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ORIGINAL PAPER

Synthesis and biological evaluation of 5-substituted benzo[*b*]thiophene derivatives as anti-inflammatory agents

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Abstract 5-Aminobenzo[*b*]thiophene-2-carboxylic acid was converted to the corresponding 5-(2-chloroacetamido)benzo[*b*]thiophene-2-carboxylic acid by reaction with chloroacetyl chloride. This acetamido product was treated with different alkyl, cycloalkyl, aryl, and heterocyclic amines to afford a series of C5-substituted benzo[*b*]thiophenes. These compounds were found to possess potent anti-inflammatory activity.

Keywords Benzo[*b*]thiophene · Acetamide · Anti-inflammatory

Introduction

The clinical application of traditional nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or indomethacin for treatment of inflammation and pain is often accompanied by adverse gastrointestinal effects. Their anti-inflammatory activity is because of inhibition of cyclooxygenases (COXs), which catalyze the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs) [1, 2]. PGs that are produced via the inducible COX-2 isozyme are responsible for inflammation, pain, and fever, whereas the constitutively expressed COX-1 isozyme produces PGs that have beneficial cytoprotective properties [3]. The initial euphoria surrounding the launch of selective cyclooxygenase-2 (COX-2) inhibitors with reduced gastrointestinal toxicity in the late 1990s [4, 5] has proven to be short-lived. The benzo[*b*]thiophene moiety is an important core structure found in biologically active small molecules. Zileuton, for example, is a potent and selective inhibitor of 5-lipoxygenase [6]. Various substituted benzothiophenes are selective estrogen receptor modulators, and one such compound, raloxifene, is used to treat osteoporosis [7]. Some inhibit serine proteases, such as thrombin [8, 9] and factor Xa [10–13], and so have potential as anticoagulants, or inhibit the cysteine protease cathepsin K, providing a potential alternative route for treatment of osteoporosis [14]. Recently, we have reported an investigation describing the design, synthesis, and anti-inflammatory and analgesic properties of novel 2-substituted benzo[*b*]thiophene derivatives [15]. We have therefore designed and synthesized a series of C5-substituted benzo[*b*]thiophenes incorporating alkyl, cycloalkyl, aryl, and heterocyclic acetamido moieties. The biological activity and structure–activity relationships (SAR) of the newly synthesized compounds were evaluated in comparison with indomethacin and the new compounds were found to possess potent anti-inflammatory activity.

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Results and discussion

Chemistry

The starting compound 5-aminobenzo[*b*]thiophene-2-carboxylic acid (**1**) was prepared according to [16–18] and converted to the corresponding 5-(2-chloroacetamido)benzo[*b*]thiophene-2-carboxylic acid **2** by reaction with chloroacetyl chloride in pyridine under cooling in an ice bath. The structure of the new compound **2** was established

on the basis of spectral data. The ^{1}H NMR spectrum of compound **2** revealed a singlet signal at $\delta = 4.46$ ppm characteristic of the methylene group of the chloroacetamido moiety. In addition, the mass spectrum revealed a peak at $m/z = 328$ corresponding to its molecular ion.

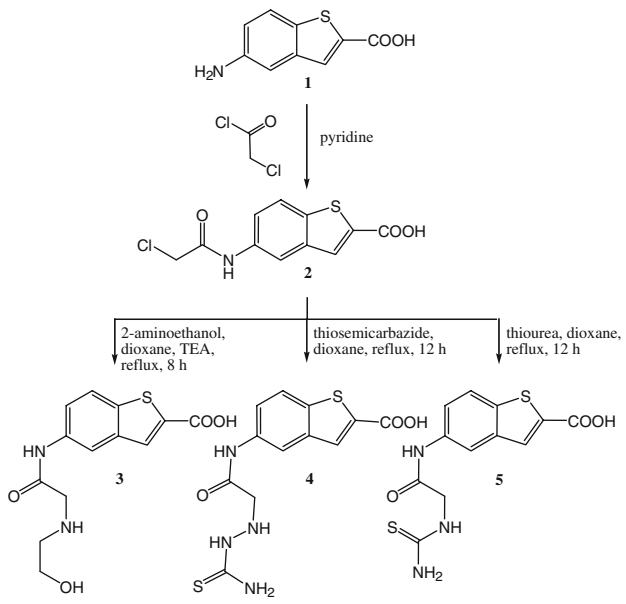
The acetamido derivative **2** was treated with different alkyl amines, such as 2-aminoethanol, thiosemicarbazide, and thiourea (Scheme 1), or cycloalkyl amines, such as 1-methylpiperazine and morpholine (Scheme 2), or aryl and heterocyclic amines, such as phenylhydrazine, 2-aminobenzothiazole, and 2-aminobenzoimidazole (Scheme 3) in dioxane–TEA under reflux for 8–12 h to afford the corresponding C5-substituted benzo[*b*]thiophene-2-carboxylic derivatives **3–10**. The structure of compounds **3–10** was established on the basis of spectral data. The ^{1}H NMR spectra of C5-substituted benzo[*b*]thiophene-2-carboxylic acid derivatives **3–10** revealed characteristic signals ranging from $\delta = 3.2$ to 4.1 ppm for the methylene group of the acetamido moiety.

It should be noted that reaction of compound **2** with thiourea as dinucleophile yields only the mononucleophilic substitution reaction product **5**; the bis isomer was excluded in accordance with the mass spectrum which revealed a molecular ion peak at $m/z = 309$.

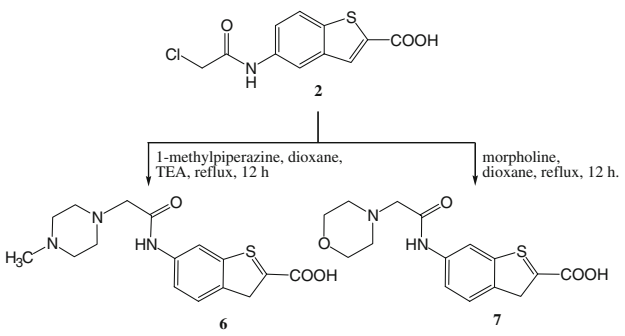
Pharmacology

Anti-inflammatory activity

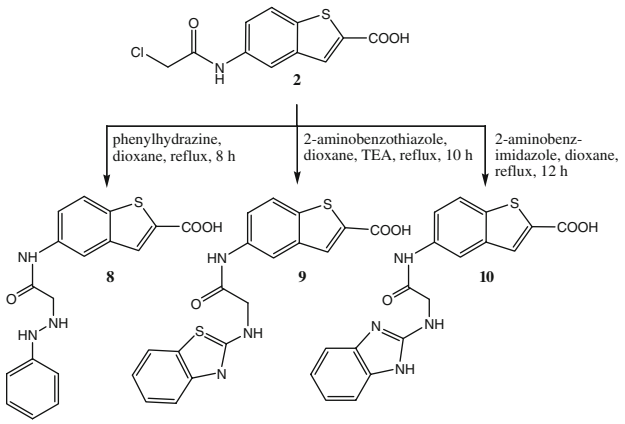
The activity of the newly synthesized compounds, compared with indomethacin as reference, was measured before and 1, 2, 3, and 4 h after carrageenan injection. Percentage oedema inhibition was calculated relative to a saline control group and the potency of the tested compounds was calculated as a percentage of the change resulting from use of indomethacin; the results are depicted in Table 1 and Fig. 1. Most of the tested compounds resulted in reasonable inhibition of oedema size in comparison with indomethacin. As shown in Table 1, 5-(2-(1*H*-benzoimidazol-2-ylamino) acetamido)benzo[*b*]thiophen-2-carboxylic acid (**10**) and compounds **3**, **5**, and **6** were found to be the most potent anti-inflammatory compounds 1 h after injection. Compounds **4** and **7** had the least anti-inflammatory effect. Compounds **7**, **8**, and **9** were most potent 4 h after injection.



Scheme 1



Scheme 2



Scheme 3

Gastric ulcerogenic studies

Oral administration of compounds **4**, **7**, and **9** at the highest dose (50 mg/kg) causes gastric mucosal lesions in the rat stomach. Compounds **2** and **8** led to a non-significant decrease of lesion numbers but a significant reduction of the severity of the lesions, by −50 and −40% relative to the control values (Table 2). In the control group, the number and the severity of gastric mucosal lesions evoked by oral administration of 100% ethanol were 12 ± 1 and 26 ± 2. This was significantly reduced by compounds **4**, **7**, and **9**. The number and severity of gastric mucosal lesions

Table 1 Time course effect of oral administration of tested compounds (50 mg/kg) and indomethacin (25 mg/kg) on rats' paw oedema formation induced by sub-plantar injection of 100 mm^3 1% carrageenan

Group	1 h		2 h		3 h		4 h	
	Oedema rate/%	Potency	Oedema rate/%	Potency	Oedema rate/%	Potency	Oedema rate/%	Potency
Control	60 ± 2	–	102 ± 8	–	101 ± 6	–	101 ± 4	–
2	41 ± 2* (−32)	0.4	50 ± 2* (−50.95)	0.7	65 ± 2* (−36)	0.5	68 ± 1* (−32)	0.5
3	30 ± 2* (−49)	0.6	53 ± 5* (−48)	0.6	66 ± 6* (−35)	0.5	74 ± 3* (−26)	0.4
4	46 ± 5* (−24)	0.3	61 ± 4* (−41)	0.5	68 ± 3* (−33)	0.5	69 ± 3* (−32)	0.5
5	31 ± 2 * (−49)	0.6	62 ± 6* (−39)	0.6	74 ± 5* (−27)	0.4	83 ± 6* (−18)	0.3
6	31 ± 3* (−48)	0.6	66 ± 7* (−35)	0.5	78 ± 6* (−23)	0.3	82 ± 4* (−18)	0.3
7	43 ± 1* (−28)	0.4	51 ± 1* (−50)	0.7	55 ± 2* (−45)	0.7	57 ± 3* (−43)	0.7
8	41 ± 2* (−32)	0.4	48 ± 4* (−53)	0.7	56 ± 4* (−44)	0.7	58 ± 3* (−42)	0.7
9	40 ± 4* (−34)	0.4	52 ± 3* (−49)	0.7	59 ± 3* (−42)	0.6	64 ± 2* (−37)	0.6
10	25 ± 2* (−58)	0.8	56 ± 5* (−45)	0.6	72 ± 5* (−29)	0.4	73 ± 7* (−27)	0.4
Indomethacin	13 ± 1* (−78)	1	26 ± 3* (−75)	1	33 ± 2* (−67)	1	38 ± 2* (−62)	1

Data represent the mean ± SE of six rats per group and the percent changes versus basal (zero min) values 1, 2, 3, and 4 h post-carrageenan injection

Percent oedema inhibition (the value in parentheses) was calculated as compared with the saline control group

Potency was calculated compared with the percentage change of the indomethacin-treated group

* Data were analyzed using one way ANOVA and Dunnett's multiple comparison test $P < 0.05$

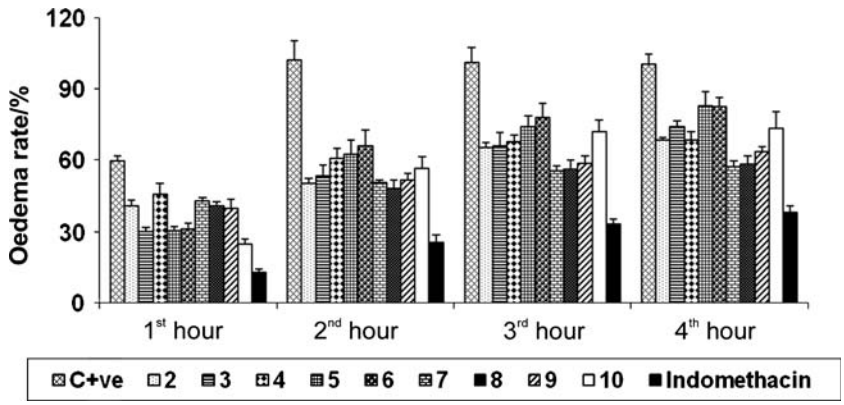


Fig. 1 Effect of oral administration of the tested compounds (50 mg/kg) and indomethacin (25 mg/kg) on rats' paw oedema formation induced by sub-plantar injection of 100 mm^3 1% carrageenan. Data represent the mean ± SE of six rats per group

Table 2 The effect of oral administration of tested compounds **2**, **4**, **7**, **8**, and **9** (50 mg/kg) on gastric mucosal injury induced by 1 cm^3 absolute ethanol in rats ($n = 6$)

Treated groups	Number of lesions/rat	Change/%	Severity of lesions/rat	Change/%
Ethanol control 100%	12. 2 ± 1.1		26.4 ± 2.3	
2	10.4 ± 0.9	−15	13.2 ± 1.2***	−50
4	8.8 ± 0.7*	−28	13.4 ± 0.5***	−49
7	7 ± 0.5**	−43	11 ± 0.9***	−58
8	12 ± 1.0	−2	15.6 ± 1.3**	−41
9	8.6 ± 0.6*	−30	9.8 ± 0.8***	−63

Data represent the mean ± SE of six rats per group

Statistical comparison of differences between the ethanol control group and treated groups was done by Student's *t* test. * $P < 0.050$, ** $P < 0.010$, and *** $P < 0.001$ significance versus control values

Percent of change was calculated compared with the ethanol control group

was reduced by −28, −49, −43, −58, −30, and 63% relative to the control values.

From the SAR viewpoint, the anti-inflammatory activity of the C5-substituted benzoimidazole moiety **10** was higher than that of the benzothiazole moiety **9**. Moreover, compound **3** with a hydroxyethylamino moiety and compound **5** with a substituted thiourea moiety showed higher activities than compound **4** with a thiosemicarbazide moiety. Compound **6** with a C5-methylpiperazinyl moiety had higher activity than compound **7** with a morpholino moiety. Compounds **2** and **8** did not cause any gastric mucosal lesions in the rat stomach at the highest dose (50 mg/kg).

Conclusions

In conclusion, we identified a series of novel C5-substituted benzothiophenes characterized by high-potency anti-inflammatory activity starting from simply prepared 5-aminobenzo[*b*]thiophene-2-carboxylic acid **1**. The biological activity and SAR of the newly synthesized compounds were evaluated in comparison with indomethacin. C5-substituted benzothiophenes were synthesized and the most active compounds were 5-(2-(1*H*-benzoimidazol-2-ylamino) acetamido)benzo[*b*]thiophene-2-carboxylic acid (**10**) and compounds **3**, **5**, and **6**. According to this study, we could point out that the C5-substituted benzothiophenes play an important role as anti-inflammatory agents. These observations may provide some predictions in order to design further substituted benzothiophene derivatives.

Experimental

Chemistry

All chemicals were purchased from commercial suppliers and used directly. Melting points were determined on a Gallenkamp melting-point apparatus. IR spectra were recorded on a Shimadzu FT-IR 8201 PC infrared spectrophotometer and are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Jeol EX-270 MHz spectrometer using DMSO-d6 as solvent and TMS as the internal standard. Mass spectra were recorded on a Finnigan-MAT SSQ-7000 GC–MS spectrometer. Analytical thin-layer chromatography (TLC) was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). 5-Aminobenzo[*b*]thiophene-2-carboxylic acid (**1**) was prepared in accordance with [16–18].

5-(2-Chloroacetamido)benzo[b]thiophene-2-carboxylic acid (**2**, $C_{11}H_8NO_3S$)

To a stirred cold solution of 3.86 g 5-aminobenzo[*b*]thiophene-2-carboxylic acid (**1**, 20 mmol) in 25 cm^3 pyridine, 2.5 cm^3 chloroacetyl chloride (22 mmol) were added portionwise over a period of 30 min at 0 °C. The reaction mixture was kept in an icebox overnight then diluted with water. The solid that precipitated was isolated by filtration, washed with water, and dried. Recrystallization from ethanol–DMF gave compound **2** in 5.10 g (95%) yield; M.p.: 238–240 °C; IR (KBr): $\bar{\nu}$ = 3,276 (COOH), 3,172 (NH), 1,734 (C=O), 1,695 (C=O) cm^{-1}; ^1H NMR (270 MHz, DMSO-d6): δ = 4.10 (s, 2H, CH_2), 7.65–7.85 (two dd, 2H, H-6, H-7), 7.73 (br s, 1H, NH, D_2O–exchangeable), 7.96 (s, 1H, H-3), 8.65 (s, 1H, H-4), 10.24 (br s, 1H, COOH) ppm;. MS (70 eV): *m*/*z* = 269 (M^+, 67), 234 (28), 220 (100), 176 (29), 147 (11), 133, (13), 91 (14), 89 (42), 75 (18), 62 (16).

*Reaction of the 5-(2-chloroacetamido)benzo[b] thiophene-2-carboxylic acid with alkyl, cycloalkyl, aryl, and heterocyclic amines: general procedure (**3–10**)*

To a stirred solution of 0.27 g 5-(2-chloroacetamido)benzo[*b*]thiophene-2-carboxylic acid (**2**, 1 mmol) in 25 cm^3 dioxane, 1 mmol of the appropriate amine was added in the presence of 0.3 cm^3 triethylamine. The reaction mixture was heated under reflux for 8–12 h (TLC-monitored) and then allowed to cool. The solid product formed was isolated by filtration, washed with ethanol, and recrystallized from EtOH–DMF to afford the corresponding C5-substituted benzo[*b*]thiophene derivatives **3–10**.

5-[2-(2-Hydroxyethylamino)acetamido]benzo[b] thiophene-2-carboxylic acid (**3**, $C_{13}H_{14}N_2O_4S$)

Yield 68%; M.p.: 120–122 °C; IR (KBr): $\bar{\nu}$ = 3,714 (OH), 3,274 (COOH), 3,212 (NH), 1,739 (C=O), 1,693 (C=O) cm^{-1}; ^1H NMR (270 MHz, DMSO-d6): δ = 2.65 (t, 2H, –$NHCH_2$), 3.27 (s, 2H, CH_2), 3.55 (t, 2H, –OCH_2), 4.7 (br s, 1H, OH), 7.62, 7.71 (two dd, 2H, H-6, H-7), 7.75 (br s, 1H, NH, D_2O– exchangeable), 7.98 (s, 1H, H-3), 8.55 (s, 1H, H-4), 10.21 (br s, 1H, COOH) ppm; MS (70 eV): *m*/*z* = 294 (M^+, 3), 280 (7), 265 (5), 238 (6), 210 (48), 182 (100), 154 (12), 105 (9), 74 (67).

5-[2-(2-Thiosemicarbazido)acetamido]benzo[b]thiophene-2-carboxylic acid (**4**, $C_{12}H_{12}N_4O_3S_2$)

Yield 83%; M.p.: >300 °C; IR (KBr): $\bar{\nu}$ = 3,421 (COOH), 3,322, 3,195 (NH, NH_2), 1,727 (C=O), 1,613 (C=O), 1,201 (C=S) cm^{-1}; ^1H NMR (270 MHz, DMSO-d6): δ = 3.85 (s, 2H, –$NHCH_2$) 4.00 (br s, 2H, NH_2), 7.32, 7.75 (br s, 2H, 2NH), 7.65, 7.73 (two dd, 2H, H-6, H-7), 7.83 (s, 1H, H-3),

8.64 (s, 1H, H-4), 11.5 (br s, NH), 11.91 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 322 (M^+−2, 76), 295 (27), 265 (15), 237 (8), 209 (5), 181 (100), 154 (37), 105 (6), 97 (67).

5-[2-(2-thioureido)acetamido]benzo[b]thiophene-2-carboxylic acid (**5**, $C_{12}H_{11}N_3O_3S_2$)
Yield 74%; M.p.: >300 °C; IR (KBr): $\bar{\nu}$ = 3,352 (COOH), 3,234, 3,198 (NH, NH_2), 1,661 (C=O), 1,596 (C=O), 1,231 (C=S) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 3.93 (s, 2H, –$NHCH_2$) 4.00 (br s, 2H, NH_2), 7.29 (br s, H, NH), 7.67, 7.76 (two dd, 2H, H-6, H-7), 7.89 (s, 1H, H-3), 8.61 (s, 1H, H-4), 11.43 (br s, NH), 11.94 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 309 (M^+, 3), 281 (33), 267 (5), 248 (69), 207 (100), 180 (35), 166 (34), 103 (6), 77 (44).

5-(2-(4-Methylpiperazin-1-yl)acetamido)benzo[b]thiophene-2-carboxylic acid (**6**, $C_{16}H_{19}N_3O_3S$)
Yield 84%; M.p.: 198–200 °C; IR (KBr): $\bar{\nu}$ = 3,298 (COOH), 3,102 (NH), 1,683 (C=O), 1,593 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 2.12 (s, 3H, –NMe) 2.55 (t, 2H, –$NHCH_2$), 2.60 (t, 2H, –$NHCH_2$), 3.15 (s, 2H, CH_2), 7.58, 7.67 (two dd, 2H, H-6, H-7), 7.76 (br s, 1H, NH, D_2O– exchangeable), 7.94 (s, 1H, H-3), 8.57 (s, 1H, H-4), 10.03 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 351 (M^+ +18, 5), 333 (M^+, 1) 280 (2), 237 (4), 223 (3), 181 (5), 141 (3), 113 (100), 70 (38).

5-(2-Morpholinoacetamido)benzo[b]thiophene-2-carboxylic acid (**7**, $C_{15}H_{16}N_2O_4S$)
Yield 90%; M.p.: 178–180 °C; IR (KBr): $\bar{\nu}$ = 3,272 (COOH), 3,082 (NH), 1,691 (C=O), 1,610(C=O) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 2.51 (t, 2H, –$NHCH_2$), 3.21 (s, 2H, CH_2), 3.65 (t, 2H, –OCH_2), 7.66, 7.81 (two dd, 2H, H-6, H-7), 7.78 (br s, 1H, NH, D_2O – exchangeable), 7.97 (s, 1H, H-3), 8.56 (s, 1H, H-4), 10.04 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 338 (M^+ +18, 5), 320 (M^+, 1) 294 (3), 252 (3), 223 (2), 181 (4), 149 (3), 100 (100), 70 (4).

5-(2-(2-Phenylhydrazinyl)acetamido)benzo[b]thiophene-2-carboxylic acid (**8**, $C_{17}H_{15}N_3O_3S$)
Yield 88%; M.p.: 260 °C; IR (KBr): $\bar{\nu}$ = 3,341 (COOH), 3,234, 3,198, 3,123 (3NH), 1,696 (C=O), 1,643 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 3.86 (s, 2H, –$NHCH_2$) 4.35 (br s, 1H, NH), 7.12–7.23 (m, 5H, Ph), 7.39 (br s, H, NH), 7.63, 7.80 (two dd, 2H, H-6, H-7), 7.93 (s, 1H, H-3), 8.55 (s, 1H, H-4), 10.32 (br s, NH), 10.45 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 341 (M^+, 6), 322 (6), 314 (9), 286 (39), 221 (11), 181 (100), 154 (23), 105 (6), 77 (42).

5-(2-(1H-Benzothiazol-2-ylamino)acetamido)benzo[b]thiophene-2-carboxylic acid (**9**, $C_{18}H_{13}N_3O_3S_2$)
Yield 84%; M.p.: >300 °C; IR (KBr): $\bar{\nu}$ = 3,264 (COOH), 3,231, 3,194 (2NH), 1,694 (C=O), 1,633 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 4.1 (s, 2H, –$NHCH_2$), 7.32–7.41 (m, 4H, benzothiazole), 7.61, 7.74 (two dd, 2H, H-6, H-7), 7.79 (br s, H, NH), 7.96 (s, 1H, H-3), 8.52 (s, 1H, H-4), 10.36 (br s, NH), 10.56 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 383 (M^+, 1), 322 (2), 286 (15), 258 (9), 223 (4), 181 (100), 154 (17), 105 (6), 77 (27).

5-(2-(1H-Benzoimidazol-2-ylamino)acetamido)benzo[b]thiophene-2-carboxylic acid (**10**, $C_{18}H_{14}N_4O_3S$)
Yield 82%; M.p.: 290 °C; IR (KBr): $\bar{\nu}$ = 3,274 (COOH), 3,242, 3,087 (2NH), 1,692 (C=O), 1,613 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d6): δ = 4.05 (s, 2H, –$NHCH_2$) 6.87 (br s, 1H, NH), 7.34–7.43 (m, 4H, benzoimidazole), 7.60, 7.73 (two dd, 2H, H–6, H-7), 7.75 (br s, H, NH), 7.97 (s, 1H, H-3), 8.56 (s, 1H, H-4), 10.3 (br s, NH), 10.4 (br s, 1H, COOH) ppm; MS (70 eV): m/z = 366 (M^+, 1), 333 (4), 319 (70), 277 (51), 246 (24), 207 (11), 181 (58), 154 (15), 105 (36), 102 (100), 77 (82).

Pharmacology

Swiss mice weighing 20–30 g were purchased from the animal house of the National Research Centre. The animals were housed in standard metal cages in an air conditioned room at 22 ± 3 °C, 55 ± 5% humidity, and provided with standard laboratory diet and water ad libitum. Experiments were performed between 0900 and 1500 hours. A group of six mice was used for each experiment. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the Local Animal Care and Use Committee. Distilled water was used as a vehicle for all tested compound and 5% sodium bicarbonate for indomethacin.

Anti-inflammatory activity

Anti-inflammatory activity in acute model was carried out according to Winter et al. [19] and Obkowicz et al. [20]. Mice were divided into 15 groups each of six. They received saline orally as control, tested compounds (50 mg/kg) and indomethacin (25 mg/kg) orally, according to Rani et al. [21], after induction of oedema by subplantar injection of 50 mm^3 1% carrageenan (Sigma, USA) in saline into the pad of right paw. The difference in hind footpad thickness was measured before and 1, 2, 3, and 4 h after carrageenan injection. The oedema was expressed as percentage of change from the control group (pre-drug) values. The doses of the tested compounds and of indomethacin were based on the rate dose converted to that of mice according to Paget et al. [22] and Souza et al. [23].

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

(% oedema inhibition) = (% oedema formation of control group − % oedema formation of treated group)/(% oedema formation of control group) × 100. The potency was calculated relative to the indomethacin-treated group according to the equation: Potency = (% oedema inhibition of treated group)/(% oedema inhibition of indomethacin treated group).

Gastric ulcerogenic studies

Gastric lesions were induced in rats by absolute ethanol (1 cm^3 orally) [24]. Animals were fasted for 24 h and then divided into six groups. One group received ethanol and served as control, and the remaining groups received tested compounds **2**, **4**, **7**, **8**, and **9** (50 mg/kg) 1 h before the ethanol was given. Rats were killed 1 h after methanol administration by cervical dislocation, after being lightly anaesthetized with ether, and the stomach was excised, opened along the greater curvature, rinsed with saline, extended on a plastic board, and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as follows: petechial lesions = 1, lesions less than 1 mm = 2, lesion between 1 and 2 mm = 3, lesions between 2 and 4 mm = 4, lesions more than 4 mm = 5. A total lesion score for each animal is calculated as the total number of lesions multiplied by the respective severity scores. Results are expressed as the severity of lesions/rat [25].

Statistical analysis

Data are expressed as mean ± SE. In the anti-inflammatory study data are expressed as mean ± SE. The results of carrageenan-induced paw oedema are expressed as percentage change relative to the control (pre-drug) values. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by multiple comparisons by the Duncan's multiple range test. A probability value <0.05 was considered statistically significant.

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