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Synthesis of 5-(4-Alkylsulfanyl-[1,2,5]Thiadiazol-3-yl)-3-Methyl-1,2,3,4-Tetrahydropyrimidine Oxalate Salts and their Evaluation as Muscarinic Receptor Agonists

The synthesis and biological test of 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)-3-methyl-1,2,3,4-tetrahydropyrimidine oxalate salts **7** as muscarinic receptor agonists are described. The key intermediate **4** was obtained by a modified Strecker reaction and cyclization, and the 3-methyl-1,2,3,4-tetrahydropyrimidines were obtained by subsequent substitution, quarternization, and reduction. The final products **7** were obtained as oxalic acid salts. The prepared compounds were examined *in vitro* for their binding affinities to the cloned human muscarinic receptor by the [^{3}H]-NMS binding assay.

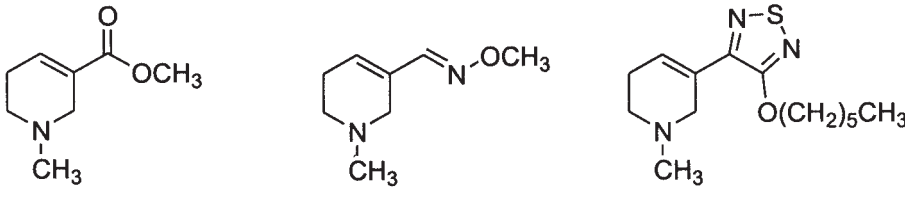
Keywords: Muscarinic receptor agonists; 1,2,3,4-tetrahydropyrimidine; Alzheimer's disease; Xanomeline

Received: September 27, 2002, Accepted: January 13, 2003 [FP730]
DOI 10.1002/ardp.200300730

Introduction

Recent work on Alzheimer's disease (AD) has focused on the development of M_1-selective agonists [1, 2]. M_1 muscarinic receptors play a role in memory function [3, 4] and stimulate phosphoinositide (PI) turnover in the mammalian forebrain [5]. An M_1 agonist is expected to bind selectively to M_1 muscarinic receptors and stimulate PI turnover in the hippocampus [6].

prove the pharmacological and pharmacokinetic properties towards a clinically meaningful profile have led to the emergence of milameline (Chart 1) [9]. On the other hand, xanomeline [10], in which 4-hexyloxy-[1,2,5]-thiadiazole ring replaced the methyl ester of arecoline and milameline, in which the alkoxyimino group replaced the methyl ester, has been reported as a potent functional M_1 selective muscarinic agonist (Chart 1).



arecoline milameline xanomeline

Chart 1. Muscarinic agonists.

The naturally occuring agonist arecoline (Chart 1) had been used for AD patients in the early clinical trial [7, 8]. However, arecoline has several major drawbacks including low efficacy, lack of subtype selectivity and poor metabolic stability. Continued researches in the efforts to improve the pharmacological and pharmacokinetic properties towards a clinically meaningful profile have led to the emergence of milameline (Chart 1) [9]. On the other hand, xanomeline [10], in which 4-hexyloxy-[1,2,5]-thiadiazole ring replaced the methyl ester of arecoline and milameline, in which the alkoxyimino group replaced the methyl ester, has been reported as a potent functional M_1 selective muscarinic agonist (Chart 1).

We have reported the synthesis and biological activities of 3-methyl-1,2,3,4-tetrahydropyrimidines bioisosteric congeners as muscarinic receptor agonists of the tetrahydropyridine derivatives (Chart 2) [11, 12], milameline and xanomeline.

Herein, we describe the synthesis of alkylsulfanyl series of bioisosteric congeners of xanomeline.

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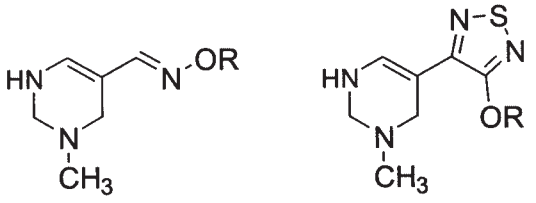
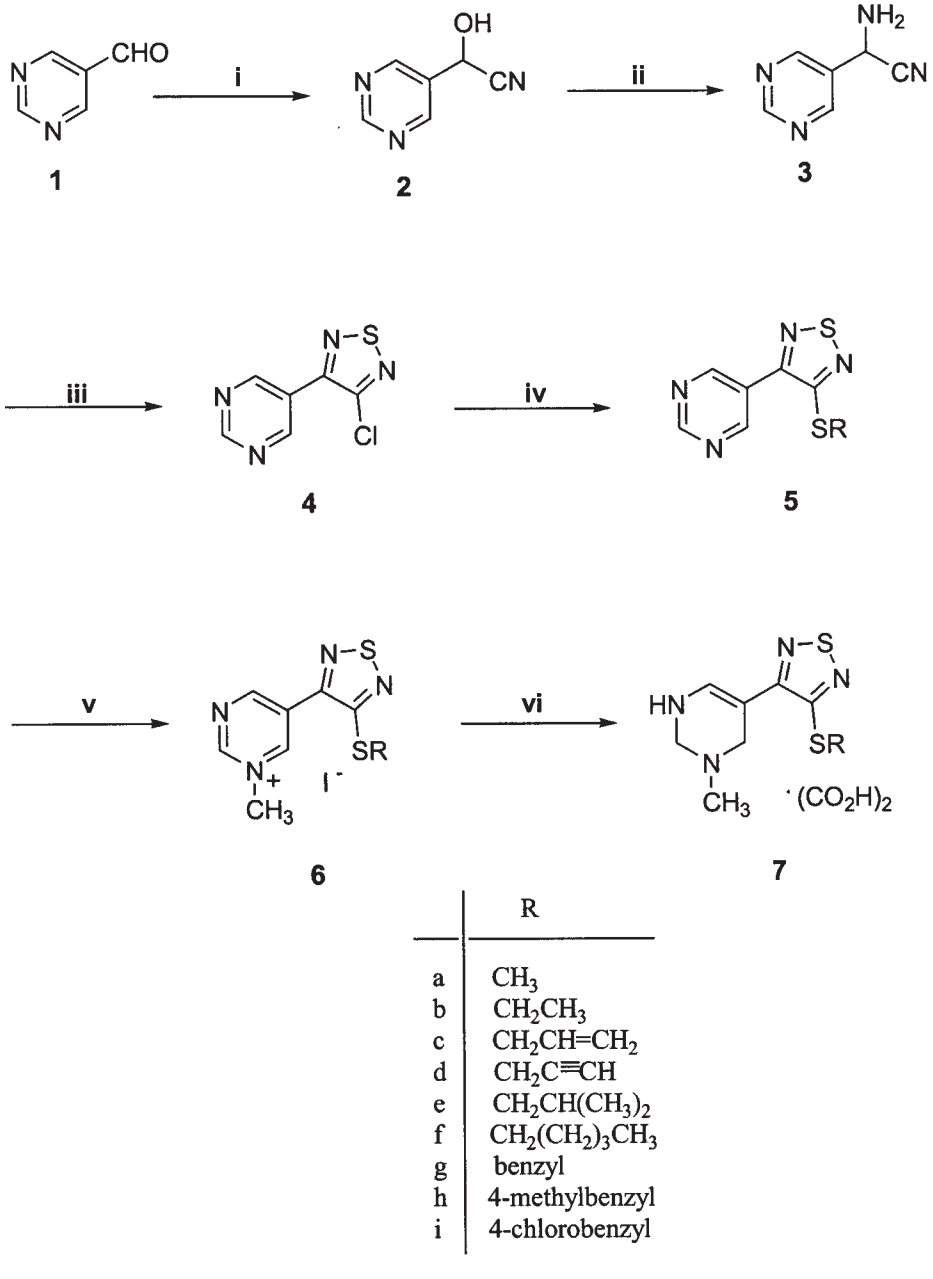


Chart 2. Bioisosteres of milameline and xanomeline.

Synthesis

As depicted in Scheme 1, the key intermediate, 5-(4-chloro-[1,2,5]thiadiazole-3-yl)pyrimidine **4** was prepared as described in our previous paper [12]. The starting material, 5-pyrimidinecarboxaldehyde **1** was synthesized from 4,6-dihydroxypyrimidine according to the known procedure [13]. The resultant **4** was treated with KCN/



	R
a	CH_3
b	CH_2CH_3
c	$CH_2CH{=}CH_2$
d	$CH_2C{\equiv}CH$
e	$CH_2CH(CH_3)_2$
f	$CH_2(CH_2)_3CH_3$
g	benzyl
h	4-methylbenzyl
i	4-chlorobenzyl

Reaction conditions: i) KCN/HOAc ii) NH_4Cl / NH_4OH iii) S_2Cl_2
iv) a. NaSH, b. RX / K_2CO_3 v) CH_3I vi) a. $NaBH_4$, b. $(CO_2H)_2$

Scheme 1

Full Paper

AcOH to give the cyanohydrin **2** and then subsequent treatment with NH_4Cl/NH_4OH in hot acetonitrile yielded the aminonitrile **3**. The one-pot Strecker conditions [14] resulted in only very low yield of **3**, but the thiadiazole **4** was obtained in high yield by treating **3** with sulfur monochloride in DMF. To introduce the requisite alkylsulfanyl groups, compound **4** was first reacted with sodium hydrosulfide hydrate and then followed by appropriate alkyl halides in the presence of potassium carbonate to give 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)pyrimidine **5** in 47–73 % yields. Compound **5** and an excess amount of methyl iodide in boiling acetone for about 48 h afforded the 3-quarternized pyrimidine salts **6** in quantitative yields. The sodium borohydride reduction of **6** in methanol at low temperature furnished 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)-3-methyl-1,2,3,4-tetrahydropyrimidine as the sole product. To enhance their stability and purity, the product was further treated with oxalic acid in methanol to give the oxalate salts **7**. The structure of **7** can be deduced from X-ray crystallographic data of pyrimidinylbenzoxazole which was previously obtained *via* identical chemical pathway [15]. ^{1}H-NMR spectra of the final compound **7** showed singlets at 7.52–7.63 ppm corresponding to the C6-H, and the signals of the two protons at C4 were observed at 3.92–4.02 ppm as singlets.

Results and discussion

Binding affinities to the M_1 receptor of the prepared compounds were sought by their ability to displace [^{3}H]-NMS from the cloned human M_1 receptor (h-M_1) expressed in CHO cells. Table 1 shows the percentages of inhibition of [^{3}H]-NMS binding to h-M_1 at 100 μM, and the IC_{50} values for [^{3}H]-NMS and [^{3}H]-Oxo-M of the compounds. The affinity values of other M_1 agonists are also presented for comparison. Most 3-methyl-1,2,3,4-tetrahydropyrimidines, **7 a**–**7 i**, synthesized as bioisosteric congeners of tetrahydropyridine derivatives, showed higher binding affinities for h-M_1 with respect to those of arecoline and milameline, but still much lower affinities to that of xanomeline. There was a rank order among compounds prepared and tested in binding affinity for h-M_1 depending on the length of the alkyl substituent, i.e. compound with longer chain showed higher affinity (**7 f** > **7 e** > **7 c** > **7 a**). Compounds with benzyl substituent, **7 g**, **7 h** and **7 i**, showed similar affinity to h-M_1.

Acknowledgement

We wish to thank the Korea Ministry of Science and Technology (KK-0205-B0) for financial grants.

Experimental

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis II FTIR. Nuclear magnetic resonance spectra were measured on a Brucker AM-300. Mass spectra were determined on JEOL JMS-DX 303 Mass Spectrometer JEOL JMA-DA 5000 mass data system focusing high resolution mass spectrometers. Although the syntheses of compounds **2**, **3**, and **4** have been reported [12], the procedures were written herein again for convenience.

Preparation of Hydroxy(pyrimidin-5-yl)acetonitrile (**2**)

KCN 8.14 g (125.00 mmol) in 50 ml water was cooled to 5 °C then to this was added portions of 5-pyrimidinecarboxaldehyde

Table 1. Inhibitory effects of 3-methyl-1,2,3,4-tetrahydropyrimidines on [^{3}H]-NMS and [^{3}H]-Oxo-M binding.

Compounds	[^{3}H]-NMS IC_{50} (μM)*	[^{3}H]-Oxo-M IC_{50} (μM)*	Ratio [^{3}H]-NMS/[^{3}H]-Oxo-M
7 a	46.60 ± 6.7	0.058 ± 0.005	803.4
7 b	8.35 ± 0.8	0.038 ± 0.006	219.7
7 c	2.65 ± 0.2	0.007 ± 0.001	378.6
7 d	5.62 ± 0.7	0.012 ± 0.002	468.3
7 e	1.76 ± 0.4	0.048 ± 0.008	36.7
7 f	0.50 ± 0.1	0.015 ± 0.004	33.3
7 g	0.73 ± 0.1	0.064 ± 0.01	11.4
7 h	2.18 ± 0.4	0.185 ± 0.03	11.8
7 i	1.84 ± 0.4	0.194 ± 0.02	9.5
xanomeline	0.13 ± 0.03	0.013 ± 0.004	10.0
milameline	17.42 ± 2.2	0.072 ± 0.01	241.9
arecoline	65.10 ± 5.4	0.03 ± 0.003	2170.0

* Data represent the mean ± S.E.M from three assays each performed in duplicate.

5.40 g (50.00 mmol) maintaining the temperature below 10 °C. When all of the aldehyde had been added, 7.16 ml (125.00 mmol) acetic acid was added dropwise at a temperature below10 °C. After stirring for 2 h at room temperature the reaction was cooled back to 5 °C, then the precipitates were collected by filtration. The filter cake was washed briefly with cold water, then dried under vacuum. Further purification by flash chromatography on silica gel (EtOAc) gave 4.55 g (67 %) of the title compound as a white powder.

mp 142–144 °C; IR (KBr) ν_{max}: 3020 (OH), 2800, 1560, 1410 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ: 9.23 (s, 1 H, C2-H), 8.99 (s, 2 H, C4-H, C6-H), 6.59 (br s, 1 H, OH), 6.06 (s, CHCN); ^{13}C NMR (75 MHz, acetone-d_6) δ: 159.6 (C2), 155.9 (C4, C6), 131.7 (C5), 119.1 (CN), 59.7 (CCN); $C_6H_5N_3O$ (135.0433), MS: *m/z* = 135.0432.

Preparation of amino(pyrimidin-5-yl)acetonitrile (**3**)

To a stirred solution of 1.35 g (10.00 mmol) hydroxy-pyrimidin-5-yl-acetonitrile and 2.67 g (50.00 mmol) NH_4Cl in 35 ml acetonitrile, 3.11 ml (20.00 mmol) 25 % NH_4OH was added and then heated under reflux for 3 h. After cooling to room temperature, the insolubles were filtered off and concentrated to dryness. The dark residue was subjected to flash chromatography on silica gel (EtOAc) to afford 0.52 g (39 %) of a red brown syrup.

IR (KBr) ν_{max}: 3400 (NH), 2200 (CN), 1560, 1410 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.27 (s, 1 H, C2-H), 8.98 (s, 2 H, C4-H, C6-H), 5.03 (s, 1 H, CHCN), 2.11 (br s, 2 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.6 (C2), 155.3 (C4, C6), 130.0 (C5), 119.1 (CN), 43.1 (CCN); $C_6H_6N_4$ (134.0591), MS: *m/z* = 134.0591.

Preparation of 5-(4-Chloro-[1,2,5]thiadiazol-3-yl)pyrimidine (**4**)

To a cooled (0 °C) and stirred solution of 0.89 ml (11.18 mmol, 3.0 eq) S_2Cl_2 in 30 ml DMF, a solution of 0.50 g (3.73 mmol) amino-pyrimidine-5-yl-acetonitrile in 10 ml DMF was added dropwise maintaining the temperature below 5 °C. The reaction was slowly warmed to 80 °C then stirring was continued for 2 h. After cooling to room temperature, the reaction was neutralized with NaOH solution and then extracted with EtOAc. The organic layer was dried over $MgSO_4$, then concentrated. The residue was purified with flash chromatography on silica gel (20 % EtOAc-Hex) to give 0.54 g (73 %) of the title compound as a white solid. Recrystallization from EtOAc yielded white needles.

mp 135–136 °C; IR (KBr) ν_{max}: 3050, 1590, 1550, 1460, 1180 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.39 (s, 2 H, C4-H, C6-H), 9.35 (s, 1 H, C2-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.2 (C2), 155.9 (C4, C6), 152.3 (C3′), 143.5 (C4′), 125.2 (C5); Anal. calcd. for $C_6H_3ClN_4S$: C, 36.3; H, 1.5; N, 28.2; S, 16.1. Found: C, 36.1; H, 1.6; N, 27.9; S, 15.9.

General procedure for the preparation of 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)pyrimidines (**5**)

5-(4-chloro-[1,2,5]thiadiazol-3-yl)pyrimidine (**4**) 0.50 g (2.52 mmol) was dissolved in 20 ml dimethylformamide. Sodium hydrosulfide hydrate (10.08 mmol) was added to this at room temperature. After heating to 100 °C stirring was continued for 24 h. The reaction was then cooled to room temperature, then K_2CO_3 (7.56 mmol) and the appropriate alkyl halide (3.78 mmol) was added, and the resulting mixture was stirred at the same temperature for 5–24 h. The reaction mixture was dissolved in water, and extrated with ethyl acetate. The organic layer was dried over $MgSO_4$ then the solvent was evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel (20 % EtOAc-Hex) to give the title compounds.

5 a: yield 47 %; mp 143–145 °C; IR (KBr) ν_{max}: 3080, 2925, 1405 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.33 (s, 2 H, C4-H, C6-H), 9.30 (s, 1 H, C2-H), 2.80 (s, 3 H, SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.3 (C2), 157.4 (C3′), 155.8 (C4, C6), 151.7 (C4′), 126.5 (C5), 15.7 (SCH_3); Anal. calcd. for $C_7H_6N_4S_2$: C, 39.99; H, 2.88; N, 26.65; S, 30.49. Found: C, 40.22; H, 2.72; N, 26.75; S, 30.54.

5 b: yield 71 %; mp 69–71 °C; IR (KBr) ν_{max}: 3050, 1590, 1550, 1460, 1180 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.33 (s, 2 H, C4-H, C6-H), 9.30 (s, 1 H, C2-H), 3.37 (q, 2 H, *J* = 7.4 Hz, SCH_2), 1.48 (t, 3 H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.8 (C2), 156.8 (C3′), 155.9 (C4, C6), 152.1 (C4′), 126.5 (C5), 27.6 (SCH_2), 14.3 (CH_3); $C_8H_8N_4S_2$ (224.0190), MS: *m/z* = 224.0191.

5 c: yield 64 %; mp 63–65 °C; IR (KBr) ν_{max}: 3060, 3010, 1560, 1430 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.32 (s, 2H, C4-H, C6-H), 9.30 (s, 1 H, C2-H), 6.00 (m, 1 H, C*H*=CH_2), 5.43 (dd, 1 H, *J* = 17.0, 1.0 Hz, C=C*H*trans), 5.31 (dd 1 H, *J* = 10.0, 1.0 Hz, C=C*H*cis), 4.01 (d, 2 H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.3 (C2), 156.0 (C4, C6), 155.9 (C3′), 152.2 (C4′), 132.0 (*C*H=CH_2), 126.5 (C5), 119.4 (CH=*C*H_2), 35.9 (SCH_2); $C_9H_8N_4S_2$ (236.0190), MS: *m/z* = 236.0193.

5 d: yield 73 %; mp 139–140 °C; IR (KBr) ν_{max}: 3200, 3030, 2970, 1400, 730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.31 (s, 1 H, C2-H), 9.29 (s, 2 H, C4-H, C6-H), 4.12 (d, 2 H, *J* = 2.5 Hz, SCH_2), 2.28 (t, 1 H, *J* = 2.5 Hz, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.0 (C2), 155.9 (C4, C6), 154.6 (C3′), 152.0 (C4′), 126.2 (C5), 78.1 (-*C*≡CH), 72.0 (-C≡*CH*), 21.7 (SCH_2); Anal. calcd. for $C_9H_6N_4S_2$: C, 46.14; H, 2.58; N, 23.91; S, 27.37. Found: C, 46.54; H, 2.52; N, 24.11; S, 27.44.

5 e: yield 79 %; mp 45–47 °C; IR (KBr) ν_{max}: 2960, 1550, 1405 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.34 (s, 2 H, C4-H, C6-H), 9.29 (s, 1 H, C2-H), 3.26 (d, 2 H, SCH_2), 2.07 (m, 1 H, CH), 1.07 (d, 6 H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.7 (C2), 157.1 (C3′), 155.9 (C4, C6), 152.0 (C4′), 126.6 (C5), 41.8 (SCH_2), 28.2 (CH), 21.9 (CH_3); $C_{10}H_{12}N_4S_2$ (252.0503), MS: *m/z* = 252.0502.

5 f: yield 73 %; mp 43–44 °C; IR (KBr) ν_{max}: 2970, 2920, 2880, 1190, 730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.33 (s, 2 H, C4-H, C6-H), 9.30 (s, 1 H, C2-H), 3.35 (t, 2 H, SCH_2), 1.81 (m, 2 H, CH_2), 1.43 (m, 4 H, 2 × CH_2), 0.92 (t, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.7 (C2), 157.0 (C3′), 155.9 (C4, C6), 152.0 (C4′), 126.7 (C5), 33.2, 30.9, 28.6, 22.1 (Pentyl CH_2), 13.8 (CH_3); Anal. calcd. for $C_{11}H_{14}N_4S_2$: C, 49.60; H, 5.30; N, 21.03; S, 24.07. Found: C, 49.86; H, 5.58; N, 21.38; S, 24.03.

5 g: yield 53 %; mp 79–80 °C; IR (KBr) ν_{max}: 3040, 2930, 1400, 980, 720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.27 (s, 3 H, C2-H, C4-H, C6-H), 7.35 (m, 5 H, aromatic H), 4.57 (s, 2 H, SCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.8 (C2), 156.2 (C3′), 155.9 (C4, C6), 152.0 (C4′), 135.9 (C5), 129.1, 127.8, 127.8, 126.4 (aromatic C), 38.1 (SCH_2); Anal. Calcd. for $C_{13}H_{10}N_4S_2$: C, 54.52; H, 3.52; N, 19.56; S, 22.39. Found: C, 54.81; H, 3.51; N, 19.96; S, 22.55.

5 h: yield 72 %; mp 107–108 °C; IR (KBr) ν_{max}: 3050, 3020, 2930, 1400, 970 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.27 (s, 3 H, C2-H, C4-H, C6-H), 7.21 (ABq, 4 H, aromatic H), 4.53 (s, 2 H, SCH_2), 2.33 (s, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.8 (C2), 156.3 (C3′), 155.9 (C4, C6), 152.0 (C4′), 137.7 (C5), 132.7, 129.3, 129.0, 126.4 (aromatic C), 37.4 (SCH_2), 21.1 (CH_3); Anal. calcd. for $C_{14}H_{12}N_4S_2$: C, 55.97; H, 4.03; N, 18.65; S, 21.35. Found: C, 56.01; H, 4.12; N, 18.94; S, 21.43.

5 i: yield 72 %; mp 88–90 °C; IR (KBr) ν_{max}: 3030, 1405 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.28 (s, 1 H, C2-H), 9.27 (s, 2 H, C4-H, C6-H), 7.30 (ABq, 4 H, aromatic H), 4.52 (s, 2 H, SCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.7 (C2), 155.8 (C4, C6), 155.6 (C3′), 151.9 (C4′), 134.5, 133.6, 130.4, 128.7 (aromatic C), 126.3 (C5), 36.7 (SCH_2); Anal. calcd for $C_{13}H_9ClN_4S_2$: C, 48.67; H, 2.83; N, 18.14; S, 19.99. Found: C, 49.03; H, 2.85; N, 17.41; S, 20.05.

General procedure for the preparation of 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)-3-methyl-1,2,3,4-tetrahydropyrimidine oxalate salts (**7**)

A solution of 3.55 mmol 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)pyrimidine (**5**) and 42.65 mmol methyl iodide in 10 ml acetone was heated under reflux for 48 h. Then the solvent and excess methyl iodide were removed on a rotary evaporator to give crude 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)-3-methylpyrimidium iodide (**6**). Without further purification, the salt was dissolved in 10 ml MeOH then cooled to low temperature (0–30 °C). To this portions of 3.91 mmol $NaBH_4$ were added. The reaction mixture was allowed to come to 0 °C, then it was concentrated *in vacuo* to dryness. The residue was dissolved in CH_2Cl_2 then washed with water. The organic layer was dried over $MgSO_4$, and concentrated. The residue was subjected to flash chromatography on silica gel (10 % MeOH-EtOAc) to give a red brown syrup. The resultant oily product, 5-(4-alkylsulfanyl-[1,2,5]thiadiazol-3-yl)-3-methyl-1,2,3,4-tetrahydropyrimidine, was dissolved in 2 ml MeOH and then to this a solution of 1.1 eq oxalic acid in 0.5 ml MeOH was added at room temperature. The resulting mixture was stirred for about 1 h. The precipitates were collected by filtration and washed with small amouts of cold MeOH and finally dried under vacuum to give the oxalate salts **7** as a pale yellow powder.

7 a: overall yield 19 %; mp 145–146 °C; IR (KBr) ν_{max}: 3290, 3020, 2620, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 8.57 (br s, 2 H, CO_2H), 7.57 (s, 1 H, C6-H), 7.28 (br s, 1 H, NH), 4.31 (s, 2 H, C2-H), 4.02 (s, 2 H, C4-H), 2.73 (s, 3 H, SCH_3), 2.72 (s, 3 H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.6 (CO_2H), 155.6 (C3′), 153.9 (C4′), 134.6 (C6), 96.6 (C5), 61.0 (C2), 51.1 (C4), 39.1 (NCH_3), 15.8 (SCH_3); $C_8H_{12}N_4S_2$ (228.0503), MS: *m/z* = 228.0503.

7 b: overall yield 26 %; mp 147–149 °C; IR (KBr) ν_{max}: 3240, 2990, 2560, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.69 (br s, 2 H, CO_2H), 7.57 (s, 1 H, C6-H), 7.23 (br s, 1 H, NH), 4.29 (s, 2 H, C2-H), 3.99 (s, 2 H, C4-H), 3.29 (q, 2 H, *J* = 7.0 Hz, SCH_2), 2.71 (s, 3 H, NCH_3), 1.35 (t, 3 H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3 (CO_2H), 155.9 (C3′), 152.8 (C4′), 134.7 (C6), 96.6 (C5), 61.1 (C2), 51.2 (C4), 39.7 (NCH_3), 27.2 (SCH_2), 14.4 (CH_3); $C_9H_{14}N_4S_2$ (242.0660), MS: *m/z* = 242.0657.

7 c: overall yield 14 %; mp 149–150; IR (KBr) ν_{max}: 3280, 3000, 2930, 2610, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.09 (br s, 2 H, CO_2H), 7.57 (s, 1 H, C6-H), 7.29 (br s, 1 H, NH), 5.97 (m, 1 H, C*H*=), 5.35, 5.16 (2 × d, 2 H, =CH_2), 4.32 (s, 2 H, C2-H), 4.02 (s, 2 H, C4-H), 3.98 (d, 2 H, SCH_2), 2.74 (s, 3 H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.5 (CO_2H), 155.8 (C3′), 152.2 (C4′), 134.7 (C6), 133.0 (CH=), 119.2 (=CH_2), 96.4 (C5), 60.9 (C2), 51.1 (C4), 39.1 (NCH_3), 35.4 (SCH_2); $C_{10}H_{14}N_4S$ (242.0660), MS: *m/z* = 242.0656.

7 d: overall yield 53 %, mp 145–147 °C; IR (KBr) ν_{max}: 3280, 2925, 1635 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.75 (br s, 2 H, CO_2H), 7.47 (s, 1 H, C6-H), 7.24 (br s, 1 H, NH), 4.28 (s, 2 H, C2-H), 4.16 (d, 2 H, *J* = 2.3 Hz, SCH_2), 3.99 (s, 2 H, C4-H), 3.20 (t, 1 H, *J* = 2.3 Hz, CH), 2.70 (s, 3 H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.4 (CO_2H), 155.9 (C3′), 151.1 (C4′), 134.9 (C6), 96.4 (C5), 79.7 (C), 74.2 (CH), 61.1 (C2), 51.1 (C4), 39.1 (NCH_3), 21.3 (SCH_2); $C_{10}H_{12}N_4S_2$ (252.0503), MS: *m/z* = 252.0503.

7 e: overall yield 28 %; mp 158–159 °C; IR (KBr) ν_{max}: 3280, 2960, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 8.07 (br s, 2 H, CO_2H), 7.63 (s, 1 H, C6-H), 7.18 (br s, 1 H, NH), 4.28 (s, 2 H, C2-H), 3.99 (s, 2 H, C4-H), 3.21 (d, 2 H, SCH_2), 2.70 (s, 3 H, NCH_3), 1.98 (m, 1 H, CH), 0.99 (d, 6 H, 2 × CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3 (CO_2H), 155.9 (C3′), 153.0 (C4′), 134.7 (C6), 96.6 (C5), 61.1 (C2), 51.2 (C4), 41.2 (SCH_2), 39.1 (NCH_3), 27.9 (CH), 21.9 (CH_3); $C_{11}H_{18}N_4S_2$ (270.0973), MS: *m/z* = 270.0973.

7 f: overall yield 9 %; mp 142-144 °C; IR (KBr) ν_{max}: 3240, 2930, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.57 (s, 1 H, C6-H), 7.05 (br s, 1 H, NH), 4.84 (br s, 2 H, CO_2H), 4.21 (s, 2 H, C2-H), 3.92 (s, 2 H, C4-H), 3.29 (t, 2 H, SCH_2), 2.65 (s, 3 H, NCH_3), 1.71 (m, 2 H, CH_2), 1.33 (m, 4 H, 2 × CH_2), 0.86 (t, 3 H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.1 (CO_2H), 155.9 (C3′), 153.0 (C4′), 134.7 (C6), 96.6 (C5), 61.1 (C2), 51.2 (C4), 39.2 (NCH_3), 32.7, 30.6, 28.3, 21.9 (pentyl CH_2), 14.1 (CH_3); $C_{12}H_{20}N_4S_2$ (284.1129), MS: *m/z* = 284.1129.

7 g: overall yield 3 %; mp 147–149 °C; IR (KBr) ν_{max}: 3290, 3030, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.52 (s, 1 H, C6-H), 7.33 (m, 5 H, aromatic H), 7.05 (br s, 1 H, NH), 5.65 (br s, 2 H, CO_2H), 4.57 (s, 2 H, SCH_2), 4.22 (s, 2 H, C2-H), 3.93 (s, 2 H, C4-H), 2.66 (s, 3 H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.8 (CO_2H), 155.9 (C3′), 152.4 (C4′), 134.9 (C6), 136.7, 129.4, 128.7, 127.7 (aromatic C), 96.6 (C5), 61.3 (C2), 51.3 (C4), 40.5 (SCH_2), 39.9 (NCH_3); $C_{14}H_{16}N_4S_2$ (304.0816), MS: *m/z* = 304.0817.

7 h: overall yield 9 %; mp 151–153 °C; IR (KBr) ν_{max}: 3280, 3020, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.52 (s, 1 H, C6-H), 7.23, (ABq, 4 H, aromatic H), 7.10 (br s, 3 H, CO_2H, NH), 4.52 (s, 2 H, SCH_2), 4.25 (s, 2 H, C2-H), 3.97 (s, 2 H, C4-H), 2.69 (s, 3 H, NCH_3), 2.25 (s, 3 H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.2 (CO_2H), 155.7 (C3′), 152.6 (C4′), 134.8 (C6), 137.0, 133.6, 129.3, 129.2 (aromatic C), 96.5 (C5), 61.1 (C2), 51.2 (C4), 39.0 (NCH_3), 36.7 (SCH_2), 20.9 (CH_3); $C_{15}H_{18}N_4S_2$ (318.0973), MS: *m/z* = 318.0974.

7 i: overall yield 25 %; mp 154–156 °C; IR (KBr) ν_{max}: 3280, 3040, 1630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 8.30 (br s, 2 H, CO_2H), 7.52 (s, 1 H, C6-H), 7.44 (ABq, 4 H, aromatic H), 7.17 (br s, 1 H, NH), 4.56 (s, 2 H, SCH_2), 4.26 (s, 2 H, C2-H), 3.97 (s, 2 H, C4-H), 2.69 (s, 3 H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3 (CO_2H), 155.8 (C3′), 152.2 (C4′), 134.9 (C6), 136.1, 132.3, 131.2, 128.6 (aromatic C), 96.5 (C5), 61.6 (C2), 51.2 (C4), 36.0 (NCH_3), 39.1 (SCH_2); $C_{14}H_{15}ClN_4S_2$ (338.0427), MS: *m/z* = 338.0426.

Biological methods

Receptor binding assay

The binding of compounds to the muscarinic M_1 receptor (M_1) was performed by the radioligand binding assay as described previously [16, 17]. Binding affinity was determined indirectly by the ability of compounds to compete with 1 nM [^3H]-N-methylscopolamine (NMS) in the suspension of cloned human M_1 ($h\text{-}_1M$) expressed in CHO cells (Biosignal Packard Inc., Montreal, Canada). Nonspecific binding was evaluated by the inclusion of 1 μM atropine in a separate set of samples. Each sample contained approximately 16 μg of protein ($h\text{-}M_1$) in 50 mM Tris buffer (pH 7.2) and varying concentrations of each compound in a final volume of 250 μl. Samples were incubated for 1 h at

27 °C in shaking incubator and then filtered through Wallac glass fiber filtermat (GF/C) using Inotech cell harvester of 96-well format. The radioactivity bound to filter was counted by Micro-β counter (Wallac, Turku, Finland).

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