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Conformationally Constrained Analogues of the Muscarinic Agonist 3-(4-(Methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine. Synthesis, Receptor Affinity, and Antinociceptive Activity

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Conformationally constrained analogues of the potent muscarinic agonist 3-(4-(methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (methylthio-TZTP, **17**) were designed and synthesized with the aim of (a) improving the antinociceptive selectivity over salivation and tremor and (b) predicting the active conformation of **17** with respect to the dihedral angle C4−C3−C3′−N2′. Using MOPAC 6.0 tricyclic analogues (**7**, **15**, **16**) with C4−C3−C3′−N2′ dihedral angles close to 180° and a rotation hindered analogue (**9**) with a C4−C3−C3′−N2′ dihedral angle close to 274° were designed, as these conformations had previously been suggested as being the active conformations. The analogues were tested for central muscarinic receptor binding affinity, for their antinociceptive activity in the mouse grid shock test, and, in the same assay, for their ability to induce tremor and salivation. The data showed that the tricyclic analogues (**7**, **15**, **16**) were equipotent with **17** as analgesics, but with no improved side effect profiles. The rotation-hindered analogue **9** had neither muscarinic receptor binding affinity nor antinociceptive activity. These results suggest that the active conformation of **17** has a C3−C4−C3′−N2′ dihedral angle close to 180°.

Introduction

Muscarinic cholinergic agonists are known from the literature to produce antinociception in a variety of species in several different antinociceptive tests.[1−4] The antinociceptive effect of muscarinic agonists were antagonized by centrally acting muscarinic antagonists, e.g., atropine, but not by peripherally acting antagonists such as methylatropine nor by opioid antagonists,[5,6] demonstrating that muscarinic antinociception was mediated by direct stimulation of centrally acting muscarinic receptors. Clinically, none of the currently available cholinergic agonists have been evaluated as analgesics, probably because of their prominent cholinergic side effects.

The potent muscarinic receptor agonist 3-(4-(methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine[7] (methylthio-TZTP, **17**) has previously been reported to be a potent antinociceptive agent, but the compound showed only little separation between doses producing antinociception and salivation.[8] One purpose of the present work was therefore to synthesize 3-(4-(methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine analogues with an improved analgesic therapeutic window. This was attempted by constructing more conformationally constrained analogues with expected improved receptor subtype selectivity. Three pharmacologically characterized (M1, M2, and M3) and five cloned (m1, m2, m3, m4, and m5) muscarinic receptor subtypes have been identified. M1 corresponds to m1, m4; M2 to m2; and M3 to m3. The function of m5 is unknown.

At present, it is not clear which of the m1−m5 muscarinic receptor subtypes is responsible for the antinociceptive response, but both the M1 and the m4 receptor subtypes have been suggested.[9−11] The aim of this study was, however, not to investigate which receptor subtype is responsible for the antinociceptive activity but to improve the in vivo therapeutic window of **17**.

A second purpose of this investigation was to determine the active conformation of **17**, which is a relatively flexible molecule. Attempts to predict the active conformation, especially with respects to the dihedral angle C4−C3−C3′−N2′, have been published previously.[12,13] The minimum-energy conformation, when rotatation was around the C3−C3′ bond, was suggested by Periyasamy et al.[12] to be the active conformation. Here, the anti (C4−C3−C3′−N2′ = 180°) form was found to have the lowest energy. Ward et al.[13] combined the Schulman model with the rotation energy analysis and predicted the active conformation to have a C4−C3−C3′−N2′ dihedral angle of 274°. This conformation was possible because of the low-energy barriers (max. 2 kcal/mol).

In the present paper we report on the synthesis and the antinociceptive activity of ring-closed analogues of **17** and 3-(4-(methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydropyridine (**18**) having C4−C3−C3′−N2′ dihedral angles equal to or close to 180°. Data on the

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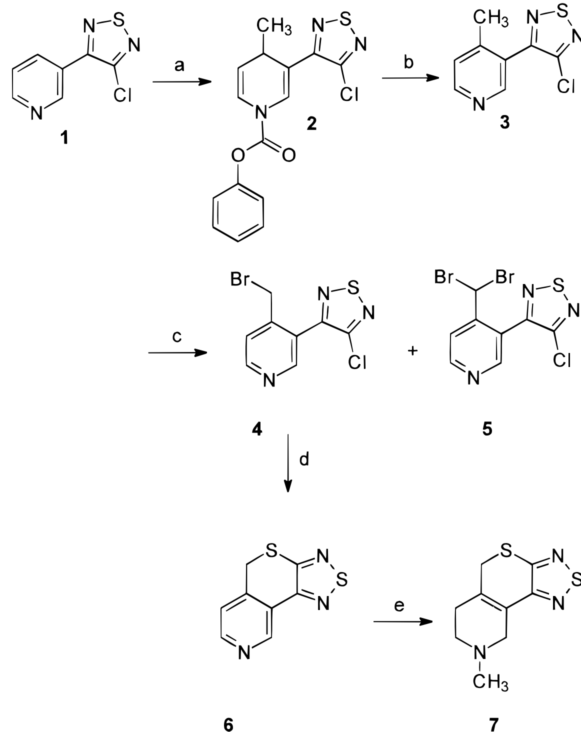
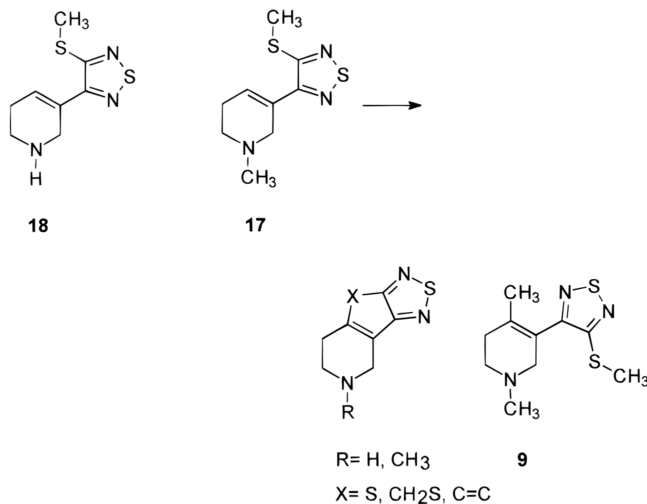
Scheme 1. Synthesis of 8-Methyl-6,7,8,9-tetrahydro-[1,2,5]thiadiazolo[3′,4′:2,3]thiopyrano[4,5-*c*]pyridine (**7**)[a]



[a] (a) PhOCOCl, CuI, CH_3MgI; (b) S_2; (c) NBS, AiBN; (d) NaSH; (e) (1) CH_3I, (2) $NaBH_4$.

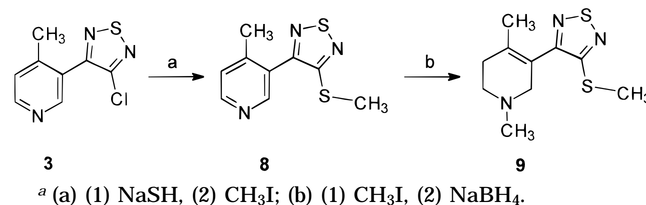
rotation-hindered 3-(4-(methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1,4-dimethylpyridine, **9**, analogue with energy minimum close to 274° will also be presented.



Chemistry

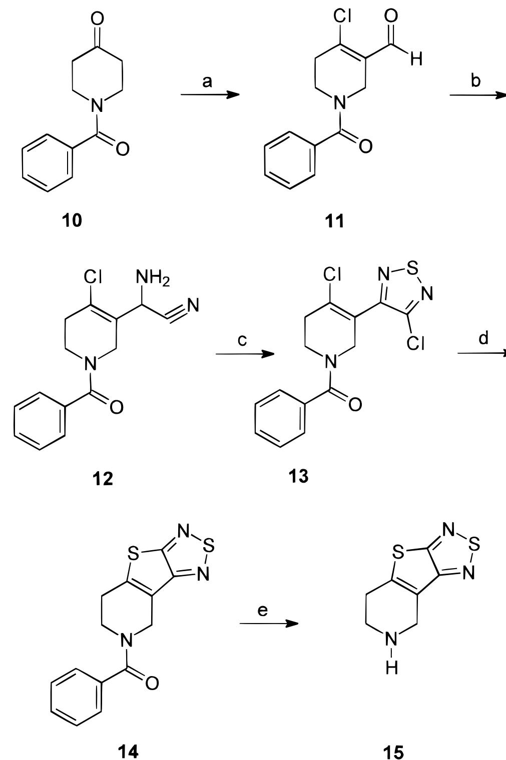
We have earlier reported on a five-step synthesis of 3-(3-chloro-1,2,5-thiadiazol-4-yl)-4-methylpyridine, **3**, starting from pyridine.[14] However, as 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine, **1**, was a readily available intermediate from the synthesis of our development candidate xanomeline,[7] we wanted to use **1** as starting material. Treating **1** with phenyl chloroformate and methyl grignard gave the dihydro-4-methyl analogue **2** as the only major product (Scheme 1). **2** was rearomatized with sulfur in refluxing Decalin to give **3** in 52% overall yield. Bromination with *N*-bromosuccinimide with 1.5 molar equiv gave the desired monobromo product **4**, starting material **3**, and the dibromo product **5** in an 1:1:1 ratio. Increasing the molar ratio of *N*-bromosuccinimide increased the yield of **5** but lowered the yield of **4**. Cyclization with sodium hydrosulfide gave the tricyclic analogue **6**, which was quaternized with methyl iodide and reduced with sodium borohydride under standard conditions to give **7**.

Scheme 2. Synthesis of 3-(3-Methylthio-1,2,5-thiadiazol-4-yl)-1,4-dimethyl-1,2,5,6-tetrahydropyridine (**9**)[a]



[a] (a) (1) NaSH, (2) CH_3I; (b) (1) CH_3I, (2) $NaBH_4$.

Scheme 3. Synthesis of 5,6,7,8-Tetrahydro[1,2,5]thiadiazolo[3′,4′:4,5]thieno[3,2-*c*]pyridine (**15**)[a]

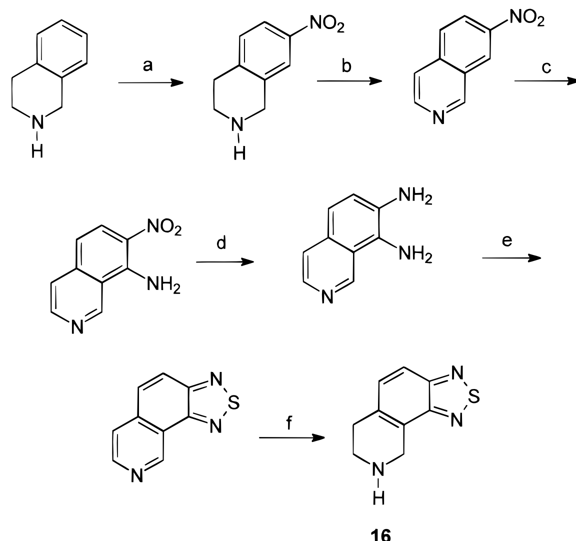


[a] (a) $POCl_3$, DMF; (b) (1) KCN, AcOH, (2) NH_4Cl, NH_4OH; (c) S_2Cl_2; (d) NaSH; (e) HCl_{conc}.

Compound **9** was synthesized analogously to a previously reported procedure[7] starting from **3**. Treating **3** with sodium hydrosulfide followed by alkylation with methyl iodide gave the methylthio analogue **8**, which was quaternized and reduced to give the desired tetrahydropyridine product **9** (Scheme 2).

Formylation of the N-protected 4-piperidone, **10**, under Vielsmeier−Haack conditions gave the known 4-chloro-3-formyl intermediate **11**.[15] The aldehyde **11** was treated with potassium cyanide and ammonium chloride in water to give the corresponding α-aminonitrile **12** (Scheme 3). Cyclization with sulfur monochloride gave the 3-chloro-1,2,5-thiadiazole analogue **13**, which was cyclized with sodium hydrosulfide to give the *N*-benzoyl-protected tricyclic product **14**. Deprotection

Scheme 4. Synthesis of 6,7,8,9-Tetrahydro[1,2,5]thiadiazolo[3,4-*h*]isoquinoline (**16**)[a]



[a] Girard et al. *J. Med. Chem.* **1989**, *32*, 1566–1571; (a) KNO_3, H_2SO_4; (b) I_2; (c) NH_2OH, KOH; (d) H_2, Pd/C; (e) S_2Cl_2; (f) $NaBH_3CN$.

of **14** in refluxing concentrated hydrogen chloride gave the target molecule **15**.

The benzo analogue of **15**, **16**, was synthesized according to published procedures[16] (Scheme 4).

Biological Evaluation

Muscarinic receptor binding assays were conducted on rat brain homogenate using [^{3}H]oxotremorine-M ([^{3}H]Oxo-M). Affinity of the compounds for receptors labeled by Oxo-M was considered to reflect their affinity for predominantly m2 and m4 receptors in the "agonist conformational state".[17,18] Displacement of [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS) binding to cloned CHO cell lines expressing m1–m5 receptors was used to evaluate the affinity of the compounds to the antagonist binding site of the individual receptor subtypes.

The mouse grid shock (MGS) assay[19] was used to assess both the antinociceptive activity and the side effect profile of the compounds. The MGS test has the advantage that since the mice respond by vocalization, even animals experiencing drug-induced tremor or other behavioral incapacitation can respond to the nociceptive stimuli.

Results

The conformation energy curves generated by MOPAC 6.0 for rotating around the C3–C3′ bond are shown in Figure 1 for the two compounds **17** and **9**. As previously published,[13] the energy barriers for **17** were very low (less than 2 kcal/mol), allowing the molecule to adopt any conformation. Contrary for **9**, the energy curve clearly showed that **9** is rotation restricted with an energy barrier of almost 10 kcal/mol at the 180° dihedral angle and with energy minima around the dihedral angles 70° and 290°. The energy barrier created by the 4-methyl substituent on the tetrahydropyridine ring makes it nearly impossible for **9** to adopt the 180° dihedral angle conformation. The ring-closed analogue of **9**, **7**, had C4–C3–C3′–N2′ dihedral angles of 168° and 192° (−168°), and the tricyclic analogues

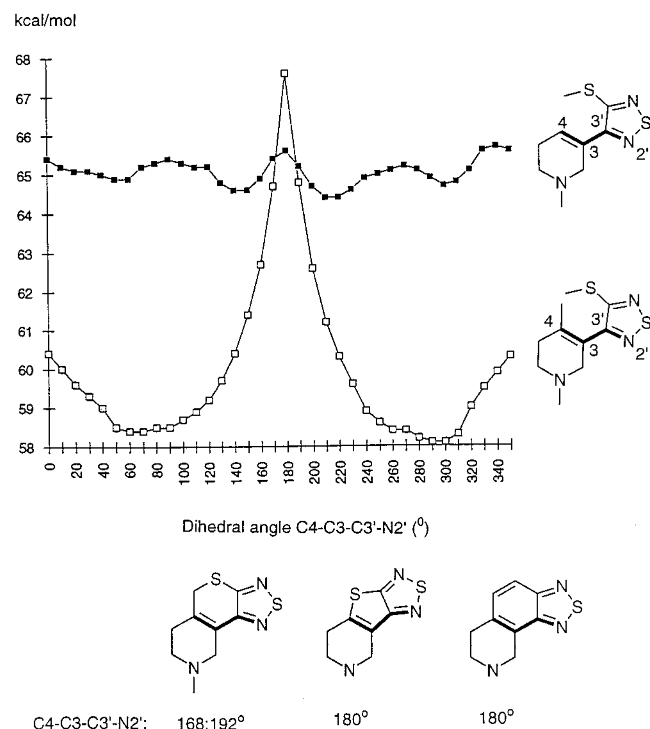


Figure 1. Upper part: Conformation energy curves of **17** (filed squares) and the rotation restricted analogue **9** (open squares) for rotation around the C3–C3′ bond. Lower part: minimum-energy conformation of the tricyclic analogues **7**, **15**, and **16**. The energy conformation analysis was performed on MOPAC 6.0 using AM1 parameters.

with either sulfur, **15**, or a carbon–carbon double bond, **16**, were planar giving dihedral angles of 180°.

The lead structure, **17**, had high affinity for centrally muscarinic receptors labeled by Oxo-M (Table 1). The *N*-des-Me analogue, **18**, had similar affinity for the Oxo-M binding site, as compared to **17**. The rotation-restricted analogue, **9**, did not have affinity (IC_{50} >500 nM) for the agonist binding sites. The Oxo-M receptor affinity was, however, to a certain extent regained for the more planar analogue of **9**, the tricyclic analogue **7**. The two planar tricyclic analogues, **15** and **16**, both had slightly lower Oxo-M receptor affinity compared to analogue **18**.

Compound **17** had potent and full efficacious antinociceptive effects in the mouse grid shock test, but had only little separation to the salivation side effect (Figure 2A; Table 1). The rotation-hindered analogue **9** had no antinociceptive effect, nor did **9** produce side effects (Figure 2B). The tricyclic analogue **7** (Figure 2C) had a pharmacological profile identical with that of **17**, but was less potent, as suggested by the lower receptor affinity. Pretreatment with scopolamine (0.5 mg/kg sc) completely blocked the antinociceptive effect of **7** (30 mg/kg sc), proving that the effect was mediated by muscarinic receptors (data not shown). Compound **18** (Figure 2D) was also a potent antinociceptive agent, but produced more tremor compared to **17**. The tricyclic analogues **15** (Figure 2E) and **16** (Figure 2F) both induced antinociceptive effects, but they had no improved side effect profile compared to **18**.

All of the tested compounds (**17**, **9**, **7**, **16**, arecoline, and oxotremorine) had lower affinity to the (NMS) antagonist binding sites in the m1–m5 CHO cell lines

Table 1. In Vitro and in Vivo Characterization of Muscarinic Ligands

compound	receptor binding to rat brain membranes, IC_{50} (nM) [^{3}H]Oxo-M ± SEM	activity in mice, ED_{50} (mg/kg, sc) MGS[a] ± SEM	salivation	tremor
17	4.5 ± 2.6	4.0 ± 1.5	6	>10
9	>500	>30	>30	>30
7	27.8 ± 10.4	10.4 ± 1.4	13	>30
18	7.6 ± 2.1	1.8 ± 1.2	7.4	4.5
15	35.5 ± 3.5	4.6 ± 1.3	8.8	15.8
16	44.5 ± 3.5	4.4 ± 2.1	5.1	6.4
arecoline[b]	62.7 ± 14.8	16	15	15
oxotremorine[b]	5.0 ± 0.6	0.038	0.2	0.2

[a] MGS = mouse grid-shock, $n = 10$ mice per group (see also Figure 2). [b] From ref 8.

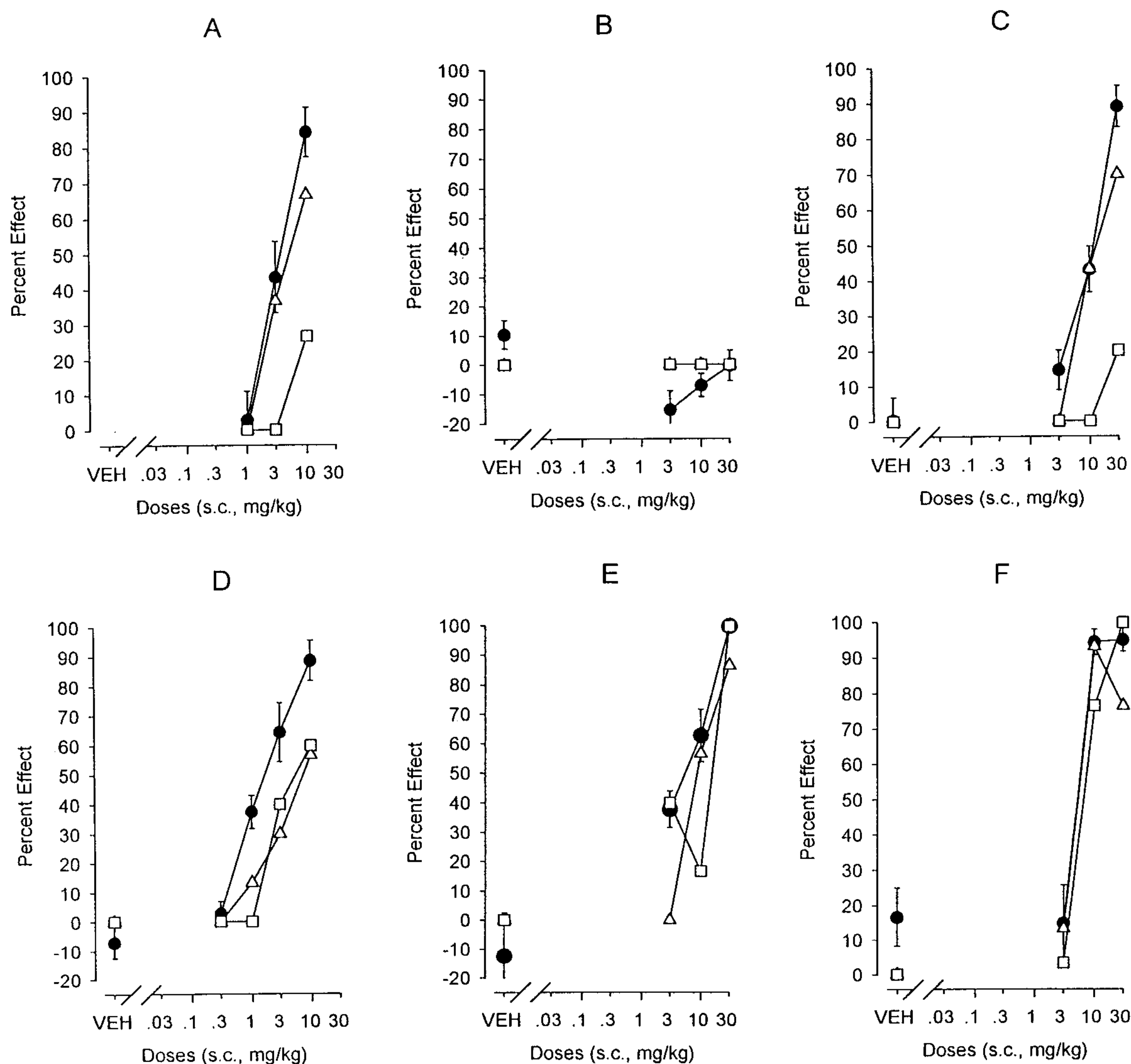


Figure 2. Dose–response curves of test compounds (panel A, **17**; B, **9**; C, **7**; D, **18**; E, **15**; F, **16**) of the antinociceptive effect (filled circles) and of the side effects, tremor (open squares) and salivation (open triangles), in the mouse grid-shock (MGS) test.

than to the agonist binding sites (Oxo-M) in brain homogenates. The lead compound **17** had 2–3 times higher affinity for the m2 receptor subtype antagonist binding site than to the other receptor subtypes (Table 2). The rotation-hindered analogue **9** had lower than 100 μM affinity for all the receptor subtypes. The tricyclic analogue **16** exhibited an approximately 15 times m2 affinity selectivity over the other receptor subtypes. The other tricyclic analogues **7** also showed highest affinity for the m2 receptor subtype.

Discussion and Conclusions

For several decades it has been known that muscarinic agonists induce antinociception in animals. However, it is only recently that attempts have been made to design muscarinic agonists that selectively produce antinociception without inducing the typical cholinergic side effects such as salivation, tremor, and diarrhea.[20,21] We have previously reported on functionally selective muscarinic analgesics of the 3-(4-(alkylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine and the

Table 2. Binding Affinity to Cloned m1−m5 CHO Cell Lines Using [^{3}H]NMS

compound	IC$_{50}$ ± SEM (μM)				
	m1	m2	m3	m4	m5
17	1.8 ± 0.3	0.48 ± 0.03	1.6 ± 0.2	1.2 ± 0.3	2.1 ± 0.2
7	20.5 ± 1.5	2.2[a]	9.7 ± 3.0	14.5 ± 0.5	23.0 ± 2.0
9	>100 (40 ± 14%)[b]	100 (51 ± 10%)	>100 (30 ± 3%)	>100 (43 ± 5%)	100 (55 ± 12%)
16	100 (52 ± 4%)	6.7 ± 1.3	100 (53 ± 5%)	100 (53 ± 1%)	100 (57 ± 3%)
arecoline	145 ± 54	4.5 ± 1.5	58 ± 10	47.5 ± 4.5	62.5 ± 16.5
oxotremorine	9.0 ± 0.1	0.40 ± 0.01	17.0 ± 4.0	5.0 ± 0.1	8.0 ± 1.0

[a] Single determination. [b] (%)= % inhibition at highest concentration: 100 μM.

corresponding azabicyclic type compounds, where selectivity was achieved by changing the alkylthio side chain to propyl/butylthio.[8,22]

The present report describes attempts to obtain the same muscarinic functional selectivity by constructing conformationally constrained analogues of **17**. At the same time these more rigid analogues would give us an indication of the active conformation of **17**.

Evaluating the functional selectivity of compounds in vivo can be difficult, but since the receptor subtype mediating the antinociceptive response is still debated,[9−11,23,24] this in vivo approach was adopted. Differences in absorption, kinetics, and metabolism of the compounds can influence the results. However, after subcutaneous administration, the difference in absorption and especially first pass metabolism is minimized. The present test compounds in general penetrate biological membranes very well, and differences in cholinergic activity are therefore, most likely, a result of different muscarinic receptor activation.

Compounds were designed to have structures as close to **17** as possible, but also being as conformationally constrained as possible. This led to the synthesis of the tricyclic analogue **7**, which is the direct analogue of **17** locked in a set conformation by a carbon−carbon bond to the 4-position of the tetrahydropyridine ring (Figure 1). Biological testing of **7** showed this compound to be nearly equally active compared to the lead compound (Figure 2C, Table 1). No improvement in analgesic selectivity over salivation was obtained, however.

As **7** showed potent activity, it was decided to make further analogues with conformations close to that of **7**. Conformation analysis using MOPAC 6.0 showed **7** to have C4−C3−C3′−C4′ dihedral angles of 168° and 192°. Two planar (C4−C3−C3′−C4′ dihedral angles equaled 180°) analogues, **15** and **16**,[16] were synthesized and tested for Oxo-M affinity and antinociceptive activity. Again potent analgesic activity and Oxo-M receptor affinity was in the same range as observed for their direct analogue **18** (Figure 2D-F, Table 1).

These observations strongly indicated that the active conformation for muscarinic antinociceptive activation was close or equal to 180°. Unfortunately, the same conformation seems to activate receptor subtypes responsible for the side effects salivation and tremor. An improved therapeutic window was therefore not obtained with any of the tricyclic analogues **7**, **15**, or **16**.

To further investigate the active conformation, MOPAC 6.0 was used to evaluate analogues of **17** with conformations close to the earlier predicted active conformation of 274°.[13] The 4-Me analogue, **9**, proved to have energy minima between 50 and 100° and between 260 and 310° (Figure 1) and was therefore synthesized. Biological testing revealed **9** to be without Oxo-M receptor affinity and without antinociceptive properties, suggesting the active conformation of **17** to be different from 274°.

Although none of the tested compounds showed any functional agonist selectivity, some of the compounds were tested for their receptor subtype affinity in cloned cell lines using the antagonist [^{3}H]NMS as ligand. Antagonist binding was used since agonist binding could not be performed on m1, m3, and m5 receptor cell lines. The functionally m1/m4 selective agonist, xanomeline (hexyloxy-TZTP), did not exhibit any receptor affinity subtype selectivity using [^{3}H]NMS,[27] but these more rigid analogues had the potential for showing some receptor subtype selectivity. Only moderate m2 receptor affinity selectivity was obtained, however, with the two tricyclic analogues **7** and **16**. Further, since the standard muscarinic agonists, arecoline and oxotremorine, also had 10 times higher affinity for the m2 receptor subtype than for the other receptor subtypes, the finding might not be relevant. Second, caution interpreting agonist affinity in antagonist binding studies should also be taken.

In summary, we have synthesized and tested planar analogues of **17** and found them to be potent antinociceptive agents, suggesting the C3−C4−C3′−C4′ dihedral angle of the active conformation of **17** to be close to 180°.

Experimental Section

Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC-200 MHz FT-NMR instrument, and mass spectra were recorded on a Finnigan 5100 mass spectrometer. Column chromatography was performed on silica gel 60 (70−230 mesh, ASTM, Merck). Elemental analysis were performed by Novo Nordisk Microanalytical Laboratory, Denmark, and were within ±0.4% of the calculated values. No particular attempts were made to optimize reaction conditions for most of the reactions described.

3-(4-Chloro-1,2,5-thiadiazol-3-yl)1-(phenoxycarbonyl)-1,4-dihydropyridine, 2. A solution of cuprous iodide (0.4 g, 2.0 mmol) in dry THF (50 mL) was stirred under nitrogen for 10 min. A solution of 3-(4-chloro-1,2,5-thiadiazol-3-yl)pyridine[7] (**1**) (9.9 g, 50 mmol) in dry THF (150 mL) was added to the reaction mixture at room temperature. The reaction mixture was cooled to −25 °C, and phenyl chloroformate (6.3 mL, 50 mmol) in THF was added slowly. The reaction mixture was stirred at −25 °C for 30 min, then allowed to warm to room temperature, and stirred for another 2.5 h. The reaction mixture was cooled to −25 °C, and methylmagnesium iodide (55 mmol, in 20 mL ether) was added. The reaction mixture was stirred for 20 min, then 20% aqueous ammonium chloride (100 mL) was added, and the mixture was extracted with ether

(200 mL). The combined organic phases were washed with 20% NH_4Cl-NH_4OH (1:1) (50 mL), water (50 mL), 4 N HCl (50 mL), and brine (50 mL). The organic phase was dried and evaporated to give crude title compound. Purification by column chromatography eluting with toluene gave **2** as an oil (11.9 g, 71% yield): ^1H NMR ($CDCl_3$) δ 1.22 (3H, d, $J = 8$ Hz), 3.84 (1H, m), 5.25 (1H, m), 6.97 (1H, m), 7.1−7.5 (5H, m), 8.17 (1H, d, $J = 2$ Hz); MS m/z 333 (M^+ of free base).

3-(4-Chloro-1,2,5-thiadiazol-3-yl)-4-methylpyridine, 3. To a solution of **2** (11.7 g, 35 mmol) in Decalin (100 mL) was added sulfur (1.4 g, 43 mmol), and the reaction mixture was refluxed for 5 h. After the mixture was cooled to room temperature, ether (100 mL) was added, and the organic phases were extracted with 1 N HCl (2 × 75 mL). The aqueous phase was made alkaline with sodium hydroxide and extracted with methylene chloride (2 × 100 mL). The methylene chloride phase was dried and evaporated to give **3** as a solid (5.47 g, 74% yield): ^1H NMR ($CDCl_3$) δ 2.33 (3H, s), 7.30 (1H, d, $J =$ 5 Hz), 8.60 (2H, m); MS m/z 211 (M^+ of free base).

4-(Bromomethyl)-3-(4-chloro-1,2,5-thiadiazol-3-yl)pyridine, 4. To a solution of **3** (0.63 g, 3 mmol) in tetrachloromethane (50 mL) was added *N*-bromosuccinimide (0.80 g, 4.5 mmol) and α,α-azoisobutyronitrile (80 mg, 0.5 mmol). The reaction mixture was stirred at 75 °C overnight. Water (50 mL) and potassium carbonate were added, and the reaction mixture was extracted with methylene chloride (3 × 100 mL). The organic phases were dried and evaporated. The residue contained a mixture of starting material (**3**), the wanted momobromo product (**4**), and the dibromo product (**5**). Separation by column chromatography eluting with ethyl acetate−methylene chloride (1:10) gave **4** as an oil (120 mg, 14% yield): ^1H NMR ($CDCl_3$) δ 4.50 (2H, s), 7.50 (1H, d, $J = 5$ Hz), 8.75 (2H, m); MS m/z 291 (M^+ of free base). The dibromo compound **5** was isolated in 180 mg (16%) yield: ^1H NMR ($CDCl_3$) δ 6.84 (1H, s), 8.03 (1H, d, $J = 5$ Hz), 8.75 (1H, s), 8.86 (1H, d, $J = 5$ Hz); MS m/z 369 (M^+ of free base).

[1,2,5]Thiadiazolo[3′,4′:2,3]thiopyrano[4,5-*c*]pyridine, 6. To a solution of **4** (400 mg, 1.5 mmol) in DMF (20 mL) was added sodium hydrosulfide monohydrate (80%) (150 mg, 1.8 mmol) and potassium carbonate (275 mg, 2 mmol). The reaction mixture was stirred at room temperature for 48 h. Water (200 mL) was added, and the mixture was extracted with ether (3 × 50 mL). The ether phases were dried and evaporated to give crude product. Purification by column chromatography eluting with ethyl acetate−methylene chloride (1:10) gave **6** as an oil (100 mg, 32% yield): ^1H NMR ($CDCl_3$) δ 4.18 (2H, s), 7.28 (1H, d, $J = 5$ Hz), 8.65 (1H, d, $J =$ 5 Hz), 9.30 (1H, s); MS m/z 207 (M^+ of free base).

8-Methyl-6,7,8,9-tetrahydro[1,2,5]thiadiazolo[3′,4′:2,3]-thiopyrano[4,5-*c*]pyridine Oxalate, 7. A solution of **6** (165 mg, 0.8 mmol) and methyl iodide (0.1 mL, 1.5 mmol) in acetone (10 mL) was stirred at room temperature overnight. The quaternized precipitate was collected by filtration (124 mg, 70% yield) and dissolved in ethanol (20 mL). Sodium borohydride (76 mg, 2 mmol) was added at 0 °C and the reaction mixture stirred at 0 °C for 3 h. Water was added, and the mixture was extracted with ether. The ether phases were dried and evaporated to give crude product. Purification by column chromatography eluting with ethyl acetate−methylene chloride (1:10) gave desired product as an oil (100 mg, 55% yield). Crystallization with oxalic acid from acetone gave **7** as white crystals: mp 144−146 °C; ^1H NMR (DMSO) δ 2.58 (2H, m), 2.75 (3H, s), 3.13 (2H, m), 3.7−4.6 (2H), 3.89 (2H, s), 3.98 (2H, s), MS m/z 225 (M^+ of free base). Anal. ($C_{11}H_{13}N_3O_4S_2$) C, H, N.

3-(4-(Methylthio)-1,2,5-thiadiazol-3-yl)-4-methylpyridine, 8. To a solution of **3** (0.42 g, 2 mmol) in DMF (10 mL) were added sodium hydrogen sulfide monohydrate (0.25 g, 3 mmol), potassium carbonate (0.7 g, 5 mmol), and methyl iodide (0.22 mL, 3.5 mmol). The reaction mixture was stirred for 1 h at room temperature. Water (20 mL) was added, and the mixture was extracted with ether (3 × 50 mL). The combined ether phases were dried and evaporated to give crude **8**. Purification by column chromatography eluting with methylene chloride−ethyl acetate (10:1) gave **8** in 350 mg (78%) yield: ^1H NMR ($CDCl_3$) δ 2.30 (3H, s), 2.72 (3H, s), 7.27 (1H, d, $J = 5$ Hz), 8.56 (1H, d, $J = 5$ Hz), 8.61 (1H, s); MS m/z 223 (M^+ of free base).

3-(4-(Methylthio)-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1,4-dimethylpyridine Oxalate, 9. A solution of **8** (350 mg, 1.5 mmol) and methyl iodide (0.3 mL, 5 mmol) in acetone (10 mL) was stirred overnight at room temperature. Ether (2 mL) was added, and the precipitate was collected by filtration (520 mg, 95%). The crystals were dissolved in methanol (10 mL) and cooled to 0 °C, and sodium borohydride (0.15 g, 4 mmol) was added. The reaction mixture was stirred at 0 °C for 3 h, after which water (10 mL) was added and the mixture extracted with ether (3 × 100 mL). The ether phases were dried, and oxalic acid (120 mg, 1.3 mmol) dissolved in acetone (2 mL) was added before the organic phase was evaporated without heating. The residue was dissolved in acetone (5 mL), and ether was added for crystallization. The crystals were collected by filtration, giving **9** in 90 mg (21%) yield: mp 121−122 °C; ^1H NMR (DMSO) δ 1.56 (3H, s), 2.50 (2H, m), 2.57 (3H, s), 2.76 (3H, s), 3.24 (2H, t, $J = 5$ Hz), 3.66 (2H, s), 6.5−9.0 (2H); MS m/z 241 (M^+ of free base). Anal. ($C_{12}H_{17}N_3O_4S_2 \cdot 1/3(C_3H_6O)$) C, H, N.

1-Benzoyl-4-chloro-3-(4-chloro-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydropyridine, 13. To a solution of **11** (42.5 g, 170 mmol) and acetic acid (11 g, 170 mmol) in ethanol (100 mL) was added potassium cyanide (12 g, 170 mmol) dissolved in water (20 mL). The reaction mixture was stirred overnight at room temperature. The precipitate was filtered and suspended in water (100 mL), and ammonium chloride (100 g, 170 mmol) and ammonium hydroxide (25% in water, 25 mL) in ethanol (100 mL) were added. The reaction mixture was stirred at room temperature for 7 h. Additional water (200 mL) was added, and the water phase was extracted with ether (3 × 200 mL). The organic extracts were dried and evaporated to give crude **12**. The residue was dissolved in DMF (100 mL) and slowly added to a solution of sulfur monochloride (25 mL, 312 mmol) in DMF (25 mL) at 0 °C. The reaction mixture was slowly heated to room temperature and stirred for another 8 h. Water (50 mL) was added carefully, and the precipitated sulfur was removed by filtration. Additional water (500 mL) was added, and the water phase was extracted with ether (2 × 200 mL). The ether phases were dried and evaporated, and the residue was purified by column chromatography, eluting with methylene chloride−ethyl acetate (20:1). The desired product **13** was isolated as an oil (7.4 g, 13% yield): ^1H NMR ($CDCl_3$) δ 2.6−2.8 (2H, br s), 3.6−4.6 (4H, m), 7.45 (5H, s); MS m/z 339 ($M^+ - 1$ of free base).

7-Benzoyl-5,6,7,8-tetrahydro[1,2,5]thiadiazolo[3′,4′:4,5]-thieno[3,2-c]pyridine, 14. To a solution of **13** (3.4 g, 10 mmol) in DMF (50 mL) was added sodium hydrosulfide (1.5 g, 20 mmol) and potassium carbonate (2.0 g, 14.5 mmol). The reaction mixture was stirred at room temperature for 3 h. Water (500 mL) was added, and the water phase was extracted with ether (2 × 15 mL). The ether phases were dried and evaporated, and the residue was purified by column chromatography eluting with methylene chloride−ethyl acetate (10:1). **14** was isolated in a 2.1 g (70%) yield: ^1H NMR ($CDCl_3$) δ 3.2−3.6 (4H, m), 5.0 (2H, m), 7.6 (5H, m).

5,6,7,8-Tetrahydro[1,2,5]thiadiazolo[3′,4′:4,5]thieno-[3,2-*c*]pyridine Oxalate, 15. A suspension of **14** (600 mg, 2 mmol) in concentrated HCl (10 mL) was heated at reflux for 16 h. The reaction mixture was diluted with water (100 mL) and made alkaline with 4 N sodium hydroxide. The water phase was extracted with ether (3 × 75 mL), and the ether phase was dried and evaporated: ^1H NMR ($CDCl_3$) δ 2.4 (1H, s), 2.6 (2H, m), 3.2 (2H, m), 3.9 (2H, s). The residue was crystallized as the oxalate salt from acetone, giving **15** in 300 mg (76%) yield: mp 209−210 °C; MS m/z 197 (M^+ of free base). Anal. ($C_9H_9N_3O_4S_2$) C, H, N.

Receptor Binding. The procedure for displacement of [^3H]-oxotremorine-M has previously been described by us.[7] Briefly, fresh cortex from male Wistar rats was homogenized for 5−10 s in 10 mL of 20 mM Hepes pH = 7.4. The suspension was

centrifuged for 15 min at 40000*g*. The pellet was washed three times with buffer. In each step the pellet was homogenized in 20 mL of Hepes pH = 7.4 (100 mL per g of original tissue) and used for binding. To 0.5 mL of tissue homogenate were added 25 μL of test solution and 25 μL of [^{3}H]Oxo-M (1.0 nM, final concentration), and the solution was mixed and incubated for 30 min at 25 °C. Nonspecific binding was determined in triplicate using arecoline (3 μM, final concentration) as test substance. After incubation, samples were filtered through a Watman glass fiber filter with ice-cold buffer, and the filter was washed with ice-cold buffer. Radioactivity of the filters was determined by scintilation counting. IC_{50} values were calculated from inhibitory effects of at least four different concentrations in triplicate using the Hill equation.

The [^{3}H]NMS SPA binding assays were run in 96-well counting plates (Corning/CoStar). To each well was added 100 μL of CHO cell membrane preparation containing 23−45 μg of protein, 50 μL of 5 nM [^{3}H]NMS, and 50 μL of test compound. The plates were incubated for 2 h, after which 50 μL of suspended WGA-coated SPA (Amersham) were added to each well, and after mixing, the plates were counted after 2 h in a Wallac MicroBeta counter.

Preparation of CHO Cell Membranes. Cell pellets were homogenized in 5 volumes of 20 mM sodium phosphate buffer pH 7.4, and homogenates were diluted to 10−20 volumes of original cell pellet. Homogenates were centrifuged at 200*g* for 15 min at 4 °C. Supernatants were stored on ice, and pellets were rehomogenized two additional times as above, pooling the supernatants. Pooled supernatants were centrifuged at 40000*g* for 45 min at 4 °C. The resulting pellets were resuspended in 1 volume of homogenization buffer, and aliquots were assayed for protein and were stored at −80 °C until assay.

Mouse Grid-Shock Analgesia. Male NMRI mice (Bomholdtgaard, Ry, Denmark) weighing 18−22 g were group housed in a large colony room with food and water available at all times, and lights on between 6:00 a.m. and 6:00 p.m. The animals were brought into the laboratory in the morning of the day of the experiments and weighed individually. All doses were administered according to body weight.

Apparatus and testing procedures were described in detail previously.[19, 20] Briefly, a transparent acrylic chamber (13 × 13 × 13 cm) was equipped with a stainless steel grid floor, and a sound detection device was attached to the top of the chamber. A shock generator could deliver scrambled shocks through the grid floor, starting at 0 mA and increasing with 10 μA every 0.01 min up to a maximal 0.5 mA at 30 s. Shocks were terminated when a 70 dB vocalization was emitted, or after 30 s had elapsed, whichever occurred first. A predrug latency to vocalization was generated for each animal individually. Animals were then given the appropriate treatment and were replaced in the holding cages. Thirty minutes later a postdrug latency to vocalization was generated. Ten animals were used per dose and for the controls. A percent increase in latency to vocalization score [(postdrug − predrug/cutoff − predrug) × 100] was generated for each animal, and the average was then calculated for each dose.

Side-Effect Scoring in Mice. For details, see ref 20. Immediately prior to the postdrug grid-shock test, the incidence and severity of salivation and tremor was assessed by assigning each animal a score from 0 to 3 corresponding to the severity of the effect (0 = no effect, 3 = maximal effect). Results were calculated by multiplying each score by the number of mice which received that score and expressing the sum of scores for each group (n = 10) as a percentage of the maximum possible sum of scores (30).

Molecular Modeling Details. All calculations were set up and analyzed using SYBYL 6.22 (R4000). Geometry optimization and torsional scans were performed initially by molecular mechanics force field from TRIPOS[25] and subsequently using the AM1 Hamiltonian in MOPAC 6.0.[26] Torsional scans were performed from 0° to 360° in 10° intervals. At each point in the scan, all coordinates were refined while the dihedral angle being investigated were constrained. An additional harmonic potential with a force constant of 100 kcal/mol × (deg)$^{-2}$ were added to maintain the dihedral angle during the initial force field minimization. The SCF convergence criteria for the MOPAC optimization was set to 1.0 × 10^{-14} using the keyword SCFCRT = 1.D-14. Geometry termination was set to a gradient norm of 1 × 10^{-4} using the keyword GNORM = 0.0001. For systems containing sulfur, MNDO parameters were utilized for this atom using the keyword PARASOK.

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