Muscarinic Agonists with Antipsychotic-like Activity: Structure−Activity Relationships of 1,2,5-Thiadiazole Analogues with Functional Dopamine Antagonist Activity

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Muscarinic agonists were tested in two models indicative of clinical antipsychotic activity: conditioned avoidance responding (CAR) in rats and inhibition of apomorphine-induced climbing in mice. The standard muscarinic agonists oxotremorine and pilocarpine were both active in these tests but showed little separation between efficacy and cholinergic side effects. Structure−activity relationships of the alkylthio-1,2,5-thiadiazole azacyclic type muscarinic partial agonists are shown, revealing the *exo*-6-(3-propyl/butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane analogues (**4a**,**b** and **9a**,**b**) to be the most potent antipsychotic agents with large separation between efficacy and cholinergic side effects. The lack of enantiomeric selectivity suggests the pharmacophoric elements are in the mirror plane of the compounds. A model explaining the potency differences of closely related compounds is offered. The data suggest that muscarinic agonists act as functional dopamine antagonists and that they could become a novel treatment of psychotic patients.

Introduction

The antipsychotic daily dose of clinically effective neuroleptics correlates with the affinity for dopamine D2 receptors.[1] It is believed that excess dopamine released in the mesolimbic and mesocortical dopamine neural circuits is blocked by dopamine antagonists and thereby reduces the hallucination (positive) symptoms of schizophrenia. Typical antipsychotic neuroleptics, like haloperidol, block not only the mesolimbic and mesocortical pathways but also the nigrostrial dopamine pathway which causes extrapyramidal side effects (EPS). The depression-like (negative) symptoms of schizophrenia, which may result from prefrontal dopaminergic hypofunction, are poorly managed by typical neuroleptics. The atypical antipsychotic clozapine is highly efficacious in treatment-resistant schizophrenics, produces fewer EPS side effects,[2] and has beneficial effects on negative symptoms.[3] New atypical antipsychotics have been introduced, which seem to be active against both positive and negative symptoms with significantly fewer EPS.[4] Despite this improvement in treatment, there is still a need for more effective antipsychotic drugs with earlier onset of action and without the side effects associated with dopamine receptor blockade, e.g., weight gain.

Muscarinic and dopaminergic receptors are to a high degree colocalized in the brain, and the muscarinic system directly or indirectly modulates dopamine pathways.[5,6] The cholinergic system has also been suggested to be involved in the pathophysiology of schizophrenia, although most attention has been on the beneficial effects of muscarinic antagonists in the treatment of EPS induced by neuroleptics.[7,8] An early study with arecoline, a selective muscarinic agonist, in schizophrenic patients indicated antipsychotic-like effects, but the cholinergic side effects precluded the therapeutic use of arecoline.[9] However, recent clinical data with xanomeline, a functional M_1,M_4-selective muscarinic receptor agonist, showed dramatically reduced psychotic symptoms in Alzheimer's patients.[10]

These findings spurred us to investigate our large library of selective muscarinic partial agonists for antipsychotic activity. Conditioned avoidance responding (CAR) in rats was used as the primary nonchemically induced antipsychotic model. All drugs with antipsychotic activity in humans are active in the CAR model.[11] Apomorphine-induced climbing in mice was further used as a test for functional dopamine antagonist activity. The rank order of potencies of known antipsychotic agents in inhibiting apomorphine climbing has been shown to correlated with the clinical potency of antipsychotics.[12]

The present paper describes the structure−activity relationships (SAR) of a series of 1,2,5-thiadiazole azacyclic muscarinic agonists leading to the identification of potent muscarinic antipsychotics with only a few cholinergic side effects. A receptor model to explain the SAR and lack of enantiomeric selectivity is also presented.

Chemistry

The synthetic routes for the compounds have been described previously. Briefly, **1** was made starting from 3-pyridinecarbaldehyde and potassium cyanide in aque-

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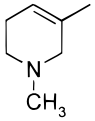
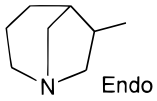
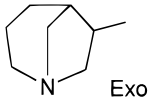
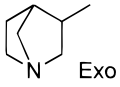
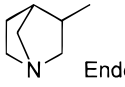
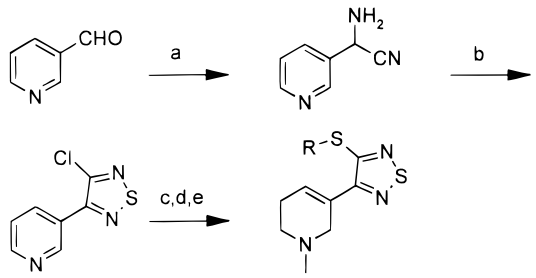
Table 1.

No	Aza	receptor binding to rat brain membranes [^{3}H]-Oxo-M IC_{50}, nM (range)[a]	conditioned avoidance responding in rats %AR/%RF (mg/kg s.c.)[b]	conditioned avoidance responding in rats ED_{50}, mg/kg s.c. ±SEM
1		1.3 (0.6-2.3)	57/0 (3)	2.8 ± 0.9
2		2.2 (2.0-2.4)	18/0 (3)	0.10 ± 0.01
3		6.1 (5.1-7.9)	97/0 (3)	>3
4		0.47 (0.36-0.60)	32/0 (1)	0.007 ± 0.011
5		1.3 (1.0-1.8)	0/57 (1)	<1
6		4.2 (4.1-4.3)	100/0 (1)	>1

[a] All values are the geometric means of results from 3−4 separate experiments using 4−6 concentrations performed in triplicate. [b] Percent avoidance responding (%AR) and percent response failures (%RF) at screening dose.

Scheme 1[a]



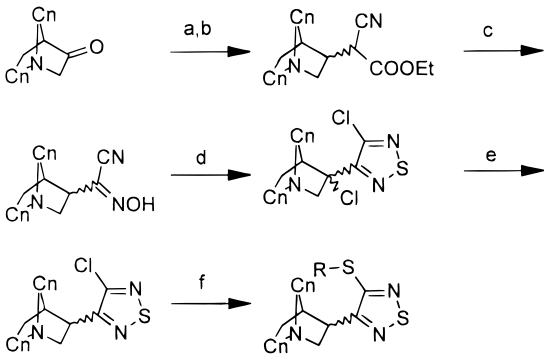
1

[a] Reagents: (a) KCN, NH_4Cl, NH_3 (aq); (b) S_2Cl_2, DMF; (c) NaSH, *n*-BuBr, DMF; (d) MeI, acetone; (e) $NaBH_4$, EtOH.

ous ammonium chloride giving the α-amino-α-(3-pyridyl)acetonitrile (Scheme 1). Cyclization of the aminonitrile with sulfur monochloride gave 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine, which was reacted with sodium hydrosulfide followed by 1-butyl bromide alkylation to give 3-(3-butylthio-1,2,5-thiadiazol-4-yl)pyridine. Quaternization with methyl iodide and sodium borohydride reduction gave **1**.[13]

Compounds **2**−**12b**[14,15] were made starting from azabicyclic ketone, either racemic or enantiomerically pure (Scheme 2). Condensation of the ketone with ethyl cyanoacetate, followed by first hydrogenation over palladium on carbon and then by nitrosation with isoamyl nitrite, gave the α-oximidonitriles. Sulfur monochloride cyclization gave the dichloro intermediate, which after hydrogenation over palladium on carbon gave the

Scheme 2[a]



2-12

[a] Reagents: (a) ethyl cyanoacetate, TEA; (b) 5% Pd/C, H_2, EtOH; (c) NaOEt, isoamyl nitrite, EtOH/MeOH; (d) S_2Cl_2, DMF; (e) 5% Pd/C, H_2, EtOH; (f) NaSH, alkyl halogenide, K_2CO_3, DMF.

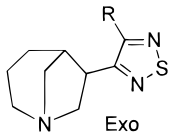
3-chloro-1,2,5-thiadiazole intermediate. Reaction with sodium hydrosulfide and alkyl bromide, as above, gave the desired products.

Biological Evaluation

Receptor binding to rat brain homogenates using the muscarinic agonist oxotremorine-M ([^{3}H]-Oxo-M) as a ligand was used as a measure of the compounds' affinity for the agonist binding site at M_2 and M_4 muscarinic receptor subtypes.[16,17]

Conditioned avoidance responding in rats was used as the primary efficacy measure of antipsychotic-like

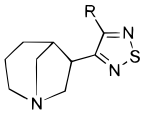
Table 2.



No	R	receptor binding to rat brain membranes [^{3}H]-Oxo-M IC_{50}, nM (range)[a]	apomorphine induced climbing in mice ED_{50}, mg/kg s.c. (95% conf.)[c]	conditioned avoidance responding in rats %AR/%RF (mg/kg s.c.)[b]	conditioned avoidance responding in rats ED_{50}, mg/kg s.c. ±SEM
7	S-Me	2.9 (2.2-4-0)	0.20[d]	34/3 (1)	0.14 ± 0.02
8	S-Et	1.3 (1.0-1.7)	0.047 (0.005-0.46)	46/30 (1)	0.045[d]
9	S-nPr	1.4 (0.9-2.0)	0.056 (0.0003-9.0)	41/0 (1)	0.056[d]
4	S-nBu	0.47 (0.36-0.60)	0.045 (0.027-0.076)	32/0 (1)	0.007 ± 0.011
10	S-nPen	2.5 (1.8-3.2)	0.054 (0.003-0.97)	3/41 (0.3)	0.065 ± 0.01
11	S-nHex	3.3 (2.4-4.0)	0.060 (0.002-1.5)	29/19 (0.3)	0.10 ± 0.01

[a,b] See corresponding footnotes to Table 1. [c] 95% confidence limits. [d] Flat dose−response curve.

Table 3.



No	Aza	R	receptor binding to rat brain membranes [^{3}H]-Oxo-M IC_{50}, nM (range)[a]	mice, ED_{50}, mg/kg, s.c. apo. climb. (95%)[c]	saliva-tion	tremor	conditioned avoidance responding in rats %AR/%RF (mg/kg, s.c.)[b]	conditioned avoidance responding in rats ED_{50}, mg/kg, s.c. ± SEM
12a	endo (S,R)	S-nPr	3.6 (2.4-5.2)	2.21 (0.88-5.5)	>30	>30	86/0 (3)	>3[d]
12b	endo (R,S)	S-nPr	13.3 (12-15)	2.2 (0.23-21)	>30	>30	51/0 (3)	≥3[d]
3a	endo (S,R)	S-nBu	2.3 (0.4-5.6)	>1[d]	>1	>1	48/20 (3)	≥3[d]
3b	endo (R,S)	S-nBu	9.9 (2.2-20)	nt	nt	nt	86/0 (3)	>3[d]
9a	exo (R,R)	S-nPr	0.64 (0.38-0.84)	0.021 (0.001-0.3)	>3	2.2	31/0 (3)	0.013 ± 0.002
9b	exo (S,S)	S-nPr	0.65 (0.42-0.84)	0.17 (0.03-0.89)	>3	1.3	39/0 (0.3)	0.03 ±0.001
4a	exo (S,S)	S-nBu	0.53 0.2-0.86)	0.026 (0.004-0.1)	>3	>3	8/0 (1)	0.018 ±0.004
4b	exo (R,R)	S-nBu	0.50 (0.20-0.78)	0.012 (0.009-0.01)	>0.3	>0.3	16/1 (0.3)	0.017 ±0.004
Oxotremorine			5.0 (3.9-6.0)	0.09 (0.001-5.0)	0.2	0.24	24/63 (0.3)	0.30 ±0.001
Pilocarpine			67 (62-72	3.87 (0.65-22)	4	>10	18/21 (10)	4.5 ±0.4
Haloperidol			nt	0.08 (0.02-0.26)	nt	nt	9/55 (0.3)	0.11 ±0.01

[a,b] See corresponding footnotes to Table 1. [c] See corresponding footnote to Table 2. [d] Tested at one dose. nt, not tested.

activity.[11] The clinically active dose of effective neuroleptics correlates with the ED_{50} for inhibiting avoidance responding in this model. Response failures, recorded in the same model, reflect motor impairment produced by the compounds. Compounds were screened at one dose sc (0.3−3.0 mg/kg), and percent avoidance responding (%AR) and response failures (%RF) were recorded. Active compounds were tested for full dose−response activity after sc administration.

Inhibition of apomorphine-induced climbing in mice was used to characterize the functional dopamine antagonist activity of the compounds. All clinically effective neuroleptics are dopamine antagonists, and their clinical potency correlates with their potency to

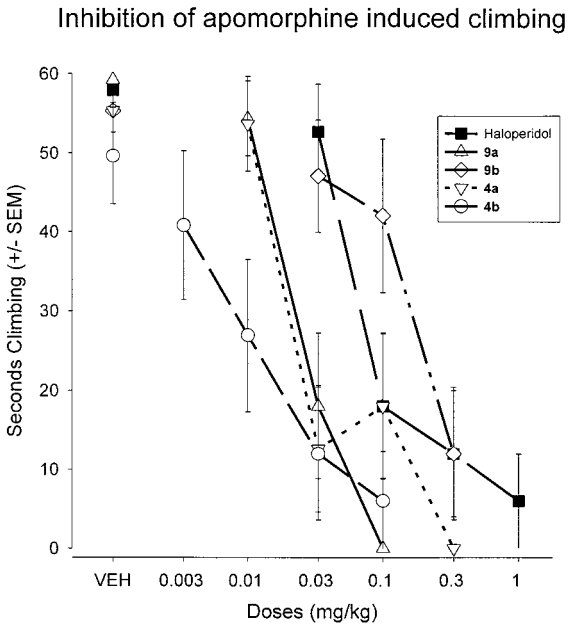


Figure 1. Dose−response curves of the two pairs of enantiomers, **4a**,**b** and **9a**,**b**, compared to haloperidol in apomorphine-induced climbing in mice. Data are expressed as the mean ± SEM of number of seconds the mice were climbing during the 60-s trial ($n = 10$).

inhibit apomorphine-induced climbing. In the same test, the parasympatomimetic effects, salivation and tremor, were recorded to measure separation between desired effect and side effects.

Results and Discussion

A large number of muscarinic agonists, represented by the butylthio-1,2,5-thiadiazole analogues **1**−**6** (Table 1), were screened for CAR activity. Although all the listed analogues, **1**−**6**, had nearly equally high affinity (IC_{50}: 0.47−6.1 nM) for inhibiting Oxo-M binding, the potency for inhibiting CAR varied substantially. The two endo analogues, **3** and **6**, were both inactive at the screening dose (3 and 1 mg/kg sc, respectively) and were therefore not investigated further. On the remaining analogues full dose−response curves were generated revealing a more than 100-fold range in potency. The tetrahydropyridine analogue **1** was the least potent, 20 times less potent than the quinuclidine derivative **2**. The [3.2.1] exo (**4**) and [2.2.1] exo (**5**) analogues were both very potent compounds, with **4** having an ED_{50} of 0.015 mg/kg sc. The pronounced response failures (RF) (57%) of **5** compared to the lack of RF with **4** at the screening dose (1 mg/kg sc) suggested that **4** would be a better drug candidate.

To find the optimal [3.2.1] exo analogue, the C_{1-6} alkylthio derivatives **7**−**11** were synthesized and tested (Table 2). Again the Oxo-M binding affinity for the series was nearly independent of the length of the side chain. The potency in CAR was, however, more sensitive to the size of the alkylthio side chain. Increasing the side chain from methylthio to butylthio increased the potency in inhibiting CAR, whereas further elongation of the side chain to pentylthio and hexylthio decreased the potency. Characteristic for the series was the low level of response failures (%RF) observed, especially compared both to the standard muscarinic agonists, oxotremorine and pilocarpine, and to the D2 antagonist, haloperidol (Table 3). All the C_{1-6} alkylthio analogues behaved as very potent functional dopamine antagonists in the apomorphine-induced climbing model (Table 2). Both the potencies and the SARs in CAR and inhibition of apomorphine climbing correlated very well (Tables 2 and 3), further suggesting that muscarinic agonists behave as functional dopamine antagonists.

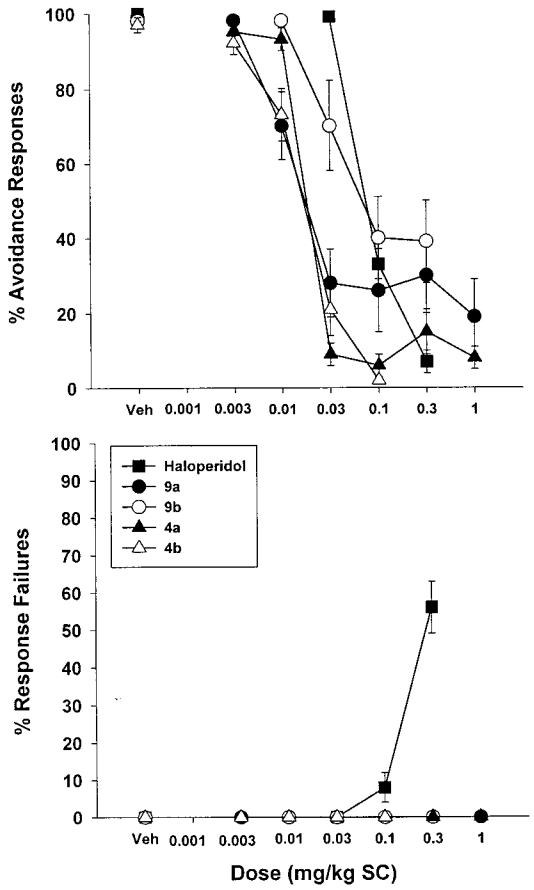


Figure 2. Dose−response curves of the two pairs of enantiomers, **4a**,**b** and **9a**,**b**, compared to haloperidol in conditioned avoidance responding in rats: upper panel, percent avoidance responses; lower panel, percent response failures. Each point represents the mean ± SEM of percentage of responses for six rats.

The compounds **2**−**11** used for optimization were all racemic mixtures, and results could therefore be misleading. To investigate the enantiomeric activity, the four isomers of the interesting propylthio and butylthio analogues **9** and **4** were synthesized and tested. The four exo enantiomers **9a**,**b** and **4a**,**b** were all very potent in the two antipsychotic models, CAR and inhibition of apomorphine-induced climbing (Table 3, Figures 1 and 2). Moreover, the side effect profiles (salivation, tremor, and response failures) in both mice and rats were improved for these [3.2.1] exo analogues, compared to the standard compounds, oxotremorine, pilocarpine, and haloperidol. Interestingly, only insignificant differences were observed between the enantiomers in CAR and in inhibition of apomorphine-induced climbing (**9a** vs **9b**, **4a** vs **4b**, **12a** vs **12b**, and **3a** vs **3b**, Table 3).

We have earlier suggested that five pharmacophoric groups in the 1,2,5-thiadiazole analogues were involved with muscarinic receptor activation: ionic interaction from the protonated nitrogen in the azacyclic ring; two

Figure 3. View of **4a**,**b** from a point perpendicular to the suggested common plane of the pharmacophoric groups (butylthio side chain, both nitrogens in thiadiazole ring, nitrogen in azacycle). Illustration of the space needed to be available at the receptor in order for both enantiomers to be equally active.

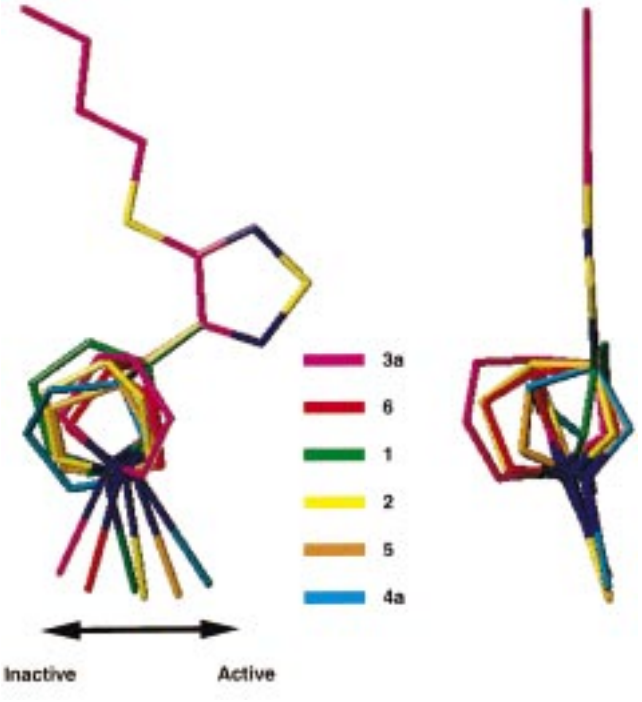


Figure 4. For all the butylthio analogues in this paper, the proposed pharmacophoric groups could be superimposed. The orientation of the azacyclic nitrogen lone pair was, however, different for the various analogues. The orientation of the extension of the lone pair correlated with the antipsychotic-like potency in conditioned avoidance responding in rats.

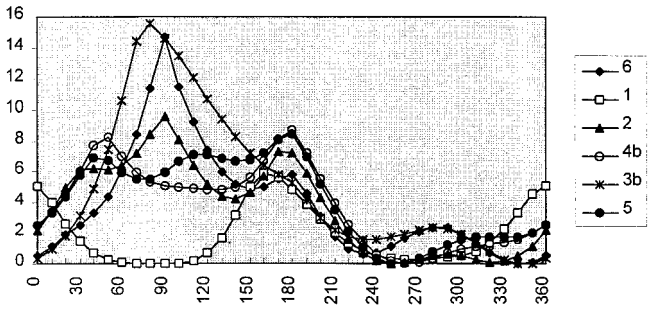


Figure 5. Conformation energies for **1**, **2**, **3b**, **4b**, **5**, and **6** when rotating around the single bond to the thiadiazole ring. The conformations close to 0° shown in Figure 4 have the lowest energies of the two possible conformations (close to 0° and close to 180°), placing the butylthio-1,2,5-thiadiazole moiety in the mirror plane.

hydrogen-bonding interactions from the nitrogen atoms in the 1,2,5-thiadiazole ring; one hydrogen-bonding interaction from the heteroatom in the alkylthio/oxy side chain; and a lipophilic interaction from the alkyl part of the side chain.[18] One explanation for the lack of enantiomeric selectivity for the [3.2.1] analogues would be if the above-mentioned pharmacophoric groups all were located in the mirror plane. If further additional space required in the vicinity of the tertiary nitrogen atom was available, then the mirror image of these molecules would be expected to provide similar biological activity (Figure 3).

Since all the 1,2,5-thiadiazole compounds mentioned in this paper could be superimposed to fit the above assumptions, this receptor model did not account for the potency differences of the butylthio compounds **1**−**6**. To address this question, we looked at the orientation of the nitrogen lone pair. If the lone pair length was increased to 2.8 Å, to indicate the possible position of the receptor functionality, and the torsion angle was adjusted to make the endpoint of the lone pair lie in the same plane as the butylthio-1,2,5-thiadiazole group, then the position of the endpoint correlated with the potency for producing antipsychotic-like activity (Figure 4). All conformations illustrated in Figure 4 (dihedral angles close to 0°) were less than 3 kcal/mol from their corresponding global energy minimum except for compound **1**, which needed more than 5 kcal/mol to adopt the required conformation (Figure 5). This additional energy might explain why the biological activity of **1** was lower than expected from Figure 4. Furthermore, the torsion angles (0°) found here were in agreement with the conformation of active constrained analogues of **1**.[19]

The very active analogues **9a**,**b** and **4a**,**b** were further characterized in other in vivo models predictive of antipsychotic-like activity: selective inhibition of A10 dopamine neurons in in vivo electrophysiology, inhibition of dopamine agonist-induced rotation in 6-OHDA-lesioned rats, inhibition of PCP-induced hypermotility, inhibition of *d*-amphetamine hyperactivity, and inhibition of methylphenidate-induced gnawing behavior. **9a**[20] and **4b** were active in all the mentioned tests without inducing catalepsy (data not shown). Receptor screening showed that the compounds were selective muscarinic ligands with no affinity for dopamine receptors. In cell lines transfected with human muscarinic receptors, **9a**

and **4b** had intrinsic activity at M_2 and M_4 receptor subtypes.[20] Further, the compounds had equally high affinity for all five muscarinic receptor subtypes using [^{3}H]NMS binding.[20]

Conclusions

We have shown that standard muscarinic agonists and muscarinic agonists of the alkylthio-1,2,5-thiadiazole azacyclic type both are active in behavioral models predictive of antipsychotic activity. SAR investigations revealed the propyl/butylthio side chain to be the optimal size for functional antipsychotic-like activity in the 1,2,5-thiadiazole azacyclic series. The azabicyclo-[3.2.1]octane ring was identified to give compounds with the most potent antipsychotic-like activity without having the undesired cholinergic side effects of salivation and tremor. These compounds, **4a**,**b**, and **9a**,**b**, were 10 times more potent than the typical antipsychotic agent haloperidol. The lack of enantiomeric selectivity of the *exo*-6-(3-propyl/butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane enantiomers suggested that the pharmacophores lie in the mirror plane of the compounds.

The described muscarinic agonists lacking the traditional cholinergic side effects have the potential of becoming a novel treatment for schizophrenia.

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 analyses were performed by Novo Nordisk Microanalytical Laboratory, Denmark, and were within ±0.4% of the calculated values.

Experimental data on preparation and physical characterization have previously been published on the following compounds: **1**;[13] **2**, **5**, **6**;[14] **4**, **4a**;[21] **9a**,**b**, **12a**,**b**.[15] The compounds below were made in the same manner:

***endo*-6-(3-Butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (3) oxalate:** ^{1}H NMR (DMSO) δ 12.0−9.0 (2H, br s), 4.1−3.85 (3H, m), 3.56−3.46 (1H, m), 3.43−3.24 (5H, m), 3.12−3.05 (1H, s), 2.04−1.84 (1H, m), 1.77−1.63 (3H, m), 1.60−1.37 (3H, m), 1.05−0.88 (4H, m); mp 123−124 °C. Anal. ($C_{15}H_{23}N_3O_4S_2$) C, H, N.

(5*S*,6*R*)-6-(3-Butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (3a) oxalate: ^{1}H NMR (DMSO) δ 10.5−8.5 (2H, br s), 4.08−3.84 (3H, m), 3.56−3.47 (1H, m), 3.43−3.23 (5H, m), 3.12−3.04 (1H, br s), 2.04−1.83 (1H, m), 1.77−1.63 (3H, m), 1.58−1.35 (3H, m), 1.05−0.86 (4H, m); mp 198−199 °C. Anal. ($C_{15}H_{23}N_3O_4S_2\cdot 0.4H_2O$) C, H, N.

(5*R*,6*S*)-6-(3-Butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (3b) hydrochloride: ^{1}H NMR (DMSO) δ 10.94 (1H, br s), 4.10−3.82 (3H, m), 3.55−3.46 (1H, m), 3.43−3.23 (5H, m), 3.11−3.05 (1H, m), 2.02−1.83 (3H, m), 1.75−1.60 (3H, m), 1.57−1.36 (3H, m), 1.04−0.88 (4H, m); mp 198−199 °C. Anal. ($C_{13}H_{22}ClN_3S_2$) C, H, N.

(5*R*,6*R*)-6-(3-Butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane (4b) tartrate: ^{1}H NMR (DMSO) δ 7.5−6.0 (4H, br s), 3.84−3.66 (2H, m), 3.63−3.56 (1H, m), 3.30 (2H, t), 3.22−3.05 (4H, m), 2.57−2.51 (1H, m), 2.13−1.94 (1H, m), 1.85−1.65 (5H, m), 1.50−1.36 (2H, m), 0.90 (3H, t); mp 140−142 °C. Anal. ($C_{17}H_{27}N_3O_6S_2$) C, H, N.

***exo*-6-(3-Methylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (7) oxalate:** ^{1}H NMR (DMSO) δ 5.0−3.0 (2H, br s), 3.96−3.80 (2H, m), 3.67−3.61 (1H, m), 3.31−3.22 (3H, m), 3.21−3.13 (1H, m), 2.74 (3H, s), 2.65−2.59 (1H, m), 2.18−2.00 (1H, m), 1.84−1.74 (3H, m); mp 144−146 °C. Anal. ($C_{12}H_{17}N_3O_4S_2$) C, H, N.

***exo*-6-(3-Ethylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (8) oxalate:** ^{1}H NMR (DMSO) δ 10−8.0 (2H, br s), 3.99−3.77 (2H, m), 3.67−3.59 (1H, m), 3.85−3.14 (6H, m), 2.63−2.57 (1H, m), 2.20−1.99 (1H, m), 1.90−1.75 (3H, m), 1.91 (3H, t); mp 159−160 °C. Anal. ($C_{13}H_{19}N_3O_4S_2$) C, H, N.

***exo*-6-(3-Pentylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (10) oxalate:** ^{1}H NMR (DMSO) δ 7.5−6.0 (2H, br s), 3.95−3.78 (2H, m), 3.68−3.49 (1H, m), 3.34−3.13 (6H, m), 2.63−2.56 (1H, m), 2.19−1.98 (1H, m), 1.90−1.67 (5H, m), 1.45−1.25 (4H, m), 0.88 (3H, t); mp 117−118 °C. Anal. ($C_{16}H_{25}N_3O_4S_2$) C, H, N.

***exo*-6-(3-Hexylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo-[3.2.1]octane (11) oxalate:** ^{1}H NMR (DMSO) δ 7.7−6.3 (2H, br s), 3.95−3.86 (2H, m), 3.68−3.49 (1H, m), 3.36−3.13 (6H, m), 2.65−2.56 (1H, m), 2.20−1.98 (1H, m), 1.90−1.65 (5H, m), 1.50−1.20 (6H, m), 0.85 (3H, t); mp 118−119 °C. Anal. ($C_{17}H_{27}N_3O_4S_2$) C, H, N.

Receptor Binding Studies. Binding to rat brain homogenates using [^{3}H]Oxo-M as ligand was performed using standard conditions as described previously.[13]

Conditioned Avoidance Responding. Male rats (Fisher-derived F344, Harlan Sprague−Dawley, Indianapolis, IN) weighing 250−300 g were trained to avoid or escape foot shock in an operant conditioning chamber. Briefly, at the start of each trial, the houselight and a tone were presented. A response within 10 s immediately terminated the trial (avoidance response). If the rat did not respond within 10 s, foot shock (2 mA) was presented. A reponse during the shock immediately terminated the trial (escape response). If the rat did not respond within 10 s after the onset of shock, the trial terminated automatically (response failure). Compounds were initially screened at a dose of 3.0 mg/kg sc, and the percent avoidance response (%AR) and the percent escape failures (%AF) were recorded. Active compounds were tested sc for full dose response. Sessions typically ended after 50 trials. Each point represents the mean ± SEM of percent of responses for six rats. The ED_{50} values were calculated as the dose producing 50% of the maximal effect produced by the drug.

Apomorphine Climbing. Groups of $n = 10$ male Bom:NMRI mice were injected with saline or a dose of drug sc, 50 min prior to the trial. Apomorphine (2 mg/kg, sc) was injected 20 min prior to the trial. Mice were placed individually in cylindrical wire cages, and the time each mouse climbed on the wire cage was automatically recorded. Data are expressed as the mean ± SEM of number of seconds the mice were climbing during the 60-s trial. ED_{50} values and 95% confidence limits were calculated using logistic nonlinear regression equations (Graph Pad Prism, Graph Pad Software Inc., San Diego, CA). Compounds were screened at 3−4 doses giving relatively large 95% confidence limits. Side effects were scored 30 min after injection of saline or a dose of drug, using three grades, and the data are expressed as the percent of the maximum possible score.[14] ED_{50} values were calculated by graphic interpolation of the dose−response curve.

Molecular Modeling. The geometry of all compounds were optimized using the AM1 Hamiltonian in SPARTAN (Wavefunction Inc., Irvine, CA 92715) and subsequently analyzed using SYBYL 6.4 (R4000, TRIPOS Inc., St. Louis, MO 63144). Torsional scans were performed from 0 to 360 in 10 intervals using the MMFF force field in MacroModel (W. Clark Still, Department of Chemistry, Columbia University, New York, NY 10027). At each point in the scan, all coordinates were refined while the dihedral angle being investigated was constrained. An additional harmonic potential with a force constant of 1000 kJ/mol × (deg)^2 was added to maintain the dihedral angle during the force field minimization. The SCF energy convergence criterion for the Spartan calculations was set to 1.0D-6. Default parameters were used for geometry optimization.

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