1-(1,2,5-Thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptanes as New Potent Muscarinic M_1 Agonists: Structure−Activity Relationship for 3-Aryl-2-propyn-1-yloxy and 3-Aryl-2-propyn-1-ylthio Derivatives

Lone Jeppesen,* Preben H. Olesen, Lena Hansen, Malcolm J. Sheardown, Christian Thomsen, Thøger Rasmussen, Anders Fink Jensen, Michael S. Christensen, Karin Rimvall, John S. Ward,† Celia Whitesitt,† David O. Calligaro,† Frank P. Bymaster,† Neil W. Delapp,† Christian C. Felder,† Harlan E. Shannon,† and Per Sauerberg

Novo Nordisk A/S, Health Care Discovery, Novo Nordisk Park, DK-2760 Måløv, Denmark, and The Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285

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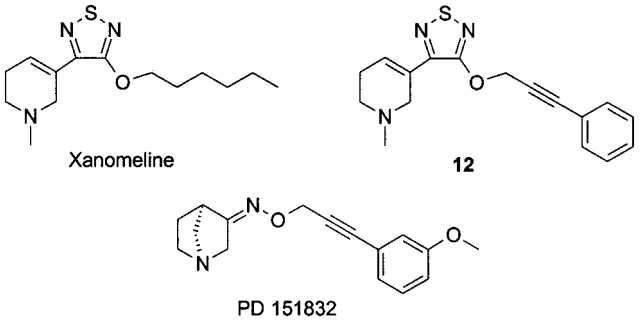
Two new series of 1-(1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptanes were synthesized and evaluated for their in vitro activity in cell lines transfected with either the human M_1 or M_2 receptor. 3-Phenyl-2-propyn-1-yloxy and -1-ylthio analogues substituted with halogen in the *meta* position showed high functional potency, efficacy, and selectivity toward the M_1 receptor subtype. A quite unique functional M_1 receptor selectivity was observed for compounds **8b**, **8d**, **8f**, **9b**, **9d**, and **9f**. Bioavailability studies in rats indicated an oral bioavailability of about 20−30%, with the *N*-oxide as the only detected metabolite.

Introduction

The observation that presynaptic cholinergic terminals degenerate whereas the postsynaptic M_1 receptors are preserved in Alzheimer's disease brain tissue led to the hypothesis that M_1-selective agonists would be beneficial in the treatment of Alzheimer's disease.[1] This hypothesis was supported by our findings that the muscarinic M_1 agonist xanomeline (3-(3-hexyloxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine) (Chart 1), in a phase II clinical trial, showed improvements in cognitive parameters in Alzheimer's disease patients.[2,3] Furthermore, behavioral symptoms associated with Alzheimer's disease, such as hallucinations, delusions, and vocal outburst, were significantly decreased with xanomeline treatment.[2,3] Xanomeline has been shown to be a partial agonist at all muscarinic receptor subtypes (M_1−M_5), but with the highest efficacy and potency at M_1 and M_4 receptors. In an attempt to identify a new drug candidate for Alzheimer's disease, the main focus was on the improvement of the M_1 receptor selectivity as well as of the oral bioavailability. An enhancement of the M_1 selectivity was expected to further increase the cognitive improvements, while minimizing peripheral side effects associated with activation of M_2 and M_3 receptors. Xanomeline has been shown to be completely absorbed after oral administration, but the compound undergoes extensive biotransformation in rat and human. In rats the oral bioavailability was estimated to be 3−7%.[3] The primary site of metabolism of xanomeline was in the hexyloxy side chain, which undergoes β-oxidation yielding hydroxy, keto, and shorter side chain carboxylic acid analogues.[4,5] The secondary site is the tetrahydropyridine ring, which undergoes N-oxidation and N-demethylation. The thiadiazole ring appears, however, to be metabolically stable.[6] Extensive structure−activity relationship (SAR) work has previously been carried out around xanomeline[7−11] showing that the hexyloxy side chain is crucial for the biological profile.[7]

Chart 1



In our search for a functionally equivalent but more stable side chain, we discovered that the 3-phenyl-2-propyn-1-yloxy group was quite unique to replace the hexyloxy group, giving a compound (**12**) with a very robust M_1 response.[12] This finding is in agreement with a previous report showing the utility of the 3-aryl-2-propynyl chain in the muscarinic agonist (−)-(*R*)-(*Z*)-3-(3-(3-methoxyphenyl)-2-propynyloxyimino)-1-azabicyclo-[2.2.1]heptane, PD 151832[13] (Chart 1). To prevent the metabolic demethylation as observed with xanomeline, the *N*-methyl group has been incorporated in a ring giving various azabicyclic and tricyclic ring systems.[12] Some of these more conformationally constricted azacycles, with various phenylpropargyloxy/thio side chains, also showed a high efficacious M_1 receptor response.[12]

We describe here two of these series (**8** and **9**), in which the *N*-methyltetrahydropyridine ring of xanomeline has been replaced with the [2.2.1.0^{2,6}]azatricycle. Variations of the substitution pattern of the aryl group were examined (**8a**−**h** and **9a**−**h**), revealing compounds with a pronounced functional M_1 receptor selectivity.

† Lilly Corp. Center.

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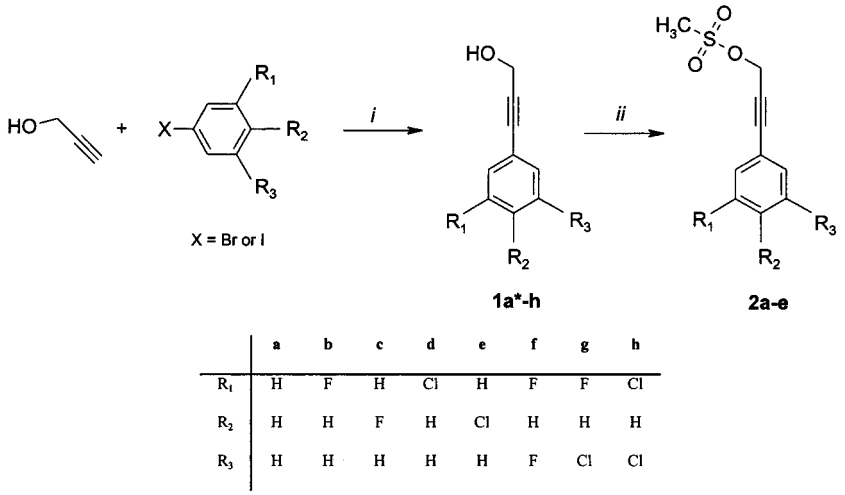
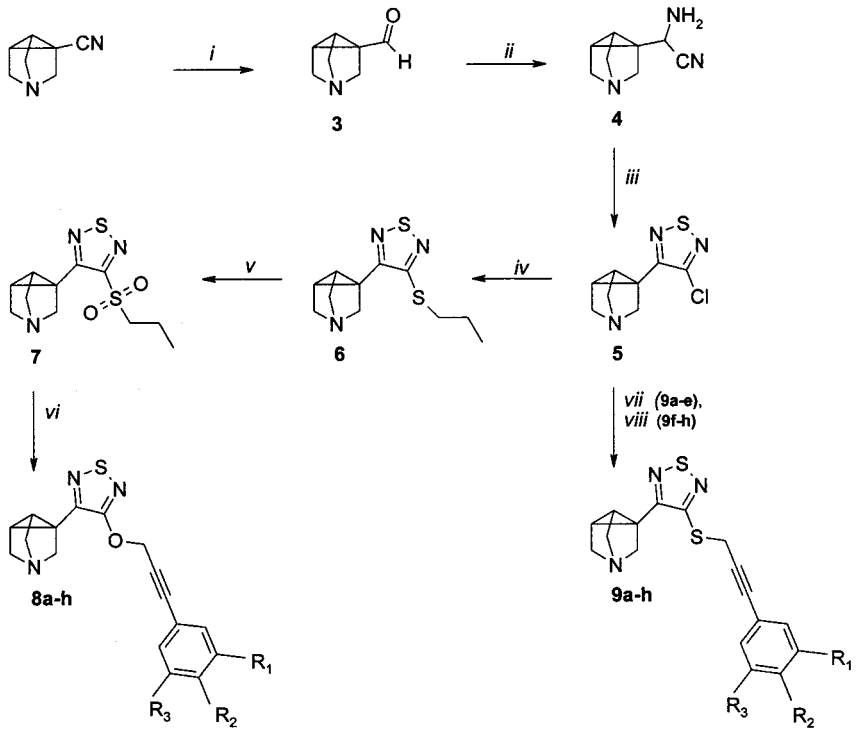
Scheme 1[a]



	a	b	c	d	e	f	g	h
R_1	H	F	H	Cl	H	F	F	Cl
R_2	H	H	F	H	Cl	H	H	H
R_3	H	H	H	H	H	F	Cl	Cl

[a] **1b**−**d,f,g**: X = Br; (i) $(Ph_3P)_2PdCl_2$, CuI, $(i\text{-}Pr)_2NH$, Et_3N. **1e,h**: X = I; (i) $(Ph_3P)_4Pd$, CuI, $(i\text{-}Pr)_2NH$. **2a**−**e**: (ii) Et_3N, CH_2Cl_2, CH_3SO_2Cl. *Commercially available.

Scheme 2[a]

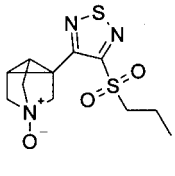


[a] (i) DIBAL, THF; (ii) KCN, H_2O, NH_4Cl, NH_3; (iii) S_2Cl_2, DMF; (iv) NaHS, DMF, $CH_3(CH_2)_2Br$, K_2CO_3; (v) oxone, HCl, H_2O; (vi) NaH, THF, **1a**−**h**; (vii) NaHS, DMF, **2a**−**e**; (viii) NaHS, THF, $(C_6H_5)_3P$, **1f**−**h**, $C_2H_5O_2CN{=}NCO_2C_2H_5$.

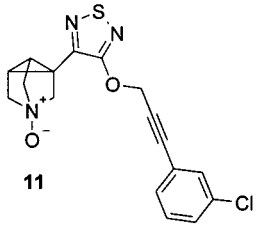
Chemistry

The syntheses of the azatricyclic thiadiazoles **8a**−**h** and **9a**−**h** began with the known 1-cyano-4-azatricyclo-[2.2.1.$0^{2,6}$]heptane[14] (Scheme 2), which is obtained by reaction of 1-azabicyclo[2.2.1]heptan-3-one[15,16] with potassium cyanide to give 3-cyano-3-hydroxy-1-azabicyclo-[2.2.1]heptane, followed by a ring closure with diethylaminosulfur trifluoride.[17] Reduction of the tricyclic nitrile, with diisobutylaluminum hydride, gave the aldehyde **3**. Aminonitrile **4** was obtained from **3** under Strecker conditions or by stepwise conversion of **3** to a cyanohydrin followed by treatment with aqueous ammonium chloride under basic conditions. Cyclization of the aminonitrile **4** with sulfur monochloride in dimethylformamide gave the 3-chloro-1,2,5-thiadiazole **5**. To obtain a better leaving group than chlorine, **5** was converted into the propylthio intermediate **6** by treating **5** with sodium hydrogen sulfide monohydrate, followed by alkylation with propyl bromide. Oxidation of **6** with an equimolar amount of oxone gave the corresponding sulfone **7**. Excess of oxone gave rise to some impurity of the corresponding *N*-oxide **10** (Chart 2), which was formed during the workup process. Further investigation showed that the pH in the reaction medium was very crucial for the *N*-oxide formation, with a pH optimum around pH 7. No amine oxidation was detected under very basic or acidic conditions. Reduction of **10** with iron powder in glacial acetic acid gave compound

Chart 2



10



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7. The target compounds **8a**−**h** were obtained by reacting **7** with an appropriate alcohol (**1a**−**h**) and a base, e.g., sodium hydride. **1a** is commercially available, whereas **1b**−**h** were synthesized by an arylalkyne cross-coupling reaction employing a Pd catalyst such as Pd-$(PPh_3)_4$ or $PdCl_2(PPh_3)_2$ and a catalytic amount of copper(I) iodide. An organic amine base, such as diisopropylamine, was used both as the reagent as well as the solvent, and no cosolvent was needed (Scheme 1). The sulfur analogues **9a**−**e** were obtained by reacting the chloro intermediate **5** with sodium hydrogen sulfide monohydrate followed by a reaction with the mesylates **2a**−**e** (Scheme 1). Conversion of the chloro intermediate **5** to the thiolate with sodium hydrogen sulfide monohydrate followed by a Mitsunobu reaction with the appropriate alcohol (**1f**−**h**) gave **9f**−**h**.

Compound **12**, the aryl-2-propyn-1-yloxy analogue of xanomeline, was synthesized via a method analogous to that which has been previously reported for xanomeline,[7] using 3-(3-chloro-1,2,5-oxadiazol-4-yl)pyridine as starting material.

Biological Evaluation

In vitro receptor binding studies using tritiated oxotremorine-M ([^{3}H]Oxo-M) were used to determine the affinity for the muscarinic agonist conformational state. Second-messenger changes in cell lines transfected with human muscarinic receptor subtypes were used to determine functional potency and efficacy. The ability of each compound to stimulate phosphoinositol (PI) hydrolysis in the A9L-M_1 cell line was determined up to a concentration of 100 μM, and the efficacy of the compound was expressed as a percentage of that produced by 100 μM carbachol. The EC_{50} for PI hydrolysis was determined for each compound that produced at least 25% increase in hydrolysis. The EC_{50}'s for less efficacious compounds could not be determined with sufficient accuracy due to the inherent variability in the assay. Potency at M_2 receptors was expressed as the EC_{50} for the compound to inhibit forskolin-induced cAMP formation by 50% in CHO cells transiently expressing the M_2 muscarinic receptor, and the efficacy was expressed as the percent of the maximal inhibition produced by carbachol.

The receptor reserve differs for the two cell lines, making comparison of potency and efficacy difficult. The reported M_2/M_1 selectivity ratios (Table 1) are therefore not absolute values, but rather relative numbers.

Results and Discussion

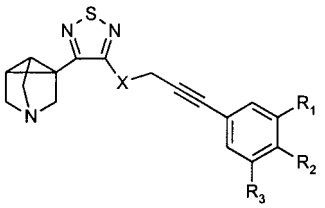
The main goal of this project was to synthesize a M_1-selective agonist with good oral bioavailability. The hexyloxy side chain in xanomeline has earlier been shown to be very crucial for the pharmacological profile, and a SAR analysis around various saturated carbon chain analogues showed that the hexyloxy group is optimal for functional M_1 selectivity.[12] On the other hand, the carbon chain underwent extensive biotransformation, and therefore an exchange of this metabolically labile hexyloxy group with a more stable side chain was essential for the design and synthesis of new analogues. This SAR work led to the discovery of **12**, which possesses the same M_1 potency and an even higher efficacy than xanomeline. In comparison with PD 151832, which contains a similar side chain, **12** was clearly far more efficacious and at least 50 times more potent in the A9L-M_1 cell line. On the basis of these findings, two series of 3-aryl-2-propyn-1-yloxy and -1-ylthio analogues, where the tetrahydropyridine ring was exchanged with the azatricycle (**8a**−**h**, **9a**−**h**), were synthesized. The rationale for choosing this very constrained heterocycle was primarily to prevent the metabolic demethylation observed with xanomeline, but also to attempt to enhance the muscarinic M_1 receptor subtype selectivity. A prevention of the conformational flexibility of a molecule can, in theory, change receptor selectivity, due to possible differences in the structural requirements for activation of the receptor subtypes. The change in the pK_a for the nitrogen in the azacycle, in this case a reduction due to the increased ring strain in the tricycle,[17] could also have an important influence on the selectivity.

Compound **12**, the direct tricyclic analogue **8a**, and the corresponding thio analogue **9a** all had similar biological profiles (Table 1). The M_2/M_1 selectivity ratios were, however, improved for **8a** and **9a** (11 and 14, respectively), compared to **12** and xanomeline (2 and 3, respectively).

Substitution on the phenyl ring with various halogens gave rise to some very interesting findings. In the oxygen series **8**, the 4-fluoro analogue **8c** had the same profile as the unsubstituted **8a**, whereas the 4-chloro compound **8e** was markedly less efficacious in the M_1 cell line. In contrast, substitution in one of the *meta* positions with either fluorine (**8b**) or chlorine (**8d**) enhanced the M_1 receptor potency approximately 10-fold. In addition to the increased M_1 potency, **8b** and **8d** both showed a distinct M_1 selectivity, with a M_2/M_1 ratio of 139 and 217, respectively. The same observation was made with the 3,5-difluoro analogue **8f**, which was a very potent full M_1 agonist with the same remarkable functional M_1 selectivity. The M_2/M_1 ratio for **8f** was calculated to be 133. The mixed 3-chloro-5-fluoro compound **8g** was less potent than the unsubstituted **8a**, and with a 3,5-dichoro substitution (**8h**) a major loss in potency was observed in the two cell lines.

In the sulfur series (**9**), there was the same trend in the influence of the substitution as described above for the oxygen series. The 4-fluoro compound **9c** was greater than 10-fold more potent in both cell lines than the unsubstituted analogue **9a**. The 4-chloro compound **9e** was, as observed for **8e**, less efficacious in the M_1 cell line. **9b** and **9d**, with a fluorine or a chlorine in the *meta* position, respectively, were more potent in the M_1 cell line and had a very favorable M_2/M_1 ratio (362 and 74, respectively). This high ratio was also observed for the 3,5-difluoro analogue **9f**, where the M_2/M_1 ratio was 218. The 3-chloro-5-fluoro analogue **9g** was equipotent

Table 1. Receptor Binding and Functional M_1 and M_2 Profiles for the Halogen-Substituted 1-(1,2,5-Thiadiazol-4-yl)-4-azatricyclo[2.2.1.$0^{2,6}$]heptanes



					[^{3}H]Oxo-M receptor binding to rat brain membranes	PI hydrolysis in A9L-M_1 cells		c-AMP formation in CHO-M_2 cells		
compd	X	R_1	R_2	R_3	IC_{50} (nM, ±SEM)	% max (±SEM)	EC_{50} (nM, ±SEM)	% max (±SEM)	EC_{50} (nM, ±SEM)	ratio $EC_{50}(M_2)$/ $EC_{50}(M_1)$
8a	O	H	H	H	16 ± 2.5	83.7 ± 17.8	70.5 ± 0.1	99 ± 9	779 ± 272	11
8b	O	F	H	H	21 ± 5.1	91.0 ± 2.6	4.9 ± 3.4	104 ± 5	683 ± 131	139
8c	O	H	F	H	10 ± 1.6	101.0 ± 7.3	71.0 ± 17.9	109 ± 5	791 ± 40	11
8d	O	Cl	H	H	47 ± 5.6	98.5 ± 1.5	10.5 ± 0.8	101 ± 14	2280 ± 720	217
8e	O	H	Cl	H	63 ± 8.3	60.5 ± 3.8	nd	102 ± 13	1210 ± 55	nd
8f	O	F	H	F	30 ± 11	107.3 ± 3.5	20.2 ± 4.2	68 ± 14	2690 ± 189	133
8g	O	F	H	Cl	92 ± 4.7	102.8 ± 11.0	110.0 ± 41.7	76 ± 17	1480 ± 101	13
8h	O	Cl	H	Cl	1688 ± 99	87.5 ± 1.8	1200 ± 900	23 ± 12	nd	nd
9a	S	H	H	H	27 ± 53	91.9 ± 5.2	61.9 ± 4.4	106 ± 1	853 ± 103	14
9b	S	F	H	H	26 ± 7.2	102.0 ± 6.2	2.5 ± 0.5	106 ± 6	904 ± 121	362
9c	S	H	F	H	20 ± 4.6	97.5 ± 9.5	4.5 ± 0.7	88 ± 1	83 ± 13	18
9d	S	Cl	H	H	49 ± 7.6	67.5 ± 6.4	9.0 ± 5.0	96 ± 2	673 ± 167	74
9e	S	H	Cl	H	242 ± 56	52.9 ± 14.6	107.0 ± 4.5	76 ± 12	80 ± 28	1
9f	S	F	H	F	28 ± 9.1	85.5 ± 4.1	7.6 ± 3.3	79 ± 16	1655 ± 391	218
9g	S	F	H	Cl	142 ± 11	101.0 ± 9.9	46.0 ± 5.4	90 ± 9	763 ± 42	17
9h	S	Cl	H	Cl	788 ± 117	57.7 ± 7.9	1810 ± 210	84 ± 17	528 ± 208	0.3
carbacol					34 ± 8.2	99 ± 2.3	4600 ± 830	100 ± 3	700 ± 85	0.2
xanomeline					16 ± 2.3	65.6 ± 14.7	134.5 ± 32.5	82 ± 8	398 ± 79	3
PD 151832					150 ± 11	37.3 ± 13.8	7121 ± 567	89 ± 11	26700 ± 3300	4
12					14 ± 2.4	111.6 ± 4.2	110.9 ± 64.0	115 ± 12	241 ± 45	2

with **9a** in the cell lines, whereas a major loss in efficacy as well as potency in the M_1 cell line was observed for the dichloro analogue **9h**.

These results clearly show that the substitution pattern in the phenyl ring is very important for the biological profile. A fluorine or a chlorine in the *meta* position in both the oxygen and sulfur series gave rise, in both the oxygen and sulfur series, to compounds with very favorable M_2/M_1 ratios, and all compounds were at the same time more potent and efficacious than xanomeline in the M_1 cell line. The same was true for the 3,5-difluoro analogues, whereas decreased potencies were observed with the disubstituted 3-chloro-5-fluoro analogues **8g** and **9g**. This was even more pronounced with the 3,5-dichloro analogues **8h** and **9h**, indicating a limitation in steric tolerance for receptor activation. A fluorine in the *para* position was tolerated in both series, whereas the more bulky chlorine caused a large loss of the efficacy in the M_1 cell line.

Due to their very favorable M_2/M_1 ratio, compounds **8b**, **8d**, **8f**, **9b**, **9d**, and **9f** were taken into further investigations. It is known that nonselective muscarinic agonists produce parasympathomimetic side effects, and a number of these effects are produced by activation of M_2 or M_3 receptors.[3,18,19] In our paradigm, compounds that induced salivation or tremor in mice were considered nonselective and were excluded in our search for a selective M_1 agonist. The side effects were determined in mice 30 min after drug administration (10 mg/kg) according to a previously described method.[19] Briefly, salivation was scored on a scale by observation according to severity (0 for no salivation, 1 for slight salivation, 2 for profuse salivation). Tremor was also scored observationally on a scale of 0−2 (0 for no tremor, up to 2 for prominent tremors). Compounds **8b** and **9d** produced profuse salivation and received a score of 2. **8b** also induced tremor with a score of 1.4. Compounds **8d**, **8f**, **9b**, and **9f**, on the other hand, did not cause any salivation or tremor.

Among compounds **8d**, **8f**, **9b**, and **9f**, compound **8f** was selected for further investigation due to the promising profile of being a fully efficacious M_1 agonist with only partial efficacy at the M_2 receptor subtype while causing no tremor or salivation. Oral bioavailability studies with **8f** in rats indicated an oral bioavailability of about 20−30%. In the HPLC traces, an additional peak with a longer retention time than that of **8f** was observed in samples from both intravenous and oral administration. This peak was more pronounced following oral administration, probably due to first-pass metabolism, and no other peaks were observed. To identify this metabolite, **11** was synthesized. **11** coeluted with the unknown peak indicating that the detected metabolite was N-oxidized **8f**. This was further confirmed by LC/MS/MS where the peak eluting later than **8f** showed the same characteristic fragment ion as **11**. This N-oxidation of the azatricycle was to some extent expected, as the increased ring strain in the azatricycle relative to the azacycle in xanomeline resulted in a substantial reduction in pK_a.[17] The assumed *N*-oxide was the only detected metabolite, indicating that the propargyl group as well as the halogen substitution results in an effective shielding of the side chain from metabolic attack. This is probably the explanation of the pronounced improvement of the bioavailability. Further pharmacological and metabolism studies will show whether this series of compounds will provide a second-generation M_1 agonist.

Summary

This study showed that 3-aryl-2-propyn-1-yloxy and -1-ylthio derivatives of 1-(1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.$0^{2,6}$]heptane are very potent and efficacious M_1 agonists. The substitution pattern in the phenyl ring in the side chain moiety had a major effect on the M_1 receptor selectivity. The *m*-fluoro, *m*-chloro, or *m*-difluoro compounds (**8b**, **8d**, **8f**, **9b**, **9d**, and **9f**) were all very selective with M_2/M_1 ratios of around 100 or more, whereas for xanomeline and its 3-phenyl-2-propyn-1-yloxy analogue **12** this ratio was 2−3. By comparing **12** with **8a**, the effect of exchanging the tetrahydropyridine ring with the tricyclic amine was an improvement in the selectivity as well. The 3,5-dichloro or 4-chloro analogues were, on the other hand, all markedly less efficacious, which was interpreted as being due to a limitation in the steric tolerance of the receptor. **8d**, **8f**, **9b**, and **9f** did not cause any salivation or tremor which indicates a selective M_1 profile. Preclinical analysis in rats demonstrated a pronounced improvement of the oral bioavailability of **8f** (20−30%) compared to xanomeline (3−7%), with the *N*-oxide as the only detected metabolite.

Experimental Section

General Chemistry. Melting points were determined with a Büchi capillary melting apparatus and are uncorrected. The 200-MHz ^{1}H NMR spectra were recorded on a Brüker AC-200 MHz FT spectrometer. The 400-MHz ^{1}H NMR spectra were recorded on a Brucker AMX2-400 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to Me_4Si, and coupling constants are in Hz. FAB/MS were recorded with a UG AutoSpec Ultima sector mass spectrometer (EBE-geometry) interfaced to a data processing system. Cesium fast ions generated with 33 kV and a 2 μA emission current were used for bombardment. Reactions were followed by either thin-layer chromatography performed on silica gel 60 F254 (Merck) TLC aluminum sheets and/or by HPLC performed on a Merck Hitachi system, containing a L-6200A Intelligen pump and a L-4000A UV detector with a Resource RPC column and/or by gas chromatography (GC) on a Chrompack CP 9001 chromatograph using a CP-Sil 5 CB, 25-m column. Flash chromatography was performed on silica gel 60 (230−400 mesh ASTM, Merck). Preparative HPLC was performed on a Dan Process system using a Knauer UV detector, with a HO50700 column containing Source 15RPC. Elemental analyses were performed by Novo Nordisk, Microanalytical Laboratory, Denmark. Freeze-drying was performed on a Hetosicc apparatus, using 0.1−0.2 mmHg vacuum and a temperature graduated from −20 °C to room temperature over 3−4 days.

Preparation of Compounds 1b−d,f,g. To a mixture of copper(I) iodide (100 mg, 0.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (210 mg, 0.3 mmol) in diisopropylamine (100 mL) and NEt_3 (20 mL) under a nitrogen atmosphere was added the appropriate fluoro- or chloro-substituted bromobenzene (30 mmol). After the mixture stirred at room temperature for 30 min, 3-propynol (1.85 g, 33 mmol) was added and the reaction mixture heated at 60 °C for 24 h. The reaction mixture was filtered and the filtrate evaporated to dryness. The product was purified by flash chromatography using CH_2Cl_2 as eluent to give the product as a viscous oil.

3-(3-Fluorophenyl)prop-2-yn-1-ol (1b): yield 72%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.88 (t, J = 6 Hz, 1 H), 4.48 (d, J = 6 Hz, 2 H), 7.00−7.35 (m, 4 H).

3-(4-Fluorophenyl)prop-2-yn-1-ol (1c): yield 23%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.33 (t, J = 6 Hz, 1 H), 4.48 (d, J = 6 Hz, 2 H), 6.93−7.05 (m, 2 H), 7.35−7.47 (m, 2 H).

3-(3-Chlorophenyl)prop-2-yn-1-ol (1d): yield 75%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.80 (t, J = 6 Hz, 1 H), 4.48 (d, J = 6 Hz, 2 H).

3-(3,5-Difluorophenyl)prop-2-yn-1-ol (1f): yield 30%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.88 (bs, 1 H), 4.50 (s, 2 H), 6.70−6.85 (m, 1 H), 6.85−7.00 (m, 2 H).

3-(3-Chloro-5-fluorophenyl)prop-2-yn-1-ol (1g): yield 55%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.80 (bs, 1 H), 4.50 (s, 2 H), 6.98−7.12 (m, 2 H), 7.22 (s, 1 H).

Preparation of Compounds 1e,h. To a solution of the appropriate chloro-substituted iodobenzene (30.0 mmol) in diisopropylamine (100 mL) under a nitrogen atmosphere were added copper(I) iodide (100 mg, 0.5 mmol) and tetrakis-(triphenylphosphine)palladium (300 mg, 0.25 mmol). After the mixture stirred for 1 h, a solution of 3-propynol (1.85 g, 33.0 mmol) in diisopropylamine (50 mL) was added. After stirring under a nitrogen atmosphere at room temperature for 16 h, the reaction mixture was filtered and the filtrate evaporated to dryness. The product was purified by flash chromatography using CH_2Cl_2 as eluent to give the product as a viscous oil.

3-(4-Chlorophenyl)prop-2-yn-1-ol (1e): yield 96%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.75 (bs, 1 H), 4.50 (s, 2 H), 7.22−7.40 (m, 4 H).

3-(3,5-Dichlorophenyl)prop-2-yn-1-ol (1h): yield 87%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.80 (t, J = 6 Hz, 1 H), 4.50 (d, J = 6 Hz, 2 H), 7.28−7.35 (m, 3 H).

Preparation of Compounds 2a−e. A solution of the appropriate alcohol (**1a−e**) (20.0 mmol) in CH_2Cl_2 and NEt_3 (2.43 g, 24 mmol) was cooled to − 20 °C. Methanesulfonyl chloride (2.25 g, 22.0 mmol) was added dropwise over 10 min under a nitrogen atmosphere. The reaction mixture was stirred for 1.5 h at − 20 °C, diluted with CH_2Cl_2 (20 mL), and washed with H_2O (3 × 20 mL). The organic phase was dried ($MgS0_4$), evaporated, and if needed submitted to flash chromatography using CH_2Cl_2 as eluent to give the pure product.

Methanesulfonic acid 3-phenylprop-2-ynyl ester (2a): yield 99%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.15 (s, 3 H), 5.08 (s, 2 H), 7.34−7.40 (m, 3 H), 7.40−7.50 (m, 2 H).

Methanesulfonic acid 3-(3-fluorophenyl)prop-2-ynyl ester (2b): yield 78%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.15 (s, 3 H), 5.08 (s, 2 H), 7.04−7.40 (m, 4 H).

Methanesulfonic acid 3-(4-fluorophenyl)prop-2-ynyl ester (2c): yield 49%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.15 (s, 3 H), 5.08 (s, 2 H), 6.96−7.10 (m, 2 H), 7.40−7.52 (m, 2 H).

Methanesulfonic acid 3-(3-chlorophenyl)prop-2-ynyl ester (2d): yield 46%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.15 (s, 3 H), 5.08 (s, 2 H), 7.24−7.48 (m, 4 H).

Methanesulfonic acid 3-(4-chlorophenyl)prop-2-ynyl ester (2e): yield 80%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.15 (s, 3 H), 5.08 (s, 2 H), 7.30−7.44 (m, 4 H).

1-Formyl-4-azatricyclo[2.2.1.$0^{2,6}$]heptane (3). 1-Cyano-4-azatricyclo[2.2.1.$0^{2,6}$]heptane[13] (5.73 g, 47.6 mmol) in dry THF (100 mL) was treated with diisobutylaluminum hydride (78.3 mL, 20% in toluene, 95.2 mmol) at 0 °C under a nitrogen atmosphere. After 3 h the reaction mixture was allowed to warm to room temperature and quenched with 2-propanol (10 mL) and 4 N HCl (pH 2−3), followed by Rochelle salt (25 g). The organic solvent was removed in vacuo and the residue extracted with Et_2O (2 × discarded). The aqueous phase was made alkaline with 6 N NaOH (pH 10−11) and the mixture extracted with CH_2Cl_2/2-propanol (9:1) (6×). The organic extracts were dried ($MgSO_4$) and filtered. Evaporation of the solvent gave 4.15 g (71%) of the title compound: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.27 (s, 2 H), 2.58 (d, J_{gem} = 10.5 Hz, 2 H), 2.66 (d, J_{gem} = 10.5 Hz, 2 H), 2.68 (s, 2 H), 9.50 (s, 1 H).

2-Amino-2-(4-azatricyclo[2.2.1.$0^{2,6}$]hept-1-yl)acetonitrile (4). To an ice-cooled solution of 1-formyl-4-azatricyclo-[2.2.1.$0^{2,6}$]heptane (**3**) (3.56 g, 29 mmol) in H_2O (5 mL) was added a solution of potassium cyanide (2.08 g, 31.9 mmol) in H_2O (5 mL) over a period of 30 min. After an additional 1 h, cooling was removed and the reaction stirred at room temperature for 16 h. To the crude cyanohydrin product were then added 25% NH_3 (4.0 mL, 52 mmol) and ammonium chloride (7.76 g, 145 mmol). The reaction mixture was stirred at room temperature for 18 h and then extracted with 2-propanol/CH_2Cl_2 (1:10) (10 × 75 mL). The organic phases were dried ($MgSO_4$), and the solvent was evaporated to give the title

compound as an oil (2.5 g, 58%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (s, 1 H), 1.72 (s, 2 H), 2.15−2.30 (m, 8 H). The compound was sufficiently pure for the next reaction step.

1-(3-Chloro-1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]-heptane (5). To a solution of sulfur monochloride (3.2 mL, 40.2 mmol) in DMF (5 mL) was added a solution of crude 2-amino-2-(4-azatricyclo[2.2.1.0^{2,6}]hept-1-yl)acetonitrile (**4**) (3.0 g, 20.1 mmol) in DMF (5 mL) at 5−10 °C over a period of 30 min. The reaction mixture was stirred for an additional 1 h at 5−10 °C, after which ice−water (12 mL) was added to the reaction. The mixture was filtered, and the filtrate was made alkaline (pH = 10) with 4 N NaOH. The product was extracted with CH_2Cl_2 (4×). The organic phases were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography on silica gel using CH_2Cl_2 graduated to CH_2Cl_2/MeOH (9:1) as eluent. The title compound was obtained as an oil in 2.1 g (48%) yield: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.12 (s, 1 H), 2.55 (d, J_{gem} = 10.5 Hz, 2 H), 2.65 (d, J_{gem} = 10.5 Hz, 2 H), 2.70 (s, 2 H); MS *m*/*z* 213 (M + 1).

1-(3-Propylthio-1,2,5-thiadiazol-4-yl)-4-azatricyclo-[2.2.1.0^{2,6}]heptane (6). A solution of 1-(3-chloro-1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptane (**5**) (700 mg, 3.3 mmol) and sodium hydrogen sulfide monohydrate (730 mg, 9.9 mmol) in DMF (20 mL) was stirred at room temperature under a nitrogen atmosphere for 1 h. 1-Propyl bromide (900 μL, 9.9 mmol) and potassium carbonate (4.6 g, 33 mmol) was added, and the reaction mixture was stirred at room temperature for 30 min. pH was adjusted with 4 N HCl (pH 2) and the mixture washed with Et_2O (2× discarded) The aqueous phase was made alkaline with 2 N NaOH (pH 10−11) and the mixture extracted with Et_2O (3×). The Et_2O phases were dried ($MgSO_4$) and evaporated. Crystallization of the residue with oxalic acid from acetone gave the title compound as the oxalate salt in 935 mg (82%) yield: mp 151−154 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (t, J = 8 Hz, 3 H), 1.80 (sextet, J = 8 Hz, 2 H), 2.13 (s, 2 H), 2.62 (d, J_{gem} = 10.5 Hz, 2 H), 2.80 (d, J_{gem} = 10.5 Hz, 2 H), 2.88 (s, 2 H), 3.25 (t, J = 8 Hz, 2 H). Anal. ($C_{13}H_{17}N_3O_4S_2$) C, H, N.

1-(3-Propylsulfonyl-1,2,5-thiadiazol-4-yl)-4-azatricyclo-[2.2.1.0^{2,6}]heptane (7). 1-(3-Propylthio-1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptane (**6**) (585 mg, 1.7 mmol) was dissolved in H_2O (20 mL) and 1 N HCl (2 mL) and the solution cooled in ice−water. Oxone (1.57 g, 2.55 mmol) in H_2O (10 mL) was added under stirring. Cooling was removed and the reaction stirred for 2.5 h. The reaction was made alkaline (pH 10−11) with 5 N NaOH and then extracted with Et_2O (6×). The organic phases were dried ($MgSO_4$), and the solvent was evaporated. Crystallization of the residue with oxalic acid from acetone gave the title compound as the oxalate salt in 545 mg (83%) yield: mp 187−191 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.13 (t, J = 8 Hz, 3 H), 1.95 (sextet, J = 8 Hz, 2 H), 2.20 (s, 2 H), 2.62 (d, J_{gem} = 10.5 Hz, 2 H), 2.86 (d, J_{gem} = 10.5 Hz, 2 H), 2.96 (s, 2 H), 3.56 (t, J = 8 Hz, 2 H). Anal. ($C_{13}H_{17}N_3O_6S_2$) C, H, N.

Preparation of Compounds 8a−h. To a solution of the appropriate alcohol (**1a−h**) (2.1 mmol) in dry THF (10 mL) was slowly added sodium hydride (80%) (80 mg, 2.7 mmol) under a nitrogen atmosphere. After 1 h 1-(3-propylsulfonyl-1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptane (**7**) (200 mg, 0.7 mmol) in dry THF (3 mL) was added. Stirring was continued for 5 days. The reaction was quenched by addition of 6 N HCl (pH 2) and the THF evaporated. The residue was washed with Et_2O (3× discarded). The aqueous phase was made basic (pH 10−11) with 25% NH_3 and extracted with Et_2O (3×). The extracts were dried ($MgSO_4$), filtered, and concentrated. The residue was taken up in acetone and precipitated with oxalic acid in acetone to give the product as the oxalate salt.

1-[3-(3-Phenyl-2-propyn-1-yloxy)-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8a): yield 76%; mp 182−185 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.30 (s, 2H), 5.30 (s, 2 H), 7.45 (m, 5 H). Anal. ($C_{19}H_{17}N_3O_5S\cdot H_2O$) C: calcd, 54.67; found, 55.02. H: calcd, 4.07; found, 4.25. N: calcd, 10.07; found, 9.98.

1-[3-[3-(3-Fluorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8b): yield 64%; mp 177−179 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2 H), 5.35 (s, 2 H), 7.32 (m, 3 H), 7.45 (m, 1 H). Anal. ($C_{19}H_{16}FN_3O_5S$) C, H, N.

1-[3-[3-(4-Fluorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8c): yield 80%; mp 185−187 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.32 (s, 2H), 5.32 (s, 2 H), 7.25 (t, 2 H), 7.55 (m, 2 H). Anal. ($C_{19}H_{16}FN_3O_5S\cdot 1.5H_2O$) C: calcd, 51.35; found, 51.31. H: calcd, 4.50; found, 4.68. N: calcd, 9.45; found, 9.17.

1-[3-[3-(3-Chlorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8d): yield 66%; mp 160−162 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2H), 5.35 (s, 2 H), 7.44 (m, 2 H), 7.52 (m, 2 H). Anal. ($C_{19}H_{16}ClN_3O_5S\cdot 0.5H_2O$) C: calcd, 51.46; found, 51.69. H: calcd, 3.83; found, 3.73. N: calcd, 9.48; found, 9.11.

1-[3-[3-(4-Chlorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8e): yield 60%; mp 172−173.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2 H), 5.35 (s, 2 H), 7.48 (m, 4 H). Anal. ($C_{19}H_{16}ClN_3O_5S$) C, H, N.

1-[3-[3-(3,5-Difluorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8f): yield 48%; mp 173−175 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2H), 5.35 (s, 2 H), 7.25 (m, 2 H), 7.38 (m, 1 H). Anal. ($C_{19}H_{15}F_2N_3O_5S\cdot 3H_2O$) C: calcd, 46.62; found, 47.01. H: calcd, 3.06; found, 3.17. N: calcd, 8.58; found, 8.44.

1-[3-[3-(3-Chloro-5-fluorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8g): yield 78%; mp 155−156 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2 H), 5.35 (s, 2 H), 7.38 (d, J = 9.0 Hz, 1 H), 7.44 (s, 1 H), 7.55 (d, J = 9.0 Hz, 1 H). Anal. ($C_{19}H_{15}ClFN_3O_5S\cdot 0.5H_2O$) C: calcd, 49.52; found, 49.65. H: calcd, 3.50; found, 3.30. N: calcd, 9.12; found, 8.95.

1-[3-[3-(3,5-Dichlorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (8h): yield 22%; mp 121−124 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.52 (s, 2 H), 3.05 (d, J_{gem} = 10.5 Hz, 2 H), 3.12 (d, J_{gem} = 10.5 Hz, 2 H), 3.35 (s, 2 H), 5.35 (s, 2 H), 7.55 (s, 2 H), 7.75 (m, 1 H). Anal. ($C_{19}H_{15}Cl_2N_3O_5S$) C, H, N.

Preparation of Compounds 9a−e. A solution of 1-(3-chloro-1,2,5-thiadiazol-4-yl)-4-azatricyclo[2.2.1.0^{2,6}]heptane (**5**) (3.0 g, 14 mmol) and sodium hydrogen sulfide monohydrate (3.14 g, 42 mmol) in dry DMF (80 mL) was stirred at room temperature under a nitrogen atmosphere for 1 h. The solvent was evaporated. H_2O was added and the pH adjusted by addition of 4 N HCl (pH 9). The mixture was cooled on ice, and 4-(4-azatricyclo[2.2.1.0^{2,6}]hept-1-yl)-1,2,5-thiadiazole-3-thiol (1.7 g, 58%) was isolated by filtration: mp 205−207 °C. A mixture of 4-(4-azatricyclo[2.2.1.0^{2,6}]hept-1-yl)-1,2,5-thiadiazole-3-thiol (106 mg, 0.5 mmol) and potassium carbonate (207 mg, 1.5 mmol) in THF (10 mL) was cooled on ice. A solution of the appropriate methanesulfonate (**2a−e**) (0.5 mmol) in THF (3 mL) was added. The reaction mixture was stirred overnight starting at 0 °C and then allowed to warm to room temperature. Ice water was added and pH adjusted by addition of 4 N HCl (pH 2) (**9d** and **9e** precipitated as the HCl salt and were isolated by filtration). THF was removed by evaporation and the residue washed with Et_2O (3× discarded). The aqueous phase was made alkaline with 25% NH_3 (pH 10−11) and the mixture extracted with Et_2O (3×). The extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was taken up in acetone and precipitated with oxalic acid in acetone to give the product as the oxalate salt.

1-[3-(3-Phenyl-2-propyn-1-ylthio)-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (9a): yield 73%; mp 154−157 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.45 (s, 2 H), 2.95 (d, J_{gem} = 10.5 Hz, 2 H), 3.08 (d, J_{gem} = 10.5 Hz, 2 H), 3.20 (s, 2 H), 4.40 (s, 2 H), 7.38 (s, 5 H). Anal. ($C_{19}H_{17}N_3O_4S_2$) C, H, N.

1-[3-[3-(3-Fluorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (9b): yield 56%; mp 148−149 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.40 (s, 2 H), 2.90 (d, J_{gem} = 10.5 Hz, 2 H), 3.02 (d, J_{gem} = 10.5 Hz, 2 H), 3.15 (s, 2 H), 4.40 (s, 2 H), 7.25 (m, 3 H), 7.40 (m, 1 H). Anal. ($C_{19}H_{16}FN_3O_4S_2$) C, H, N.

1-[3-[3-(4-Fluorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane oxalate (9c): yield 62%; mp 149−150 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.45 (s, 2 H), 2.95 (d, J_{gem} = 10.5 Hz, 2 H), 3.08 (d, J_{gem} = 10.5 Hz, 2 H), 3.20 (s, 2 H), 4.40 (s, 2 H), 7.20 (m, 2 H), 7.42 (m, 2 H). Anal. ($C_{19}H_{16}FN_3O_4S_2$) C, H, N.

1-[3-[3-(3-Chlorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane hydrochloride (9d): yield 66%; mp 175−177 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.77 (s, 2 H), 3.43 (d, J_{gem} = 10.5 Hz, 2 H), 3.53 (d, J_{gem} = 10.5 Hz, 2 H), 3.70 (s, 2 H), 4.40 (s, 2 H), 7.40 (m, 4 H), 11.45 (bs, 1 H). Anal. ($C_{17}H_{15}Cl_2N_3S_2$) C, H, N.

1-[3-[3-(4-Chlorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane hydrochloride (9e): yield 72%; mp 154−156 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.77 (s, 2 H), 3.43 (d, J_{gem} = 10.5 Hz, 2 H), 3.53 (d, J_{gem} = 10.5 Hz, 2 H), 3.70 (s, 2 H), 4.40 (s, 2 H), 7.40 (dd, J = 8.0 Hz, 4 H), 11.45 (bs, 1 H). Anal. ($C_{17}H_{15}$ $Cl_2N_3S_2$·$1H_2O$) C: calcd, 49.27; found, 49.31. H: calcd, 4.11; found, 3.98. N: calcd, 10.14; found, 9.69.

Preparation of Compounds 9f−h. To a solution of 4-(4-azatricyclo[2.2.1.0^{2,6}]hept-1-yl)-1,2,5-thiadiazole-3-thiol (211 mg, 1.0 mmol) (prepared as described under **9a**−**e**) in dry THF (10 mL) was added triphenylphosphine (262 mg, 1.0 mmol), and the mixture was stirred under a nitrogen atmosphere. The mixture was cooled on ice, and the appropriate alcohol (**1f**,**g**,**h**) (1.5 mmol) dissolved in dry THF (5 mL) was added followed by diethyl azodicarboxylate (174 mg, 1.0 mmol). The mixture was stirred for 16 h starting at 0 °C and then allowed to warm to room temperature. Ice water was added and pH adjusted with 4 N HCl to pH 2.0; THF was removed in vacuo. By addition of Et_2O the product precipitated and was isolated by filtration.

1-[3-[3-(3,5-Difluorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane hydrochloride (9f): yield 88%; mp 194−196 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.77 (s, 2 H), 3.43 (d, J_{gem} = 10.5 Hz, 2 H), 3.53 (d, J_{gem} = 10.5 Hz, 2 H), 3.70 (s, 2 H), 4.42 (s, 2 H), 7.15 (m, 2 H), 7.35 (m, 1 H), 11.50 (bs, 1 H). Anal. ($C_{17}H_{14}F_2ClN_3S_2$·$0.33H_2O$) C: calcd, 50.50; found, 50.52. H: calcd, 3.47; found, 3.54. N: calcd, 10.40; found, 10.04.

1-[3-[3-(3-Chloro-5-fluorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane hydrochloride (9g): yield 59%; mp 201−203 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.77 (s, 2 H), 3.43 (d, J_{gem} = 10.5 Hz, 2 H), 3.53 (d, J_{gem} = 10.5 Hz, 2 H), 3.70 (s, 2 H), 4.42 (s, 2 H), 7.25 (d, J = 9.0 Hz, 1 H), 7.30 (s, 1 H), 7.50 (d, J = 8 Hz), 11.70 (bs, 1 H). Anal. ($C_{17}H_{14}FCl_2N_3S_2$) C, H, N.

1-[3-[3-(3,5-Dichlorophenyl)-2-propyn-1-ylthio]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane hydrochloride (9h): yield 60%; mp 184−187 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.77 (s, 2 H), 3.43 (d, J_{gem} = 10.5 Hz, 2 H), 3.53 (d, J_{gem} = 10.5 Hz, 2 H), 3.70 (s, 2 H), 4.42 (s, 2 H), 7.43 (s, 2 H), 7.65 (s, 1 H), 11.75 (bs, 1 H). Anal. ($C_{17}H_{14}$ $Cl_3N_3S_2$) C, H, N.

1-(3-Propylsulfonyl-1,2,5-thiadiazol-4-yl)-4-azatricyclo-[2.2.1.0^{2,6}]heptane 4-Oxide (10). To a suspension of **7** (150 mg, 0.5 mmol) in H_2O (5 mL) was added a solution of oxone (360 mg, 0.58 mmol) in H_2O. pH was adjusted to pH 7 with 1 N NaOH until the reaction was completed. The solution was acidified with 1 N HCl to pH 2, and the product was purified by HPLC using 0.1% TFA in H_2O and acetonitrile graduated from 5% to 12% as eluent. The title compound was isolated after freeze-drying: MS FAB$^+$ m/z 302 $(M + H)^+$.

1-[3-[3-(3-Chlorophenyl)-2-propyn-1-yloxy]-1,2,5-thiadiazol-4-yl]-4-azatricyclo[2.2.1.0^{2,6}]heptane 4-Oxide (11). To a solution of **8d** (105 mg, 0.24 mmol) in EtOH (50 mL) and H_2O (50 mL) was added a solution of oxone (162 mg, 0.26 mmol) in H_2O (25 mL). pH was adjusted to pH 7 with 1 N NaOH until the reaction was completed after 1.5 h. The solution was acidified with 1 N HCl. By removing EtOH in vacuo the product precipitated and was isolated by filtration to give 60 mg (70%) of the title compound: MS FAB$^+$ m/z 360 $(M + H)^+$.

3-[3-(3-Phenyl-2-propyn-1-yloxy)-1,2,5-thiadiazol-4-yl]-1,2,5,6-tetrahydro-1-methylpyridine (12). Under a nitrogen atmosphere, sodium hydride (80%) (300 mg, 10.0 mmol) was added to an ice-cooled solution of 3-phenyl-2-propyn-1-ol (1.32 g, 10.0 mmol) in THF (30 mL). After the mixture stirred 1 h at room temperature, a solution of 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine[7] (980 mg, 5.0 mmol) in THF (15 mL) was added. The reaction mixture was stirred at 50 °C for 2 h and evaporated. The residue was dissolved in H_2O (50 mL) and extracted with Et_2O (3 × 100 mL). The combined organic phases were dried and evaporated to give crude 3-[3-(3-phenyl-2-propyn-1-yloxy)-1,2,5-thiadiazol-4-yl]pyridine. The product was redissolved in acetone (20 mL) and treated with methyl iodide (1 mL, 15.0 mmol). The reaction mixture was stirred at room temperature for 18 h and evaporated. The residue was dissolved in MeOH (40 mL), and sodium borohydride (570 mg, 15.0 mmol) was added under a nitrogen atmosphere at 0 °C. The reaction mixture was stirred for 15 min and evaporated. The residue was dissolved in H_2O (40 mL) and the product extracted with Et_2O (3 × 200 mL). The dried Et_2O phases were evaporated, and the residue was purified by column chromatography on silica gel using EtOAc/MeOH (4:1) as eluent. The product was crystallized as the oxalate salt from acetone to give 930 mg (60% overall yield) of the title compound: mp 150−151 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.62 (m, 2 H), 2.82 (s, 3 H), 3.1 (m, 2 H), 4.00 (s, 2 H), 5.44 (s, 2 H), 7.14 (m, 1 H), 7.45 (m, 5 H). Anal. ($C_{19}H_{19}N_3O_5S$·$0.5H_2O$).

Radioligand Binding Assays. Fresh or frozen rat brain tissue (cortex) was homogenized in assay buffer (20 mM Hepes, pH 7.4) and centrifuged for 10 min at 40000*g*. Pellets were reconstituted in assay buffer, and an appropriate amount of tissue sample was mixed in tubes with [^{3}H]oxotremorine-M (Oxo-M; NEN, NET-671; final concentration 1 nM) and test drug. The tubes were incubated at room temperature for 60 min. Unbound ligand was separated from bound ligand by vacuum filtration through GF/C filters presoaked in 0.5% poly(ethylenimine). Filters were washed once with 10 mL of wash buffer and transferred to vials; 4 mL of scintillation fluid was added, and the radioactivity was measured by scintillation counting. Nonspecific binding was measured with 10 μM arecoline.

Stimulation of Phosphoinositol Hydrolysis in A9L-M_1 Cells. A mouse fibroblast cell line (A9L) stably expressing the M_1 receptor subtype was obtained from Mark Brann (University of Vermont). A9L cells were incubated at 37 °C in a humidified atmosphere (5% CO_2) as a monolayer culture in DMEM supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), 100 units/mL each of penicillin G and streptomycin, and 4 mM glutamine (M.A. Bioproducts, Walkersville, MD). A9L cells were grown to confluence in the presence of 1.3 μCi/mL [^{3}H]inositol for 48 h prior to assaying. On the day of inositol phosphate determination, cells were detached by rinsing with Ca^{2+}-free Dulbecco's phosphate-buffered saline and then incubated for 5 min in 0.25% trypsin. The detached cells were collected by centrifugation (300*g* for 2 min) and resuspended in oxygenated HEPES/LiCl buffer (Hepes 30 mM, NaCl 142 mM, KCl 5.6 mM, $CaCl_2$ 2.2 mM, $NaHCO_3$ 1.8 mM, $MgCl_2$ 1.0 mM, glucose 5.6 mM, and LiCl 10 mM, pH 7.4 at 37 °C). Cells (0.5−1.0 mg of protein) were incubated at 37 °C for 45 min in the presence of 100 μM carbachol or increasing concentrations of drugs. The total water-soluble inositol phosphate fraction was separated from [^{3}H]inositol by ion-

exchange chromatography as previously described.[20] [^{3}H]IP was eluded directly into scintillation vials for counting with 4 mL of 0.1 ammonium formate/0.01 mM formic acid/5 mM sodium borate. Data are expressed as the percent of total [^{3}H]-IP stimulated in the presence of 100 μM carbachol stimulation, approximately 8000 dpm. All drugs were dissolved in distilled water and added to the incubation mixture in a 50-μL aliquot. Basal inositol phosphate hydrolysis was the amount isolated in the presence of 50 μL of distilled water, between 600 and 800 dpm. EC_{50} values for dose−response curves were determined using a four-parameter logistic model (GraphPAD, California). Affinity constants were determined using linear regression.

Measurements of cAMP Formation in Chinese Hamster Ovary (CHO) Cells Expressing the Human Muscarinic M_2 Receptor. Plasmids containing the human M_2 receptor were transfected into CHO cells which were cultured in 150-mm Petri dishes with DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mg/mL streptomycin, 0.1 mU/mL penicillin, 1 μM atropine, and 1 μM methotrexate in an incubator at 37 °C (95% air, 5% CO_2). One day before the experiment, cells were washed twice with 20 mL of growth media without atropine and left in this medium overnight. On the day of the assay, cells were washed twice with phosphate-buffered saline, pH 7.4, and incubated in phosphate-buffered saline + 1 mM EDTA, pH 7.4, for 5 min at 37 °C. The detached cells were transferred to 50-mL vials and washed twice with assay buffer (composition: DMEM + 20 mM HEPES (pH 7.4) + 0.5 mM 3-isobutyl-1-methylxanthine) by centrifugation (1000*g*, 5 min). Cells were resuspended in ice-cold assay buffer and preincubated for 30 min at 0 °C. Test substances, buffer, and cells were added to test tubes in a volume of 450 μL, and the incubation was started by adding 50 μL of forskolin (10 μM final concentration). After the tubes were incubated for 10 min at 37 °C, ice-cold ethanol (1 mL/tube, 96%) was added and the cells were transferred to an ice bath. Samples were centrifuged (4500 rpm, 4 °C, 15 min), and the supernatant was transferred to fresh tubes. The ethanol was evaporated under a stream of nitrogen at 70 °C, and pellets were dissolved for measurements of cAMP using a radioimmunoassay kit (Amersham, U.K.). EC_{50} values were calculated from dose−response curves (5 points minimum) by a nonlinear regression analysis using the GraphPad Prism program (GraphPad Software).

Bioavailability. 8f was screened for oral bioavailability in rats. Following intravenous and oral administration of **8f**, blood samples were taken and plasma was separated. **8f** was extracted from plasma using solid-phase extraction (SPEC PLUS, C8/SCX) and quantified by HPLC with UV detection (282 nm). The stationary phase was a C18, 250 × 4.5-mm column (Symmetry, Waters) and the mobile phase acetonitrile: 0.1 M H_3PO_4 buffer, pH 3:TFA (40:60:0.1, v/v/v).

References

(1) Mash, D. C.; Flynn, D. D.; Potter, L. T. Loss of M2 Muscarinic Receptors in the Cerebral Cortex in Alzheimer's Disease and Experimental Cholinergic Denervation. *Science* **1985**, *228*, 1115−1117.

(2) Bodick, N. C.; Offen, W.; Levey, A. I.; Cutler, N. R.; Gauthier, S. G.; Satlin, A.; Shannon, H. E.; Tollefson, G. D.; Rasmussen, K.; Bymaster, F. P.; Hurley, D. J.; Potter, W. Z.; Paul, S. M. Effects of Xanomeline, a Selective Muscarinic Receptor Agonist, on Cognitive Function and Behavioral Symptoms in Alzheimer Disease. *Arch. Neurol.* **1997**, *54*, 465−473.

(3) Bymaster, F. P.; Whitesitt, C. A.; Shannon, H. E.; DeLapp, N.; Ward, J. S.; Calligaro, D. O.; Shipley, L. A.; Buelke-Sam, J. L.; Bodick, N. C.; Farder, L.; Sheardown, M. J.; Olesen, P. H.; Hansen, K. T.; Suzdak, P. D.; Swedberg, M. D. B.; Sauerberg, P.; Mitch, C. H. Xanomeline: A Selective Muscarinic Agonist for the Treatment of Alzheimer's Disease. *Drug Dev. Res.* **1997**, *40*, 158−170.

(4) Vanggard Andersen, J.; Hansen, K. T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarinic M1 receptor agonist Xanomeline. *Xenobiotica* **1997**, *27* (9), 901−912.

(5) DeLong, A. F.; Bonate, P. L.; Gillespie, D. P.; Henry, D. P.; Satterwhite, J. H. Absorption, Distribution, Metabolism, and Elimination of Radiolabled Xanomeline in Healthy Male Subjects. Presented at the Alzheimer's and Parkinson's Disease Recent Developments Third International Conference, Chicago, IL, Nov 1−6, 1993.

(6) Gillespie, T. A.; Goodwin, R. M.; DeLong, A.; Swanson, S. Electrospray MS and MS/MS for Metabolite Identification in various Animal and Human Biotransformation Studies. Presented at the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, May 30−June 4, 1993.

(7) Sauerberg, P.; Olesen, P. H.; Nielsen, S.; Treppendahl, S.; Sheardown, M. J.; Honoré, T.; Mitch, C. H.; Ward, J. S.; Pike, A. J.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E. Novel Functional M1 Selective Muscarinic Agonists. Synthesis and Structure−Activity Relationships of 3-(1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines. *J. Med. Chem.* **1992**, *35*, 2274−2283.

(8) Ward, J. S.; Merritt, L.; Klimkowski, V. J.; Lamb, M. L.; Mitch, C. H.; Bymaster, F. P.; Sawyer, B.; Shannon, H. E.; Olesen, P. H.; Honore, T.; Sheardown, M. J.; Sauerberg, P. Novel Functional M1 Selective Muscarinic Agonists. 2. Synthesis and Structure−Activity Relationships of 3-Pyrazinyl-1,2,5,6-tetrahydro-1-methylpyridines. Construction of a Molecular Model for the M1 Pharmacophore. *J. Med. Chem.* **1992**, *35*, 4011−4019.

(9) Sauerberg, P.; Olesen, P. H.; Suzdak, P. D.; Sheardown, M. J.; Mitch, C. H.; Quimby, S. J.; Ward, J. S.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E. Synthesis And Structure−Activity Relationships Of Heterocyclic Analogues Of The Functional M1 Selective Muscarinic Agonist Hexyloxy-TZTP. *Bioorg. Med. Chem Lett.* **1992**, *2* (8), 809−814.

(10) Quimby, S. J.; Shannon, H. E.; Bymaster, F. P.; Sauerberg, P.; Olesen, P. H.; Sheardown, M. J.; Suzdak, P. D.; Mitch, C. H. Synthesis And Structure Activity Relationships Of Alkyl Substituted Analogues Of The Functional M1 Selective Muscarinic Receptor Agonist Xanomeline. *Bioorg. Med. Chem. Lett.* **1994**, *4* (18), 2205−2210.

(11) Ward, J. S.; Merritt, L.; Calligaro, D. O.; Bymaster, F. P.; Shannon, H. E.; Sawyer, B. D.; Mitch, C. H.; Deeter, J. B.; Peters, S. C.; Sheardown, M. J.; Olesen, P. H.; Swedberg, M. D. B.; Sauerberg, P. Functionally Selective M1 Muscarinic Agonists: 3. Side Chains and Azacycles Contributing to Functional Muscarinic Selectivity among Pyrazinylazacycles. *J. Med. Chem.* **1995**, *38* (18), 3469−3481.

(12) Sauerberg, P.; Jeppesen, L.; Olesen, P. H.; Sheardown, M. J.; Fink-Jensen, A.; Rasmussen, T.; Shannon, H. E.; Bymaster, F. P.; DeLapp, N. W.; Calligaro, D. O.; Ward, J. S.; Whitesitt, C. A.; Thomsen, C. Identification of Side Chains on 1,2,5-Thiadiazole-azacycles Optimal for Muscarinic M1 Receptor Activation. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2897−2902.

(13) Jaen, J.; Barrett, S.; Brann, M.; Callahan, M.; Davis, R.; Doyle, P.; Eubanks, D.; Lauffer, D.; Lauffer, L.; Lipinski, W.; Moreland, D.; Nelson, C.; Raby, C.; Schwarz, R.; Spencer, C.; Tecle, H. In vitro and in Vivo Evaluation of the Subtype-Selective Muscarinic Agonist PD 151832. *Life Sci.* **1995**, *56* (11/12), 845−852.

(14) Macleod, A. M.; Herbert, R.; Hoogsteen, K. WO 92/11261.

(15) Olesen, P. O.; Sauerberg, P.; Petersen, T. G.; Treppendahl, S.; Bentzen, B.; Deeter, J.; Ward, J. S.; Mitch, C. H.; Lehmann, S. V. Synthesis and Structural Determination of Stereoisomeres of Muscarinic Ligands of the (3-Propylthio-1,2,5-Thiadiazol-4-yl)-1-Azabicycloalkane Type. *Chirality* **1997**, *9* (8), 739−749.

(16) Jenkins, S. M.; Wadsworth, H. J.; Bromidge, S.; Orlek, B. S.; Wyman, P. A.; Riley, G. J.; Hawkins, J. Substituent variation in azabicyclic triazole- and tetrazole-based muscarinic receptor ligands. *J. Med. Chem.* **1992**, *35*, 2392−2406.

(17) MacLeod, A. M.; Herbert, R.; Hoogsteen, K. Facile Synthesis of the 4-Azatricyclo[2.2.1.0^{2,6}]heptane System. *J. Chem Soc., Chem. Commun.* **1990**, 100−102.

(18) Bymaster, F. P.; Carter, P. A.; Peters, S. C.; Zhang, W.; Ward, J. S.; Mitch, C. H.; Calligaro, D. O.; Whitesitt, C. A.; DeLapp, N.; Shannon, H. E.; Rimvall, K.; Jeppesen, L.; Sheardown, M. J.; Fink-Jensen, A.; Sauerberg, P. Xanomeline compared to other muscarinic agent on stimulation of phosphoinositide hydrolysis in vivo and other cholinomimetic effects. *Brain Res.* **1998**, 179−190.

(19) Shannon, H. E.; Bymaster, F. P.; Calligaro, D. O.; Greenwood, B.; Mitch, C. H.; Sawyer, B. D, Ward, J. S.; Wong, D. T.; Olesen, P. H.; Sheardown, M. J.; Swedberg, M. D. B.; Suzdak, P. D.; Sauerberg, P. Xanomeline: A Novel Muscarinic Receptor Agonist with Functional Selectivity for M1 Receptors. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 271−281.

(20) Schoepp, D. D.; Johnson, B. G. Selective inhibition of excitatory amino acid-stimulated phosphoinositide hydrolysis in the rat hippocampus by activation of protein kinase C. *Biochem. Pharmacol.* **1988**, 4299−4305.

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