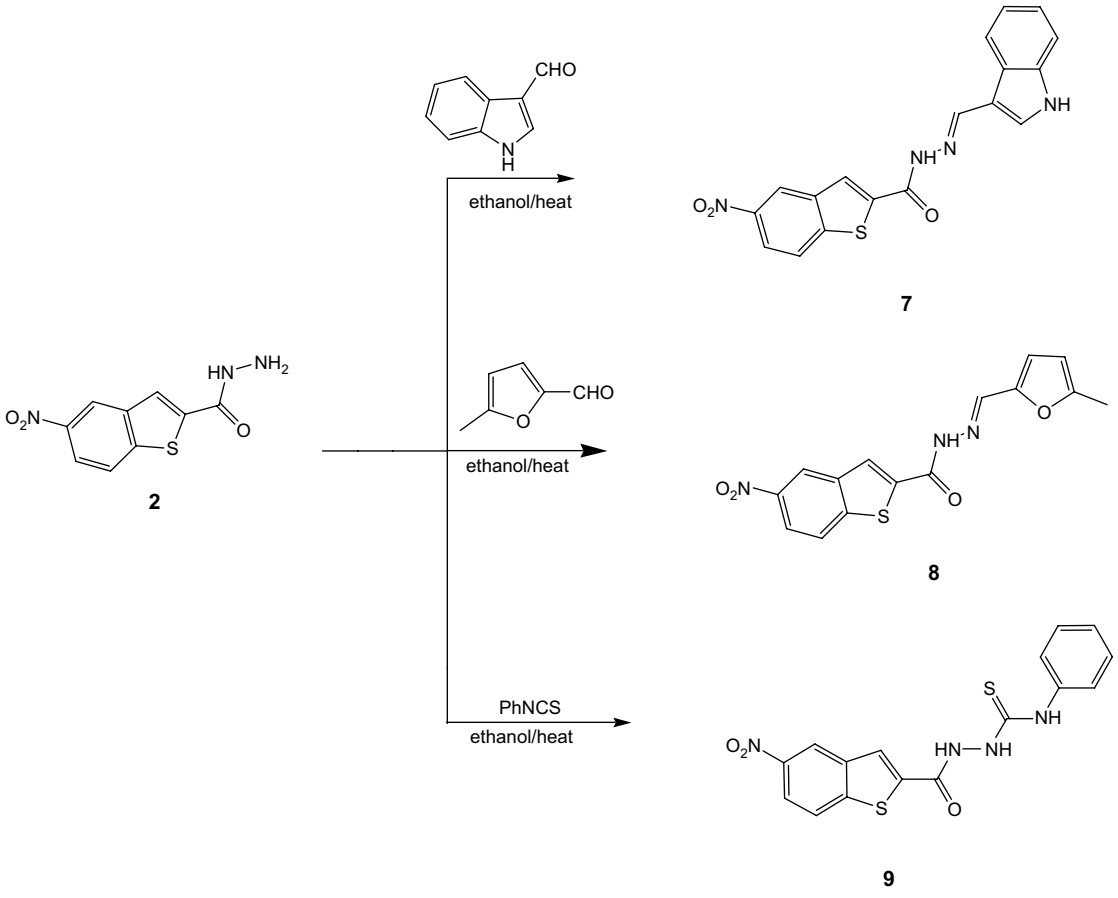
absolute ethanol afforded *N*-((1*H*-indol-3-yl)methylene)-5-nitrobenzo[*b*]thiophene-2-carbohydrazide **7** and *N*-((5-methylfuran-2-yl)methylene)-5-nitrobenzo[*b*]thiophene-2-carbohydrazide **8**, respectively. In addition, the reaction of carbohydrazide **2** with phenylisothiocyanate in refluxing absolute ethanol provided 1-(5-nitrobenzo[*b*]thiophene-2-carbonyl)-4-phenylthiosemicarbazide **9** (Scheme 2). The structures of compounds **7**–**9** were assigned on the basis of its elemental analysis and spectral data (cf. Section 4).

2.2. Pharmacology

2.2.1. Anti-inflammatory activity

All tested compounds **2**, **6**–**9** were screened for anti-inflammatory activity using the carrageenan-induced paw oedema assay in rats. Their activity was compared with the classic NSAID piroxicam. Percent oedema inhibition was calculated as a regard to saline control group. After, s.c., administration, all compounds, except for the small dose of compound **8** induced remarkable anti-inflammatory activity as depicted in (Table 1, Fig. 2). Compound **2** at the dose of 25 mg/kg inhibited the paw oedema response compared with the control group at 2, 3 and 4 h post-carrageenan (−48, −50.1 and −52.7%), vs control values at corresponding time point. The higher dose (50 mg/kg) of compound **2** inhibited oedema at all time points in this test (−25.3, −57.7, −58.7 and −56.6%). The control drug piroxicam used in the study inhibited the oedema response by −30, −57.1, −54.1 and −56.5% at 1, 2, 3 and 4 h post-carrageenan, respectively. The oedema response was less considerably reduced by prior administration of compound **6**, Zileuton analogue, compared with piroxicam at 2, 3 and 4 h post-carrageenan, the percentages of inhibition were −28.7, −29.5 and −32.2% or −54, −51.5 and −52.3 at doses of 25 or 50 mg/kg, respectively. The small dose of compound **7** inhibited the oedema response by −19.6 and −23.6 at 3 and 4 h post-carrageenan. The higher dose of 50 mg/kg exhibited more marked inhibition of the oedema response and the percentages of maximal inhibition were −27.9, −53.4, −58.5 and −58% at 1, 2, 3 and 4 h time points. Only at the higher dose of 50 mg/kg of compound **8** caused significant inhibition of paw oedema formation and the percentages of maximal inhibition were −26.8, −58.3, −62.9 and −64.7% at 1, 2, 3 and 4 h time points. Compound **9** observably inhibited the paw oedema compared with saline-treated control group, with a maximal reduction of paw oedema of −21.8, −52.8, −51.3 and −50.3% by 25 mg/kg and −30.3, −57.3, −59.5 and −60.8% by 50 mg/kg of the compound, observed 1, 2, 3 and 4 h post-carrageenan, respectively.

In conclusion, compounds **9**, **2** and **6**, respectively, were more potent among the tested compounds as anti-inflammatory and anti-nociceptive agents at dose 25 or 50 mg/kg.



Scheme 2.

Table 1
Effect of compounds **2**, **6**–**9** on paw oedema response to carrageenan injection in the rat

Group	Paw oedema (%)			
	1 h	2 h	3 h	4 h
Control	51.2 ± 1.6	99.9 ± 3.3	112.0 ± 4.7	118.2 ± 3.4
Piroxicam (10 mg/kg)	35.9 ± 3.4*	42.9 ± 4.0*	51.4 ± 3.5*	51.4 ± 3.4*
Compound **2** (25 mg/kg)	49.4 ± 1.5	51.9 ± 2.4*	55.9 ± 2.7*	55.9 ± 2.8*
Compound **2** (50 mg/kg)	38.2 ± 2.6*	42.2 ± 3.2*	46.3 ± 4.3*	51.3 ± 1.8*
Compound **6** (25 mg/kg)	59.7 ± 2.1	71.2 ± 3.5*	79.0 ± 3.8*	80.0 ± 3.7*
Compound **6** (50 mg/kg)	45.9 ± 3.1	45.9 ± 3.2*	54.3 ± 2.7*	56.4 ± 2.6*
Compound **7** (25 mg/kg)	63.2 ± 3.5	89.1 ± 3.9	90.1 ± 4.4*	90.0 ± 4.7*
Compound **7** (50 mg/kg)	36.9 ± 2.3*	46.5 ± 0.9*	46.6 ± 1.2*	49.6 ± 1.3*
Compound **8** (25 mg/kg)	55.9 ± 3.3	77.2 ± 6.1	92.2 ± 3.8*	92.0 ± 3.7*
Compound **8** (50 mg/kg)	37.5 ± 1.9*	41.6 ± 3.2*	41.3 ± 3.0*	40.7 ± 3.2*
Compound **9** (25 mg/kg)	40.0 ± 2.2*	48.1 ± 3.2*	54.6 ± 3.5	58.7 ± 2.4*
Compound **9** (50 mg/kg)	35.7 ± 1.8*	42.7 ± 2.2*	45.3 ± 0.6*	46.2 ± 2.1*

Data are expressed as mean ± SE, $n = 6$ per group. The values indicate the percentage increase of paw volume (oedema) from control (basal pre-drug) values. Drugs were, s.c., administered 30 min before the injection of carrageenan. Statistically significant from vehicle control at corresponding time point: *$P < 0.05$.

2.2.2. Analgesic activity

Next, the analgesic activity of the synthesized 2-substituted benzo[*b*]thiophenes was also investigated. It was assessed by two different models: the acetic acid-induced writhing test and hot-plate test.

2.2.2.1. Hot-plate assay. The mean reaction time on the hot plate was significantly delayed after the administration of tested compounds **2**, **6**, **8** and **9**, compared with basal values, denoting decreased nociception. The percentage increase in the pain threshold for these compounds were 24.7 and 31.6% for compound **2** at 1 and 2 h post-treatment, 19.4 and 37.5% for compound **6** at 1 and 2 h post-drug, 19.8% for compound **8** at 1 h post-treatment and 36.5% for compound **9** at 1 h post-drug. Compounds **8** and **9** showed analgesic activity only 1 h post-treatment. Compound **7** showed weak analgesic activity that did not reach statistical significance. The NSAID piroxicam increased hot-plate latency by 21.4% 1 h post-treatment (Table 2, Fig. 3). Compounds **2**, **6**, and **9** showed more analgesic activity in comparison to piroxicam.

2.2.2.2. Acetic acid-induced writhing. Acetic acid-induced writhing was significantly reduced in mice receiving the compounds **2**, **6**, **8** and **9**. The degree of inhibition of the writhing response by these compounds was −88.2, −43.4, −43.1 and −63.4%, respectively, compared to the saline-treated control group. The analgesic activity of the compound **2** was significantly higher than that of the control drug piroxicam (−88.2 vs −56.9%). Compound **7** did not exhibit analgesic activity in this test (Table 3, Fig. 4).

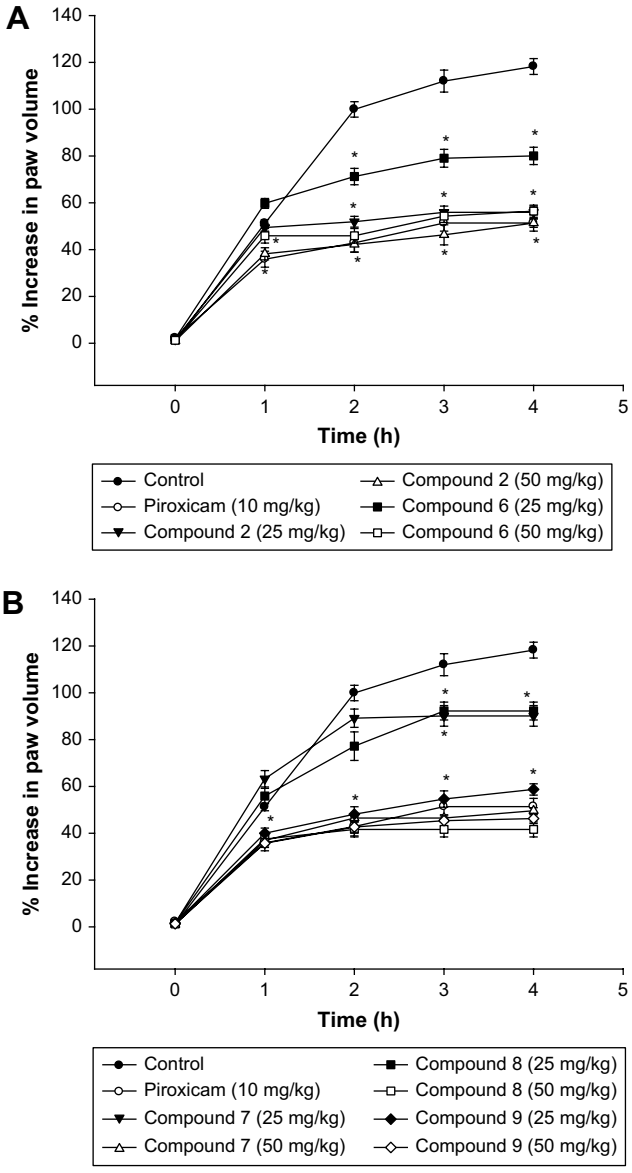


Fig. 2. (A) The anti-inflammatory effect of compounds **2** and **6** administered at doses of 25 and 50 mg/kg. The control drug piroxicam was administered at 10 mg/kg. Drugs were given, s.c., 30 min prior to subplantar carrageenan injection. Results are expressed as a percentage change from control (pre-drug) values, each point represents mean ± SE of six rats per group. Asterisks indicate significant change from control value at respective time points (ANOVA and Duncan's multiple comparison test). (B) The anti-inflammatory effect of compounds **7**–**9** administered at doses of 25 and 50 mg/kg. The control drug piroxicam was administered at 10 mg/kg. Drugs were given, s.c., 30 min prior to subplantar carrageenan injection. Results are expressed as a percentage change from control (pre-drug) values, each point represents mean ± SE of six rats per group. Asterisks indicate significant change from control value at respective time points (ANOVA and Duncan's multiple comparison test).

According to the structure–activity relationship (SAR) point of view it is clear that the activity of phenylthiosemicarbazide **9**, carbohydrazide **2** and semicarbazide **6**, systems is more potent than carbohydrazide **7**, **8** (which contains

Table 2
Analgesic effect of compounds **2**, **6**–**9** and piroxicam on thermal pain by using hot-plate test

Treatment group	Latency (s)		
	Basal	1 h (% change)	2 h (% change)
Saline control	14.61 ± 1.0	15.0 ± 0.8	15.3 ± 0.6
Piroxicam (10 mg/kg)	16.42 ± 0.7	19.94 ± 0.8 (21.4%)*	17.78 ± 1.0 (8.3%)
Compound **2** (25 mg/kg)	15.2 ± 1.0	18.95 ± 0.6 (24.7%)*	20.0 ± 0.8 (31.6%)*
Compound **6** (25 mg/kg)	16.0 ± 0.8	19.1 ± 0.7 (19.4%)*	22.0 ± 1.0 (7.5%)*
Compound **7** (25 mg/kg)	14.66 ± 1.1	17.1 ± 0.9 (16.6%)	14.72 ± 0.9
Compound **8** (25 mg/kg)	17.54 ± 0.6	21.02 ± 0.8 (19.8%)*	15.88 ± 0.7 (22.39%)
Compound **9** (25 mg/kg)	14.96 ± 0.9	20.42 ± 0.7 (36.5%)*	16.66 ± 0.8

Basal, 1 and 2 h values of hot-plate latency (seconds) of saline (control) and compounds **2**, **6**–**9**-treated rats. Drugs were, s.c., administered 1 h before testing. Data represent the mean value ± SE of six rats per group. Statistical comparisons are made between basal and post-drug values and denoted by $^*P < 0.05$.

azomethine moiety, CH═N, connected to indole and furan ring, respectively).

2.2.3. Gastric ulcerogenic studies

The oral administration of compounds **2**, **6**–**9** did not cause any gastric mucosal lesions in the rat stomach at the highest dose (50 mg/kg), indicating that these compounds are devoid of gastric irritant properties.

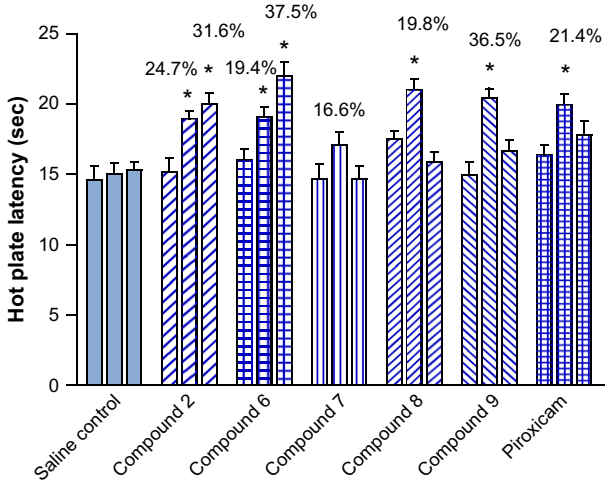


Fig. 3. Reaction time on the hot plate in seconds after the administration of compounds **2**, **6**–**9** (25 mg/kg) and piroxicam (10 mg/kg), respectively. Basal, 1 and 2 h latencies were determined for each treatment group (saline, piroxicam, compounds **2**, **6**–**9**). Each column represents mean ± SE of six rats per group. The first column represents the basal (pre-drug) latencies and the second and third columns represent the 1 and 2 h (post-drug) values of each treated group, respectively. Saline or drugs were, s.c., administered 1 h prior to testing. Percent increase in hot-plate latencies (%) compared to the baseline is also shown in top of bars. Hot-plate latencies comparisons were made between baseline paw withdrawal latencies (pre-drug values) as compared with the respective post-drug values. Asterisks indicate significant change from the corresponding baseline value (ANOVA and Duncan's multiple comparison test).

Table 3
Analgesic effect of compounds **2**, **6**–**9** and piroxicam on abdominal constrictions induced by acetic acid in mice

Treatment	Number of abdominal constrictions (% inhibition)
Saline control	124.0 ± 4.4
Piroxicam (10 mg/kg)	53.4 ± 3.5 (−56.9%)*
Compound **2** (25 mg/kg)	14.6 ± 0.8 (−88.2%)*
Compound **6** (25 mg/kg)	70.2 ± 3.1 (−43.41%)*
Compound **7** (25 mg/kg)	145.0 ± 2.7 (16.9%)
Compound **8** (25 mg/kg)	70.6 ± 3.4 (−43.1%)*
Compound **9** (25 mg/kg)	45.4 ± 0.8 (−63.4%)*

Data represent the mean value ± SE of six mice per group. Drugs were, s.c., administered 30 min before testing. Statistical comparisons are made between saline control group and drug-treated groups and denoted by $^*P < 0.05$.

2.2.4. Assessment of liver enzymes

Serum levels of AST and ALT were not significantly changed by the oral administration of compounds **2**, **6**–**9** at the highest dose (50 mg/kg) (Table 4).

3. Conclusions

In the present study we have described a straightforward and efficient synthesis of new bioactive 2-substituted benzo[*b*]thiophene derivatives, Zileuton analogues, starting from simply prepared 5-nitrobenzo[*b*]thiophene-2-carboxylic acid **1**. The biological activities and structure–activity relationship (SAR) of the newly synthesized compounds were evaluated in comparison with classic NSAID piroxicam drug. Compounds **9**, **2** and **6** were more potent as anti-inflammatory and anti-nociceptive agents; they are more potent in some times than piroxicam.

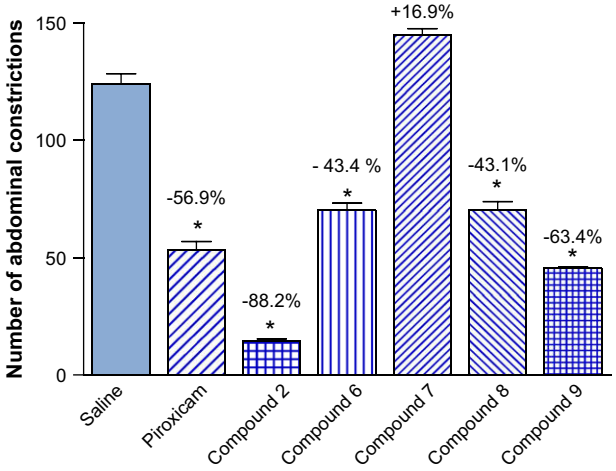


Fig. 4. Effect of compounds **2**, **6**–**9** (25 mg/kg) and piroxicam (10 mg/kg), on the number of abdominal constrictions caused by i.p. acetic acid in mice. $^*P < 0.05$ compared to control (ANOVA and Duncan's multiple comparison test). Data represent means of six observations (±SE). Percent inhibition (%) compared to the control animals is also shown on top of bars.

Table 4
Effect of compounds **2**, **6–9** on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats

Treatment	ALT	AST
Saline control	36.0 ± 3.4	77.2 ± 6.54
Compound **2** (50 mg/kg)	28.3 ± 3.0	65.7 ± 5.8
Compound **6** (50 mg/kg)	40.0 ± 3.6	74.7 ± 8.1
Compound **7** (50 mg/kg)	29.3 ± 2.0	66.2 ± 5.7
Compound **8** (50 mg/kg)	34.2 ± 2.4	89.9 ± 9.4
Compound **9** (50 mg/kg)	42.0 ± 4.4	79.8 ± 8.8

Data represent the mean value ± SE of six rats per group. Drugs were administered orally for 1 week.

4. Experimental section

4.1. Chemistry

4.1.1. General

All chemicals were purchased from commercial suppliers and used directly. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8201 PC infrared spectrophotometer and expressed in cm^{-1}. ^1H NMR spectra were recorded on a Jeol EX-270 MHz spectrometer using DMSO-d_6 as solvent and TMS as the internal standard. Mass spectra were recorded on a Finnigan mat. SSQ-7000 GC–MS spectrometer. Microanalyses were performed at the Microanalytical Center of Cairo University. Analytical thin-layer chromatography (TLC) was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). 5-Nitrobenzo[*b*]thiophene-2-carboxylic acid **1** was prepared following the procedures reported in the Refs. [18,19].

4.1.2. Synthesis of the 5-nitrobenzo[b]thiophene-2-carbohydrazide (2)

A solution of 5-nitrobenzo[*b*]thiophene-2-carboxylic acid **1** (2.23 g, 10 mmol) in absolute ethanol (20 mL) in the presence of concentrated H_2SO_4 (5 mL) was gently heated for 10 h to give the intermediate ester; subsequently, hydrazine hydrate (2 mL) was added to the reaction mixture which was further refluxed for 3 h. Then the reaction mixture was left to cool at room temperature and poured over ice/water. The formed solid product was filtered off, washed with ethanol to afford 5-nitrobenzo[*b*]thiophene-2-carbohydrazide **2** as white solid (2.1 g, 88.6%). Recrystallization from EtOH/DMF gave analytically pure material; mp 239–241 °C. IR (KBr) ν_{max} 3178 (NH), 3172 (NH), 1658 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.45 (br s, 2H, NH_2), 7.47 (br s, 1H, NH, D_2O-exchangeable), 8.3–8.4 (dd, 2H, H-6, H-7), 8.45 (s, 1H, H-3), 8.9 (s, 1H, H-4). EIMS: *m/z* (%) 237, M^+ (15), 222 (69), 206 (100), 193 (18), 177 (22), 160 (79), 149 (21), 93 (25), 89 (43), 75 (17), 62 (19). Anal. Calcd for $C_9H_7N_3O_3S$: C, 45.57; H, 2.97; N, 17.71; O, 20.23; S, 13.52. Found: C, 45.53; H, 2.99; N, 17.75; O, 20.27; S, 13.50.

4.1.3. Synthesis of the 5-nitrobenzo[b]thiophene-2-carboazide (3)

To a mixture of carbohydrazide **2** (2.37 g, 10 mmol) and concentrated hydrochloric acid (5 mL), sodium nitrite solution (10 mL, 10 mmol) was added at 0 °C with stirring for a period of 30 min. The mixture was filtered off to leave the corresponding 5-nitrobenzo[*b*]thiophene-2-carboazide **3** as a white solid (1.85 g, 75%). Recrystallization from EtOH gave analytically pure material; mp 210–211 °C. IR (KBr) ν_{max} 1698 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.25–8.35 (dd, 2H, H-6, H-7), 8.40 (s, 1H, H-3), 8.95 (s, 1H, H-4). EIMS: *m/z* (%) 248, M^+ (17), 220 (52), 206 (100), 192 (24), 176 (22), 160 (74), 147 (34), 91 (26), 89 (39), 75 (18), 62 (12). Anal. Calcd for $C_9H_4N_4O_3S$: C, 43.55; H, 1.62; N, 22.57; O, 19.34; S, 12.92. Found: C, 43.50; H, 1.68; N, 22.54; O, 19.56; S, 12.91.

4.1.4. Synthesis of the ethyl (5-nitrobenzo[b]thiophene-2-yl) carbamate (5)

A mixture carboazide **3** (2.48 g, 10 mmol) and (25 mL) absolute ethanol was refluxed for 3 h. After cooling, the mixture was filtered off to furnish ethyl (5-nitrobenzo[*b*]thiophene-2-yl) carbamate **5** as a pall yellow solid (1.45 g, 55%). Recrystallization from EtOH gave analytically pure material; mp 155–157 °C. IR (KBr) ν_{max} 3197 (NH), 1713 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 8.35–8.40 (dd, 2H, H-6, H-7), 8.42 (br s, 1H, NH, D_2O-exchangeable), 8.45 (s, 1H, H-3), 9.1 (s, 1H, H-4). EIMS: *m/z* (%) 266, M^+ (13), 221 (23), 209 (100), 194 (21), 164 (76), 148 (33), 91 (16), 89 (45), 75 (14), 62 (11). Anal. Calcd for $C_{11}H_{10}N_2O_4S$: C, 49.62; H, 3.79; N, 10.52; O, 24.03; S, 12.04. Found: C, 49.56; H, 3.73; N, 10.56; O, 24.11; S, 12.01.

4.1.5. Synthesis of the 4-(5-nitrobenzo[b]thiophene-2-yl) semicarbazide (6)

A mixture ethyl carbamate **5** (2.66 g, 10 mmol) and hydrazine hydrate (2 mL) in ethanol (20 mL) was refluxed for 4 h. After cooling, the mixture was filtered off to furnish 4-(5-nitrobenzo[*b*]thiophene-2-yl)semicarbazide **6** as white solid (2 g, 79%). Recrystallization from EtOH/DMF gave analytically pure material; mp 245–247 °C. IR (KBr) ν_{max} 3170–3260 (2NH) and (NH_2), 1670 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.9 (br s, 2H, NH_2), 8.25–8.35 (two dd, 2H, H-6, H-7), 8.43 (br s, 1H, NH, D_2O-exchangeable), 8.47 (s, 1H, H-3), 8.85 (s, 1H, H-4). EIMS: *m/z* (%) 252, M^+ (11), 237 (17), 222 (61), 206 (100), 191 (23), 175 (25), 160 (83), 144 (32), 91 (14), 89 (41), 75 (19), 62 (16). Anal. Calcd for $C_9H_8N_4O_3S$: C, 42.85; H, 3.20; N, 22.21; O, 19.03; S, 12.71. Found: C, 42.78; H, 3.17; N, 22.19; O, 19.11; S, 12.69.

4.1.6. General method for preparation of compounds (7) and (8)

A mixture of 5-nitrobenzo[*b*]thiophene-2-carbohydrazide **2** (0.18 g, 0.76 mmol) and the appropriate heterocyclic carboxyaldehydes, such as indole-3-carboxyaldehyde and 5-methylfuran-2-carboxyaldehyde (0.76 mmol) was refluxed in

absolute ethanol for a period of 4 h. After cooling, the mixture was filtered off to provide the corresponding product (compounds **7** and **8**), which was crystallized from the appropriate solvent.

*4.1.6.1. N-((1H-indol-3-yl)methylene)-5-nitrobenzo[b]thiophene-2-carbohydrazide (**7**).* Yellow solid (0.25 g, 90%); mp 90–92 °C (from EtOH/DMF). IR (KBr) ν_{max} 3220–3310 (2NH), 1677 (C=O), 1574 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.17 (br s, 1H, NH), 7.21 (m, 2H, H-5′, H-6′, indole), 7.82 (d, 1H, H-7′, indole), 8.2 (s, 1H, H-2′, indole), 8.25–8.35 (two dd, 2H, H-6, H-7, benzothiophene), 8.41 (s, 1H, H-3, benzothiophene), 8.45 (d, 1H, H-4′, indole), 8.75 (s, 1H, H-4, benzothiophene), 8.9 (s, 1H, CH=N), 12.01 (s, 1H, NH). MS: *m/z* (%) 364 (M^+, 7), 318 (15), 222 (13), 208 (14), 156 (72), 110 (100), 77 (67). Anal. Calcd for $C_{18}H_{12}N_4O_3S$: C, 59.33; H, 3.32; N, 15.38; O, 13.17; S, 8.80. Found: C, 59.26; H, 3.28; N, 15.34; O, 13.26; S, 8.76.

*4.1.6.2. N-((5-methylfuran-2-yl)methylene)-5-nitrobenzo[b]thiophene-2-carbohydrazide (**8**).* Yellow (0.23 g, 92%); mp 78–80 °C (from EtOH/DMF). IR (KBr) ν_{max} 3214 (NH), 1694 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.7 (s, 3H, CH_3), 6.1 (d, 1H, H-4, furan proton), 6.7 (d, 1H, H-3, furan proton), 7.2 (br s, 1H, NH), 8.24–8.38 (two dd, 2H, H-6, H-7, benzothiophene), 8.40 (s, 1H, H-3, benzothiophene), 8.74 (s, 1H, CH=N), 8.8 (s, 1H, H-4, benzothiophene). MS: *m/z* (%) 329 (M^+, 24), 283 (41), 234 (69), 222 (12), 208 (19), 167 (18), 136 (31), 103 (19), 92 (32), 77 (100), 64 (52). Anal. Calcd for $C_{15}H_{11}N_3O_4S$: C, 54.71; H, 3.37; N, 12.76; O, 19.43; S, 9.74. Found: C, 54.63; H, 3.32; N, 12.75; O, 19.48; S, 9.70.

*4.1.7. Synthesis of the 1-(5-nitrobenzo[b]thiophene-2-carbonyl)-4-phenylthiosemicarbazide (**9**)*

A mixture of 5-nitrobenzo[*b*]thiophene-2-carbohydrazide **2** (0.237 g, 1 mmol) and phenylisothiocyanate (0.135 g, 1 mmol) was refluxed in absolute ethanol for 4 h. After cooling, the mixture was filtered off to afford 1-(5-nitrobenzo[*b*]thiophene-2-carbonyl)-4-phenyl-thiosemicarbazide (**9**). Recrystallization from EtOH/DMF gave analytically pure material; Yellow (0.34 g, 91%); mp 217–219 °C. IR (KBr) ν_{max} 3164–3325 (3NH), 1687 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.3 (br s, 2H, 2NH), 7.15–7.4 (m, 5H, Ph), 8.20–8.35 (two dd, 2H, H-6, H-7), 8.45 (s, 1H, H-3), 9.0 (s, 1H, H-4), 11.1 (br s, 1H, NH, D_2O-exchangeable). EIMS: *m/z* (%) 373, M^+ (5), 267 (25), 222 (8), 208 (12), 167 (10), 135 (40), 104 (19), 93 (42), 77 (100), 64 (22). Anal. Calcd for $C_{16}H_{12}N_4O_3S_2$: C, 51.60; H, 3.25; N, 15.04; O, 12.89; S, 17.22. Found: C, 51.51; H, 3.22; N, 15.01; O, 12.94; S, 17.19.

4.2. Pharmacological assay

4.2.1. Animals

Sprague–Dawley strain rats weighing 120–130 g or Swiss albino mice 20–25 g body weight (National Research Centre, Cairo) was used. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised 1985). Equal groups of six rats or mice/group were used in all experiments. Experiments were performed between 9 am and 3 pm.

4.2.2. Tests of inflammation: carageenin-induced paw oedema assay

All tested compounds **2**, **6–9** were screened for anti-inflammatory activity using the carrageenan-induced paw oedema assay in rats. Their activity was compared with the classic NSAID piroxicam. Paw oedema was induced by subplantar injection of 100 μL of 1% sterile carrageenan lambda in saline into the right hind paw [20]. Contra-lateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). The oedema component of inflammation was quantified by measuring the increase in paw volume (mL) at before carrageenan injection and at 1, 2, 3 and 4 h after carrageenan injection with respect to the pre-injection value for each animal. Oedema was expressed as a percentage of change from control (pre-drug) values. The effect of systemic administration of each of the test drugs at doses of 25 or 50 mg/kg (0.5 mL, s.c., $n = 6$ per group) or piroxicam (10 mg/kg, s.c., 0.5 mL) given as a 30 min pretreatment was studied. The control groups received saline (0.5 mL, $n = 6$ per group, s.c.) instead.

In anti-inflammatory study, the percentage of oedema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\%\ \text{Oedema inhibition} = \{[(\%\ \text{oedema formation of control group} - \%\ \text{oedema formation of treated group})]/[\%\ \text{oedema formation of control group}]100\}$$

4.2.3. Anti-nociceptive activity

This activity was determined by measuring the responses of animals to the Koster test and hot-plate test.

4.2.3.1. Hot-plate assay. The hot-plate test was performed on rats by using an electronically controlled hot plate (Ugo Basile, Italy) heated to 52 °C (±0.1 °C) [21]. The cut-off time was 30 s. Groups of rats ($n = 6$ per group) were given different compounds **2**, **6**, **7**, **8** or **9** at the dose of 25 mg/kg, s.c., saline (control), or piroxicam at 10 mg/kg, s.c., 1 h minimum prior to testing. The experimenter was blind to dose and treatment. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups. Pain thresholds were measured sequentially before and at 1 and 2 h post-treatment.

4.2.3.2. Acetic acid-induced writhing (Koster test). Separate groups of six mice each were administered vehicle and/or

drug under study, s.c., at the dose of 25 mg/kg. After 30 min pretreatment interval, an i.p. injection of 0.6% acetic acid (0.4 mL) was administered [22]. Each mouse was then placed in an individual clear plastic observational chamber, and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration (Fig. 4).

4.2.4. Gastric ulcerogenic studies

Rats received either saline (0.2 mL/rat, s.c., $n = 6$) (control) or drugs under study at the dose of 50 mg/kg orally daily for 1 week [23]. Rats were killed and gastric mucosa inspected for the presence of any lesions.

4.2.5. Assessment of liver enzymes

Rats received either saline (0.2 mL/rat, s.c., $n = 6$) (control) or drugs under study at the dose of 50 mg/kg orally daily for 1 week. At the end of the experiments, blood samples were obtained from the retro-orbital vein plexuses, under ether anaesthesia. ALT and AST activities in serum were measured according to Reitman–Frankel colorimetric transaminase procedure [24] using commercially available kits (BioMérieux, France).

4.2.6. Statistical analyses

Data are expressed as mean ± SE. The results of carrageenan-induced paw oedema experiments are expressed as a percentage of change from control (pre-drug) values. Differences between vehicle control and treatment groups were tested using one- and two-way ANOVA followed by multiple comparisons by the Duncan's multiple comparison test. A probability value less than 0.05 was considered to be significant.

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