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Original scientific paper

Screening for antidepressant, sedative and analgesic activities of novel fused thiophene derivatives

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This study was aimed at the synthesis of fused benzothiophene derivatives containing heterocyclic moiety. The reaction of the tetrahydrobenzo[b]thiophene derivatives **1a,b** with ethoxycarbonylisothiocyanate afforded the thiourea derivatives **2a,b**. Cyclization of the latter products gave the annulated benzo[b]thienopyrimidine derivatives **3a,b**. Compounds **2a,b** and **3a** underwent a series of heterocyclization reactions through the reaction with some chemical reagents to give the new benzo[b]thienopyrimidine derivatives **5a,b** to **8a-c**. Also, this work was extended to study the potential role of the novel synthesized thiourea derivative **2a** and benzo[b]thienopyrimidine derivatives **3a**, **5b**, **6a** and **8b** as antidepressant, sedative or analgesic agents at two doses (15 or 30 mg kg^{-1} body mass). Some compounds (**2a**, **3a** and **5b**) showed mild antidepressant activity in the forced-swimming test. No compound showed sedative effect. Visceral pain evoked by *i.p.* injection of acetic acid in mice was significantly inhibited by all compounds at a high doses.

Keywords: tetrahydrobenzo[b]thiophene, pyrimidine, thiourea, antidepressant, sedative, analgesic

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Heterocyclic antidepressants were the mainstay of antidepressant treatment from their inception in the late 1950's until the mid-1980's. The development of synthetic heterocyclic compounds as antidepressant, sedative or analgesic agents progressed considerably during the past decade (1–3). In recent years, the role of antidepressant drugs in the management of different pain syndromes has elicited considerable interest (4, 5). Pyrimidine derivatives possessing anti-inflammatory, antidepressant and analgesic activities have been reported in literature (6–8). Over the past few years, our research group was conducting a comprehensive program towards the synthesis of thiophenes and their fused derivatives (9–12). The importance of such compounds is based on their use as anti-flammatory (13, 14), anti-protozoal (15), and antitumor agents (16), templates for

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serine protease inhibition (17) and alternate substrate inhibitors of cholesterol eastrase (18). In view of all these observations, we report here in on the use of 2-amino-4,5,6,7--tetrahydrobenzo[b]thiophene derivatives (**1a,b**) (19) in the synthesis of novel benzo[b]thienopyrimidine derivatives. The systemic anti-depressant, sedative or analgesic activities of the newly synthesized compounds were also investigated.

EXPERIMENTAL

Synthetic methods, analytical and spectral data

Melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. Elemental analyses were determined on a Yanaco CHN Corder elemental analyzer (Japan). IR spectra (ν cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer in DMSO--d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75e V) MS equipment (Germany). Elemental analyzes were performed on a Yanaco CHN Corder elemental analyzes (Japan).

The analytical and spectral data of the newly synthesized products are presented in Tables I and II, respectively. The pharmacological data are given in Tables III, IV and V.

Compounds **2a** and **2b** were synthesized according to our recently published work (20).

*3-Cyano-2-(*N*-ethoxycarbonylthiouryl)-4,5,6,7-tetrahydrobenzo[b]thiophene (**2a**) and ethyl 2-(*N*-ethoxycarbonylthiouryl)-4,5,6,7-tetrahydrobenzo[b]thiophen-3-carboxylate (**2b**)*

General procedure. – To a solution of either **1a** (1.78 g, 0.01 mol) or **1b** (2.25 g, 0.01 mol) in 1,4-dioxan (30 mL), ethoxycarbonyl isothiocyanate (1.31 g, 0.01 mol) [prepared by adding ammonium isothiocyanate (0.01 mol) to a solution of ethyl chloroformate (0.01 mol) in 1,4-dioxan (20 mL) and heating for 1/2 h followed by isolation of the by-product, ammonium chloride] was added. The whole reaction mixture, in each case, was stirred at room temperature overnight and the solid product formed upon pouring onto ice/water was collected by filtration to give either **2a** or **2b**, crystallized from acetic acid.

Ethyl 1-imino-3-thioxo-4[H]*-6,7,8,9-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-2-carboxylate (**3a**) and ethyl 1-oxo-3-thioxo-4*[H]*-6,7,8,9-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-2-carboxylate (**3b**)*

General procedure. – A suspension of either **2a** (3.09 g, 0.01 mol) or **2b** (3.56 g, 0.01 mol) in sodium ethoxide (0.01 mol) [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (40 mL)] was heated in a boiling water bath for 6 h and then left to cool. The solid product, formed upon pouring onto ice/water containing hydrochloric acid (to pH = 6), was collected by filtration to give either **3a**, crystallized from 1,4-dioxane or **3b**, crystallized from ethanol.

*3-Cyano-2-(*N*-phenylaminocarbonylthiourayl-4,5,6,7-tetrahydrobenzo[b]thiophene (**4a**) and ethyl2-(*N*-phenylaminocarbonylthiourayl-4,5,6,7-tetrahydrobenzo[b]thiophen-3-carboxylate (**4b**)*

General procedure. – To a dry solid of either **2a** (3.09 g, 0.01 mol) or **2b** (3.56 g, 0.01 mol), aniline oil (0.94 g, 0.01 mol) was added. The reaction mixture was heated in an oil

Table I. Physico-chemical data for the synthesized compounds

Compd. No.	Yield (%)	M.p. (°C)	Mol. formula (M_r)	Found/calcd. (%)			
				C	H	N	S
2a	70	188–190	$C_{13}H_{15}N_3O_2S_3$	50.46	4.89	13.58	20.73
			(309.41)	50.07	5.42	13.88	20.57
2b	66	105	$C_{15}H_{20}N_2O_4S_2$	50.54	5.66	7.86	17.99
			(356.46)	50.87	5.24	8.31	18.44
3a	62	233–235	$C_{13}H_{15}N_3O_2S_2$	50.46	4.89	13.58	20.73
			(309.41)	50.22	5.31	13.88	21.12
3b	55	233–235	$C_{13}H_{14}N_2O_3S_2$	50.30	4.54	9.02	20.66
			(310.40)	50.07	4.88	20.38	20.38
4a	84	158–162	$C_{17}H_{16}N_4OS_2$	57.28	4.52	15.72	17.99
			(356.47)	56.89	4.33	15.92	18.31
4b	73	229–233	$C_{19}H_{21}N_3O_3S_2$	56.55	5.25	10.41	15.89
			(403.53)	56.39	5.09	10.22	15.89
5a	61	> 300	$C_{17}H_{16}N_4OS_2$	57.28	4.52	15.72	17.99
			(356.47)	56.79	4.42	16.02	18.33
5b	52	266–270	$C_{17}H_{15}N_3O_2S_2$	57.12	4.23	11.76	17.94
			(357.46)	56.79	4.09	11.46	18.31
6a	74	198–200	$C_{25}H_{22}N_4O_2S_2$	63.27	4.67	11.80	13.51
			(474.61)	62.99	4.82	11.67	13.72
6b	62	145–147	$C_{25}H_{21}N_3O_3S_2$	63.14	4.45	8.84	13.48
			(475.59)	62.89	4.75	9.24	13.69
7a	56	222–226	$C_{21}H_{22}N_4O_3S_2$	56.99	5.01	12.66	14.49
			(442.56)	56.89	4.76	12.93	14.83
7b	75	189–191	$C_{21}H_{21}N_3O_4S_2$	56.87	4.77	9.47	14.46
			(443.55)	56.67	5.08	9.34	14.33
8a	52	136–138	$C_{17}H_{18}N_6OS$	57.61	5.12	23.71	9.05
			(354.44)	57.33	5.63	23.59	8.88
8b	63	220–223	$C_{23}H_{22}N_6OS$	64.16	5.15	9.52	17.45
			(430.53)	63.77	4.98	19.78	7.72
8c	70	170–172	$C_{17}H_{17}N_5O_2S$	57.44	4.82	19.70	9.02
			(355.42)	57.28	4.59	19.58	8.81
8d	50	> 300	$C_{23}H_{21}N_5O_2S$	64.02	4.90	16.23	7.43
			(431.52)	63.89	5.38	16.39	8.59

Table II. Spectral data of the synthesized products

Compd. No.	IR (ν, cm^{-1})	^1H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	MS (M^+)
2a	3460–3324 (2NH), 2980, 2888 (CH_3, CH_2), 2225 (CN), 1687 (CO), 1638 (C=C), 1205–1196 (C=S)	1.61 (t, 3H, J = 7.02 Hz, CH_3), 2.14–2.16 (m, 4H, $2CH_2$), 2.23–2.26 (m, 4H, $2CH_2$), 4.11 (s, 1H, NH), 4.24 (q, 2H, J = 7.02, CH_2), 8.32 (s, 1H, NH)	14.88 (ester CH_3), 20.0, 23.3, 23.9, 24.7 ($4CH_2$), 60.5 (ester CH_2), 118.8 (CN), 122.3, 136.7, 135.6, 140.8 (thiophene-C), 154.7 (amide C=O), 178.8 (C=S)	309
2b	3456–3339 (2NH), 2986, 2893 (CH_3, CH_2), 1690, 1685 (2 CO), 1636 (C=C), 1205–1196 (C=S)	1.62, 1.65 (2t, 6H, 2 CH_3), 2.16–2.19 (m, 4H, $2CH_2$), 2.25–2.29 (m, 4H, $2CH_2$), 4.10 (s, 1H, NH), 4.22, 4.25 (2q, 4H, 2 CH_2), 8.30 (s, 1H, NH)	14.87, 16.58 (two ester CH_3), 20.1, 23.5, 23.6, 24.4 ($4CH_2$), 60.6, 62.7 (two ester CH_2), 118.6 (CN), 122.1, 136.3, 135.9, 140.1 (thiophene-C), 154.6 (amide C=O), 179.5 (C=S)	356
3a	3442–3326 (2NH), 2982, 2887 (CH_3, CH_2), 1688 (CO), 1665 (exocyclic C=N), 1639 (C=C), 1207–1193 (C=S)	1.36 (t, 3H, J = 7.66 Hz, CH_3), 1.69–1.72 (m, 4H, $2CH_2$), 2.20–2.23 (m, 4H, $2CH_2$), 4.13 (s, 1H, NH), 4.20 (q, 2H, J = 7.66 Hz, CH_2), 8.26 (s, 1H, NH)	13.8 (ester CH_3), 23.3, 23.9, 24.7 ($4CH_2$), 52.7 (ester CH_2), 121.3, 134.3, 136.9, 147.9.1 (thiophene-C), 159.8 (C=O), 164.7 (C=N), 179.5 (C=S)	309
3b	3456–3336 (NH), 2980, 2890 (CH_3, CH_2), 1693, 1685 (2 CO), 1636 (C=C), 1204–1190 (C=S)	1.38 (t, 3H, J = 7.21 Hz, CH_3), 1.66–1.70 (m, 4H, $2CH_2$), 2.22–2.26 (m, 4H, $2CH_2$), 4.22 (s, 1H, NH), 4.27 (q, 2H, J = 7.21 Hz, CH_2)		310
4a	3489–3326 (3NH), 3053 (CH-aromatic), 2218 (CN), 1682 (CO), 1637 (C=C)	1.64–1.68 (m, 4H, $2CH_2$), 2.25–2.28 (m, 4H, $2CH_2$), 4.33, 5.42 (2s, 2H, 2NH), 7.03–7.35 (m, 5H, C_6H_5), 8.81 (s, 1H, NH)	20.3, 22.3, 23.9, 24.9 ($4CH_2$), 118.6 (CN), 120.8, 121.7, 122.8, 124.7, 137.9 (phenyl C), 130.7, 132.2, 138.7, 144.8 (thiophene C), 155.8 (CO), 178.3 (C=S)	356
4b	3487–3320 (3NH), 3056 (CH-aromatic), 2988, 2873 (CH_3, CH_2), 1692, 1686 (2 CO), 1637 (C=C)	1.34 (t, 3H, J = 6.77 Hz, CH_3), 1.66–1.72 (m, 4H, $2CH_2$), 2.23–2.27 (m, 4H, $2CH_2$), 4.22 (q, 2H, J = 6.77 Hz, CH_2), 4.30, 5.30 (2s, 2H, 2NH), 7.33–7.42 (m, 5H, C_6H_5), 8.94 (s, 1H, NH)		403
5a	3462–3336 (3NH), 3059 (CH-aromatic), 1681 (CO), 1670 (exocyclic C=N), 1644 (C=C), 1205–1192 (C=S)	1.68–1.70 (m, 4H, $2CH_2$), 2.25–2.29 (m, 4H, $2CH_2$), 4.32, 5.36 (2s, 2H, 2NH), 7.22–7.36 (m, 5H, C_6H_5), 9.05 (s, 1H, NH)		356

5b	3462–3336 (2 NH), 3049 (CH-aromatic), 1693, 1685 (2 CO), 1637 (C=C), 1203–1196 (C=S)	1.65–1.74 (m, 4H, $2CH_2$), 2.22–2.27 (m, 4H, $2CH_2$), 4.36 (s, 1H, NH), 7.28–7.38 (m, 5H, C_6H_5), 9.17 (s, 1H, NH)		357
6a	3455–3330 (2NH), 3064 (CH-aromatic), 1690, 1687 (2CO), 1677 (exocyclic C=N), 1639 (C=C)	1.65–1.70 (m, 4H, $2CH_2$), 2.22–2.31 (m, 4H, $2CH_2$), 4.68 (s, 2H, CH_2), 7.33–7.39 (m, 10H, $2C_6H_5$), 8.38, 8.94 (2s, 2H, 2NH)	20.4, 22.3, 24.4, 24.5 ($4CH_2$), 128.6 (CN), 120.6, 121.9, 122.5, 124.2, 138.4, 139.5, 140.3 (two phenyl C), 130.4, 132.8, 134.8, 149.8 (thiophene C), 156.8, 160.3 (2 CO), 168.7 (C=O)	474
6b	3442–3321 (NH), 3052 (CH-aromatic), 1693–1685 (3CO), 1636 (C=C)	1.66–1.73 (m, 4H, $2CH_2$), 2.24–2.28 (m, 4H, $2CH_2$), 4.58 (s, 2H, CH_2), 7.31–7.40 (m, 10H, 2 C_6H_5), 8.33 (s, 1H, NH)		475
7a	3456–3318 (2 NH), 3058 (CH-aromatic), 2978, 2869 (CH_3, CH_2), 1690, 1688 (2 CO), 1660 (C=N), 1636 (C=C)	1.33 (t, 3H, J = 7.11 Hz, CH_3), 1.64–1.71 (m, 4H, $2CH_2$), 2.25–2.32 (m, 4H, $2CH_2$), 4.21 (q, 2H, J = 7.11 Hz, CH_2), 4.92 (s, 2H, CH_2), 7.26–7.37 (m, 5H, C_6H_5), 8.26, 8.36 (2s, 2H, 2NH)		422
7b	3447–3322 (NH), 3060 (CH-aromatic), 2982, 2871 (CH_3, CH_2), 1691, 1688–1685 (3 CO), 1656 (C=N), 1634 (C=C)	1.31 (t, 3H, J = 7.04 Hz, CH_3), 1.65–1.73 (m, 4H, $2CH_2$), 2.24–2.36 (m, 4H, $2CH_2$), 4.24 (q, 2H, J = 7.04 Hz, CH_2), 4.86 (s, 2H, CH_2), 7.28–7.40 (m, 5H, C_6H_5), 8.33 (s, 1H, NH)		443
8a	3521–3312 (3NH, NH_2), 3056 (CH-aromatic), 1683 (CO), 1670 (exocyclic C=N), 1637 (C=C)	1.65–1.70 (m, 4H, $2CH_2$), 2.22–2.31 (m, 4H, $2CH_2$), 2.88 (s, 1H, NH), 4.46 (s, 2H, NH_2), 6.58 (s, 1H, NH), 7.31–7.39 (m, 5H, C_6H_5), 8.21 (s, 1H, NH).	20.9, 22.6, 24.4, 24.9 ($4CH_2$), 120.8, 121.7, 122.8, 123.5, 134.8 (phenyl C), 133.5, 133.7, 134.4, 147.9, 158.4 (thiophene, pyrimidine C), 158.9 (2 CO), 169.3 (C=N)	354
8b	3542–3319 (4NH, NH_2), 3062 (CH-aromatic), 1687 (CO), 1668 (exocyclic C=N), 1636 (C=C)	1.63–1.68 (m, 4H, $2CH_2$), 2.24–2.36 (m, 4H, $2CH_2$), 2.87, (s, 1H, 1NH), 6.55 (s, 1H, NH), 7.26–7.38 (m, 10H, $2C_6H_5$), 8.20, 8.23 (2s, 2H, 2NH)		430
8c	3560–3335 (2NH, NH_2), 3052 (CH-aromatic), 1691, 1688 (2 CO), 1657 (C=N), 1644 (C=C)	1.60–1.66 (m, 4H, $2CH_2$), 2.20–2.32 (m, 4H, $2CH_2$), 2.87 (s, 1H, NH), 4.68 (s, 2H, NH_2), 7.32–7.37 (m, 5H, C_6H_5), 8.20 (s, 1H, NH)		355

8d	3348–3320 (3NH), 3058 (CH-aromatic), 1684, 1680 (2 CO), 1662 (C=N), 1637 (C=C)	1.62–1.69 (m, 4H, $2CH_2$), 2.22–2.30 (m, 4H, $2CH_2$), 2.90 (s, 1H, NH), 7.31–7.42 (m, 10H, $2C_6H_5$), 8.22, 8.24 (2s, 2H, 2NH)	431

bath (140 °C) for 1 h. Then, it was left to cool and the remaining product was triturated with diethyl ether and the formed solid product was collected by filtration to give either **4a** or **4b**, crystallized from acetic acid.

1-Imino-2-phenylaminocarbonyl-4[H]*-3-thioxo-6,7,8,9-tetrahydrobenzo*[*b*]*thieno*[*2,3-d*]*pyrimidine* (***5a***) *and 1-oxo-2-phenylaminocarbonyl-4*[H]*-3-thioxo-6,7,8,9-tetrahydrobenzo*[*b*]*thieno-*[*2,3-d*]*pyrimidine* (***5b***)

Method A. – A suspension of either **3a** (3.56 g, 0.01 mol) or **3b** (4.03 g, 0.01 mol), in sodium ethoxide/ethanol solution [prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (60 mL)] was heated in a boiling water bath for 12 h. The solid product formed upon pouring onto ice/water containing hydrochloric acid (till pH 6) was collected by filtration to give either **5a** or **5b**, crystallized from DMF.

Method B. – To the dry solid of either **3a** (3.09 g, 0.01 mol) or **3b** (3.10 g, 0.01 mol), aniline oil (0.94 g, 0.01 mol) was added. It was reaction mixture was heated in an oil bath (140 °C) for 1 h. It was left to cool and the remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

3-Benzoylmethylsulphido-1-imino-2-phenylaminocarbonyl-6,7,8,9-tetrahydrobenzo[*b*]*-thieno*[*2,3-d*]*-pyrimidine* (***6a***), *3-benzoylmethylsulphido-1-oxo-2-phenylaminocarbonyl-6,7,8,9-tetrahydrobenzo*[*b*]*thieno*[*2,3-d*]*pyrimidine* (***6b***), *1-imino-2-phenylaminocarbonyl-3-(ethyl acetatosulfyl)-6,7,-8,9-tetrahydrobenzo*[*b*]*thieno*[*2,3-d*]*pyrimidine* (***7a***) *and 1-oxo-2-phenylamino-carbonyl-3-(ethyl-acetatosulfyl)-6,7,8,9-tetrahydrobenzo*[*b*]*thieno*[*2,3-d*]*pyrimidine* (***7b***)

General procedure. – To a solution of either **5a** (3.56 g, 0.01 mol) or **5b** (3.57 g, 0.01 mol) in 1,4-dioxane (40 mL), either ω-bromoacetophenone (2.0 g, 0.01 mol) or ethyl chloroacetate (1.22 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h, then poured onto ice/water containing a few drops of sodium hydroxide (to pH 6) and the formed solid product was collected by filtration to give either **6a**, **6b**, **7a** or **7b**, crystallized from 1,4-dioxane.

3-Hydrazino-2-phenylaminocarbonyl-1-imino-6,7,8,9-tetrahydrobenzo[*b*]*thieno*[*2,3-d*]*pyrimidine* (***8a***), *3-phenylhydrazino-2-phenylaminocarbonyl-1-imino-6,7,8,9-tetrahydrobenzo*[*b*]*thieno* [*2,3-d*]*pyrimidine* (***8b***), *3-hydrazino-2-phenylaminocarbonyl-1-oxo-6,7,8,9-tetrahydrobenzo*[*b*] *thieno*[*2,3-d*]*pyrimidine* (***8c***) *and 3-phenylhydrazino-2-phenylaminocarbonyl-1-oxo-6,7,8,9-tetrahydrobenzo*[*b*]*thieno*[*2,3-d*]*pyrimidine* (***8d***)

General procedure. – To a solution of either **7a** (4.42 g, 0.01 mol) or **7b** (4.43 g, 0.01 mol) in 1,4-dioxane (40 mL), either hydrazine hydrate (0.5 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h, then poured onto ice/water containing hydrochloric acid (to pH 6) and the formed solid product was collected by filtration to give either **8a** or **8b**, crystallized from 1,4-dioxane or **8c** or **8d**, crystallized from DMF.

Animals

Swiss albino mice of either sex, weighing 20–25 g, aged 6–8 weeks, were supplied by the Animal House at National Research Centre (Giza, Egypt). Animals were maintained under 12/12 h light/dark cycle at 20 ± 2 and fed the standard laboratory diet and water *ad libitum*. In accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985), groups of 6 mice for group were used in all experiments.

Screening for antidepressant activity

Porsolt's forced-swimming test. – Each mouse was placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water to a height of 12 cm. Water temperature was maintained at 22–23 °C. The animal was forced to swim for 6 min and the duration of immobility was measured. The mouse was considered to be immobile when it stopped struggling and moved only to remain floating in water, keeping its head above water. The floating time, which was the measure of despair (21), was recorded 60 min after treatment with each drug (15 or 30 mg kg^{-1}, *i.p.*), saline or imipramine (15 mg kg^{-1}, *i.p.*). Test compounds were dissolved using a few drops of Tween 80 and further dilutions were done using saline to get the necessary doses. The negative control was the vehicle solution (Tween 80 in saline).

Screening for sedative effect

Mice were observed in a commercially available motor activity apparatus (Ugo Basel, Italy) in which locomotor and exploratory activity could be monitored. In these experiments, each mouse was intraperitoneally injected with the drug (30 mg kg^{-1}) and 30 min later it was placed in the activity monitor in which activity was monitored for 6 min.

Screening for analgesic effect

Acetic acid-induced writhing was performed for separate groups of 6 mice each, to which *i.p.* vehicle, compounds **2a**, **3a**, **5b**, **6a** and **8b** (15 and 30 mg kg^{-1}) or indomethacin were administered (20 mg kg^{-1}). After 30 min pretreatment interval, an *i.p.* injection of 0.6% acetic acid was administered (22). Each mouse was then placed in an individual clear plastic observational chamber and the total number of writhes (abdomen constriction, trunk twisting and extension of hind legs) made by each mouse was counted for 30 min after acetic acid administration.

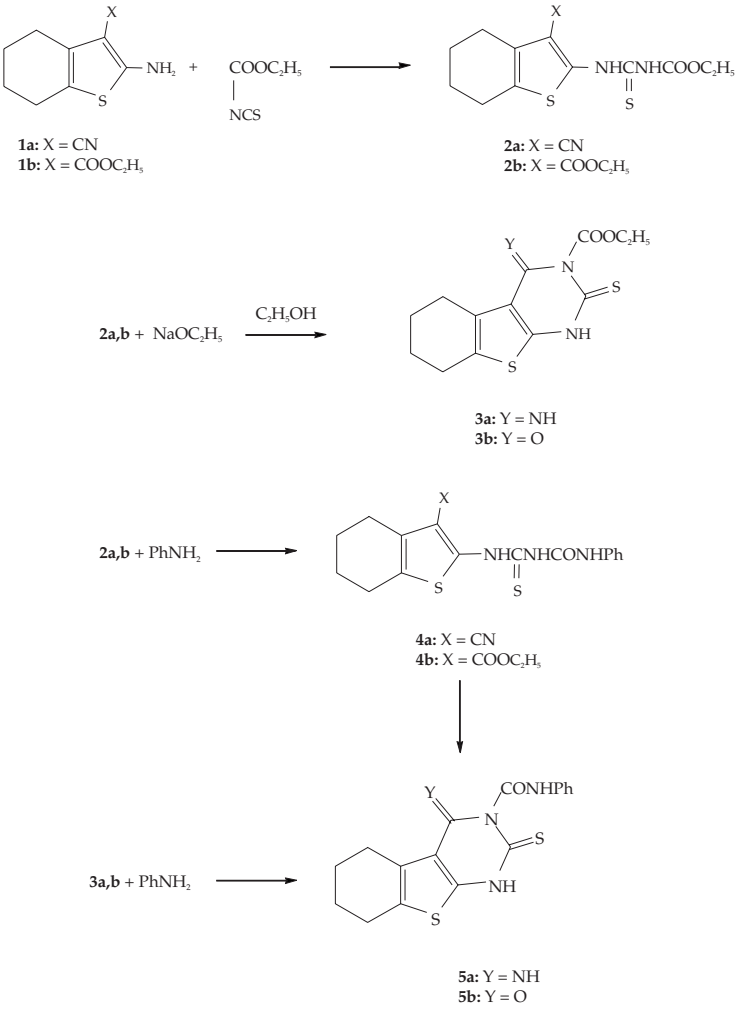
Statistics

Data are presented as mean ± SEM. Data were analyzed by ANOVA followed by the Duncan and multiple group comparison test.

RESULTS AND DISCUSSION

Chemistry

The reaction of 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene derivative **1a,b** with ethoxycarbonylisothiocyanate in 1,4-dioxane at room temperature gave the *N*-ethoxycarbonylthiourea derivatives **2a** and **2b**, respectively. The structures of compounds **2a,b** were based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **2a** showed the presence of a triplet at δ 1.61 ppm corresponding to the ester CH_3 group, a multiplet at δ 2.14–2.16 and 2.23–2.26 ppm corresponding to the four CH_2 groups of cyclohexene



Sheme 1

ring, two singlets at δ 4.11 and 8.32 for the two NH groups and a quartet at δ 4.24 ppm for the ester CH_2 group. The reactions of isothiocyanates with NH_2 compounds were reported earlier (23). Compounds **2a,b** underwent ready cyclization when heated in sodium ethoxide/ethanol solution in a boiling water bath to give the tetrahydro[b]thieno-[2,3-d]pyrimidine derivatives **3a** and **3b**, respectively.

The reaction of **2a,b** with aniline in an oil bath (at 140 °C) gave the anilide derivatives **4a** and **4b**, respectively. Structures of compounds **4a,b** were based on the obtained analytical and spectral data (see experimental section). In the reaction of **2b**, aniline reacts with the ethyl *N*-carboxylate rather than the ethyl carboxylate group attached to C-thiophene based on the cyclization of **4b** into **5b** *via* ethanol elimination. Moreover, formation of the same cyclized product *via* another reaction route was noted. Thus, compounds **4a** and **4b** underwent ready cyclization when heated in sodium ethoxide/ethanol solution in a boiling water bath to give the 4,5,6,7-tetrahydrobenzo[b]thieno[2,3-d]-pyrimidine derivatives **5a** and **5b**, respectively. Structures of the latter products were established on the basis of analytical and spectral data and on their synthesis using another reaction route. Thus, the reaction of either **3a** or **3b** with aniline oil at 140 °C gave the same products **5a** and **5b**, respectively (m.p., mixed m.p. and fingerprint IR spectrum) (Scheme 1).

The reaction of either compound **5a** or **5b** with ω-bromoacetophenone in 1,4-dioxane gave the thioether-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine derivatives **6a** and **6b**, respectively. Structures of the latter products were based on analytical and spectral data. The ^{1}H NMR spectrum of **6a** showed the presence of two multiplets with δ 1.65–1.70 ppm and 2.22–2.31 ppm for the 4 CH_2 groups, a singlet with δ 4.68 ppm for the side

Table III. Effect of tested compounds on the duration of immobility in Porsolt's forced-swimming test

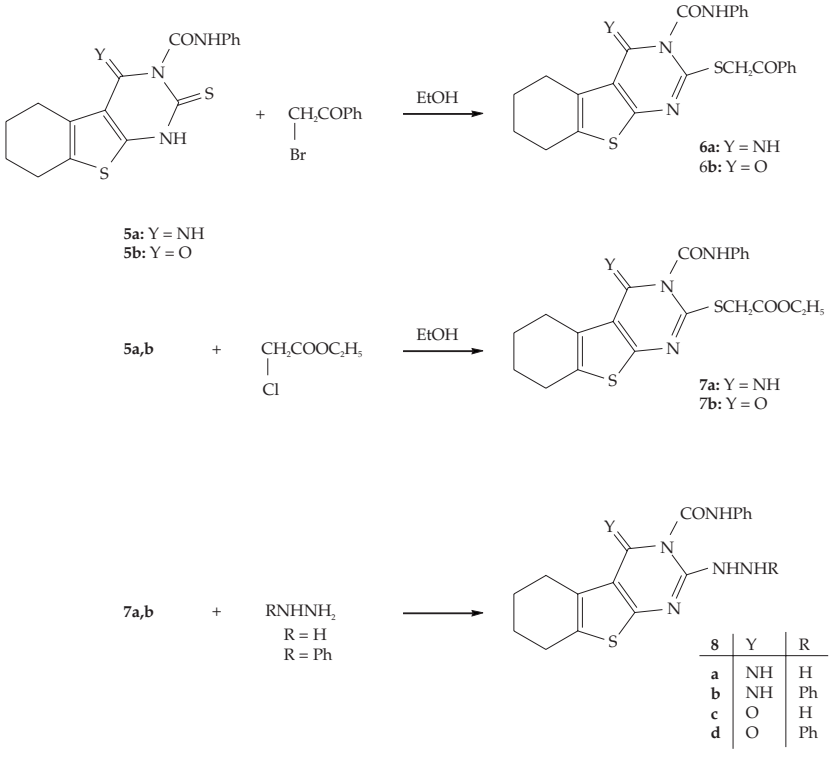
Treatment/compd.	Duration of immobility (s)[a]
Saline	289.9 ± 7.1
Imipramine (15 mg kg^{-1})	237.0 ± 14.0[b]
2a (15 mg kg^{-1})	279.7 ± 11.4
2a (30 mg kg^{-1})	265.5 ± 15.8
3a (15 mg kg^{-1})	269.3 ± 9.6
3a (30 mg kg^{-1})	266.1 ± 10.3
5b (15 mg kg^{-1})	271.7 ± 8.5
5b (30 mg kg^{-1})	266.7 ± 17.6
6a (15 mg kg^{-1})	286.8 ± 7.8
6a (30 mg kg^{-1})	273.3 ± 13.6
8b (15 mg kg^{-1})	283.9 ± 11.8
8b (30 mg kg^{-1})	301.0 ± 9.4

[a] Mean ± SEM (n = 6).
[b] Significant difference *vs*. saline-treated control group ($p < 0.05$).

chain CH_2 group, a multiplet with δ 7.33–7.39 ppm for the two phenyl groups and two singlets with δ 8.38 and 8.94 ppm corresponding to two NH groups. On the other hand, the reaction of either **5a** or **5b** with ethyl chloroacetate gave the thioether derivatives **7a** and **7b**, respectively. The spectral data of the latter are consistent with the proposed structures (Tables I and II). The reaction of either compound **7a** or **7b** with either hydrazine hydrate or phenylhydrazine afforded the corresponding 2-hydrazino-4,5,6,7-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine derivatives **8a-d**, respectively (Scheme 2). The analytical and spectral data of the latter products agree well with the proposed structures (Tables I and II).

Screening for antidepressant activity

After 60 min of *i.p.* administration, some compounds (**2a**, **3a** and **5b**) showed mild, non-significant antidepressant activity at high doses and were found active, compared with negative control group. The other compounds failed to display antidepressant properties in the swimming test (Table III).



8	Y	R
a	NH	H
b	NH	Ph
c	O	H
d	O	Ph

Sheme 2

Screening for sedative activity

No sedative effect could be observed in mice treated with compounds **2a**, **5b**, **6a** and **8b**; even more exploratory movements compared to the saline treated group were observed (Table IV).

All tested compounds at the two doses (15 or 30 mg kg^{-1}), except for the lower dose of **8b**, significantly reduced the number of abdominal writhes induced by *i.p.* injection of

Table IV. Effect of tested compounds on the number of exploratory movements in mice[a]

Treatment/compd.	Number of movements[b]
Saline	27.8 ± 2.3
2a (30 mg kg^{-1})	36.3 ± 3.9[c]
3a (30 mg kg^{-1})	25.0 ± 1.9
5b (30 mg kg^{-1})	51.3 ± 3.0[c]
6a (30 mg kg^{-1})	39.3 ± 4.4[c]
8b (30 mg kg^{-1})	63.5 ± 3.9[c]

[a] Number of movements in 6 minutes.
[b] Mean ± SEM (n = 6).
[c] Significant difference *vs.* saline-treated control group ($p < 0.05$).

Table V. Inhibition of abdominal constrictions caused by injection of acetic acid[a]

Treatment/compd.	Number	Inhibition (%)
Saline	92.8 ± 6.0	–
2a (15 mg kg^{-1})	44.8 ± 4.0[c]	51.7
2a (30 mg kg^{-1})	2.5 ± 0.29[c]	97.3
3a (15 mg kg^{-1})	28.3 ± 3.5[c]	69.5
3a (30 mg kg^{-1})	26.8 ± 1.2[c]	71.7
5b (15 mg kg^{-1})	31.5 ± 2.2[c]	66.1
5b (30 mg kg^{-1})	27.5 ± 3.2[c]	70.4
6a (15 mg kg^{-1})	50.0 ± 4.0[c]	46.1
6a (30 mg kg^{-1})	23.5 ± 2.6[c]	74.7
8b (15 mg kg^{-1})	81.7 ± 6.8	11.9
8b (30 mg kg^{-1})	57.3 ± 4.1[c]	38.3
Indomethacin (20 mg kg^{-1})	50.3 ± 5.4[c]	45.8

[a] Constriction during 30 min.
[b] Mean ± SEM (n = 6).
[c] Significant difference *vs.* saline-treated control group ($p < 0.05$).

acetic acid in mice (Table V). Compound **2a** was the most potent in this respect, inhibiting the number of abdominal writhes by 97.3% at high dose (30 mg kg^{-1}), compared to saline as the negative control group. Meanwhile, compounds **6a**, **3a** and **5b** at high doses inhibited the number of abdominal writhes by 74.7, 71.7 and 70.4%, respectively. These compounds, at low and high doses, were even more potent than indomethacin in this respect.

From the structure-activity relationship viewpoint, the thiourea tetrahydrobenzo-[b]thiophene derivative **2a** has the strongest antidepressant and analgesic activity. Replacement of ethoxycarbonylthiouryl side chain with fused pyrimidine moiety decreased these activities in the other compounds. The presence of benzoylmethylsulphide side chain attached to pyrimidine moiety in compound **6a** increased its activity as analgesic agent.

CONCLUSIONS

In this study, we have described efficient synthesis of novel benzo[b]thienopyrimidine derivatives derived from the thiourea derivatives of tetrahydrobenzo[b]thiophenes **2a** and **2b**. The newly synthesized thiophene derivatives **2a**, **3a**, **5b**, **6a** and **8b** showed mild antidepressant and analgesic activity of various intensities. However, compound **2a** had the strongest antidepressant and analgesic activity. Compounds **2a**, **6a**, **3a** and **5b**, as analgesic agents, at low and high doses, were more potent than indomethacin.

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S A Ž E T A K

Ispitivanje antidepresivnog, sedativnog i analgetskog djelovanja novih fuzioniranih derivata tiofena

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U radu je opisana sinteza fuzioniranih derivata benzotiofena koji sadrže heterociklički ostatak bitan za farmakološko djelovanje. Tiourea derivati **2a,b** dobiveni su reakcijom derivata tetrahidrobenzo[b]tiofena **1a,b** s etoksikarbonilizotiocijanatom. Iz njih su dalje priređeni anulirani derivati benzo[b]tienopirimidina **3a,b**. Spojevi **2a,b** i **3a** su reakcijama heterociklizacije prevedeni u benzo[b]tienopirimidine **5a,b-8a-c**. Ispitivano je antidepresivno, sedativno i analgetsko djelovanje novosintetiziranih derivata tiouree **2a** i benzo[b]tienopirimidina **3a**, **5b**, **6a** i **8b** u dvije doze (15 ili 30 mg kg^{-1} tjelesne mase). Spojevi **2a**, **3a** i **5b** pokazali su blago antidepresivno djelovanje u testu forsiranog plivanja, dok sedativni učinak nije pokazao niti jedan ispitivani spoj. Visceralna bol inducirana *i.p.* injekcijom octene kiseline u miševa značajno je inhibirana sa svim spojevima, ali u visokim dozama.

Ključne riječi: tetrahidrobenzo[b]tiofen, pirimidin, tiourea, antidepresiv, sedativ, analgetik

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