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## Synthesis of new [1,3,4]thiadiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidinone derivatives with antiinflammatory activity

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New thiadiazolothienopyrimidinones were synthesized in continuation of efforts to prepare thienopyrimidine derivatives with analgesic and antiinflammatory activities. In this study, the effect of various substituents in the thiophene ring on the pharmacological activity of the compounds was investigated.

### 1. Introduction

In previous papers [1–6] we reported the synthesis of polycyclic derivatives containing the thienopyrimidine moiety to test their analgesic and antiinflammatory activities. We prepared many substances containing the thiazolo[3,2-*a*]thieno[2,3-*d*] or [3,2-*d*]pyrimidin-5-one [4], the [1,3,4]thiadiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidin-5-one [1–3] and the thieno[2',3':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazine systems [5, 6]. Among them, several of the thiadiazolothienopyrimidinones showed interesting analgesic and antiinflammatory effects, as well as a very low acute toxicity and absence of ulcerogenic activity. As a consequence of these results, the present study reports the synthesis of new thiadiazolothienopyrimidinones **9–20** to investigate further the effect of variation of substituents in the thiophene ring on both analgesic and antiinflammatory activities.

### 2. Investigations, results and discussion

Compounds **9–20** were obtained by reaction of monopotassium salts of 3-amino-2,3-dihydro-2-thioxothieno[2,3-*d*]pyrimidin-4(1*H*)-one (**4**) [7], of 3-amino-6-ethyl (**5**) [8], of 3-amino-6-propyl-2,3-dihydro-2-thioxothieno[2,3-*d*]pyrimidin-4(1*H*)-one (**6**) and of the ethyl ester of 5-amino-3-methyl-2,3-dihydro-6-thioxothieno[2,3-*d*]pyrimidin-4(1*H*)-one-2-carboxylic acid (**7**) [8] with acetic, propionic and benzoic acid in the presence of phosphorus oxychloride under reflux; the same compounds **9–20** were obtained by reaction of the respective thioxo derivative under the same conditions, but the yields were lower by this route. Salt **6** was obtained by cyclization under reflux in an ethanolic potassium hydroxide solution of the ethyl ester of 2-[(hydrazinothioxomethyl)amino]-5-propyl-3-thiophenecarboxylic acid (**3**), which was prepared from the ethyl ester of 2-isothiocyanato-5-propyl-3-thiophenecarboxylic acid (**2**) with hydrazine monohydrate in dichloromethane at room temperature. The structure of compound **6** was confirmed by analytical and spectral data of the corresponding thioxo derivative **8** obtained by two routes, by acidification with concentrated hydrochloric acid of a suspension of the potassium salt **6** and by reaction of isothiocyanate **2** with an excess of hydrazine monohydrate in benzene under reflux. Derivative **2** was prepared from the ethyl ester of 2-amino-5-propyl-3-thiophenecarboxylic acid (**1**) [9] with thiophosgene in dichloromethane and water, and in presence of sodium hydrogen carbonate (Scheme).

The structures of the compounds synthesized were confirmed by analytical data, IR spectra, and <sup>1</sup>H NMR spectra of representative samples.

Some of the compounds described in this paper were screened for their analgesic and antiinflammatory activities, as well as for their potential ulcerogenic activity and for behavioural effects according to a previously reported procedure [6] (Table).

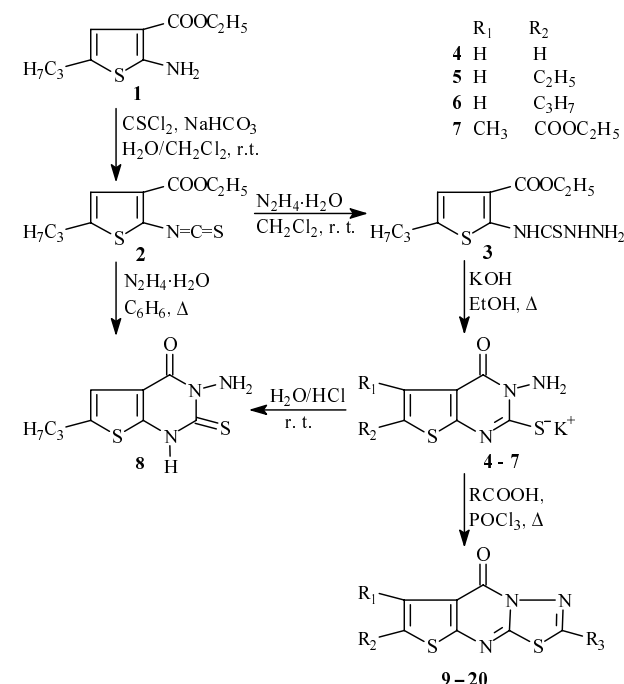
Some of the test compounds (**14**, **15**, **17**, and **20**) did not cause remarkable behaviour modifications according to Irwin's assay at any of the doses administered, either orally or intraperitoneally. Other compounds showed marked sedation about 15 min after administration for a period of 1–3 h. Death generally occurred at 1–3 h postdrug in 40–60% of animals. The surviving mice appeared normal and remained so throughout the seven-day observation period.

In the mouse phenylquinone-induced writhing test, compounds **14** and **20** exhibited a remarkable analgesic action at 10 mg/kg p.o., while the rest showed lower activity. The most potent compounds were more effective than phenylbutazone (PBZ) at the same dose level.

In the carrageenin paw oedema test, the compounds **14** and **15** exhibited good antiinflammatory activities at 100 mg/kg p.o. with a potency lesser than that of PBZ.

In the peritonitis acetic acid test, the compounds **9**, **15**, and **20** tested at 10 mg/kg p.o. had a fairly good antiexu-

### Scheme



**Table: Physical data of compounds 9–20 and pharmacological data of compounds 9, 11, 12, 14, 15, 17, 18, and 20**

Compd.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	M.p. (°C)	Recry. solv. Yield (%)	Molecular formula	IR (KBr) (cm <sup>-1</sup> ) C=O	<sup>1</sup> H NMR chemical shifts (DMSO-d <sub>6</sub> ) (ppm from TMS)	Acute toxicity Approximate LD <sub>50</sub> (mg/kg)		Analgesic activity Phenyl-quinone Writting-test (% protection ± SE) (10 mg/kg)	Anti-inflammatory activity Carra-geenin Paw oedema (% inhibition ± SE) <sup>a</sup> (mg/kg)		Ulcerogenic index (mg/kg × 2)
									po	ip		100	10	
<b>9</b>	H	H	CH <sub>3</sub>	202 (dec.)	EtOH 47	C <sub>8</sub> H <sub>5</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1675	2.71 (s, 3 H), 7.47 (d, J = 6 Hz, 1 H), 7.55 (d, J = 6 Hz, 1 H).	~ 700	~300	33 ± 5*	38 ± 19*	44 ± 5*	300
<b>10</b>	H	H	C <sub>2</sub> H <sub>5</sub>	152 (dec.)	EtOH 39	C <sub>9</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1685	—	—	—	—	—	—	—
<b>11</b>	H	H	C <sub>6</sub> H <sub>5</sub>	219–221 (dec.)	Dioxane 57	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1690	—	~ 700	~300	25 ± 8*	—	9 ± 7	300
<b>12</b>	H	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	160–162 (dec.)	EtOH 39	C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1685	—	~ 700	~300	35 ± 13*	—	7 ± 7	250
<b>13</b>	H	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	102–103	EtOH 39	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1710	1.28 (t, J = 7.4 Hz, 3 H), 1.33 (t, J = 7.2 Hz, 3 H), 2.87 (q, J = 7.2 Hz, 2 H), 3.06 (q, J = 7.4 Hz, 2 H), 7.20 (s, 1 H)	—	—	—	—	—	—
<b>14</b>	H	C <sub>2</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	189–190 (dec.)	EtOH 35	C <sub>15</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1715	—	~1000	~600	40 ± 5*	41 ± 18*	33 ± 5*	200
<b>15</b>	H	C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	177–179 (dec.)	EtOH 45	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1700	—	>1000	>800	23 ± 13*	46 ± 25*	49 ± 5*	40
<b>16</b>	H	C <sub>3</sub> H <sub>7</sub>	C <sub>2</sub> H <sub>5</sub>	129–130	EtOH 57	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1705	—	—	—	—	—	—	—
<b>17</b>	H	C <sub>3</sub> H <sub>7</sub>	C <sub>6</sub> H <sub>5</sub>	182–183	EtOH 44	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1700	0.95 (t, J = 7.4 Hz, 3 H), 1.67 (m, 2 H), 2.84 (t, J = 7.4 Hz, 2 H), 7.25 (s, 1 H), 7.58–8.03 (m, 5 H).	~1000	~600	3 ± 11	26 ± 28*	22 ± 7*	250
<b>18</b>	CH <sub>3</sub>	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	209–210	EtOH/ dioxane 60	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1720 1700	—	~ 700	~300	32 ± 3*	28 ± 25*	33 ± 7*	300
<b>19</b>	CH <sub>3</sub>	COOC <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	152	EtOH/ dioxane 50	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1720 1680	—	—	—	—	—	—	—
<b>20</b>	CH <sub>3</sub>	COOC <sub>2</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	221–223 (dec.)	EtOH/ dioxane 58	C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	1710	1.32 (t, J = 7 Hz, 3 H), 2.91 (s, 3 H), 4.32 (q, J = 7 Hz, 2 H), 7.64–8.04 (m, 5 H).	~1000	~600	51 ± 4*	36 ± 21*	40 ± 5*	250
<b>PBZ</b>									~ 700	~300	21 ± 5*	59 ± 12*	10 ± 7	300

Oral administration for all tests

<sup>a</sup> Values are percent of controls. P < 0.05 Student-Newman-Keuls test versus controls

ductive effect, being much more effective than PBZ at the same dose.

All compounds, except **15**, when tested at a total oral dose of 200 mg/kg, produced severe ulcerogenic effects. These results (Table) clearly indicate that only compound **15** shows a significant antiinflammatory activity with low acute toxicity and ulcerogenic effects much lower than that of PBZ although its analgesic action is not remarkable. The antiinflammatory activity of compound **15** appears to be higher than that of the thienopyrimidothiadiazine derivatives [5, 6] and is comparable to that of the most active thiadiazolo and thiazolothienopyrimidine derivatives [1–4]. From the studies carried out so far we may deduce that the thiadiazolothienopyrimidine system, compared to the other systems considered, is the one with the best antiinflammatory agents, without ulcerogenic effects: in this series, the best results were in the derivatives with a phenyl in position 7 [2] and with a tetramethylene chain in position 6, 7 [1]. Often, these compounds also show excellent analgesic activity. Further studies are in progress in order to obtain some information about the mechanism of the antiulcerogenic and analgesic activities of our most powerful compounds.

### 3. Experimental

Melting points were determined in open capillary tubes on a Gallenkamp apparatus and are uncorrected. Elemental analyses for C, H, N, and S were obtained on a Fisons-Carlo Erba EA1108 Elemental Analyzer instrument and were within 0.4% of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1600 FT-IR spectrometer in KBr disks. <sup>1</sup>H NMR spectra were obtained at 200 MHz on a Varian Inova-Unity 200 spectrometer in DMSO-d<sub>6</sub> solution and are expressed as δ units (ppm)

relative to TMS as the internal standard; coupling constants (J) are in Hertz. The purity of the compounds was checked by TLC on Merck silica gel 60 F-254 plates.

#### 3.1. Ethyl ester of 2-isothiocyanato-5-propyl-3-thiophenecarboxylic acid (2)

A solution of amino ester **1** (2 g, 9.39 mmol) in dichloromethane (25 ml) was added with stirring at room temperature for 15 min to a solution of thiophosgene (1 ml) and sodium hydrogen carbonate (1.5 g, 17.86 mmol) in dichloromethane (50 ml) and water (10 ml). The organic layer was then separated and washed twice with water. The organic extract was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield **2** (1.43 g, 60%) purified by column chromatography as a yellow oil, IR (cm<sup>-1</sup>): 2080 (C=N=S), 1715 (C=O). C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 0.90 (t, J = 7.2 Hz, 3 H), 1.31 (t, J = 7.2 Hz, 3 H), 1.59 (m, 2 H), 2.72 (t, J = 7.2 Hz, 2 H), 4.27 (q, J = 7.2 Hz, 2 H), 7.03 (s, 1 H).

#### 3.2. Ethyl ester of 2-[(hydrazinothioxomethyl)amino]-5-propyl-3-thiophenecarboxylic acid (3)

A solution of isothiocyanate **2** (2 g, 7.84 mmol) in dichloromethane (30 ml) was added with stirring at room temperature to a solution of hydrazine monohydrate (0.39 ml, 98%) in dichloromethane (20 ml) and the suspension was stirred at room temperature for 1 h. The solid was collected, washed with ethanol, dried and recrystallized from ethanol to give compound **3** (0.9 g, 40%), m.p. 195–197 °C, IR (cm<sup>-1</sup>): 1670 (C=O), 3355, 3195 and 3185 (NH). C<sub>11</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 0.90 (t, J = 7.2 Hz, 3 H), 1.29 (t, J = 7 Hz, 3 H), 1.59 (m, 2 H), 2.63 (t, J = 7.2 Hz, 2 H), 4.26 (q, J = 7 Hz, 2 H), 6.86 (s, 1 H), 9.74 (s, 1 H).

#### 3.3. Monopotassium salt of 3-amino-2,3-dihydro-6-propyl-2-thioxothieno[2,3-d]pyrimidin-4(1H)-one (6)

Derivative **3** (4.8 g, 16.72 mmol) was added to a solution of potassium hydroxide (0.94 g, 16.78 mmol) in ethanol (69 ml) and the suspension was refluxed with stirring for 1 h. The solid was collected while hot and washed with warm absolute ethanol to give salt **6** (3.16 g, 68%).

### 3.4. 3-Amino-2,3-dihydro-6-propyl-2-thioxothieno[2,3-d]pyrimidin-4(1H)-one (**8**)

A suspension of potassium salt **6** (0.6 g, 2.15 mmol) in water (50 ml) was acidified with concentrated hydrochloric acid and stirred at room temperature for 30 min. The white solid was collected, washed with water, dried and recrystallized from ethanol to give the thioxo derivative **8** (0.3 g, 58%), m.p. 213–215 °C, IR (cm<sup>-1</sup>): 1660 (C=O), 3285 and 3170 (NH). C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>OS<sub>2</sub>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 0.90 (t, *J* = 7 Hz, 3H), 1.60 (m, 2H), 2.74 (t, *J* = 7 Hz, 2H), 6.31 (br s, 2H), 6.99 (s, 1H), 13.8° (br s, 1H). This compound has analytical and spectral data like those of the compound obtained in the following manner:

A mixture of isothiocyanate **2** (0.6 g, 2.35 mmol) and hydrazine monohydrate (2 ml) was kept under reflux and with stirring in benzene (15 ml) for 2 h. After cooling, the solid was collected, washed with ethanol, dried and recrystallized from ethanol to yield compound **8** (0.25 g, 44%), m.p. 213–214 °C, IR (cm<sup>-1</sup>): 1660 (C=O), 3285 and 3170 (NH). C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>OS<sub>2</sub>.

### 3.5. 8H-[1,3,4]Thiadiazolo[3,2-a]thieno[2,3-d]pyrimidin-5-one derivatives (**9–20**)

A mixture of potassium salts **4–7** (3 mmol) and acetic, propionic or benzoic acid (6 mmol), and phosphorus oxychloride (10 ml) was refluxed for 40 min. After cooling, the suspension was poured into cold water and neutralized with 10% sodium hydroxide solution. The precipitate was collected, washed with water dried and recrystallized from a suitable solvent (Table).

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