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Synthesis and spasmolytic action of 2-substituted thienopyrimidin-4-one derivatives

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Abstract

In the search for novel compounds to treat disorders of smooth muscle function, efforts have focused on some 2-substituted thieno[2,3-d]pyrimidin-4-one derivatives that show interesting spasmolytic action. Our laboratories have developed a new series of quaternary salts of 2-substituted thieno[2,3-d]pyrimidin-4-one and thieno[3,2-d]pyrimidin-4-one isomers with therapeutic potential. These substances were prepared starting from simple derivatives of thiophene. Their spasmolytic activity was evaluated on transmurally stimulated guinea-pig ileum. The most active compounds (IC50 1.12–2.71 μM) **7f–7h, 12d** and **12f** had the terminal piperidino nucleus in the thioalkyl chain and lacked two methyl groups in the thiophene ring. Their relaxant activity on the isolated ileum was potentiated (approx. 20–25%) by phosphodiesterase inhibitors. Compounds **7f–h, 12d** and **12f** were less effective in inhibiting contractions of the guinea-pig ileum induced by acetylcholine (IC50 26.7–41.4 μM) or histamine (IC50 41.5–63.4 μM) and had a moderate binding activity to muscarinic receptors in membrane homogenates from the rat heart (M_2 sites; pK_i values between 5.55 ± 0.08 and 5.14 ± 0.12; n = 3) and submaxillary gland (M_3 sites; pK_i values between 6.15 ± 0.07 and 5.76 ± 0.08; n = 3). Action involving soluble guanylyl cyclase or any potential binding to guinea-pig ventricular L-type calcium channels was not considered likely. It is concluded that at least two different mechanisms of action contribute to their spasmolytic activity.

Introduction

Several drugs with different mechanisms of action are employed for the treatment of gastrointestinal and biliary spasms (for review see Ruoff et al 1991; De Ponti & Malagelada 1998). Among others, antimuscarinics (Ehlert et al 1999; Wallis & Napier 1999), calcium antagonists (Elorriaga et al 1996) and antispasmodic agents acting through different mechanisms (Parker et al 1987; Nardi et al 1993; Santicioli et al 1999) have been widely investigated. We previously reported the synthesis and spasmolytic properties of a series of compounds containing a thieno[2,3-d]pyrimidin-4-one system corresponding to structure A (Russo et al 1990) (Figure 1). The relaxant action of these derivatives on intestinal smooth muscle prompted us to extend our research to investigate the pharmacological mechanism underlying their activity. The choice of the 2-(*N*-diethylamino)ethylthio-3-phenyl-5,6-dimethyl-thieno[2,3-d]pyrimidin-4-one methyl iodide (**1**) as lead compound was made because, among the previously reported derivatives, it was shown to have the best relaxant activity on intestinal smooth muscle in-vivo and in-vitro. On the basis of the structural features of the described compounds and in order to obtain more

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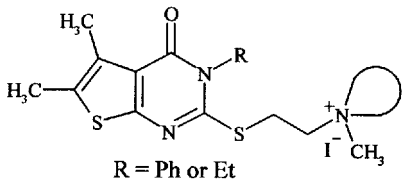

A

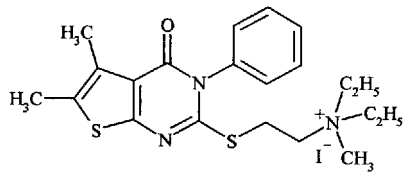

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Figure 1 Structure of analogues of thieno[2,3-d]pyrimidin-4-one.

active derivatives, we performed the following modifications: (i) substitution of the diethylamino group with the piperidine ring; (ii) insertion of a substituent *para* to the aromatic ring; (iii) removal of the methyl groups on the thiophene nucleus; and (iv) substitution of the thieno[2,3-d]pyrimidin-4-one nucleus with the corresponding thieno[3,2-d]pyrimidin-4-one isomers.

The spasmolytic activity of these novel derivatives was evaluated on transmurally stimulated guinea-pig ileum. The most promising compounds were evaluated more extensively to check the involvement of phosphodiesterases of the soluble guanylyl cyclase or of the muscarinic receptors. We also measured their binding activity to muscarinic receptors in membrane homogenates from the rat heart (M_2 sites) and submaxillary gland (M_3 sites), or any potential binding to guinea-pig ventricular L-type calcium channels.

Derivatives **7a**–**l** were synthesized by the action of the appropriate arylisothiocyanate derivative on starting amines **2** and **3** to give the thiourea derivatives **4a**–**e**. Cyclization of the thiourea derivatives yielded the 2-mercapto-thienopyrimidin-4-one compounds **5a**–**e**. Base-catalysed alkylation of these thienopyrimidin-4-one derivatives with the appropriate 2-chloroethyl amine gave compounds **6a**–**l**. Quaternization of the bases with methyl iodide provided the final compounds **7a**–**l** (Figure 2). The corresponding thieno[3,2-d]pyrimidin-4-one isomers **12a**–**f** were synthesized by the same procedure, starting from the methyl 3-aminothiophene-2-carboxylate **8** (Figure 3). Compound **2** was prepared as reported by Gewald et al (1966). Methyl 2-amino-3-thiophenecarboxylate (**3**) and methyl 3-amino-2-thiophenecarboxylate (**8**) are commercially available.

Materials and Methods

Chemistry

Reagents used for synthesis were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise specified. Thin-layer chromatography was done on pre-coated silica gel 60 F_{264} aluminium sheets (Merck) with visualization under UV light. Melting points were determined in open capillary tubes with a Büchi melting point apparatus, and are uncorrected. IR spectra were obtained using a 1600 FT-IR Perkin-Elmer spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Varian Inova 200 spectrometer. Elemental analyses (C, H, N, S) were determined on a Carlo Erba Model 1106 elemental analyser and were within 0.4% of the theoretical values.

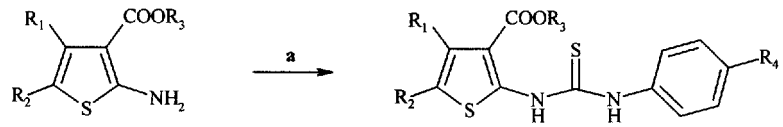
General procedure for the preparation of ethyl 2({[(phenyl)amino]carbonothioyl}amino)-4,5-dimethyl-thiophene-3-carboxylate (4a–b), methyl 2({[(phenyl)amino]carbonothioyl}amino)thiophene-3-carboxylate (4c–e) and methyl 3({[(phenyl)amino]carbonothioyl}amino)thiophene-2-carboxylate (9a–c)

The appropriate arylisothiocyanate (0.015 mol) was added, dropwise, to a hot and stirred solution of thiophene amino ester derivative **2**, **3** or **8** (0.013 mol) in ethanol (40 mL) The mixture was then refluxed for a reaction time of between 0.5 and 4 h. When the mixture was cooled, the crystals were separated by filtration. After washing with ethanol, the final compounds were recrystallized from ethanol.

*Ethyl 2-({[(4-chlorophenyl)amino]carbonothioyl}amino)-4,5-dimethyl-thiophene-3-carboxylate (**4a**)*
(80%) mp 192–194°C; IR (KBr) 1660. Reaction time: 2 h. Analysis: calc. for $C_{16}H_{17}ClN_2O_2S_2$.

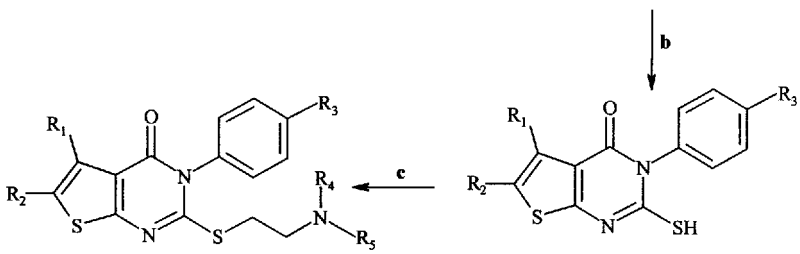
*Ethyl 4,5-dimethyl-2-({[(4-methylphenyl)amino]carbonothioyl}amino)thiophene-3-carboxylate (**4b**)*
(80%) mp 161–162°C; IR (KBr) 1660. Reaction time: 3 h. Analysis: calc. for $C_{17}H_{20}N_2O_2S_2$.

*Methyl2-[(anilinocarbonothioyl)amino]thiophene-3-carboxylate (**4c**)*
(40%) mp 165–167°C; IR (KBr) 1660. Reaction time: 4 h. Analysis: calc. for $C_{13}H_{12}N_2O_2S_2$.



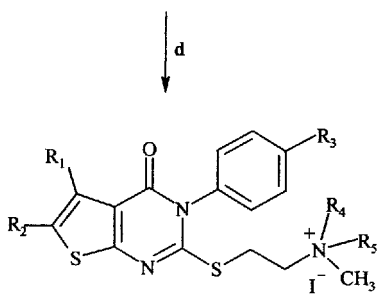
2, $R_1 = R_2 = CH_3$, $R_3 = C_2H_5$
3, $R_1 = R_2 = H$, $R_3 = CH_3$

4a, $R_1 = R_2 = CH_3$, $R_3 = C_2H_5$, $R_4 = Cl$
4b, $R_1 = R_2 = CH_3$, $R_3 = C_2H_5$, $R_4 = CH_3$
4c, $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = H$
4d, $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = Cl$
4e, $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = CH_3$



6a, $R_1 = R_2 = CH_3$, $R_3 = Cl$, $R_4 = R_5 = C_2H_5$
6b, $R_1 = R_2 = CH_3$, $R_3 = CH_3$, $R_4 = R_5 = C_2H_5$
6c, $R_1 = R_2 = H$, $R_3 = H$, R_4, $R_5 = C_2H_5$
6d, $R_1 = R_2 = H$, $R_3 = Cl$, R_4, $R_5 = C_2H_5$
6e, $R_1 = R_2 = H$, $R_3 = CH_3$, R_4, $R_5 = C_2H_5$
6f, $R_1 = R_2 = H$, $R_3 = H$, $R_4 = R_5 = -(CH_2)_5-$
6g, $R_1 = R_2 = H$, $R_3 = Cl$, $R_4 = R_5 = -(CH_2)_5-$
6h, $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = R_5 = -(CH_2)_5-$
6i, $R_1 = R_2 = CH_3$, $R_3 = Cl$, $R_4 = R_5 = -(CH_2)_5-$
6l, $R_1 = R_2 = CH_3$, $R_3 = CH_3$, $R_4 = R_5 = -(CH_2)_5-$

5a, $R_1 = R_2 = CH_3$, $R_3 = Cl$
5b, $R_1 = R_2 = CH_3$, $R_3 = CH_3$
5c, $R_1 = R_2 = H$, $R_3 = H$
5d, $R_1 = R_2 = H$, $R_3 = Cl$
5e, $R_1 = R_2 = H$, $R_3 = CH_3$



7a-l

7a, $R_1 = R_2 = CH_3$, $R_3 = Cl$, $R_4 = R_5 = C_2H_5$
7b, $R_1 = R_2 = CH_3$, $R_3 = CH_3$, $R_4 = R_5 = C_2H_5$
7c, $R_1 = R_2 = H$, $R_3 = H$, R_4, $R_5 = C_2H_5$
7d, $R_1 = R_2 = H$, $R_3 = Cl$, R_4, $R_5 = C_2H_5$
7e, $R_1 = R_2 = H$, $R_3 = CH_3$, R_4, $R_5 = C_2H_5$
7f, $R_1 = R_2 = H$, $R_3 = H$, $R_4 = R_5 = -(CH_2)_5-$
7g, $R_1 = R_2 = H$, $R_3 = Cl$, $R_4 = R_5 = -(CH_2)_5-$
7h, $R_1 = R_2 = H$, $R_3 = CH_3$, $R_4 = R_5 = -(CH_2)_5-$
7i, $R_1 = R_2 = CH_3$, $R_3 = Cl$, $R_4 = R_5 = -(CH_2)_5-$
7l, $R_1 = R_2 = CH_3$, $R_3 = CH_3$, $R_4 = R_5 = -(CH_2)_5-$

Figure 2 Synthesis of compounds **7a–l**. Reagents and conditions: a. $S=C=NC_6H_4R_4$, C_2H_5OH, reflux; b. i. 2 M KOH, ii. 3 M HCl; c. $ClC_2H_4N(C_2H_5)_2$ or 1-(2-chloroethyl)piperidine, 2 M NaOH; d. CH_3I, dioxane.

*Methyl 2-({[(4-chlorophenyl)amino]carbonothioyl}amino)thiophene-3-carboxylate (**4d**)*
(52%) mp 190–192°C; IR (KBr) 1660. Reaction time: 2.5 h. Analysis: calc. for $C_{11}H_{13}ClN_2O_2S_2$.

*Methyl 2-({[(4-methylphenyl)amino]carbonothioyl}amino)thiophene-3-carboxylate (**4e**)*
(46%) mp 186–189°C; IR (KBr) 1660. Reaction time: 3 h. Analysis: calc. for $C_{14}H_{14}N_2O_2S_2$.

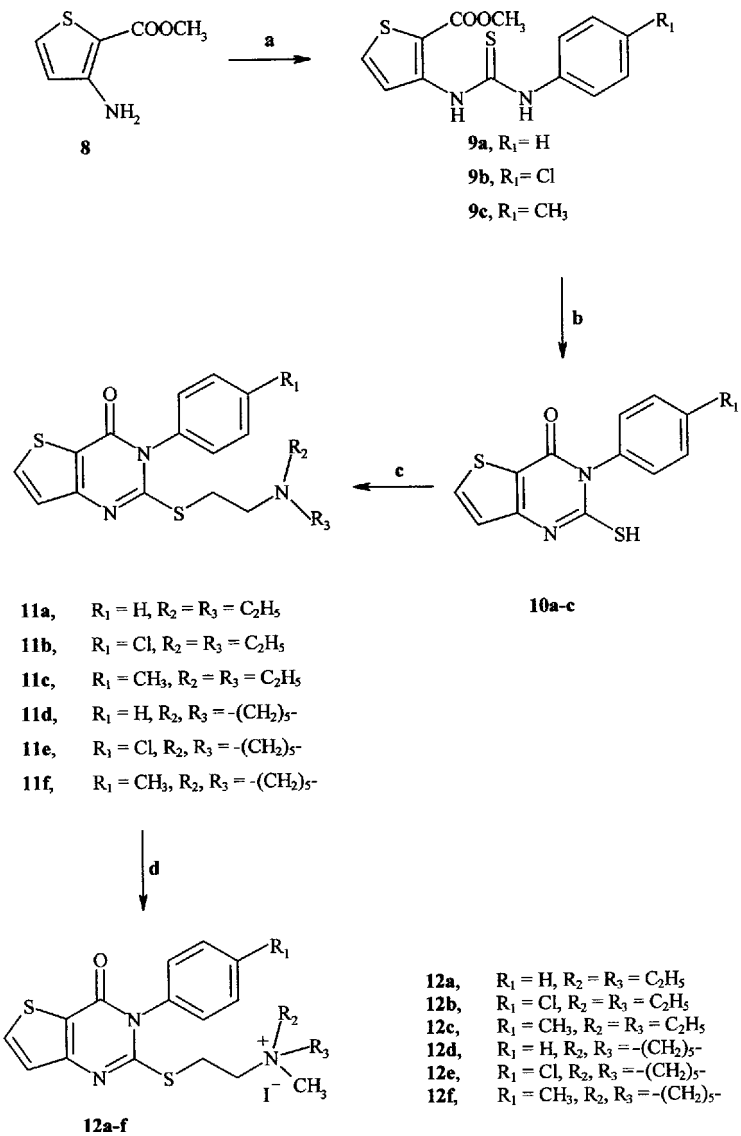


Figure 3 Synthesis of compounds **12a–f**. Reagents and conditions: a. $S=C=NC_6H_4R_4$, C_2H_5OH, reflux; b. i. 2 M KOH, ii. HCl; c. $ClC_2H_4N(C_2H_5)_2$ or 1-(2-chloroethyl)piperidine, 2 M NaOH; d. CH_3I, dioxane.

*Methyl3-[(anilinocarbonothioyl) amino] thiophene-2-carboxylate (**9a**)*
(85%); mp 260–262°C; IR (KBr) 1660. Reaction time: 3 h. Analysis: calc. for $C_{12}H_8N_2OS_2$.

*Methyl3-({ [(4-chlorophenyl) amino] carbonothioyl} amino) thiophene-2-carboxylate (**9b**)*
(80%); mp 282–285°C; IR (KBr) 1660. Reaction time: 2 h. Analysis: calc. for $C_{12}H_7ClN_2OS_2$.

*Methyl3-({ [(4-methylphenyl) amino] carbonothioyl} amino) thiophene-2-carboxylate (**9c**)*
(88%); mp 249–251°C; IR (KBr) 1660. Reaction time: 0.5 h. Analysis: calc. for $C_{13}H_{10}N_2OS_2$.

General procedure for the preparation of 3-(phenyl)-2-mercapto-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (5a–b), 2-mercapto-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one (5c–e) and 2-mercapto-3-phenylthieno[3,2-d]pyrimidin-4-(3H)-one (10a–c)

Compound of type **4** or **9** (0.015 mol) was added to a solution of 2 M KOH (100 mL), and the reaction mixture was stirred under reflux for 1 h until complete dissolution. After cooling and acidification with 3 M HCl, the precipitate obtained was removed by filtration and washed with water (3 × 100 mL). It was then recrystallized from ethanol, giving solid as white crystals.

*3-(4-Chlorophenyl)-2-mercapto-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**5a**)*
(85%); mp 293–295°C; IR (KBr) 1670; Analysis: calc. for $C_{14}H_{11}ClN_2OS_2$.

*2-Mercapto-5,6-dimethyl-3-(4-methylphenyl)-thieno[2,3-d]pyrimidin-4(3H)-one (**5b**)*
(79%); mp 269–271°C; IR (KBr) 1670; Analysis: calc. for $C_{15}H_{14}N_2OS_2$.

*2-Mercapto-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one (**5c**)*
(89%); mp 279–282°C; IR (KBr) 1670; Analysis: calc. for $C_{12}H_8N_2OS_2$.

*3-(4-Chlorophenyl)-2-mercaptothieno[2,3-d]pyrimidin-4(3H)-one (**5d**)*
(90%); mp 288–290°C; IR (KBr) 1670; Analysis: calc. for $C_{12}H_7ClN_2OS_2$.

*2-Mercapto-3-(4-methylphenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**5e**)*
(90%); mp 285–287°C; IR (KBr) 1670; Analysis: calc. for $C_{13}H_{10}N_2OS_2$.

*2-Mercapto-3-phenylthieno[3,2-d]pyrimidin-4-(3H)-one (**10a**)*
(85%); mp 260–262°C; IR (KBr) 1680; Analysis: calc. for $C_{12}H_8N_2OS_2$.

*3-(4-Chlorophenyl)-2-mercaptothieno[3,2-d]pyrimidin-4(3H)-one (**10b**)*
(80%); mp 282–285°C; IR (KBr) 1680; Analysis: calc. for $C_{12}H_7ClN_2OS_2$.

*2-Mercapto-3-(4-methylphenyl)thieno[3,2-d]pyrimidin-4(3H)-one (**10c**)*
(88%); mp 249–251°C; IR (KBr) 1680; Analysis: calc. for $C_{13}H_{10}N_2OS_2$.

General procedure for the preparation of 3-(phenyl)-2-{[2-(diethylamino)ethyl]thio}-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (6a–b, i–l), 2-{[2-(diethylamino)ethyl]thio}-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one (6c–h) and 2-{[2-diethylamino)ethyl]thio}-3-phenylthieno[3,2-d]pyrimidin-4(3H)-one (11a–f)

The suitable chloroethyl tertiary amine hydrochloride (0.014 mol) in water (40 mL) was added to an aqueous solution of 2 M NaOH (40 mL) containing the appropriate mercapto derivatives (0.014 mol) in water (100 mL). The reaction mixture was stirred at 50°C for 0.5 h. After cooling, the solid was filtered, washed with water (2 × 40 mL) and recrystallized from ethanol giving the desired product.

*3-(4-Chlorophenyl)-2-{[2-(diethylamino)ethyl]thio}-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**6a**)*
(85%) mp 113–116°C; Analysis: calc. for $C_{20}H_{24}ClN_3OS_2$.

*2-{[2-(diethylamino)ethyl]thio}-5,6-dimethyl-3-(4-methylphenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**6b**)*
(80%); mp 97–100°C; Analysis: calc. for $C_{21}H_{27}N_3OS_2$.

*2-{[2-(diethylamino)ethyl]thio}-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one (**6c**)*
(66%); mp 72–74°C; Analysis: calc. for $C_{18}H_{21}N_3OS_2$.

*3-(4-Chlorophenyl)-2-{[2-(diethylamino)ethyl]thio}thieno[2,3-d]pyrimidin-4(3H)-one (**6d**)*
(90%); mp 130–133°C; Analysis: calc. for $C_{18}H_{20}ClN_3OS_2$.

*2-{[2-(Diethylamino)ethyl]thio}-3-(4-methylphenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**6e**)*
(77%); mp 100–102°C; Analysis: calc. for $C_{19}H_{23}N_3OS_2$.

*3-Phenyl-2-[(2-piperidin-1-ylethyl)thio]thieno[2,3-d]pyrimidin-4(3H)-one (**6f**)*
(78%); mp 130–132°C; Analysis: calc. for $C_{19}H_{21}N_3OS_2$.

*3-(4-Chlorophenyl)-2-[(2-piperidin-1-ylethyl)thio]thieno[2,3-d]pyrimidin-4(3H)-one (**6g**)*
(95%); mp 140–142°C; Analysis: calc. for $C_{19}H_{20}ClN_3OS_2$.

*3-(4-Methylphenyl)-2-[(2-piperidin-1-ylethyl)thio]thieno[2,3-d]pyrimidin-4(3H)-one (**6h**)*
(65%); mp 127–129°C; Analysis: calc. for $C_{20}H_{23}N_3OS_2$.

*3-(4-Chlorophenyl)-5,6-dimethyl-2-[(2-piperidin-1-ylethyl)thio]thieno[2,3-d]pyrimidin-4(3H)-one (**6i**)*
(85%); mp 157–160°C; Analysis: calc. for $C_{21}H_{24}ClN_3OS_2$.

*5-6-Dimethyl-3-(4-methylphenyl)-2-[(2-piperidin-1-ylethyl)thio]thieno[2,3-d]pyrimidin-4(3H)-one (**6l**)*
(90%); mp 122–124°C; Analysis: calc. for $C_{22}H_{27}N_3OS_2$.

*2-{[(2-Diethylamino)ethyl]thio}-3-phenylthieno[3,2-d]pyrimidin-4(3H)-one (**11a**)*
(82%); mp 85–87°C; Analysis: calc. for $C_{18}H_{21}N_3OS_2$.

*3-(4-Chlorophenyl)-2-{[(2-diethylamino)ethyl[thio}thieno[3,2-d]pyrimidin-4(3H)-one (**11b**)*
(69%); mp 268–271°C; Analysis: calc. for $C_{18}H_{20}ClN_3OS_2$.

*2-{[(2-Diethylamino)ethyl]thio}-3-(4-methylphenyl)thieno[3,2-d]pyrimidin-4(3H)-one (**11c**)*
(92%); mp 143–145°C; Analysis: calc. for $C_{19}H_{23}N_3OS_2$.

*3-Phenyl-2-[(2-piperidin-1-ylethyl)thio]thieno[3,2-d]pyrimidin-4(3H)-one (**11d**)*
(85%); mp 153–155°C; Analysis: calc. for $C_{19}H_{21}N_3OS_2$.

*3-(4-Chlorophenyl)-2-[(2-piperidin-1-ylethyl)thio]thieno[3,2-d]pyrimidin-4(3H)-one (**11e**)*
(90%); mp 145–148°C; Analysis: calc. for $C_{19}H_{20}ClN_3OS_2$.

*3-(4-Methylphenyl)-2-[(2-piperidin-1-ylethyl)thio]thieno[3,2-d]pyrimidin-4(3H)-one (**11f**)*
(90%); mp 121–123°C; Analysis: calc. for $C_{20}H_{23}N_3OS_2$.

General procedure to obtain quaternary derivatives *7a–l* and *12a–f*

CH_3I (0.05 mol) was added to a stirred solution of the suitable tertiary amine (0.05 mol) in dioxane (25 mL) and the reaction mixture was refluxed for 0.5 h. The precipitate was filtered, washed with $(C_2H_5)_2O$ (3 × 10 mL) and recrystallized from ethanol, giving the desired final product.

2-{[3-(4-Chlorophenyl)-5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}-N,N-*diethyl*-N-*methylethanaminium iodide (**7a**)*
(50%); mp 194–197°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.16–1.35 (m, 6H, CH_3), 2.33 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.18–3.43 (m, 11H), 7.41–7.71 (m, 4H, Ar-H); Analysis: calc. for $C_{21}H_{27}ClIN_3OS_2$.

2-{[5,6-Dimethyl-3-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}-N,N-*diethyl*-N-*methylethanaminium iodide (**7b**)*
(75%); mp 154–157°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.18–1.35 (m, 6H, CH_3), 2.33 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.26–3.50 (m, 8H), 7.15–7.40 (m, 4H, Ar-H); Analysis: calc. for $C_{22}H_{30}IN_3OS_2$.

N,N-*Diethyl*-N-*methyl*-2-*{[4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidin2-yl]thio}ethanaminium iodide (**7c**)*
(73%); mp 207–209°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.19–1.38 (m, 6H, CH_3), 2.97 (s, 3H, CH_3), 3.26–3.50 (m, 8H), 7.36–7.63 (m, 7H, thiophene H, Ar-H); Analysis: calc. for $C_{19}H_{24}IN_3OS_2$.

2-{[3-(4-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}-N,N-*diethyl*-N-*methylethanaminium iodide (**7d**)*
(90%); mp 94–95°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.17–1.36 (m, 6H, CH_3), 2.97 (s, 3H, CH_3), 3.26–3.50 (m, 8H), 7.39 (d, H, thiophene H), 7.44–7.71 (m, 5H, thiophene H, Ar-H); Analysis: calc. for $C_{19}H_{23}ClIN_3OS_2$.

N,N-*Diethyl*-N-*methyl*-2-*{[3-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}ethanaminium iodide (**7e**)*
(75%); mp 198–200°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.18–1.37 (m, 6H, CH_3), 2.39 (s, 3H, CH_3), 2.97 (s, 3H,

CH_3), 3.27–3.50 (m, 8H), 7.25–7.42 (m, 5H, thiophene H, Ar-H). 7.54 (d, H, thiophene H); Analysis: calc. for $C_{20}H_{26}IN_3OS_2$.

*1-Methyl-1-{2-[(4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl)thio]ethyl}piperidinium iodide (**7f**)*
(95%); mp 250–252°C; Analysis: ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.62 (m, 2H, piperidine H), 1.68–1.93 (m, 4H, piperidine H), 3.09 (s, 3H, CH_3), 3.27–3.60 (m, 8H), 7.30–7.68 (m, 7H, thiophene H, Ar-H); Analysis: calc. for $C_{20}H_{24}IN_3OS_2$.

*2-{[(3(4-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}ethyl-piperidinium iodide (**7g**)*
(95%); mp 292–294°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.61 (m, 2H, piperidine H), 1.71–1.93 (m, 4H, piperidine H), 3.09 (s, 3H, CH_3), 3.23–3.61 (m, 8H), 7.32–7.71 (m, 6H, thiophene H, Ar-H); Analysis: calc. for $C_{20}H_{23}ClIN_3OS_2$.

*1-Methyl-1-(2-{[(3(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}ethyl)piperidinium iodide (**7h**)*
(95%); mp 235–237°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.46–1.63 (m, 2H, piperidine H), 1.71–1.93 (m, 4H, piperidine H), 2.39 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 3.26–3.61 (m, 8H), 7.22–7.58 (m, 6H, thiophene H, Ar-H); Analysis: calc. for $C_{21}H_{26}IN_3OS_2$.

*2-{[3-(4-Chlorophenyl)-5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}ethyl)-1-methylpiperidinium iodide (**7i**)*
(80%); mp 132–134°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.61 (m, 2H, piperidine H), 1.70–1.92 (m, 4H, piperidine H), 2.33 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 3.18–3.52 (m, 8H), 7.43–7.70 (m, 4H, Ar-H); Analysis: calc. for $C_{22}H_{27}ClIN_3OS_2$.

*2-{[5,6-Dimethyl-3-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl]thio}ethyl)-1-methylpiperidinium iodide (**7l**)*
(80%); mp 231–233°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.61 (m, 2H, piperidine H), 1.72–1.90 (m, 4H, piperidine H), 2.33 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.06 (s, 3H, CH_3), 3.20–3.52 (m, 8H), 7.22–7.41 (m, 4H, Ar-H); Analysis: calc. for $C_{23}H_{30}IN_3OS_2$.

N,N-*Diethyl*-N-*methyl-2-[{4oxo-3-phenyl-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl]thio}ethanamonium iodide (**12a**)*
(90%); mp 181–182°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.21–1.35 (m, 6H, CH_3), 2.98 (s, 3H, CH_3), 3.28–3.46 (m, 8H), 7.36 (d, 1H, thiophene H), 7.41–7.68 (m, 4H, Ar-H) 8.27 (d, 1H, thiophene H); Analysis: calc. for $C_{19}H_{24}IN_3OS_2$.

*2-{[3-(4-Chlorophenyl)-4oxo-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl]thio}*N,N-*diethyl*-N-*methylethanamonium iodide (**12b**)*
(80%); mp 214–216°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.21–1.34 (m, 6H, CH_3), 2.97 (s, 3H, CH_3), 3.28–3.46 (m, 8H), 7.33 (d, 1H, thiophene H), 7.42–7.71 (m, 4H, Ar-H), 8.23 (d, 1H, thiophene H); Analysis: calc. for $C_{19}H_{23}ClIN_3OS_2$.

N,N-*Diethyl*-N-*methyl-2-[{3-(4-methylphenyl)-4oxo-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl]thio}ethanamonium iodide (**12c**)*
(95%); mp 219–220°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.18–1.38 (m, 6H, CH_3), 2.39 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.28–3.48 (m, 8H), 7.26–7.43 (m, 5H, thiophene H, Ar-H) 8.26 (d, 1H, thiophene H); Analysis: calc. for $C_{20}H_{26}IN_3OS_2$.

*1-Methyl-1{2-[(4-oxo-3-phenyl-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl)thio]ethyl}piperidinium iodide (**12d**)*
(90%); mp 243–245°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.46–1.61 (m, 2H, piperidine H), 1.73–1.92 (m, 4H, piperidine H), 3.10 (s, 3H, CH_3), 3.28–3.59 (m, 8H), 7.37–7.63 (m, 5H, thiophene H, Ar-H), 8.26 (d, 1H, thiophene H); Analysis: calc. for $C_{20}H_{24}IN_3OS_2$.

*1-2-{[3-(4-Chlorophenyl)-4-oxo-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl]thio}ethyl-1-methylpiperidinium iodide (**12e**)*
(75%); mp 221–223°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.61 (m, 2H, piperidine H), 1.73–1.92 (m, 4H, piperidine H), 3.10 (s, 3H, CH_3), 3.30–3.58 (m, 8H), 7.38 (d, 1H, thiophene H), 7.48–7.72 (m, 4H, Ar-H), 8.26 (d, 1H, thiophene H); Analysis: calc. for $C_{20}H_{23}ClIN_3OS_2$.

*1-Methyl-1-2-{[3-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl]thio}ethyl piperidinium iodide (**12f**)*
(95%); mp 231–233°C; ^{1}H NMR (DMSO-d_6) δ, ppm: 1.47–1.61 (m, 2H, piperidine H), 1.72–1.92 (m, 4H,

piperidine H), 2.40 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 3.31–3.60 (m, 8H), 7.26–7.42 (m, 5H, thiophene H, Ar-H), 8.25 (d, 1H, thiophene H); Analysis: calc. for $C_{21}H_{26}IN_3OS_2$.

Materials

1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), an inhibitor of soluble guanylyl cyclase (Hebeiss & Kilbinger 1998) was obtained from ICN Biomedicals (Milan, Italy). Trequinsin and rolipram, inhibitors of phosphodiesterases (Souness & Rao 1997), atropine sulfate, nifedipine, isdradipine, acetylcholine hydrochloride, histamine dihydrogen phosphate, isoprenaline and phenyl-methyl-sulphonylfluoride, were purchased from Sigma-Aldrich. [^{3}H]isradipine (sp. act. 80 Ci mmol^{-1}) and [^{3}H]*N*-methylscopolamine ([^{3}H]NMS; sp. act. 79.5 Ci mmol^{-1}) were obtained from NEN Life Science Products (Milan, Italy). All other reagents were either of analytical grade or of the highest purity available.

Animals

Male Hartley guinea-pigs (300–400 g), male Sprague–Dawley rats (250–300 g) and male Swiss mice (20–25 g) were purchased from Charles River (Calco, Como, Italy). The animals were kept in air-conditioned rooms (21 ± 2°C; 60 ± 10% relative humidity) under a constant 12-h light–dark cycle and received standard diet and tap water. Experiments were carried out in accordance with the internationally accepted guidelines and all procedures followed the guidelines issued by Animal Care and Use Committee of the University of Bologna (Bologna, Italy; D. Leg. 116/92).

Isolated guinea-pig ileum

Sections of ileum (approx. 10 cm) from the ileo–caecal junction were resected from guinea-pigs and placed in 5-mL tissue baths containing modified Tyrode's solution kept at 35°C, oxygenated with 95% O_2/5% CO_2. The Tyrode's solution had the following composition (mM) 137 NaCl; 2.7 KCl; 1.0 $MgCl_2$; 1.8 $CaCl_2$; 0.4 NaH_2PO_4; 11.9 $NaHCO_3$; 5.5 glucose. The electrode was made of platinum and the intraluminal electrode was used as anode. The ileum was stimulated electrically with rectangular pulses of 0.5-ms duration, at the frequency of 0.15 Hz and at a high enough voltage to give a maximal response (70–80 V). Responses were recorded isometrically with an initial tension of 1 g. After 30–60 min equilibration, 0.2 mL of the test compounds (dissolved in methanol) was applied during electrical stimulation. Each compound was assayed at four concentrations. In some experiments, the ileum's responses to isoprenaline, histamine and acetylcholine were evaluated; test compounds were applied 2 min before the agonist. All experiments were repeated at least three times on different strips of ileum from three different animals. The data are expressed as mean percentage inhibition $\pm$ s.e.m.

M_2 and M_3 muscarinic receptor binding assays

Rats were killed and the hearts were rapidly removed and weighed. Hearts were minced in 5 vols homogenizing buffer (50 mM Tris, 4 mM EDTA pH 7.4, 4°C) using an Ultraturrax and homogenized with a tissue homogenizer (Wheaton; 10 strokes). The suspension was filtered through gauze and centrifuged (1000 *g*, 4°C, 10 min). The supernatant was kept on ice and the pellet was washed with 2 vols homogenizing buffer and centrifuged as before. The two supernatants were then combined and centrifuged (45000 *g*, 4°C, 12 min). The pellet was suspended in 5 vols assay buffer (50 mM Tris, 0.5 mM EDTA pH 7.4, 4°C) and centrifuged as above. The pellet was washed a second time with 5 vols assay buffer and centrifuged as before. After the second wash, a portion of the suspension was retained for protein assay (Lowry et al 1951).

Submaxillary glands were rapidly removed, weighed, minced in 10 vols homogenizing buffer (50 mM Tris, 5 mM EDTA pH 7.4, 4°C), using the Ultraturrax, and homogenized as described for the hearts. The suspension was filtered through gauze and centrifuged (1000 *g*, 4°C, 10 min). The supernatant was kept on ice and the pellet was washed with 2 vols homogenizing buffer and centrifuged as before. The two supernatants were combined and centrifuged (45000 *g*, 4°C, 12 min). The pellet was suspended in 10 vols assay buffer and centrifuged as indicated. The pellet was washed a second time with 10 vols assay buffer and centrifuged as before. After the second suspension, a portion was retained for protein assay (Lowry et al 1951). Pellets were stored at -80°C until used.

The detailed methods of the binding assay are published elsewhere (Waelbroeck et al 1986; Melchiorre et al 1993). [^{3}H]NMS was used to evaluate binding sites in rat heart homogenates (expressing M_2 muscarinic receptors; K_d 0.32 ± 0.04 nM; B_{max} = 77.8 ± 15.3 fmol (mg protein)$^{-1}$) and submaxillary gland homogenates (expressing M_3 muscarinic receptors; K_d 0.48 ± 0.03 nM;

B_{max} = 1102±85 fmol (mg protein)$^{-1}$). Competition binding studies were done in incubation buffer (50 mM sodium phosphate, pH 7.4; enriched with 2 mM $MgCl_2$ and 1% bovine serum albumin). Homogenates (500 μg protein) were incubated for 2 h at 25°C in 1 mL incubation buffer and in the indicated concentration of tracer (0.8 nM). Non-specific binding was assessed in the presence of 10 μM atropine sulfate. Binding assays were terminated by filtration on Whatman GF/C glass-fibre filters previously soaked in 0.1% poly(ethylenimine) and rinsed four times with 5 mL ice-cold 50 mM phosphate buffer (pH 7.4).

Data were analysed using the LIGAND program (Munson & Rodbard 1980). Differences in the slopes of the curves were determined by the test of parallelism described by Tallarida & Murray (1991), and were not significantly different from unity ($P > 0.05$). Hill numbers (n_H) were not significantly different from unity ($P > 0.05$). Equilibrium dissociation constants (K_i) were derived from the Cheng–Prusoff equation (Cheng & Prusoff 1973), $K_i = IC50/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of [^{3}H]NMS. pK_i values are the mean±s.e.m. of three separate experiments run in duplicate.

Binding to L-type calcium channels

Membranes were prepared according to the method of Glossmann & Ferry (1985), using guinea-pig heart ventricles. All tissue preparation steps were done at 4°C, unless otherwise specified. The tissues were finely minced and homogenized with a Polytron homogenizer in 3 vols ice-cold buffer solution containing 20 mM $NaHCO_3$ and 0.1 nM phenyl-methyl-sulfonylfluoride, pH 7.4. The homogenate was diluted 1:7 in the same buffer solution and centrifuged at 1500 *g* for 15 min. The supernatant was collected and centrifuged at 48000 *g* for 15 min; the pellet was washed twice by homogenization under the conditions already described in 7 vols ice-cold 50 mM Tris/HCl, 0.1 mM phenyl-methyl-sulfonylfluoride buffer, pH 7.4, with centrifugation at 48000 *g* for 15 min. The final pellet was resuspended in 3 vols of the same buffer, and the membrane suspension was divided into aliquots and stored at −80°C. On the day of the experiment, aliquots of membranes were thawed, resuspended in 50 mM Tris/HCl, 1 mM $CaCl_2$ buffer, pH 7.4 and used for the binding assay (100 μg protein/tube). Protein was determined by the method of Lowry et al (1951).

Binding experiments were done in near-darkness. Saturation binding experiments were done at concentrations ranging from 0.05 to 5 nM [^{3}H]isradipine (K_d = 4.3×10^{-11}). Specific binding was determined in the presence of 1 μM nifedipine. Ventricular membranes were incubated at 25°C for 120 min in a final volume of 1 mL and incubation was stopped by vacuum filtration over Whatman GF/C filters. For competition binding experiments, 0.2 nM isradipine was used. Drugs were prepared as stock solutions (10^{-2} M) in ethanol and protected from light. Drug dilutions were made in assay buffer. Bound radioactivity was measured in a Beckman Ls 1701 scintillation counter and the data were analysed using the LIGAND program (Munson and Rodbard 1980). At least three independent experiments with assays in duplicate were done for each compound.

Charcoal meal

Mice were fasted for 24 h. Test compounds, suspended in 1% carboxymethylcellulose, were administered intraperitoneally (50 mg kg^{-1}) and, 20 min later, 0.2 mL charcoal meal (5% charcoal in 1% carboxymethylcellulose, w/v) was given orally. Mice were killed 30 min later using sodium pentobarbital (100 mg kg^{-1}, i.p.) and the length of the small intestine traversed by the charcoal was measured in mm. The mean values of both treated groups (Xt; n = 6) and the vehicle-treated group (Xc; n = 6) were calculated, and the percentage inhibition induced by each compound was evaluated in comparison with controls: $(Xc - Xt)/Xc \times 100$.

Data analysis

Data are expressed as mean±s.e.m. Statistical comparisons were done by analysis of variance and post hoc Dunnett's multiple comparison test, with differences of $P < 0.05$ being considered significant (GraphPad Software, San Diego, CA). The concentrations needed to produce 50% inhibitory effects (IC50) were calculated from log concentration–response curves (Tallarida & Murray 1991).

Results and Discussion

The isolated guinea-pig ileum was used as a functional assay to evaluate spasmolytic activity, by measuring the inhibitory effect of compounds on the response to the transmural electrical stimulation. Electrical stimulation of the ileum induced regular and reproducible contractions, which were not affected by administration of 0.2 mL methanol (data not shown).

Table 1 Effect of compounds **1**, **7a–l**, and **12a–f** and isporenaline on spasmolytic activity induced by electrical stimulation in the isolated guinea-pig ileum in the presence of either the vehicle or the phosphodiesterase inhibitors rolipram and trequinsin (10^{-7} M; added 30 min before test compounds).

Compound	IC50 (μM)		
	Vehicle	Rolipram	Trequinsin
1	18.72±0.11	13.41±0.04	14.3±0.09
7a	28.76±0.98	n.d.	n.d.
7b	48.64±1.24	n.d.	n.d.
7c	33.83±2.1	n.d.	n.d.
7d	496.12±22.4	n.d.	n.d.
7e	52.42±0.45	n.d.	n.d.
7f	1.12±0.24	0.73±0.14	0.82±0.17
7g	1.92±0.15	1.03±0.11	1.25±0.16
7h	2.35±0.21	1.77±0.10	1.84±0.18
7i	16.11±0.19	12.5±0.09	14.5±0.32
7l	29.16±1.15	n.d.	n.d.
12a	812.43±13.5	n.d.	n.d.
12b	66.34±0.88	n.d.	n.d.
12c	73.43±0.76	n.d.	n.d.
12d	1.48±0.11	0.59±0.04	0.66±0.13
12e	65.80±1.28	n.d.	n.d.
12f	2.71±0.28	2.23±0.04	2.26±0.17
Isoprenaline	7.6±0.8	3.4±0.4*	3.971±0.3*

Values are the mean±s.e.m. of three different experiments. n.d. = not determined.
*$P < 0.01$ vs vehicle (Dunnett's test after analysis of variance).

As shown in Table 1, the most active compounds (IC50 < 5.0 μM) **7f–h**, **12d** and **12f** were approximately 10-times more active than the lead compound **1**, and had the terminal piperidino nucleus in the thioalkyl chain and lacked two methyl groups in the thiophene ring. The spasmolytic activity displayed by these compounds was not related to the isomer considered or to the substituent on the thiophene ring. In fact, the spasmolytic activity of the isomeric couples **12d**/**7f** and **12f**/**7h** was comparable. Compound **7i** and the lead compound **1** had lower activity (IC50 = 16.7 and 18.1 μM, respectively). The latter was the only compound, among those with most spasmolytic activity, with a diethyl group in the lateral chain. The *para* substituent on the aromatic ring (**7 g–7i**, **12f**) did not significantly influence the activity.

Compounds **7a–c**, **7e**, **7l**, **12b–c** and **12e** were the least potent of this series, and compounds **7d** and **12a** were the less effective (Table 1).

The test compounds maintained their spasmolytic action as long as they were left in contact with the isolated tissue (2 min), and it was reversible after a single wash by overflow.

Compounds **1**, **7f–i**, **12d** and **12f**, which had strong spasmolytic activity, were further investigated. Trequinsin and rolipram (10^{-7} M), blockers of phosphodiesterase activity (Souness & Rao 1997), potentiated their spasmolytic activity by approximately 20–25% and were not active by themselves (data not shown). These selective inhibitors significantly potentiated the spasmolytic action of isoprenaline, by approximately 50% (Table 1). Therefore, any inhibition of phosphodiesterase activity in the spasmolytic effect of the test compounds was less marked than for the potent β-adrenoceptor agent isoprenaline. In preliminary experiments, the most effective compounds potentiated the spasmolytic action

Table 2 Spasmolytic activity of compounds **1**, **7f–i**, **12d** and **12f** on acetylcholine- or histamine-stimulated guinea-pig ileum. Test compounds were added 10 min before acetylcholine chloride (10^{-7} M) or histamine dihydrogen phosphate (10^{-7} M).

Compound	Inhibition of acetylcholine-induced contractions IC50 (μM)	Inhibition of histamine-induced contractions IC50 (μM)
1	44.3±3.0	66.4±6.4
7f	26.7±2.5	41.5±3.2
7g	32.7±4.9	58.7±4.1
7h	31.7±4.1	53.3±2.8
7i	41.4±2.8	63.4±5.1
12d	30.4±1.8	51.0±2.8
12f	33.7±3.5	59.3±3.4
Atropine sulfate	0.8±0.3*	n.d.

Values are the mean±s.e.m. of three different experiments. n.d. = not determined.
*$P < 0.01$ vs test compounds (Dunnett's test after analysis).

Table 3 Affinity estimates, expressed as pK_i values, in the rat heart (M_2) and submaxillary gland (M_3) muscarinic receptors subtypes of compounds **1**, **7f–i**, **12d** and **12f** and atropine sulfate.

Compound	PK_i	
	M_2	M_3
1	5.02±0.12	5.53±0.08
7f	5.55±0.08	6.15±0.07
7g	5.50±0.13	5.80±0.05
7h	5.31±0.12	5.84±0.08
7i	5.26±0.10	5.82±0.11
12d	5.45±0.18	6.01±0.06
12f	5.14±0.12	5.76±0.08
Atropine sulfate	8.89±0.05	9.44±0.09

Values are the mean±s.e.m. of three separate experiments performed in duplicate.

Table 4 Effect of compounds **1**, **7f–i**, **12d** and **12f** on a charcoal meal test in the mouse.

Compound	Inhibition vs control (%)
1	35±3.7
7f	55±6.1
7g	50±4.3
7h	47±3.6
7i	37±1.8
12d	48±3.1
12f	32±1.8

Values are the mean±s.e.m. of six mice.

of isoprenalime (10^{-6} M) (compound **7f**: from 24.5±2.24 to 40.5±3.7, n = 3, $P < 0.01$; compound **7c**: from 24.5±2.24 to 39.5±2.3, n = 3, $P < 0.01$; compound **12d**: from 24.5±2.24 to 38.2±1.9, n = 3, $P < 0.01$).

The selective soluble guanylyl cyclase inhibitor ODQ (10^{-6} M) (Heibeiss & Kilbinger 1998), which blocked the relaxation evoked by 100 μM sodium nitroprusside in the transmurally stimulated guinea-pig ileum, had no effect on the spasmolytic action of the test compounds (data not shown). Compounds **1**, **7f–i**, **12d** and **12f**, as well as atropine sulfate, inhibited contractions induced by acetylcholine, atropine being the most effective (Table 2). IC50 values of test compounds were significantly different compared with atropine sulfate (Table 2), and these compounds also inhibited histamine-induced contractions of the guinea-pig ileum (Table 2). The potential affinity of compounds **1**, **7f–i**, **12d** and **12f** towards muscarinic receptors was explored. We assayed their binding to the muscarinic M_2 and M_3 receptors, which are found in intestinal smooth muscle (Ehlert et al 1997), employing rat heart and submaxillary gland homogenates. These compounds displayed moderate affinity for M_3 sites as well as for M_2 receptors (Table 3). Compounds **7f** and **12d** were the most active. In displacement binding studies on guinea-pig ventricular homogenates labelled with [^{3}H]isradipine, these compounds (up to 10 μM) did not bind to L-type calcium channels (data not shown), whereas the calcium channel antagonist nifedipine was effective (K_i = 1.9±0.05 nM, n = 3).

The relaxant activity displayed by the eight selected compounds was confirmed in-vivo. The compounds, administered intraperitoneally at the dose of 50 mg kg^{-1}, inhibited the transit of a charcoal meal through the mouse gastrointestinal tract (Table 4). Compounds **7f** and **12d** were the most effective, reducing transit by approximately 50%.

We describe a novel series of 2-substituted thienopyrimidin-4-one derivatives, which display spasmolytic activity through different mechanisms. The most active compounds, **7f** and **12d**, which have the terminal piperidino nucleus in the thioalkyl chain and lack the substituent on the thiophene and aromatic ring, act at least partially as M_2/M_3 muscarinic receptor antagonists. The involvement of these receptors in intestinal activity is well known (Ehlert et al 1999; Wallis & Napier 1999). Spasmolytic activity may also involve different second messengers (Parker et al 1987; Young et al 1996). These compounds may possibly influence phosphodiesterase activity as well and, by raising cAMP levels, may contribute to the inhibition of intestinal smooth muscle function (Parker et al 1987). Furthermore, they reduce histamine-induced contractions. Taken together, these results suggest that the spasmolytic action of these compounds was, at least partially, post-synaptic and they have only weak activity on muscarinic receptors. Therefore, an atropine-like action cannot be the primary explanation of their spasmolytic effect. However, functional interactions among muscarinc receptors and other relaxants such as isporenaline and forskolin, or compounds that elicit contractions of the gastrointestinal smooth muscle (such as histamine) have been described (Ehlert et al 1999).

In conclusion, the relaxant effects of compounds **1**, **7f–i**, **12d** and **12f** on visceral smooth muscle may be the result of their interaction with muscarinic receptors, besides being mediated by the cAMP cascade. Spasmolytic compounds with a weak antimuscarinic activity that seems to block smooth muscle contraction, through different mechanisms, have already been described

(Choo et al 1986; Tonini et al 1987; Abbiati et al 1988; Evangelista 1999). These drugs are used to treat several gastrointestinal disorders and cause fewer unpleasant side-effects than the antimuscarinic drugs (De Ponti & Malagelada 1998; Wallis & Napier 1999). This study confirms that some 2-substituted thienopirimidin-4-one derivatives may serve as novel spasmolytic agents to treat disorders of smooth muscle function. Further studies will be necessary to clarify their real therapeutic potential.

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