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Antinociceptive properties of new coumarin derivatives bearing substituted 3,4-dihydro-2*H*-benzothiazines

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Abstract

Background: Coumarins are an important class of widely distributed heterocyclic natural products exhibiting a broad pharmacological profile. In this work, a new series of coumarins bearing substituted 3,4-dihydro-2*H*-benzothiazines were described as potential analgesic agents. The clinical use of NSAIDs as traditional analgesics is associated with side effects such as gastrointestinal lesions and nephrotoxicity. Therefore, the discovery of new safer drugs represents a challenging goal for such a research area.

Results: The target compounds 3-(3-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]thiazin-3-yl)-2*H*-chromen-2-ones **2a-u** were synthesized and characterized by spectral data. The antinociceptive properties of target compounds were determined by formalin-induced test and acetic acid-induced writhing test in mice. Among the tested compounds, compound **2u** bearing 2-(4-(methylsulfonyl)benzoyl)- moiety on benzothiazine ring and 4-(methylsulfonyl)phenacyloxy- group on the 7 position of coumarin nucleus showed better profile of antinocecieption in both models. It was more effective than mefenamic acid during the late phase of formalin-induced test as well as in the acetic acid-induced writhing test.

Conclusion: Considering the significant antinoceciptive action of phenacyloxycoumarin derivatives, compound **2u** prototype might be further used as model to obtain new more potent analgesic drugs.

Keywords: Analgesic activity, Antinociception, Coumarin, Benzothiazine, Formalin test, Writhing test

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Introduction

Pain is an uncomfortable sensation that alerts the human organs about a current or potential damage to tissues [1]. It has been accepted that pain can widely affect human life quality, and its management is considered as a main challenge in pharmacotherapy [2]. NSAIDs are one of major classes of traditional analgesics for treatment of pain. The clinical use of NSAIDs is associated with side effects such as gastrointestinal lesions and nephrotoxicity [3]. Therefore, the discovery of new safer drugs represents a challenging goal for such a research area.

Coumarins are an important class of widely distributed heterocyclic natural products exhibiting a broad pharmacological profile [4]. Several coumarin derivatives have been synthesized with diverse biological activities [5-9] especially analgesic/anti-inflammatory activity [10-13]. Recently, the synthesis and anti-inflammatory/analgesic activities of several coumarin derivatives with various substitutions on 3-position of coumarin nucleus have been reported [14-16]. On the other hand, benzothiazine derivatives are also important heterocyclic compounds with wide spectrum of biological activities [17,18]. In view of the above facts and in continuation of our research program on the synthesis of biologically active heterocyclic compounds [19,20], we introduced herein the new coumarin derivatives bearing substituted 3,4-dihydro-2*H*-benzothiazines as analgesic agents. The antinociceptive properties of target compounds were determined by formalin-induced paw licking test and

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acetic acid-induced writhing test in mice. Indeed, the formalin-induced paw licking method is used to investigate both peripheral and central mechanisms whereas the acetic acid test is believed to demonstrate the involvement of peripheral mechanisms in the control of pain [21,22].

Materials and methods

Chemistry

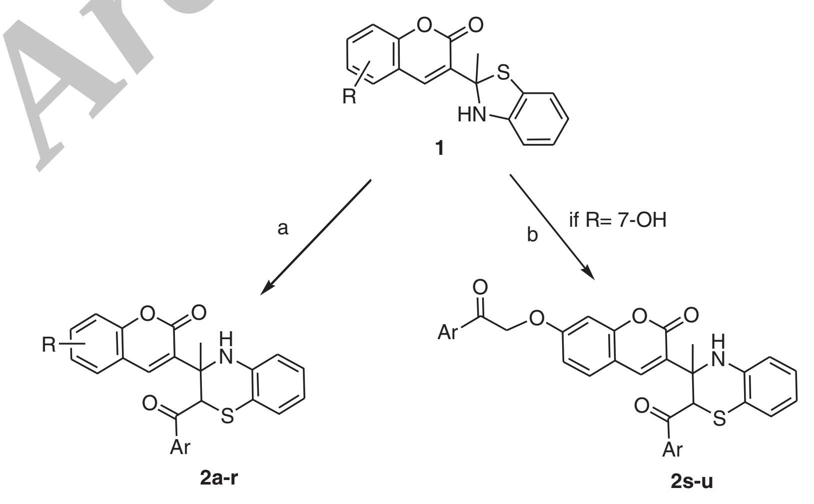
The target compounds 3-(3-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]thiazin-3-yl)-2*H*-chromen-2-ones **2a-r** (Additional file 1: Table S1) were synthesized according to the pathway outlined in Scheme 1 [23]. All reagents and chemicals were commercially available and used as received. Alumina-supported potassium fluoride (KF/Al_2O_3) was prepared by literature method [24]. The dihydrobenzothiazole derivatives **1** were prepared as reported method by us [19,20]. The synthesis of compounds **2a-d**, **2f-i** and **2p-r** was described in our previous paper [23]. Column chromatography was carried out on silica gel (70–230 mesh). TLC was conducted on silica gel 250 micron, F254 plates. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrographs (KBr disks). ^1H NMR spectra were recorded on a Bruker 400 or 500 MHz NMR instruments. The chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million and Hertz, respectively. Mass spectra of the products were obtained with an HP (Agilent technologies) 5937 Mass Selective Detector. Elemental analyses were carried out by a CHN-Rapid Heraeus elemental analyzer. The results of elemental analyses (C, H, N) were within ± 0.4% of the calculated values.

General procedure for the synthesis of compounds 2

A suspension of dihydrobenzothiazole derivatives **1** (1.0 mmol), KF/Al_2O_3 (0.7 g), and quinine hydrochloride (10 mol%) in ethanol (3.0 mL) was stirred at room temperature for 5 min. Then, appropriate phenacyl halide (1.2 mmol) was added to the mixture and stirring was continued. After completion of the reaction (3–5 h), the solvent was removed under reduced pressure. The residue was mixed with ethyl acetate (5 mL) and the catalyst was filtered and washed with ethyl acetate (3 × 5 mL). After evaporation of the solvent at reduced pressure, the crude product was purified by column chromatography (*n*-hexane/ethyl acetate, 9:1) and crystallized from ethanol for further purification.

3-(2-(3,4-Dichlorobenzoyl)-3-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2e)

Yellow solid (361 mg, 75%); *syn*-isomer; mp 91–93°C; IR (KBr, cm^{-1}) 3382 (NH), 1708 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ ^1H NMR (500 MHz, $CDCl_3$) δ 1.94 (s, 3H, CH_3 benzothiazine), 4.49 (s, 1H, NH), 5.77 (s, 1H, C-H benzothiazine), 6.75 (dt, *J* = 7.2 and 1.2 Hz, 1H, H_7 benzothiazine), 6.95 (m, 2H, $H_{5,8}$ benzothiazine), 7.14 (dt, *J* = 7.2 and 1.2 Hz, 1H, H_6 benzothiazine), 7.22 (t, *J* = 8.0 Hz, 1H, H_6 chromene), 7.28 (dd, *J* = 8.0 and 1.9 Hz, 1H, H_5 benzoyl), 7.33 (d, *J* = 8.0 Hz, 1H, H_6 benzoyl), 7.40 (m, 2H, $H_{5,8}$ chromene), 7.43 (d, *J* = 1.9, 1H, H_3 benzoyl), 7.49 (dt, *J* = 8.0 and 1.2 Hz, 1H, H_7 chromene), 7.77 (s, 1H, H_4 chromene); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.3, 42.9, 58.0, 110.9, 116.1, 117.1, 119.1, 119.3, 124.3, 127.0, 127.1, 128.4, 128.7, 130.1, 130.8, 131.2, 131.4, 132.0, 136.9, 137.1, 139.8, 141.0, 153.2, 160.9, 192.6; Anal. calcd for



Scheme 1 Synthesis of coumarin based dihydrobenzothiazines 2a-u. Reagents and conditions: (a) phenacyl halide (1.2 mmol), KF/Al_2O_3 (0.7 g), quinine hydrochloride (10 mol%), EtOH (3 mL), r.t. (b) phenacyl halide (2.5 mmol), KF/Al_2O_3 (1.5 g), quinine hydrochloride (10 mol%), EtOH (3 mL), r.t.

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$C_{25}H_{17}Cl_2NO_3S$: C, 62.25; H, 3.55; N, 2.90. Found: C, 62.41; H, 3.67; N, 3.15.

3-(2-(4-Fluorobenzoyl)-3-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2j)

Yellow solid (336 mg, 78%); *syn*-isomer; mp 161–163°C; IR (KBr, cm^{-1}) 3413 (NH), 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.76 (s, 3H, CH_3 benzothiazine), 4.50 (s, 1H, NH), 5.97 (s, 1H, C-H benzothiazine), 6.73 (t, *J* = 7.4 Hz, 1H, H_7 benzothiazine), 6.93 (m, 2H, $H_{5,6}$ benzothiazine), 7.14 (m, 3H, H_8 benzothiazine and $H_{3,5}$ benzoyl), 7.28 (t, *J* = 7.4 Hz, 1H, H_6 chromene), 7.35 (d, *J* = 7.4 Hz, 1H, H_8 chromene), 7.40 (d, *J* = 7.4 Hz, 1H, H_5 chromene), 7.50 (t, *J* = 7.4 Hz, 1H, H_7 chromene), 7.79 (s, 1H, H_4 chromene), 8.05 (m, 2H, $H_{2,6}$ benzoyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.6, 37.5, 57.6, 111.9, 115.7, 115.9, 116.1, 119.1, 119.4, 124.4, 126.7, 128.4, 128.5, 131.2, 131.3, 131.5, 133.2, 139.5, 141.1, 153.3, 161.3, 164.7, 166.7, 191.3; Anal. calcd for $C_{25}H_{18}FNO_3S$: C, 69.59; H, 4.20; N, 3.25. Found: C, 69.42; H, 4.03; N, 3.47.

3-(3-Methyl-2-(thiophene-2-carbonyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2k)

Yellow solid (356 mg, 85%); as mixture of diastereomers (*anti/syn:* 15/85); IR (KBr, cm^{-1}) 3389 (NH), 1707 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.77$_{syn}$ (s, CH_3 benzothiazine), 1.87$_{anti}$ (s, CH_3 benzothiazine), 4.48$_{syn}$ (s, NH), 4.53$_{anti}$ (s, NH), 5.50$_{anti}$ (s, C-H benzothiazine), 5.87$_{syn}$ (s, C-H benzothiazine), 6.77$_{syn}$ (t, *J* = 8.0, H_7 benzothiazine), 6.81$_{anti}$ (t, *J* = 8.0, H_7 benzothiazine), 6.92$_{anti}$ (d, *J* = 8.0 Hz, H_5 benzothiazine), 6.95$_{syn}$ (d, *J* = 8.0 Hz, H_5 benzothiazine), 7.07-7.10$_{anti}$ (m, $H_{6,8}$ benzothiazine), 7.10-7.13$_{syn}$ (m, $H_{6,8}$ benzothiazine), 7.20$_{syn}$ (t, *J* = 7.6 Hz, H_7 chromene), 7.25$_{syn}$ (t, *J* = 7.6 Hz, H_6 chromene), 7.25-7.29$_{anti}$ (m, H_4 thiophene and H_7 chromene), 7.38$_{syn}$ (d, *J* = 7.6 Hz, H_5 chromene), 7.42$_{syn}$ (d, *J* = 7.6 Hz, H_8 chromene), 7.48-7.54 (m, $H_{5,7,8}$ chromene (anti) and H_4 thiophene (syn)), 7.56$_{anti}$ (d, *J* = 4.0, H_3 thiophene), 7.74$_{syn}$ (d, *J* = 4.0, H_3 thiophene), 7.79$_{anti}$ (d, *J* = 4.0, H_5 thiophene), 7.80$_{syn}$ (s, H_4 chromene), 7.97$_{syn}$ (d, *J* = 4.0, H_5 thiophene), 8.15$_{anti}$ (s, H_4 chromene); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 27.5, 45.3, 54.2, 116.1, 117.0, 118.6, 119.0, 120.1, 124.5, 126.2, 127.1, 128.2, 128.4, 131.4, 131.7, 132.2, 134.4, 140.2, 140.9, 143.5, 153.1, 160.1, 186.8; Anal. calcd for $C_{23}H_{17}NO_3S_2$: C, 65.85; H, 4.08; N, 3.34. Found: C, 65.92; H, 3.91; N, 3.29.

3-(2-(5-Bromothiophene-2-carbonyl)-3-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2l)

Yellow solid (378 mg, 77%); as mixture of diastereomers (*anti/syn:* 26/74); IR (KBr, cm^{-1}) 3390 (NH), 1712 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.75$_{syn}$ (s, CH_3 benzothiazine), 1.87$_{anti}$ (s, CH_3 benzothiazine), 4.45$_{syn}$ (s, NH), 4.55$_{anti}$ (s, NH), 5.48$_{anti}$ (s, C-H benzothiazine), 5.78$_{syn}$ (s, C-H benzothiazine), 6.76$_{syn}$ (t, *J* = 8.0, H_7 benzothiazine), 6.81$_{anti}$ (t, *J* = 8.0, H_7 benzothiazine), 6.92$_{syn}$ (d, *J* = 8.0 Hz, H_5 benzothiazine), 6.94$_{anti}$ (d, *J* = 8.0 Hz, H_5 benzothiazine), 6.92$_{anti}$ (d, *J* = 8.0 Hz, H_8 benzothiazine), 6.96$_{syn}$ (d, *J* = 8.0 Hz, H_8 benzothiazine), 7.09$_{anti}$ (t, *J* = 8.0 Hz, H_6 benzothiazine), 7.12$_{syn}$ (t, *J* = 8.0 Hz, H_6 benzothiazine), 7.16$_{syn}$ (d, *J* = 4.0 Hz, H_4 thiophene), 7.23$_{anti}$ (d, *J* = 4.0 Hz, H_4 thiophene), 7.24$_{syn}$ (t, *J* = 7.5, H_6 chromene), 7.27-7.29$_{anti}$ (m, $H_{6,8}$ chromene), 7.36$_{syn}$ (d, *J* = 7.5 Hz, H_8 chromene), 7.40$_{syn}$ (d, *J* = 7.5 Hz, H_5 chromene), 7.50 $_{syn}$ (t, *J* = 7.5 Hz, H_7 chromene), 7.51-7.53$_{anti}$ (m, $H_{5,7}$ chromene), 7.56$_{anti}$ (d, *J* = 4.0 Hz, H_3 thiophene), 7.69$_{syn}$ (d, *J* = 4.0 Hz, H_3 thiophene), 7.77$_{syn}$ (s, H_4 chromene), 8.15$_{anti}$ (s, H_4 chromene); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 24.8, 38.7, 57.4, 112.3, 116.1, 116.9, 119.1, 119.5, 123.6, 124.4, 126.6, 128.1, 128.4, 130.8, 131.5, 131.6, 132.4, 139.2, 141.0, 145.9, 153.3, 161.2, 185.6; MS, *m/z* (%) 499 ($[M + 2]^+$, 40%), 497 (M^+, 37), 375 (47), 373 (44), 308 (100), 294 (51), 280 (84); Anal. calcd for $C_{23}H_{16}BrNO_3S_2$: C, 55.43; H, 3.24; N, 2.81 Found: C, 55.22; H, 3.47; N, 2.73.

3-(3-Methyl-2-(thiophene-3-carbonyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2m)

Yellow solid (335 mg, 80%); as mixture of diastereomers (*anti/syn:* 32/68); IR (KBr, cm^{-1}) 3374 (NH), 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.77$_{syn}$ (s, CH_3 benzothiazine), 1.87$_{anti}$ (s, CH_3 benzothiazine), 4.50$_{anti}$ (s, NH), 4.55$_{syn}$ (s, NH), 5.48$_{anti}$ (s, C-H benzothiazine), 5.81$_{syn}$ (s, C-H benzothiazine), 6.74$_{syn}$ (t, *J* = 7.5, H_7 benzothiazine), 6.80$_{anti}$ (t, *J* = 7.5, H_7 benzothiazine), 6.93$_{anti}$ (d, *J* = 7.5 Hz, H_5 benzothiazine), 6.97$_{syn}$ (d, *J* = 7.5 Hz, H_5 benzothiazine), 7.08$_{anti}$ (d, *J* = 7.5 Hz, H_8 benzothiazine), 7.12$_{syn}$ (t, *J* = 7.5 Hz, H_6 benzothiazine), 7.13$_{syn}$ (t, *J* = 7.2 Hz, H_6 chromene), 7.24-7.25$_{syn}$ (m, H_8 benzothiazine and H_8 chromene), 7.26-7.29$_{anti}$ (m, H_6 benzothiazine and $H_{6,8}$ chromene), 7.35-7.37$_{syn}$ (m, H_4 thiophene and $H_{5,7}$ chromene), 7.37-7.40$_{anti}$ (m, H_6 chromene and H_4 thiophene), 7.50-7.53$_{anti}$ (m, H_7 chromene and H_5 thiophene), 7.58$_{syn}$ (d, *J* = 5.0 Hz, H_5 thiophene), 7.79$_{anti}$ (s, H_2 thiophene), 8.04$_{syn}$ (s, H_2 thiophene), 8.14$_{anti}$ (s, H_4 chromene), 8.25$_{syn}$ (s, H_4 chromene); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 27.8, 45.7, 54.4, 116.1, 116.8, 119.1, 119.9, 124.6, 126.3, 126.5, 127.3, 128.2, 128.4, 130.6, 131.5, 131.7, 132.6, 140.0, 140.2, 141.2, 153.1, 160.2, 187.7; MS, *m/z* (%) 419 (M^+, 68%), 404 (12), 386 (12), 308 (97), 295 (100), 280 (64), 111 (63); Anal. calcd for $C_{23}H_{17}NO_3S_2$: C, 65.85; H, 4.08; N, 3.34. Found: C, 65.98; H, 3.82; N, 3.60.

8-Methoxy-3-(3-methyl-2-(4-methylbenzoyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-2H-chromen-2-one (2n)

Yellow solid (343 mg, 75%); *syn*-isomer; mp 145–147°C; IR (KBr, cm^{-1}) 3360 (NH), 1700 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.77 (s, 3H, CH_3 benzothiazine), 2.43 (s, 3H, CH_3 benzoyl), 3.90 (s, 3H, O-CH_3 chromene), 4.52 (s, 1H, NH), 6.00 (s, 1H, C-H benzothiazine), 6.78 (dt, *J* = 7.5 and

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1.3 Hz, 1H, H_7 benzothiazine), 6.93 (dd, *J* = 8.0 and 1.3 Hz, 1H, H_7 chromene), 7.02-7.11 (m, 4H, $H_{5,6,8}$ benzothiazine and H_6 chromene), 7.18 (m, 3H, H_5 chromene and $H_{3,5}$ benzoyl), 7.74 (d, *J* = 8.3 Hz, 2H, $H_{2,6}$ benzoyl), 8.13 (s, 1H, H_4 chromene); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 27.9, 43.6, 54.6, 56.1, 113.3, 114.8, 118.9, 119.4, 119.7, 119.9, 124.3, 126.3, 127.4, 128.5, 129.3, 131.1, 133.7, 140.0, 140.2, 142.8, 143.8, 146.7, 159.5, 192.9 cm^{-1}; Anal. calcd for $C_{27}H_{23}NO_4S$: C, 70.88; H, 5.07; N, 3.06. Found: C, 70.64; H, 5.23; N, 3.22.

3-(2-(4-Fluorobenzoyl)-3-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-8-methoxy-2H-chromen-2-one (2o)

Yellow solid (323 mg, 70%); *syn*-isomer; mp 236–238°C; IR (KBr, cm^{-1}) 3398 (NH), 1690 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 1.78 (s, 3H, CH_3 benzothiazine), 3.98 (s, 3H, O-CH_3 chromene), 4.51 (s, 1H, NH), 5.98 (s, 1H, C-H benzothiazine), 6.74 (t, *J* = 7.4 Hz, 1H, H_7 benzothiazine), 6.93 (m, 2H, $H_{5,6}$ benzothiazine), 6.98 (d, *J* = 8.0 Hz, 1H, H_7 chromene), 7.05 (m, 2H, $H_{5,6}$ chromene), 7.16 (m, 3H, H_8 benzothiazine and $H_{3,5}$ benzoyl), 7.77 (s, 1H, H_4 chromene), 8.05 (m, 2H, $H_{2,6}$ benzoyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.5, 37.4, 56.3, 57.6, 111.9, 113.3, 115.7, 115.9, 116.9, 119.3, 119.8, 124.2, 126.7, 128.4, 131.2, 131.3, 131.4, 133.1, 139.5, 141.2, 146.8, 160.7, 164.7, 166.7, 191.2; Anal. calcd for $C_{26}H_{20}FNO_4S$: C, 67.67; H, 4.37; N, 3.04. Found: C, 67.43; H, 4.18; N, 3.25.

3-(2-(4-Bromobenzoyl)-3-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-7-(2-(4-bromophenyl)-2-oxoethoxy)-2H-chromen-2-one (2s)

Yellow solid (507 mg, 72%); as mixture of diasteromers (*anti*/*syn*: 18/82); IR (KBr, cm^{-1}) 3382 (NH), 1697 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 1.74_{syn} (s, CH_3 benzothiazine), 1.85_{anti} (s, CH_3 benzothiazine), 4.48_{anti} (s, NH), 4.51_{syn} (s, NH), 5.29_{anti} (s, O-CH_2), 5.31_{syn} (s, O-CH_2), 5.60_{anti} (s, C-H benzothiazine), 5.91_{syn} (s, C-H benzothiazine), 6.71_{anti} (t, *J* = 7.5, H_7 benzothiazine), 6.73_{syn} (t, *J* = 7.5, H_7 benzothiazine), 6.80_{anti} (s, H_8 chromene), 6.82_{syn} (s, H_8 chromene), 6.87_{syn} (d, *J* = 8.5 Hz, H_6 chromene), 6.90_{anti} (d, *J* = 8.5 Hz, H_6 chromene), 6.93_{syn} (d, *J* = 8.0 Hz, $H_{3,5}$ phenyl-2-oxoethoxy), 7.05_{anti} (d, *J* = 8.0 Hz, $H_{3,5}$ phenyl-2-oxoethoxy), 7.07_{anti} (t, *J* = 7.5 Hz, H_6 benzothiazine), 7.12_{syn} (t, *J* = 7.5 Hz, H_6 benzothiazine), 7.33_{syn} (d, *J* = 8.5, H_5 chromene), 7.44_{anti} (d, *J* = 8.5, H_5 chromene), 7.52_{anti} (d, *J* = 8.5, $H_{3,5}$ benzoyl), 7.63_{syn} (d, *J* = 8.0 Hz, $H_{3,5}$ benzoyl), $7.64\text{-}7.67_{anti}$ (m, $H_{5,8}$ benzothiazine), 7.68_{syn} (m, $H_{5,8}$ benzothiazine), 7.72_{syn} (s, H_4 chromene), $7.82\text{-}7.85_{anti}$ (m, $H_{2,6}$ benzoyl and $H_{2,6}$ phenyl-2-oxoethoxy), $7.85\text{-}7.89_{syn}$ (m, $H_{2,6}$ benzoyl and $H_{2,6}$ phenyl-2-oxoethoxy), 8.06_{anti} (s, H_4 chromene); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 24.5, 37.6, 57.4, 70.6, 101.1, 111.8, 112.9, 116.9, 118.7, 119.3, 126.7, 128.3, 128.4, 129.5, 129.6, 129.9, 130.0, 131.9, 132.0, 132.3, 132.8, 135.5, 139.6, 140.9, 154.7, 160.6, 161.3, 191.6, 192.4; Anal. calcd for $C_{33}H_{23}Br_2NO_5S$: C, 56.19; H, 3.29; N, 1.99. Found: C, 56.21; H, 4.31; N, 2.09.

3-(3-Methyl-2-(4-methylbenzoyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-7-(2-oxo-2-p-tolylethoxy)-2H-chromen-2-one (2t)

Yellow solid (397 mg, 70%); as mixture of diastereomers (*anti/syn: 30/70*); IR (KBr, cm^{-1}) 3397 (NH), 1702 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 1.74_{syn} (s, CH_3 benzothiazine), 1.85_{anti} (s, CH_3 benzothiazine), 2.36_{anti} (s, CH_3 phenyl-2-oxoethoxy), 2.42_{syn} (s, CH_3 phenyl-2-oxoethoxy), 2.44_{anti} (s, CH_3 benzoyl), 2.45_{syn} (s, CH_3 benzoyl), 4.50_{syn} (s, NH), 4.61_{anti} (s, NH), 5.31_{anti} (s, O-CH_2), 5.35_{syn} (s, O-CH_2), 5.61_{anti} (s, C-H benzothiazine), 5.96_{syn} (s, C-H benzothiazine), 6.69_{anti} (d, *J* = 2.1 Hz, H_8 chromene), 6.72_{syn} (t, *J* = 7.3 Hz, H_7 benzothiazine), 6.75_{anti} (t, *J* = 7.3 Hz, H_7 benzothiazine), 6.81_{syn} (d, *J* = 2.1 Hz, H_8 chromene), 6.87_{syn} (dd, *J* = 8.0 and 2.1 Hz, H_6 chromene), $6.90\text{-}6.93_{syn/anti}$ (m, $H_{5,8}$ benzothiazine), 7.05_{anti} (t, *J* = 7.3 Hz, H_6 benzothiazine), 7.11_{syn} (t, *J* = 7.3 Hz, H_6 benzothiazine), 7.17_{anti} (d, *J* = 8.0 Hz, $H_{3,5}$ phenyl-2-oxoethoxy), 7.28-7.33 (m, H_5 chromene (syn/anti), $H_{3,5}$ benzoyl (syn/anti) and $H_{3,5}$ phenyl-2-oxoethoxy (syn)), 7.34_{anti} (d, *J* = 8.0 Hz, $H_{2,6}$ phenyl-2-oxoethoxy), 7.73_{syn} (s, H_4 chromene), 7.79_{anti} (d, *J* = 8.0 Hz, $H_{2,6}$ benzoyl), 7.87_{syn} (d, *J* = 8.0 Hz, $H_{2,6}$ phenyl-2-oxoethoxy), 7.94_{syn} (d, *J* = 8.0 Hz, $H_{2,6}$ benzoyl), 8.06_{anti} (s, H_4 chromene); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 24.0, 27.7, 32.6, 43.3, 56.0, 80.2, 110.4, 110.6, 113.6, 116.1, 118.7, 118.9, 119.9, 122.4, 124.5, 126.2, 127.2, 128.4, 129.2, 131.6, 140.1, 154.2, 161.2, 161.4, 192.1, 192.6; MS, *m/z* (%) 575 (M^+, 8%), 557 (64), 542 (43), 410 (35), 264 (44), 239 (29), 119 (100); Anal. calcd for $C_{35}H_{29}NO_5S$: C, 73.02 ; H, 5.08; N, 2.43. Found: C, 73.21; H, 5.12; N, 2.54.

3-(3-Methyl-2-(4-(methylsulfonyl)benzoyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)-7-(2-(4-(methylsulfonyl)phenyl)-2-oxoethoxy)-2H-chromen-2-one (2u)

Yellow solid (576 mg, 82%); as mixture of diastereomers (*anti/syn: 28/72*); IR (KBr, cm^{-1}) 3394 (NH), 1688 (C=O), 1320 (SO_2), 1153 (SO_2); ^1H NMR (500 MHz, $CDCl_3$) δ 1.78_{syn} (s, CH_3 benzothiazine), 1.89_{anti} (s, CH_3 benzothiazine), 3.02_{anti} (s, SO_2-CH_3 phenyl-2-oxoethoxy), 3.04_{anti} (s, SO_2-CH_3 benzoyl), 3.09_{syn} (s, SO_2-CH_3 phenyl-2-oxoethoxy), 3.12_{syn} (s, SO_2-CH_3 benzoyl), 4.50_{anti} (s, NH), 4.69_{syn} (s, NH), 5.37_{syn} (s, O-CH_2), 5.41_{anti}(s, O-CH_2), 5.61_{anti} (s, C-H benzothiazine), 5.95_{syn} (s, C-H benzothiazine), 6.69_{anti} (s, H_8 chromene), 6.74_{syn} (t, *J* = 7.5, H_7 benzothiazine), 6.80_{anti} (t, *J* = 7.5, H_7 benzothiazine), 6.83_{syn} (s, H_8 chromene), $6.88\text{-}6.95_{syn}$ (m, H_6 chromene and $H_{5,8}$ benzothiazine), 7.08_{anti} (d, *J* = 8.0, H_6 chromene), 7.11_{anti} (t, *J* = 7.5, H_6 benzothiazine), 7.13_{syn} (t, *J* = 7.5 Hz, H_6 benzothiazine), 7.36_{syn} (d, *J* = 8.0, H_5 chromene), 7.50_{anti} (d, *J* = 8.0, H_5 chromene), 7.59_{anti} (d, *J* = 7.5 Hz, H_8

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benzothiazine), 7.74_{syn} (s, H_4 chromene), 7.82_{anti} (d, *J* = 7.5 Hz, H_5 benzothiazine), 7.94_{anti} (s, H_4 chromene), 8.04-$8.19_{syn/anti}$ (m, $H_{2,3,5,6}$ phenyl-2-oxoethoxy and $H_{2,3,5,6}$ benzoyl); ^{13}C NMR (*syn*-isomer, 125 MHz, $CDCl_3$) δ 24.5, 44.2, 44.3, 57.5, 65.5, 70.9, 101.0, 110.9, 113.0, 116.9, 119.4, 126.9, 127.4, 127.9, 128.1, 128.5, 129.1, 129.3, 129.8, 138.0, 139.6, 140.8, 141.0, 143.9, 145.1, 154.7, 160.4, 161.2, 190.6, 192.6; Anal. calcd for $C_{35}H_{29}NO_9S_3$: C, 59.73 ; H, 4.15; N, 1.99. Found: C, 59.59; H, 4.31; N, 2.30.

Pharmacology

Animals

Male NMRI mice weighing 20–30 g were used for studying in vivo antinociceptive activities of target compounds. Animals were maintained under standard conditions (24 ± 2°C, 60-70% humidity) and allowed food and water *ad libitum*. They were housed in appropriate cages with 12 h light/dark cycle. Before each experiment animals randomly selected and allocated into groups. The whole protocol was approved by the Ethics Committee of the Faculty of Pharmacy at Tehran University of Medical Sciences.

Formalin-induced pain test

All target compounds **2a-u** were subjected for testing their analgesic activity using formalin paw test [25]. The compounds or standard drug mefenamic acid were administered i.p. (30 mg/kg, 0.2 mL/20 g body weight) as a suspension in saline and tween 80 (4% w/v). Each group of mice (n = 6 animals per group) were pretreated by test compounds, mefenamic acid or vehicle, 30 minutes before injection of formalin (20 μL, 0.5%, s.c.) into the planar surface of the right hind paw. The amount of time that the animal spent licking injected paw was measured during the first 10 minutes (phase 1, neurogenic) and 10–30 minutes (phase 2, inflammatory) after formalin injection.

Table 1 Antinociception activity of target compounds 2a-u and mefenamic acid (30 mg/kg, i.p.) assessed by formalin test in mice

Compounds	Phase 1			Phase 2		
	Licking time[a]	Inhibition[b] (%)	Relative activity[c]	Licking time	Inhibition (%)	Relative activity
2a	58 ± 3.46	48.10**	0.54	37.33 ± 3.93	44.28**	0.52
2b	51.33 ± 2.96	54.06***	0.61	50.33 ± 4.91	24.88	0.29
2c	68.33 ± 4.05	38.85**	0.44	38 ± 1.73	43.28**	0.51
2d	55 ± 2.74	50.78***	0.57	57.33 ± 6.35	14.43	0.17
2e	44 ± 2.89	60.63***	0.68	54 ± 4.93	19.40	0.23
2f	60.33 ± 3.76	46.01**	0.52	38.33 ± 4.63	42.79**	0.50
2g	56.66 ± 8.74	49.29**	0.55	55.66 ± 3.92	16.92	0.20
2h	70.25 ± 2.95	37.14**	0.42	38 ± 1	43.28**	0.51
2i	51.33 ± 2.40	54.06***	0.61	37 ± 1.15	44.78**	0.53
2j	46.25 ± 2.56	58.61***	0.66	50.33 ± 0.33	24.88	0.29
2k	51.66 ± 2.18	53.77***	0.60	51.66 ± 5.54	22.89	0.27
2l	70 ± 11.13	37.36**	0.42	37 ± 4.35	44.78**	0.53
2m	63.33 ± 8.21	43.33**	0.49	18.33 ± 0.33	72.64***	0.85
2n	69 ± 9.16	38.26**	0.43	40.66 ± 1.20	39.30**	0.46
2o	53.8 ± 3.21	51.85**	0.58	65 ± 6.41	2.98	0.03
2p	53.9 ± 3.18	51.76**	0.58	64.8 ± 4.19	3.28	0.04
2q	61.33 ± 5.78	45.12**	0.51	49.5 ± 2.02	26.12	0.31
2r	94.4 ± 4.89	25.86**	0.29	11 ± 1.7	93.64***	1.1
2s	50 ± 3.22	33.33**	0.37	5.2 ± 2.78	96.99***	1.14
2t	46 ± 2.4	38.86**	0.43	3 ± 1.04	98.26***	1.15
2u	34.8 ± 2.65	53.6***	0.61	14.8 ± 1.92	91.44***	1.07
Control	111.75 ± 6.94	-	-	67 ± 3.14	-	-
Mefenamic acid	12.33 ± 3.93	88.96***	1	10 ± 2.52	85.07***	1

[a] Data are expressed as mean ± S.E.M (number of animals in each group, n = 6).
[b] The percentage inhibition was determined by using the following formula: Inhibition % = 100 × (control − experiment)/control. The asterisks denote the levels of significance in comparison with control groups (**P* <0.05, ***P* <0.01 and ****P* <0.001).
[c] Activity relative to mefenamic acid was determined by using the following formula: Relative Activity = Inhibition % of compound/Inhibition % of mefenamic acid.

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Acetic acid-induced writhing test

The analgesic activity was also determined *in vivo* by the abdominal constriction test induced by acetic acid (0.6%; 0.1 mL/10 g) in mice [21]. An acetic acid solution was administered i.p. 30 minutes after administration of compounds or mefenamic acid. After the treatment, pairs of mice were placed in separate boxes and the numbers of constrictions of the abdominal muscles, together with stretching, were counted cumulatively over a period of 60 minutes. Antinociceptive activity was expressed as the percentage of inhibition of constrictions when compared with the vehicle control group.

Statistical analysis

The nociception data are expressed as means ± SEM. Variance analysis (ANOVA) followed by Bonferroni's test was used to compare means. *P*-values less than 0.05 were considered to be statistically significant.

Results and discussion

Chemistry

The dihydrobenzothiazole derivatives **1** were quantitatively obtained by reaction of 3-acetylcoumarins with 2-aminothiophenol derivatives in the presence of acetic acid under reflux condition or microwave irradiation [19,20]. The intramolecular Mannich-type reaction of compounds **1** with different phenacyl halides in the present of KF/Al_2O_3 and catalyzing by quinine hydrochloride in ethanol afforded 3,4-dihydro-2*H*-benzothiazine derivatives **2a-r** via a ring expansion. When 7-hydroxy-3-(benzothiazol-2-yl) coumarin derivative **1e** was treated with 2.5 equivalents of phenacyl halides, without protection of hydroxyl group, *O*-phenacyl derivatives **2s-u** was obtained in excellent yields (Scheme 1). The physicochemical and spectral data of new compounds **2e**, **2j-o**, and **2s-u** are described in experimental section.

Biological activity

Formalin-induced nociception test

All target compounds **2a-u** were tested using formalin-induced pain test in mice [25]. The obtained results were reported as mean ± SEM of licking time and as percent of inhibition in Table 1. In general, the results showed that most of compounds were significantly able to reduce the licking time with percent of inhibition in the range of 25% to 60% at the first phase. The standard drug mefenamic acid showed 89% reduction of the licking time during the first phase. Amongst the tested compounds, **2a**, **2c**, **2f**, **2h**, **2i**, **2l-n** and **2r-u** significantly reduced the formalin induced licking time in the range of 39-98% as compared to mefenamic acid with 85% of inhibition during the second phase. Compounds **2m** and **2r-u** showed more effective antinociceptive activity in the second phase rather than first phase, indicating their ability to inhibit nociception associated with inflammatory response. Indeed, 7-hydroxy- and 7-phenacyloxy-coumarin derivatives (**2r** and **2s-u**, respectively) were more effective than mefenamic acid. Compounds **2s** and **2t** were the most effective compounds at the dose of 30 mg/kg.

Table 2 Antinociception activity of selected compounds in comparison with mefenamic acid (30 mg/kg, i.p.) assessed by acetic acid-induced writhing test in mice

Compound	Nociception (Mean ± SEM)	Inhibition (%)[a]	Relative activity[b]
2b	0.6 ± 0.24***	99	1.4
2c	38 ± 4.04***	49	0.7
2d	9.6 ± 2.54***	87	1.3
2g	3.5 ± 1.09***	96	1.37
2h	3 ± 1.84***	97	1.38
2i	4.6 ± 2***	94	1.34
2k	20 ± 2.48***	73	1.04
2o	6 ± 3.2***	92	1.31
2r	29 ± 2.12***	63	0.9
2s	14 ± 2.28***	80	1.14
2t	30 ± 7.6***	60	0.85
2u	2 ± 1.3***	98	1.4
Control[c]	75 ± 3.2		
Mefnamic acid	23 ± 1.3***	70	

[a] The percentage inhibition was determined by using the following formula: Inhibition% = 100 × (control − experiment)/control.
[b] Activity relative to mefenamic acid was determined by using the following formula: Relative Activity = Inhibition % of compound/Inhibition % of mefenamic acid.
[c] Tween 80 in saline (4% w/v).
***P <0.001 vs. control.

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Acetic acid-induced writhing test

The analgesic activity of compounds **2b-d**, **2g-i**, **2k**, **2o** and **2r-s** was also evaluated in vivo by using abdominal constriction test induced by acetic acid in mice [21]. The abdominal constriction response induced by acetic acid is sensitive procedure to establish efficacy of peripherally acting analgesics. The analgesic activity was expressed as the percentage of inhibition of constrictions when compared with the control group. The results are summarized in Table 2.

Significant protection against writhing was observed in animals treated with all test compounds where the mean numbers of writhes after 1 h were less than 38 compared to 75 in the control group. The percent of inhibition was in the range of 49-99%. All tested compounds were more effective than standard drug mefenamic acid with the exception of **2c**, **2r** and **2t**. Compounds **2b** and **2u** with percent of inhibition ≥98% were the most effective compounds in acetic acid-induced writhing test. Moreover, compounds **2g-i** and **2o** exhibited high protection against writhing (percent of inhibition > 90%).

Structure–activity relationships

From the structure–activity relationships of unsubstituted coumarin series (compound **2a-m**) based on the late stage of formalin-induced test, it was inferred that 3-thienylcarbonyl group is more favorable for activity. By comparing the activity of 7-substituted coumarin compounds **2r-u** with those of other compounds it is appeared that the 7-hydroxy or 7-phenacyloxy groups dramatically increase the effectiveness of compounds and their ability to inhibit nociception associated with inflammatory response. On contrary, compounds **2r-u** showed low level of inhibition at early phase of formalin test.

By comparing the percent of inhibition of 4-(methylsulfonyl)benzoyl derivatives **2d**, **2r** and **2u**, it is revealed that the introduction of hydroxyl group on 7-position of coumarin ring diminished the antinociception activity, while the introduction of 4-(methylsulfonyl)phenacyloxy- group increased the activity as resulted from writhing test. In the 7-phenacyloxy-coumarin derivatives **2s-u**, methylsulfonyl substituent was more favorable than bromo and methyl groups. The observed results of unsubstituted coumarin derivatives in Table 2 demonstrate that electron donating or bulky groups (for example, methoxy or phenyl, respectively) can increase antinociceptive activity in writhing test.

Conclusion

In summary, a series of 3-(3-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]thiazin-3-yl)-2*H*-chromen-2-one derivatives **2a-u** bearing different aroyl group on the 2-position of benzothiazine ring were described as potential analgesic agents. The antinociceptive properties of target compounds were determined by formalin-induced test and acetic acid-induced writhing test in mice. The effect of substituent on aroyl moiety was explored by introduction of various electron withdrawing, electron donating or bulky groups. Surprisingly, compound **2u** bearing 2-[4-(methylsulfonyl)benzoyl]- moiety on benzothiazine ring and 4-(methylsulfonyl)phenacyloxy- group on the 7 position of coumarin nucleus showed better profile of antinociception in both models. It was more effective than mefenamic acid during the late phase of formalin-induced test as well as in the acetic acid-induced writhing test. However, unsubstituted coumarin derivative **2b** containing 4-methylbenzoyl moiety on benzothiazine ring, fully protected animals against writhing and was moderately able to inhibit the both phases of the formalin test. Considering the significant antinociceptive action of phenacyloxy-coumarin derivatives, compound **2u** prototype might be further used as model to obtain new more potent analgesic drugs.

Additional file

Additional file 1: Table S1. Chemical structure of coumarin compounds **2a-u**.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MA: Synthesis of target compounds. MK: Synthesis of target compounds. SE: Collaboration in identifying of the structures of target compounds, manuscript preparation. SF: Collaboration in determination of antinociceptive properties. SFG: Collaboration in determination of antinociceptive properties. MA: Supervision of the pharmacological part, AF: Collaboration in identifying of the structures of target compounds. AS: Design of target compounds and supervision of the synthetic and pharmacological parts. All authors read and approved the final manuscript.

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