partment. Completion of the electro-reduction was marked by the complete disappearance of the purple color of **2**. An aliquot of the reduced solution was then mixed with DMPO to give a final concentration of 10–40 mM of the spin-trapping agent. These solutions were continuously purged with N_2 gas. A sample of the resulting solution was then transferred to an ESR flat cell using a syringe. Both the syringe and flat cell were thoroughly purged with N_2 before use. After the flat cell was placed in the cavity of the ESR spectrometer, the solution was exposed to the air and rapidly turned purple. Control solutions were prepared in exactly the same fashion except that **1** or **2**, as appropriate, was not present. None of the control solutions gave any ESR spectrum.

Isolation and Characterization of 3-(2-Aminoethyl)-6-[3-(2-aminoethyl)-1,7-dihydro-5-hydroxy-7-oxo-6*H*-indol-6-ylidene]-1*H*-indole-5,7(4*H*,6*H*)-dione (4). The most convenient method to prepare sufficient quantities of **4** was to oxidize **1** (1.5 mM in 60 mL of phosphate buffer, pH 7.4, $\mu = 0.1$) in the presence of peroxidase (0.08 mg/mL) and H_2O_2 (0.5 mM) at ambient temperature for ca. 5 h. Repetitive 10-mL injections of the resulting product solution using HPLC method III were employed to separate **4** (t_R = 15.2 min) from the other products (primarily **2**, **3**, **5**, and **6**). The eluent containing **4** was immediately collected in a flask maintained at −80 °C (dry ice bath). The combined solutions containing **4** were freeze-dried to give an orange solid. In pH 7.4 phosphate buffer the bright yellow solution of **4** exhibited a characteristic spectrum with λ_{max}, nm (log ϵ_{max}): 378 (4.08), 300 (3.98), 232 sh (4.07). FAB-MS (thioglycerol matrix) showed m/e = 381 (MH^+, 100%). Accurate mass measurements on MH^+ gave m/e = 381.1550 ($C_{20}H_{21}N_4O_4$; calcd m/e = 381.1563). ^1H NMR (Me_2SO-d_6): δ 12.60 (d, $J_{1,2}$ = 2.1 Hz, 1 H, N(1)-H), 12.12 (d, $J_{1',2'}$ = 2.1 Hz, 1 H, N(1')-H), 8.10 (br s, ca. 4 H, NH_3^+ and OH), 7.83 (br s, ca. 3 H, NH_3^+), 7.34 (d, $J_{1,2}$ = 2.7 Hz, 1 H, C(2)-H), 7.14 (d, $J_{1',2'}$ = 2.1 Hz, 1 H, C(2')-H), 5.67 (s, 1 H, C(4)-H), 2.95 (m, 4 H, CH_2CH_2), 2.65 (m, 4 H, CH_2CH_2), 2.53 (s, 2 H, C(4')-H). Addition of D_2O caused the resonances at δ 12.6, 12.12, 8.10, and 7.83 to disappear and the doublets at δ 7.34 (C(2)-H) and 7.14 (C(2')-H) to collapse into singlets. ^{13}C NMR using the attached proton test (APT) experiment showed positive resonances (carbons with even or zero attached protons) at δ 183.40 (C(5')=0), 176.9 (C(7')=0), 172.7 (C(7)=0), 139.2 (C-5), 129.7, 129.2, 125.2, 119.3, 117.6, 117.4, 116.9, 114.9, 49.8 (C-4'), and 24.6, and negative resonances (carbons with an odd number of attached protons) at δ 126.9 (C-2), 123.7 (C-2'), and 97.8 (C-4).

Spectroscopic evidence in support of the structures of **2** and **3** has been presented elsewhere.[10,11]

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Registry No. 1, 31363-74-3; 2, 100513-78-8; 3, 124620-49-1; 4, 141272-05-1; catalase, 9001-05-2; superoxide dismutase, 9054-89-1; cerulieplasmin, 9031-37-2; tyresinase, 9002-10-2; peroxidase, 9003-99-0.

Novel Functional M_1 Selective Muscarinic Agonists. Synthesis and Structure–Activity Relationships of 3-(1,2,5-Thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines

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A series of novel 3-(3-substituted-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines (substituted-TZTP; **5a–l**, **7a–h**, **8**, **9c–n**, **11**, **13j**) were synthesized and tested for central muscarinic cholinergic receptor affinity by using [^{3}H]-oxotremorine-M (Oxo-M) and [^{3}H]-pirenzepine (Pz) as ligands. The potency and efficacy of the compounds for the pharmacological defined M_1 and M_2 muscarinic receptors were determined on isolated electrically stimulated rabbit vas deferens and on spontaneously beating isolated guinea pig atria, respectively. Selected compounds were also tested for M_3 activity in the isolated guinea pig ileum. The C_{1-8} alkoxy-TZTP **5a–l** analogues all displaced [^{3}H]-Oxo-M and [^{3}H]-Pz with low nanomolar affinity. Depicting chain length against Oxo-M binding and against Pz binding the unbranched C_{1-8} alkoxy-TZTP (**5a–h**) derivatives produced U-shaped curves with butoxy- (**5d**) and (pentyloxy)-TZTP (**5e**) as the optimum chain length, respectively. This U-shaped curve was also seen in the ability of the compounds **5a–h** to inhibit the twitch height in the vas deferens preparation. The (pentyloxy)- (**5e**) and the (hexyloxy)-TZTP (**5f**) analogues produced an over 90% inhibition of the twitch height with IC_{50} values in the low picomolar range. In both the atria and in the ileum preparations **5f** had low efficacy and potency. With the (alkylthio)-TZTP (**7a–h**) analogues the structure–activity relationship was similar to the one observed with the alkoxy (**5a–h**) analogues, but generally **7a–h** had higher receptor affinity and was more potent than the corresponding **5a–h**. However, the C_{3-8} alkyl-TZTP (**9c,e,g,h**) analogues had 10–100 times lower affinity for the central muscarinic receptors than the corresponding alkoxy and alkylthio derivatives, and their efficacy in the vas deferens preparation was too low to obtain IC_{50} values. The unsubstituted TZTP (**11**) compound was a potent but nonselective muscarinic agonist. The two 3-(3-butoxy/(hexyloxy)-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines (butoxy/(hexyloxy)-OZTP; **19a/b**) were also synthesized and tested. Both **19a** and **19b** had much lower affinity for the central muscarinic receptors than **5d** and **5f**, and the efficacy of **19a,b** was too low to give IC_{50} values in the vas deferens preparation. Therefore, the C_{5-6} (alkyloxy)/(alkylthio)-TZTP's represent a unique series of potent functional M_1 selective muscarinic agonists.

The deficits in central cholinergic transmission, which occur in patients with Alzheimer's disease,[1] have increased the attention on muscarinic pharmacology. Neurochemical examination of brain material from Alzheimer's patients has demonstrated loss of the presynaptic marker enzyme, choline acetyltransferase and of muscarinic receptors of

[†] Novo Nordisk.
[‡] Eli Lilly and Company.

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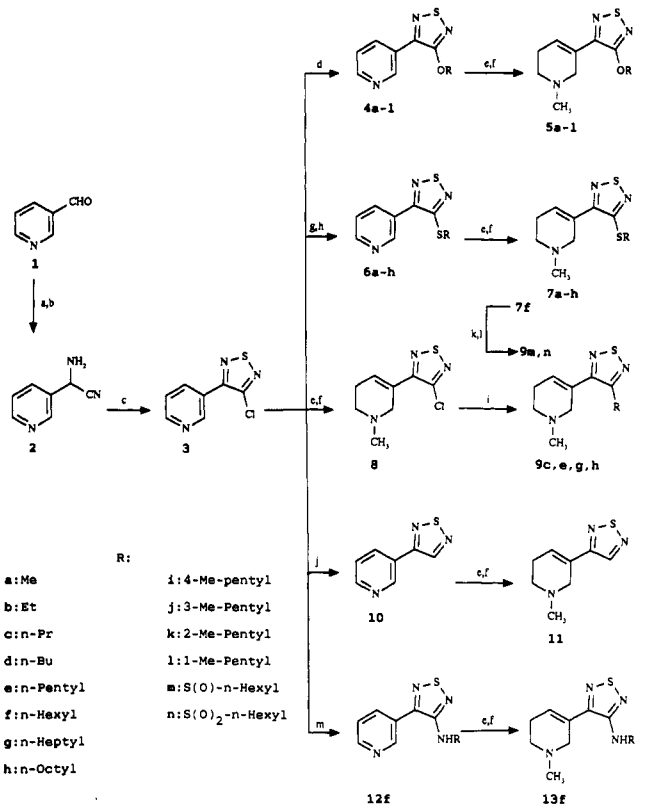
the M_2 subtype.[1-3] The M_2 muscarinic receptors appear to be located predominantly presynaptically, where they may function as autoreceptors.[4,5] The postsynaptic muscarinic receptors, which primarily are of the M_1 subtype, seem to a large extent to survive the loss of cholinergic nerve endings.[6] These findings have led to attempts at restoring cholinergic function by means of cholinomimetic drugs such as acetylcholinesterase inhibitors and muscarinic agonists, the hypothesis being that enhancement of cholinergic neurotransmission would alleviate the symptoms of the disease, particularly the deficits in cognition and memory. Clinical studies with the muscarinic agonists arecoline,[7] RS 86,[8] and pilocarpine[9] have produced disappointing results probably partially due to the agonists' lack of M_1 selectivity and efficacy and partially due to the dose limiting side effects associated with M_2 and M_3 muscarinic receptor subtype stimulation.

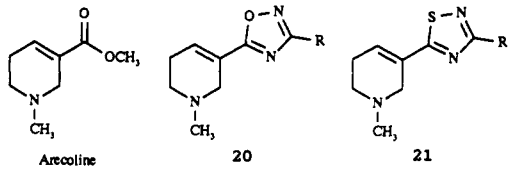
Pharmacological investigation of muscarinic receptor subtypes using both functional and binding studies has identified three distinct muscarinic receptor subtypes, M_1, M_2, and M_3,[10] and molecular cloning efforts have identified five genetically distinct muscarinic receptor subtypes, m_1–m_5.[11] Functional expression of the cloned genes has suggested a correlation between the genetically and pharmacologically defined subtypes such that $M_1 = m_1$, m_4, and m_5; $M_2 = m_2$; and $M_3 = m_3$.[12]

Scheme I.[a]



[a] Reagents: (a) $KCN/H_2O/AcOH$; (b) $NH_4Cl/NH_3(aq)$; (c) S_2Cl_2/DMF; (d) NaOR/ROH; (e) MeI/acetone; (f) $NaBH_4/EtOH$; (g) NaSH/DMF; (h) RCl (Br, I), K_2CO_3; (i) RMgBr/THF; (j) NaSBu/THF; (k) $NaIO_4/H_2O$; (l) $KHSO_5/H_2O$; (m) 1-hexylamine/DMSO.

We have focused our research on identifying M_1 selective muscarinic agonists capable of crossing the blood–brain barrier (BBB). The muscarinic agonist arecoline has



Arecoline 20 21

previously been used as a lead structure to design centrally active muscarinic agents. Replacement of the ester functionality with either the 3-alkyl-1,2,4-oxadiazole **20** or the 3-alkyl-1,2,4-thiadiazole **21** have produced very potent muscarinic agonists.[13,14] However, systematic removal of the heteroatoms in the 3-methyl-1,2,4-oxadiazole giving oxazoles and furans caused a decrease in affinity for the agonist binding site. The two isomers 2-methyl-1,3,4-ox-

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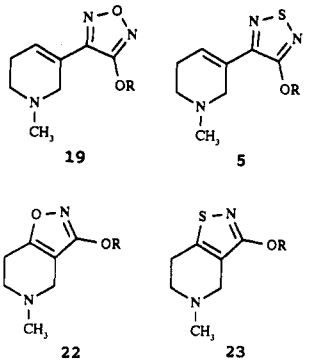
adiazole and 5-methyl-1,2,4-oxadiazole also had lower affinity for muscarinic receptors.[13,14] No muscarinic M_1 subtype selectivity has been reported for the above-mentioned agonists.

The 1,2,5-oxadiazoles and the 1,2,5-thiadiazoles that would also be isomers of the 1,2,4-oxadiazoles **20** and the 1,2,4-thiadiazoles **21**, respectively, were not investigated in these earlier studies.[15] This could partly be a consequence of the negative results obtained with the 2-methyl-1,3,4-oxadiazole and the 5-methyl-1,2,4-oxadiazole and partly because the substituent would be in another relative position, compared to the active 1,2,4-oxadiazoles/thiadiazoles (**20**/**21**). However, the structural resemblance of the 1,2,5-oxadiazoles **19** and the 1,2,5-thiadiazoles **5** with the 3-alkoxyisoxazoles **22** and the 3-alkoxyisothiazoles **23** utilized as ester bioisosters in conformationally restricted arecoline analogues[16,17] encouraged us to pursue these analogues. Furthermore, the 3-alkoxyisoxazoles/isothiazoles **22**/**23** were perceived to be a better model on which to base substituent selection. Consequently, we focused initially on the alkoxy analogues rather than the alkyl analogues, which were active in the 1,2,4-oxadiazole series.

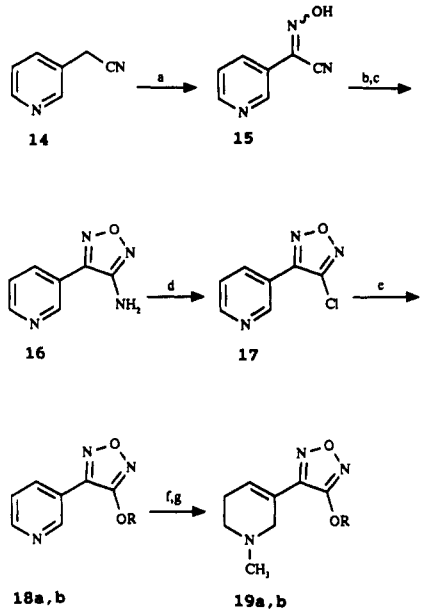


In this paper, we report on the synthesis and the in vitro biological evaluation of 3-(3-substituted-1,2,5-oxadiazol/thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines, of which several are potent, functional M_1 selective muscarinic agonists.

Chemistry

The α-amino-α-(3-pyridyl)acetonitrile (**2**) intermediate was initially synthesized according to the published procedure[18] adding the 3-pyridinecarbaldehyde (**1**) to a stirring aqueous solution of potassium cyanide and ammonium chloride (Strecker condition). The yield was highly improved by changing to a two step synthesis, reacting the carbaldehyde **1** with potassium cyanide generating the cyanohydrin, which after isolation immediately was reacted with ammonium chloride under basic aqueous conditions to give the aminonitrile **2** (Scheme I). Cyclization of **2** with sulfur monochloride in DMF gave the desired 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine (**3**). This key intermediate, **3**, was crystalline and could be stored without decomposition. Nucleophilic substitution of the chlorine of **3** with the appropriate sodium alkoxide gave the compounds **4a–h**, which were quaternized with methyl iodide in acetone and then reduced to the tetrahydropyridine product **5a–h**, with sodium borohydride in ethanol. The yields of **5a–h** were between 20 and 40% based on **3** (Table III).

Scheme II.[a]



[a] Reagents: (a) MeONO/NaOH/MeOH; (b) NH_2OH/NaOAc/EtOH; (c) PCl_5/ether; (d) $NaNO_2/CuCl_2/Cu/HCl$/AcOH; (e) NaOR, ROH; (f) MeI/acetone; (g) $NaBH_4$/MeOH.

The hexylaminothiadiazole **13f** was also obtained from **3** by heating with hexylamine, quaternizing the pyridine with methyl iodide and reducing the product with sodium borohydride. The same approach was used in attempts to synthesize the alkylthio analogues **6a–h**. Adding **3** to a solution of sodium hydride and 1-butanethiol in THF gave one product, not the expected **6d**, but instead the reduced analogue **10**. Several modifications of the reaction conditions showed that sodium butanethiolate always acted as a reducing agent on **3**. Compound **10** was quaternized with methyl iodide and reduced with sodium borohydride to give the unsubstituted analogue **11** under standard conditions.

The alkylthio analogues **6a–h** were obtained by treating **3** with sodium hydrosulfide in DMF followed by the appropriate alkyl halide. Quaternization and reduction gave the desired products **7a–h** in 30–50% yields based on **3** (Table IV). Oxidation of **7f** with $NaIO_4$ or $KHSO_5$ gave sulfoxide **9m** or sulfone **9n**, respectively. Attempts to prepare the 3-(3-alkyl-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines **9** in an analogous way failed, due to ring opening of the 3-alkyl-1,2,5-thiadiazoles upon reduction of the quaternary pyridines with borohydride. Instead, the chloro intermediate **3** was reduced to the other interesting intermediate **8**, which when subjected to Grignard reagents in THF gave the alkyl analogues **9c,e,g,h** (Table V). No addition of the Grignard reagent to the double bond in the tetrahydropyridine ring was observed in this reaction.

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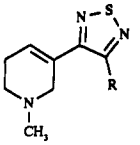
Table I. In Vitro Data on 3-(3-Substituted-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines



compd	R	receptor binding to rat brain membranes: IC_{50} ± SEM, nM [^{3}H]-Oxo-M	[^{3}H]-Pz	inhibition of twitch height in rabbit vas deferens: IC_{50}, nM	% max ±SEM	inhibition of force of contraction in guinea pig atria: IC_{30}, nM	% max ±SEM
5a	OMe	22 ± 0.8	148 ± 19	(364)	47 ± 22	1100	50 ± 3
5b	OEt	5.7 ± 0.24	48 ± 1		43 ± 20	>75000	0
5c	O-*n*-Pr	1.6 ± 0.06	18 ± 0.2		25 ± 31	90	100
5d	O-*n*-Bu	1.4 ± 0.12	5.0 ± 0.7	3	68 ± 7	200	71 ± 4
5e	O-*n*-pentyl	2.0 ± 0.25	4.0 ± 0.6	0.004	93 ± 1	2800	43 ± 6
5f	O-*n*-hexyl	9.7 ± 1.1	7.0 ± 0.7	0.008	94 ± 1	>27000	20 ± 2
5g	O-*n*-heptyl	6.2 ± 0.02	12 ± 3	9	69 ± 5	3900	100
5h	O-*n*-octyl	30 ± 7.5	40 ± 5		0	>75000	0
5i	O-4-Mepentyl	8.0 ± 0.8	9.7 ± 1.3	0.006	86 ± 1	7300	73 ± 10
5j	O-3-Mepentyl	10 ± 2.2	8.4 ± 0.7	47	65 ± 16	>75000	0
5k	O-2-Mepentyl	13 ± 1.3	9.0 ± 0.9		32 ± 6	>75000	0
5l	O-1-Mepentyl	7.0 ● 0.11	6.9 ± 0.8	7	79 ± 5	>25000	15 ± 1
7a	SMe	2.3 ± 0.6	43 ± 5	3	70 ± 10	300	100
7b	SEt	1.9 ± 0.8	18 ± 1	22	71 ± 14	70	100
7c	S-*n*-Pr	0.9 ± 0.01	4.0 ± 0.3		42 ± 12	50	100
7d	S-*n*-Bu	1.7 ± 0.15	1.3 ± 0.2	0.003	83 ± 6	100	45 ± 3
7e	S-*n*-pentyl	4.8 ± 1.4	2.4 ± 0.2	0.002	90 ± 5	5400	33 ± 5
7f	S-*n*-hexyl	6.5 ± 2.2	5 ± 0.7	0.001	92 ± 1	9700	56 ± 7
7g	S-*n*-heptyl	3.0 ± 0.9	12 ● 0.9	108	63 ± 9	7500	40 ± 6
7h	S-*n*-octyl	140 ± 20	49 ± 5		42 ± 8	>72000	5 ± 4
9c	*n*-Pr	80 ± 4.4	84 ± 8		13 ± 6	>75000	7 ± 6
9e	*n*-pentyl	146 ± 18	170 ± 16		40 ● 8	>75000	12 ± 3
9g	*n*-heptyl	187 ● 37	73 ± 9		26 ± 6	5400	78 ± 9
9h	*n*-octyl	428 ± 20	56 ± 5		26 ± 6	>75000	14 ± 1
9m	S(O)-*n*-hexyl	109 ± 1.5	860 ± 73	48	80 ± 8	25000	45 ± 5
9n	$S(O)_2$-*n*-hexyl	29 ± 0.8	62 ± 5	43	73 ● 7	5500	76 ± 6
13f	NH-*n*-hexyl	60 ± 3.0	105 ± 20	3000	65 ± 10	13000	84 ± 10
11	H	2.0 ± 0.04	145 ± 31	26	59 ± 18	10	100
8	Cl	10.8 ± 2.1	375 ± 48	537	84[a]	29400	67 ± 8
arecoline		77 ± 13	1300 ± 260	545	95 ± 1	600	100
carbachol		23 ± 3.7	1380 ± 140	460	77 ± 5	500	100
McN-A-343		355 ± 40	955 ± 75	659	91 ± 2	>100000	0

[a] n = 2 tissues.

Table II. In Vitro Data on 3-(3-Alkoxy-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines

compd	R	receptor binding to rat brain membranes: IC_{50} ± SEM, nM [^{3}H]-Oxo-M	[^{3}H]-Pz	inhibition of twitch height in rabbit vas deferens: IC_{50}, nM	% max ±SEM	inhibition of force of contraction in guinea pig atria: IC_{30}, nM	% max ±SEM
19a	O-*n*-Bu	24 ± 4.3	345 ± 45		12 ± 21	>70000	0
19b	O-*n*-hexyl	84 ± 25	277 ± 31	(10000)	47 ± 20	>70000	0

The 3-(3-alkoxy-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines **19** were obtained as outlined in Scheme II. 3-Pyridylacetonitrile dissolved in methanol was treated with freshly generated methylnitrite under alkaline conditions to give the α-oxime derivative, **15**, in good yield. Reacting the α-oximidonitrile **15** with hydroxylamine gave the corresponding acetamidoxime, as a crude material, which was dehydrated with PCl_5 to give the desired 3-(3-amino-1,2,5-oxadiazol-4-yl)pyridine **16**. Diazotization of **16** in hydrochloric acid with sodium nitrite in the presence of copper and $CuCl_2$ led to the key intermediate 3-(3-chloro-1,2,5-oxadiazol-4-yl)pyridine (**17**). In a similar manner as described above for the thiadiazoles, **17** was converted to the 3-(3-alkoxy-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines **19**.

Evaluation of Biological Effects

The affinity of the compounds for muscarinic receptor sites in the rat brain was determined by in vitro receptor binding studies. The ability of the compounds to displace tritiated oxotremorine-M (Oxo-M), a potent subtype nonselective muscarinic agonist, was interpreted as the affinity for the "agonist conformational state" of the muscarinic receptor sites. Displacement of pirenzepine (Pz), a selective antagonist for M_1 muscarinic receptors, was used to estimate the affinity for M_1 receptor sites in

Table III. Data for 3-(3-Alkoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines

compd	yield based on 3,[a] %	mp, °C	MS, m/z (M^+)	formula[b]
5a	34	150–151	211	$C_9H_{13}N_3OS$, $C_2H_2O_4$
5b	35	137–139	225	$C_{10}H_{15}N_3OS$, $C_2H_2O_4$
5c	41	148–150	239	$C_{11}H_{17}N_3OS$, $C_2H_2O_4$
5d	33	158–160	253	$C_{12}H_{19}N_3OS$, $C_2H_2O_4$
5e	32	160–161	267	$C_{13}H_{21}N_3OS$, $C_2H_2O_4$
5f	38	148–150	281	$C_{14}H_{23}N_3OS$, $C_2H_2O_4$
5g	34	152–153	295	$C_{15}H_{25}N_3OS$, $C_2H_2O_4$
5h	28	144–145	309	$C_{16}H_{27}N_3OS$, $C_2H_2O_4$
5i	25	72–74	281	$C_{14}H_{23}N_3OS$, $C_4H_4O_4$
5j	29	133–134	281	$C_{14}H_{23}N_3OS$, t-$C_4H_4O_4$
5k	26	120–122	281	$C_{14}H_{23}N_3OS$, t-$C_4H_4O_4$
5l	25	143–144	281	$C_{14}H_{23}N_3OS$, t-$C_4H_4O_4$

[a] No attempt was made to optimize yield. [b] All compounds gave satisfactory microanalyses for C, H and N.

Table IV. Data for 3-(3-(Alkylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates

compd	yield based on 3,[a] %	mp, °C	MS, m/z (M^+)	formula[b]
7a	38	169–170	227	$C_9H_{13}N_3S_2$, $C_2H_2O_4$
7b	49	145–146	241	$C_{10}H_{15}N_3S_2$, $C_2H_2O_4$
7c	30	138–139	255	$C_{11}H_{17}N_3S_2$, $C_2H_2O_4$
7d	33	148–150	269	$C_{12}H_{19}N_3S_2$, $C_2H_2O_4$
7e	38	136–138	283	$C_{13}H_{21}N_3S_2$, $C_2H_2O_4$
7f	30	126–128	297	$C_{14}H_{23}N_3S_2$, $C_2H_2O_4$
7g	36	122–123	311	$C_{15}H_{25}N_3S_2$, $C_2H_2O_4$
7h	32	121–122	325	$C_{16}H_{27}N_3S_2$, $C_2H_2O_4$

[a,b] See the footnotes for Table III.

Table V. Data for 3-(3-Alkyl-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates

compd	yield based on 3,[a] %	mp, °C	MS, m/z (M^+)	formula[b]
9c	75	141–142	223	$C_{11}H_{17}N_3S$, $C_2H_2O_4$
9e	24	156–157	251	$C_{13}H_{21}N_3S$, $C_2H_2O_4$
9g	72	151–152	279	$C_{15}H_{25}N_3S$, $C_2H_2O_4$
9h	75	157–158	293	$C_{16}H_{27}N_3S$, $C_2H_2O_4$

[a,b] See the footnotes for Table III.

hippocampus. The atria has been reported to have muscarinic receptors primarily of the M_2 subtype;[19] therefore, the spontaneously beating isolated guinea pig atria model was used to access the functional efficacy and potency of the compounds at the M_2 receptors. As most of the test compounds had low intrinsic activity on the atria, IC_{30} values were calculated in order to do SAR. The maximum effect of the compounds was expressed as percent of that produced by the full agonist acetylcholine.

The inhibition of the twitch height in the electrically stimulated rabbit vas deferens has been reported[20–23] to be very sensitive to pirenzepine and probably mediated by muscarinic M_1 receptors. Furthermore, immunoprecipitation from rabbit vas deferens showed a mixture of m_1 and m_2 receptors,[24] in agreement with functional studies characterizing a presynaptic M_1 heteroreceptor mediating inhibition and a postsynaptic M_2 receptor enhancing neurogenic contraction in this tissue. To evaluate the in vitro functional efficacy and potency of the compounds at the M_1 receptors, the isolated vas deferens assay was used. Selected compounds were tested for their M_3 efficacy and potency in the isolated guinea pig ileum preparation.

Results and Structure–Activity Relationship

In the 3-(3-alkoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (alkoxy-TZTP) series all the derivatives with unbranched C_{1-8} alkoxy substituents (**5a–h**, Table I) had high affinity for the muscarinic agonist conformational state as measured by Oxo-M binding. Affinity initially increased with carbon chain length (IC_{50} = 22 nM for methoxy, **5a**) with optimum activity being detected with the butoxy-TZTP, **5d**, derivative (IC_{50} = 1.4 nM). Thereafter, affinity declined with chain length, but the affinities of the propoxy **5c** and the pentyloxy **5e** analogues were comparable to **5d**.

A slight diminution in affinity for the agonist conformational state was produced by branching in the alkoxy chain, but the position of the branching did not dramatically affect this property. For instance, methyl substitution of the pentyloxy at the 1, 2, 3, and 4 position of the alkoxy chain increased the IC_{50} from 2 nM (**5e**) to 7–13 nM (**5i,j,k,l**).

All the alkoxy-TZTP derivatives tested (**5a–l**) displaced [^{3}H]-Pz with IC_{50} values in the low nanomolar range. For the straight-chain C_{1-8} alkoxy-TZTP (**5a–h**) analogues the affinity of the compounds again increased with chain length, reaching maximum with the pentyloxy **5e** (IC_{50} = 4 nM), and then decreased with increasing chain length (Table I). In the methyl-branched alkoxy-TZTP series **5i–l**, affinity for displacing Pz was only slightly decreased compared to the unbranched pentyloxy derivative, **5e**.

The ability of the unbranched alkoxy-TZTP compounds to inhibit the twitch height in the vas deferens gave the same SAR as obtained in the binding assays. The pentyloxy **5e** and the hexyloxy **5f** analogues produced an over 90% inhibition of the twitch height with IC_{50} values in the low picomolar range. The butoxy **5d** and the heptyloxy **5g** derivatives were less potent and produced an approximately 70% inhibition with an IC_{50} of a few nanomolar. The methoxy **5a** and ethoxy **5b** analogues were only partial agonists in this assay, and IC_{50} values could not be obtained. In the methyl-substituted pentyloxy series **5i–l**, the isohexyloxy analogue **5i** was considerably more potent than isomers with methyl in either the 1 (**5l**) or the 3 (**5j**) position. A methyl in the 2 position (**5h**) reduced the efficacy of the compound below 50%. Pirenzepine dose-dependently produced a parallel shift to the right in the concentration–response curves, with a K_B of 10 nM for **5f**. Neither pirenzepine nor AFDX-116 affected twitch height when tested alone.

The ability of the alkoxy-TZTP derivatives to inhibit the inotropic activity in the guinea pig atria gave a SAR that was different from the SAR observed in the receptor binding and vas deferens assays. Generally, the com-

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pounds had low potency and efficacy. The methoxy (**5a**) analogue was a weak partial agonist, whereas the ethoxy (**5b**) analogue was an antagonist. The most potent compound in the alkoxy series was the propoxy (**5c**) analogue, which was a full agonist with an IC_{50} of 90 nM. The potency and efficacy then declined with increasing chain length, with the exception of heptyloxy (**5g**) that was a weak, but full agonist. The hexyloxy (**5f**) analogue only inhibited the force of contraction by 20% at very high concentrations. The methyl-branched (pentyloxy)-TZTP (**5j–l**) compounds were all antagonists, whereas the (isohexyloxy)-TZTP **5i** was a weak partial agonist. The ability of the compounds to produce negative inotropic effects in the guinea pig atria was blocked by atropine (100 nM) whereas pirenzepine (1 μM) had no effect on the concentration–response curves.

As oxygen often can be exchanged for sulfur without loss of biological activity we decided to synthesize the corresponding (alkylthio)-TZTP analogues.

The (alkylthio)thiadiazoles **7a–h** (Table I), in general, had equal or greater affinity for Oxo-M receptors than the 3-alkoxythiadiazoles **5a–h** of corresponding chain length. In this series of compounds, the relationship between alkyl chain length and affinity was difficult to interpret because of the consistently high potency of the compounds, although alkyl chain lengths beyond seven carbons markedly decreased affinity. Oxidation of the sulfur in the side chain of **7f**, i.e., **9m,n**, significantly decreased affinity for this receptor. In the Pz binding assay and in the vas deferens preparation the SAR for the (alkylthio)-TZTP analogues **7a–h** was essentially the same as for the corresponding alkoxy analogues **5a–h**, but again the alkylthio compounds were generally more potent. As for the alkoxy analogues, pirenzepine (30 nM) produced a parallel shift to the right of the concentration–response for the (alkylthio)-TZTP analogous in the vas deferens preparation. The sulfoxy (**9m**) and sulfone (**9n**) analogues had much lower affinity for the Pz site and were much less potent in the vas deferens preparation than **7f**. On the atria the C_{1-3} alkylthio derivatives **7a–c** were full agonists with increasing potency with increasing chain length. From butylthio **7d** to octylthio **7h** the potency decreased with increasing chain length, and the compounds were only partial agonists on the atria. Both atropine and the relatively M_2 selective antagonist, AFDX-116, concentration dependently inhibited the effects on the guinea pig atria.

The alkyl and hexylamino analogues were also synthesized to test whether the oxygen/sulfur was essential to the potency and selectivity of the ligands.

The alkyl-TZTP's **9c,e,g,h** and the hexylamino derivative **13f** had significantly lower affinity for the receptor sites labeled by Oxo-M and Pz than either the alkoxy- (**5b–g**) or (alkylthio)-TZTP (**7b–g**) compounds. However, the unsubstituted thiadiazole **11** and the chloro-TZTP **8** analogues both had high affinity for the Oxo-M receptor site but low affinity for the Pz receptor site. None of the alkyls **9c,e,g,h** was efficacious enough to obtain an IC_{50} value in the vas deferens or an IC_{30} in the atria model and the hexylamino **13** and chloro **8** ligands both had high IC_{50} and IC_{30} values in these assays. The unsubstituted thiadiazole **11** was a weak partial agonist on the vas deferens, but a very potent (IC_{30} = 10 nM), full agonist on the guinea pig atria preparation.

The most interesting compounds, (hexyloxy)-TZTP (**5f**) and (hexylthio)-TZTP (**7f**) were tested for M_3 activity in the guinea pig ileum preparation. The hexyloxy (**5f**) analogue had an EC_{50} of 65 nM and produced a maximum contraction of 65%, compared to acetylcholine. The

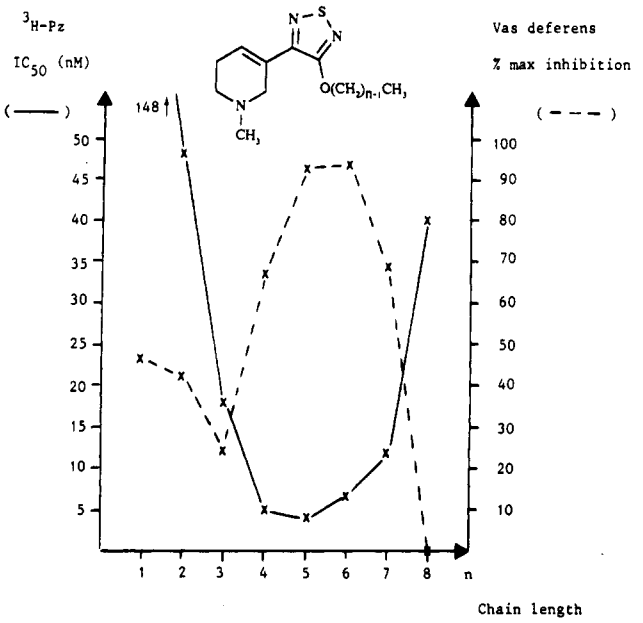

Figure 1.

hexylthio compound **7f** was virtually inactive, producing a 10% contraction at 1 μM. In the same preparation arecoline was a full agonist with an EC_{50} of 70 nM.

The interesting results obtained with the 1,2,5-thiadiazoles prompted us to synthesize the 1,2,5-oxadiazole analogues. Having the above-mentioned SAR knowledge we decided to make the interesting C_4 and C_6 alkoxy derivatives.

The 3-(3-butoxy/(hexyloxy)-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (butoxy/(hexyloxy)-OZTP) analogues **19a,b** (Table II) had lower affinity for the Oxo-M binding site than the corresponding butoxy/(hexyloxy)-TZTP (**5d/f**) analogues. Likewise, the ability of these 1,2,5-oxadiazole compounds to displace Pz was very poor and the intrinsic activities in the vas deferens were too low to obtain IC_{50} values. In the atria assay the compounds appeared to be antagonists.

Discussion

An interesting U-shaped relationship between the inhibition of both Pz binding and twitch height in the vas deferens and the alkyl chain length in the unbranched alkoxy- and (alkylthio)-TZTP series (Table I, Figure 1) was found. In both series the optimum chain length for M_1 receptor binding affinity and functional M_1 efficacy was four to six carbon chains. These C_{4-6} alkoxy/alkylthio analogues showed weak functional M_2 activity, as measured by suppression of inotropic activity in the guinea pig atria. Maximum M_2 potency occurred with the three carbon side chains in both series of compounds. This M_2 activity could account for the apparently abnormally low ability of the propoxy- and (propylthio)-TZTP (**5c**, **7c**) derivatives to inhibit the twitch height in the vas deferens preparation (Table I, Figure 1). Stimulation of the M_2 receptors increases the twitch height in the vas deferens and thereby competes with the M_1-mediated twitch height inhibition.[20] The M_2 efficacy and potency of the remaining compounds in the alkoxy and alkylthio series was not sufficient to diminish markedly the M_1-mediated response in this tissue. Maximum M_1 selectivity occurred with the five- and six-carbon side chains in both the alkoxy and alkylthio series. Not only did these compounds lack M_2 efficacy but they also had low picomolar potencies in the vas deferens. The reasons for the picomolar potency of these compounds requires further study, but is most likely due to their lack

of M_2 efficacy and the highly efficient M_1 receptor–effector coupling in the rabbit vas deferens.[23]

The methyl-branched (pentyloxy)-TZTP analogues **5i–l** had nearly the same affinity to the M_1 receptor as (pentyloxy)-TZTP (**5e**), but the functional efficacy of these compounds at the M_1 receptor in the vas deferens preparation was markedly decreased when the methyl group was closer to the thiadiazole ring. Exchange of the oxygen/sulfur in the alkoxy/(alkylthio)-TZTP (**5**, **7**) derivatives for carbon (**9c–h**), nitrogen (**13f**), sulfoxide (**9m**), or sulfone (**9n**) also reduced the M_1 receptor binding affinity and the functional M_1 potency drastically.

These results indicate that affinity and especially potency and efficacy at the M_1 receptors in the rabbit vas deferens is highly dependent on the substituent directly attached to the 3 position of the 1,2,5-thiadiazole ring and on the length and space-filling properties of the alkyl chain. The optimum substituents were found to be the unbranched C_{5-6} alkoxy/alkylthio (**5e,f**, **7e,f**) side chain. It is probable that this chain fits into a widening lipophilic cavity in the receptor whose occupancy is highly beneficial for activating the M_1 receptor. Oxygen and sulfur directly attached to the 1,2,5-thiadiazole ring probably influence the electronic and conformational properties of the 1,2,5-thiadiazole to obtain optimum agonist receptor interaction. The low M_1 receptor binding affinity and functional M_1 efficacy of the butoxy and hexyloxy 1,2,5-oxadiazoles **19a,b** support the assumption that the 1,2,5-thiadiazole has unique physical properties and that the 1,2,5-thiadiazole ring in the TZTP ligands is heavily involved in the receptor stimulation.

The length of the alkyl chain is probably also responsible for the separation of the M_1 and M_2 (and M_3) functional agonist activity of the alkoxy/(alkylthio)-TZTP derivatives. Generally, the shorter chain derivatives were effective in suppressing the force of contraction in the guinea pig atria (M_2 agonist response) while the longer chain (C_{5-8} alkoxy/(alkylthio)-TZTP, **5e–h**, **7e–h**) analogues were poor M_2 agonists, suggesting that the longer alkyl chains either interfere with binding to M_2 receptor or prevent their activation. The most interesting compounds, the (hexyloxy)- and the (hexylthio)-TZTP (**5f** and **7f**), also had low functional activity at M_3 receptors, as measured by their ability to contract the guinea pig ileum.

Another unique feature about the alkoxy-TZTP (**5a–l**) and (alkylthio)-TZTP (**7a–h**) ligands was the lack of tremor and salivation when a dose of 10 mg/kg was administered i.p. to mice. Very high doses of **5f** and **7f** were required to produce salivation and tremor, both compounds having ED_{50}'s > 100 mg/kg i.p. for either effect. In the same test the unsubstituted compound, **11**, induced severe tremor, salivation, and body temperature drop, killing the animals at a dose of 1 mg/kg i.p.

The aim of our research was to identify M_1 selective muscarinic agonists capable of crossing the BBB. The (hexyloxy)/thio-TZTP compounds **5f**/**7f** seem likely to fulfill some of these criteria. They show a high degree of functional M_1 selectivity, and the pK_a = 7.8 and the log P = 3.9 (pH = 7.4) for the hexyloxy (**5f**) analogue suggest that good BBB penetration is likely. However, M_1 receptor subtype selectivity has not been proven by the here-reported receptor binding data; and preliminary [^{3}H]-NMS binding data in cloned m_1–m_5 receptors have not shown the subtype selectivity observed in functional assays. The two compounds **5f** and **7f** are on the other hand selective muscarinic ligands, since affinity for other receptor systems was not detected in an extensive receptor screening. Therefore, the functional M_1 selectivity of **5f** and **7f** is probably due to the efficacy at the various muscarinic receptor subtypes rather than the affinity.

Conclusions

The 3-alkoxy/(alkylthio)-1,2,5-thiadiazole moieties represent unique isosteres to the arecoline ester functionality producing potent functional M_1 selective muscarinic agonists. Unbranched C_{5-6} alkoxy/alkylthio are the preferred sizes of the lipophilic substituent for displacing [^{3}H]-Pz in brain tissues and inhibiting twitch height in the vas deferens preparation, but without reducing the force of contraction in the atria model. The preliminary in vivo data on these ligands indicate only minor peripheral side effects making them potential candidates for the treatment of Alzheimer's disease. Full pharmacological evaluation of these compounds will be reported in the near future.

Experimental Section

Chemistry. Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-248 spectrometer, and mass spectra were recorded with a Finnigan 5100 mass spectrometer. Column chromatography was performed on silica gel 60 (70–230 mesh, ASTM, Merck). Radial chromatography was performed on a Harrison Research Chromatotron Model 7924 T using Analtech precast silica gel rotors. Elemental analyses were performed by Novo Microanalytical Laboratory, Denmark, and were within ±0.4% of calculated values.

2-Amino-2-(3-pyridyl)acetonitrile (2). To a solution of potassium cyanide (71.63 g, 1.10 mol) in water (300 mL) was added 3-pyridinecarboxaldehyde (93.96 mL, 1.0 mol) over 30 min at 5–10 °C. To the reaction mixture was added acetic acid (62.86 mL, 1.10 mol) over 30 min at 5–10 °C. The reaction mixture was stirred at room temperature for a further 18 h and then cooled to 5 °C. The 2-hydroxy-2-(3-pyridyl)acetonitrile product was collected by filtration washed with water, and the crystals were used immediately after: ^{1}H NMR ($CHCl_3$) δ 5.65 (1 H, s), 6.95 (1 H, br s), 7.40 (1 H, dd, J = 5 and 9 Hz), 7.95 (1 H, d, J = 9 Hz), 8.52 (1 H, d, J = 5 Hz), 8.62 (1 H, s). The cyanohydrin product (98 g, 0.73 mol) was added slowly, at room temperature, to a solution of ammonium chloride (196 g, 3.66 mol) in water (550 mL) and 25% aqueous ammonia (98 mL, 1.31 mol). The reaction mixture was stirred at room temperature for 18 h and then extracted with methylene chloride (5 × 500 mL). The organic phases were dried and evaporated to give the title compound **2** as a red-brown oil (82 g, 61%): ^{1}H NMR ($CDCl_3$) δ 2.35 (2 H, br s), 5.00 (1 H, s), 7.35 (1 H, dd, J = 5 and 9 Hz), 7.90 (1 H, d, J = 9 Hz), 8.55 (1 H, d, J = 5 Hz), 8.75 (1 H, s). **2** was used without further purification.

3-(3-Chloro-1,2,5-thiadiazol-4-yl)pyridine (3). To a solution of sulfur monochloride (80 mL, 992 mmol) in DMF (135 mL) was added over 1 h a solution of crude **2** (66 g, 496 mmol) in DMF (65 mL) at 5–10 °C. The reaction mixture was stirred for an additional 30 min at 5–10 °C after which ice–water (250 mL) was added to the reaction to keep the temperature below 20 °C. Sulfur precipitated, and the mixture was filtered. To the filtrate was added an aqueous sodium hydroxide solution (9 M, 200 mL) while keeping the temperature below 20 °C. The mixture was cooled to 5–10 °C, and the product **3** precipitated (59 g, 60%). An additional 5.5 g (5.6%) was collected from the mother liquor after extraction. The intermediate **3** was used without further purification, but an analytical sample was recrystallized from *n*-heptane to give yellow crystals: mp 48–49 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (1 H, m), 8.30 (1 H, m), 8.75 (1 H, dd, J = 5 and 2 Hz), 9.25 (1 H, d, J = 2 Hz). Anal. ($C_7H_4ClN_3S$) C, H, N.

General Procedure for the Synthesis of 3-(3-Alkoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines 5a–l (Table III). 3-(3-Methoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (5a) Oxalate Salt. Compound **3** (750 mg, 3.8 mmol) was added to a solution of sodium (460 mg, 20 mg atom) in methanol (10 mL). The reaction mixture was stirred at 50 °C for 1 h and then evaporated. The residue was dissolved in water (20 mL) and extracted with methylene chloride (3 × 100 mL). The combined organic phases were dried and evaporated to give compound **4a** (630 mg, 86%) as a white solid:

^{1}H NMR ($CDCl_3$) δ 4.25 (3 H, s), 7.40 (1 H, m), 8.45 (1 H, m), 8.70 (1 H, m), 9.45 (1 H, pert. s). Crude **4a** (620 mg, 3 mmol) was dissolved in acetone (10 mL) and treated with methyl iodide (370 μL, 6 mmol). The reaction mixture was stirred at room temperature for 18 h, and the methylpyridinium iodide product was collected by filtration (1.0 g, 100%). The methylpyridinium product (1.0 g, 3 mmol) was dissolved in absolute ethanol (20 mL), and sodium borohydride (460 mg, 12 mmol) was added to the solution at −10 °C. The reaction mixture was stirred at −10 °C for 1 h and then evaporated. The residue was dissolved in water (20 mL) and extracted with methylene chloride (3 × 100 mL). The dried combined organic phases were evaporated and the residue purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent. The free base of **5a** was isolated as an oil in 47% (300 mg) yield: ^{1}H NMR ($CDCl_3$) δ 2.2–2.6 (4 H, m), 2.45 (3 H, s), 3.45 (2 H, m), 4.15 (3 H, s), 7.05 (1 H, m). Crystallization with oxalic acid from acetone gave the title compound **5a** in 43% (390 mg) yield. Overall yield **3** to **5a**: 34%; mp 150–151 °C; MS m/z 211 (M^+ of free base). Anal. ($C_9H_{13}N_3OS$, $C_2H_2O_4$) C, H, N.

General Procedure for the Synthesis of 3-(3-(Alkylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines 7a–h (Table IV). 3-(3-(Ethylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (7b) Oxalate Salt. Sodium hydrosulfide monohydrate (250 mg, 3.3 mmol) was added to a solution of **3** (590 mg, 3.3 mmol) in DMF (20 mL) at room temperature, and the reaction mixture was stirred for 30 min. Potassium carbonate (1.24 g, 9 mmol) and ethyl iodide (360 μL, 4.5 mmol) were added, and the reaction mixture was stirred for additional 10 min. Water (50 mL) was added to the reaction mixture and the aqueous phase extracted with ether (3 × 100 mL). The combined ether phases were dried and evaporated to give crude **6b**. The residue (**6b**) was dissolved in acetone (5 mL), and methyl iodide (0.5 mL, 7.5 mmol) was added. The reaction mixture was stirred at room temperature for 20 h and evaporated. After the residue was dissolved in absolute ethanol (20 mL), sodium borohydride (270 mg, 7 mmol) was added to the solution at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, and water (20 mL) was added. The aqueous phase was extracted with ethyl acetate (3 × 100 mL), and the dried organic phases were evaporated. The residue was purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent to give the free base of **7b** in 370-mg yield: ^{1}H NMR ($CDCl_3$) δ 1.40 (3 H, t, J = 6 Hz), 2.2–2.6 (4 H, m), 2.45 (3 H, s), 3.25 (2 H, q, J = 6 Hz), 3.45 (2 H, m), 6.75 (1 H, m). Crystallization with oxalic acid from acetone gave the title compound **7b** in 49% (490 mg) yield based on **3**: mp 145–146 °C; MS m/z 241 (M^+ of free base). Anal. ($C_{10}H_{15}N_3S_2$, $C_2H_2O_4$) C, H, N.

3-(3-Chloro-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (8) Oxalate Salt. A solution of **3** (850 mg, 4.3 mmol) and methyl iodide (0.8 mL, 13 mmol) in acetone (10 mL) was stirred at room temperature for 18 h. The precipitate was collected by filtration (1.46 g, 100%) and dissolved in ethanol (20 mL). Sodium borohydride (330 mg, 8.6 mmol) was added to the solution at 0 °C, and the reaction mixture was stirred for 1 h. Water (20 mL) was added to the reaction mixture, and the aqueous phase was extracted with ethyl acetate (3 × 100 mL). The combined and dried organic phases were evaporated and the residue purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent. The free base of **8** was obtained as an oil (880 mg, 95%) which upon treatment with oxalic acid crystallized from acetone in 1.17-g (89%) yield: mp 124–125 °C; MS m/z 215 and 217 (M^+ of the isotopes of the free base); ^{1}H NMR (DMSO) δ 2.5–2.4 (2 H, m), 2.80 (3 H, s), 3.1–3.4 (2 H, m), 4.05 (2 H, m), 7.10 (1 H, m), 9.5–10.2 (2 H, br s). Anal. ($C_8H_{10}ClN_3S$, $C_2H_2O_4$) C, H, N.

General Procedure for the Synthesis of 3-(3-Alkyl-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines 9c,e,g,h (Table V). 3-(3-Propyl-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (9c) Oxalate Salt. The free base of **3** (450 mg, 1.5 mmol) was dissolved in dry tetrahydrofuran (20 mL), and a solution of propylmagnesium bromide (3 mL, 1.5 mmol) in tetrahydrofuran was added dropwise at 0 °C. The reaction mixture was stirred for 10 min, and then water (20 mL) was added. The aqueous phase was extracted with ether (3 × 100 mL) and the dried ether phases evaporated. The residue was crystallized as the oxalate salt of **9c** from acetone in 350-mg (75%) yield: mp 141–142 °C; MS m/z 223 (M^+ of the free base); ^{1}H NMR (DMSO) δ 0.90 (3 H, t, J = 6 Hz), 1.4–1.9 (2 H, m), 2.3–2.8 (2 H, m), 2.78 (3 H, s), 2.9–3.3 (4 H, m), 3.80 (2 H, pert. s), 6.50 (1 H, m), 10–11 (2 H, br s). Anal. ($C_{11}H_{17}N_3S$, $C_2H_2O_4$) C, H, N.

3-(3-(Hexylsulfinyl)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (9m) Oxalate Salt. To a solution of **7f** and HCl (700 mg, 2.1 mmol) in water (20 mL) was added $NaIO_4$ (1.35 g, 6.3 mmol) under argon atmosphere. After 1 h saturated aqueous $NaHCO_3$ (20 mL) was added, and the mixture was extracted with methylene chloride (2 × 50 mL). The combined extracts were dried and evaporated. The residue was purified by column chromatography on silica gel using chloroform–ethanol–ammonium hydroxide (96.7:3:0.3) as eluent to gain the free base of the title compound as a colorless product in 96% (630 mg) yield: ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, m), 1.23 (4 H, m), 1.42 (1 H, m), 1.63 (1 H, s), 1.77 (1 H, m), 2.46 (3 H, s), 2.59 (4 H, m), 3.22 (4 H, m), 3.34 (1 H, m), 6.61 (1 H, s).

Crystallization with oxalic acid from ethyl acetate–ethanol gave the title compound **9m**: mp 138–140 °C. Anal. ($C_{14}H_{23}N_3OS_2$, $C_2H_2O_4$) C, H, N.

3-(3-(Hexylsulfonyl)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (9n) Oxalate Salt. A solution of **7f** (837 mg, 2.5 mmol) in water (15 mL) kept under argon atmosphere was cooled to 0 °C as $KHSO_5$ (2.3 g, 3.7 mmol) in water (15 mL) was added dropwise with stirring. The reaction was allowed to go to room temperature, and after 4 h $NaHSO_3$ (260 mg, 2.5 mmol) in water (5 mL) was added. The solution was made basic with 5 N sodium hydroxide and extracted with methylene chloride (3 × 25 mL). The extracts were dried, and the solvent was evaporated to give a yellow liquid that was purified by radial chromatography eluting with chloroform–ethanol–ammonium hydroxide (97.25:2.5:0.25). The resulting yellow liquid (0.78 g) was converted to the oxalate salt and recrystallized from ethyl acetate to give **9n** as a slightly yellow solid in 69% (720 mg) yield: mp 122–123 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, t, J = 6 Hz), 1.25–1.40 (4 H, m), 1.47–1.54 (2 H, m), 1.88–1.98 (2 H, m), 2.76–2.96 (2 H, m), 3.0 (3 H, s), 3.3–3.5 (2 H, m), 3.60–3.67 (2 H, m), 4.10–4.35 (2 H, m), 7.16 (1 H, m). Anal. ($C_{14}H_{23}N_3O_2S_2$, $C_2H_2O_4$) C, H, N.

3-(1,2,5-Thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (11) Oxalate Salt. To a solution of 1-butanethiol (2.7 g, 30 mmol) and sodium hydride dispersion (60%) (1.2 g, 30 mmol) in dry tetrahydrofuran (50 mL) was added at −10 °C a solution of **3** (1.2 g, 6 mmol) in dry tetrahydrofuran (20 mL). The reaction mixture was stirred for 30 min, and water (30 mL) was added. The aqueous phase was extracted with ether, and the organic phases were dried and evaporated. The residue was purified by column chromatography on silica gel using ethyl acetate–methylene chloride (1:1) as eluent. The product **10** was dissolved in acetone (5 mL) and treated with methyl iodide (1 mL, 15 mmol). The reaction mixture was stirred for 18 h at room temperature, and the methylpyridinium iodide product was collected by filtration (1.2 g, 74%). The precipitate was dissolved in ethanol (20 mL), and sodium borohydride (380 mg, 10 mmol) was added to the solution at −10 °C. The reaction mixture was stirred at −10 °C for 30 min, and water (20 mL) was added. The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The dried organic phases were evaporated, and the residue was purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent. The free base of **11** was obtained as an oil (310 mg, 39%): ^{1}H NMR ($CDCl_3$) δ 2.3–2.7 (4 H, m), 2.45 (3 H, s), 3.45 (2 H, pert. s), 6.65 (1 H, m), 8.60 (1 H, s). Crystallization with oxalic acid from acetone gave the title compound **11** in 430 mg (36%) yield: mp 189–190 °C; MS m/z 181 (M^+ of free base). Anal. ($C_8H_{11}N_3S$, $C_2H_2O_4$) C, H, N.

3-(3-(Hexylamino)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (13f) Oxalate Salt. A solution of **3** (590 mg, 3.0 mmol) and n-hexylamine (1.52 g, 15 mmol) in DMSO (5 mL) was stirred at 100 °C for 48 h. After evaporation, water (20 mL) was added to the residue and the aqueous phase was extracted with ether (3 × 100 mL). The combined organic extracts were dried and evaporated to give the crude hexylamine-substituted product **12f**. The residue was dissolved in acetone (5 mL), and methyl iodide (0.6 mL, 9.6 mmol) was added. The reaction mixture was stirred at room temperature for 18 h and then

evaporated. The crude methylpyridinium product was dissolved in ethanol (99.9%, 25 mL), and sodium borohydride (380 mg, 10 mmol) was added to the solution at −10 °C. The reaction mixture was stirred at −10 °C for 1 h and then evaporated. The residue was dissolved in water (20 mL) and extracted with ethyl acetate. The dried combined organic phases were evaporated and the residue purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent. Crystallization with oxalic acid from acetone gave the title compound **13f** in 44% (490 mg) yield based on **3**: mp 102–103 °C; MS *m/z* 280 (M^+ of free base); ^1H NMR (DMSO) δ 0.85 (3 H, t, *J* = 6 Hz), 1.1–1.7 (8 H, m), 2.3–2.8 (2 H, m), 2.85 (3 H, s), 3.0–3.5 (4 H, m), 4.0 (2 H, pert. s), 6.4–6.6 (1 H, m), 6.6–7.0 (1 H, br s), 9.0 (2 H, br s). Anal. ($C_{14}H_{24}N_4S$, 1.15 $C_2H_2O_4$) C, H, N.

α-Oximido-3-pyridylacetonitrile (15). 3-Pyridylacetonitrile (**14**) (47.2 g, 400 mmol) was dissolved in a solution of sodium hydroxide (16 g, 400 mmol) in methanol (100 mL). Methyl nitrite, generated by adding dropwise a solution of concentrated sulfuric acid (12.8 mL) and water (26 mL) to a solution of sodium nitrite (33.2 g, 480 mmol) in water (20 mL) and methanol (20 mL), was bubbled through the 3-pyridylacetonitrile solution at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and the precipitate collected by filtration. The precipitate was washed with a little methanol to give the sodium salt of the wanted product **15** in 60% (41.1 g) yield: mp >260 °C; MS *m/z* 147 (M^+); ^1H NMR (DMSO) δ 7.20 (1 H, m), 7.75 (1 H, m), 8.15 ($^2/_3$ H, m), 8.40 ($^1/_3$ H, m), 8.67 ($^2/_3$ H, d, *J* = 3 Hz), 9.25 ($^1/_3$ H, d, *J* = 3 Hz). An analytical sample was dissolved in water and the free base precipitated upon addition of acetic acid: mp 224–226 °C. Anal. ($C_7H_5N_3O$, 0.2 CH_3COOH) C, H, N.

3-(3-Amino-1,2,5-oxadiazol-4-yl)pyridine (16). A mixture of **15** (41.0 g, 279 mmol), hydroxylamine hydrochloride (21.5 g, 310 mmol), and sodium acetate (50.8 g, 620 mmol) in ethanol (99.9%, 500 mL) was refluxed for 4 h. After the mixture was cooled, the precipitate was collected by filtration and dried. The precipitate consisted of α-oximido-3-pyridylacetamidoxime and sodium acetate (85 g, 168%): MS *m/z* 180 (M^+). Crude oximido-3-pyridylacetamidoxime (5 g) and phosphorus pentachloride (5 g) were refluxed in dry ether (250 mL) for 6 h. Water and potassium carbonate to alkaline pH were added and the phases separated. The aqueous phase was extracted with ether, and the combined ether phases were dried. Evaporation of the ether phase gave the title compound **16** as a white solid in 850-mg yield: mp 149–151 °C; MS *m/z* 162 (M^+); ^1H NMR (DMSO) δ 3.20 (2 H, br s), 7.55 (1 H, m), 8.15 (1 H, m), 8.70 (1 H, m), 9.05 (1 H, m). Anal. ($C_7H_6N_4O$) C, H, N.

3-(3-Chloro-1,2,5-oxadiazol-4-yl)pyridine (17). To a solution of **16** (1.0 g, 6.2 mmol) in acetic acid (16 mL) and concentrated hydrochloric acid (5.2 mL) was added $CuCl_2$ (938 mg, 7 mmol) and copper coils (100 mg) at 0 °C. After 10 min a solution of sodium nitrite (483 mg, 7 mmol) in water (3 mL) was added dropwise at 5 °C. The reaction mixture was stirred for an additional 30 min at 0 °C. Aqueous sodium hydroxide (2 N) was added to alkaline pH, and the mixture was extracted with ether (3 × 100 mL). The ether phases were dried and evaporated and the residue purified by column chromatography on silica gel using ethyl acetate as eluent. The chloro compound **17** was obtained as an oil in 230-mg (20%) yield: MS *m/z* 181 and 183 (M^+ of the two isotopes); ^1H NMR (DMSO) δ 7.45 (1 H, dd, *J* = 8 and 6 Hz), 8.00 (1 H, m), 8.60 (1 H, dd, *J* = 6 and 3 Hz), 8.80 (1 H, d, *J* = 3 Hz).

General Procedure for the Synthesis of 3-(3-Alkoxy-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines 19a,b. 3-(3-Butoxy-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (19a) Oxalate Salt. To a solution of sodium (150 mg, 6.5 mtom) in 1-butanol (5 mL) was added **17** (350 mg, 1.9 mmol), and the reaction mixture was stirred at 25 °C for 2 h and evaporated. The residue was dissolved in water (20 mL) and extracted with ether (3 × 50 mL). The combined organic phases were dried and evaporated to give crude **18a**. The residue, **18a**, was dissolved in acetone (10 mL) and treated with methyl iodide (1 mL, 15 mmol). The reaction mixture was stirred at room temperature for 18 h and evaporated. The residue was dissolved in methanol (20 mL), and sodium borohydride (148 mg, 3.8 mmol) was added to the solution at 0 °C. The reaction mixture was stirred for 15 min and evaporated. The residue was dissolved in water (20 mL) and then extracted with ether (3 × 100 mL). The dried ether phases were evaporated and the residue purified by column chromatography on silica gel using ethyl acetate–methanol (4:1) as eluent. The free base of **19a** [^1H NMR ($CDCl_3$) δ 1.0 (3 H, t, *J* = 6 Hz), 1.2–2.1 (4 H, m), 2.3–2.7 (4 H, m), 2.42 (3 H, s), 3.33 (2 H, d, *J* = 3 Hz), 4.47 (2 H, t, *J* = 6 Hz), 6.80 (1 H, m)] was crystallized as the oxalate salt from acetone in 120-mg (19% based on **17**) yield: mp 132–135 °C; MS *m/z* 237 (M^+ of free base). Anal. ($C_{12}H_{19}N_3O_2$, $C_2H_2O_4$, 0.3 H_2O) C, H, N.

3-(3-(Hexyloxy)-1,2,5-oxadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (19b) Oxalate Salt. Synthesized as described above: mp 143–147 °C; MS *m/z* 265 (M^+ of free base); ^1H NMR ($CDCl_3$, free base) δ 0.90 (3 H, t, *J* = 6 Hz), 1.1–2.1 (8 H, m), 2.2–2.6 (4 H, m), 2.40 (3 H, s), 3.30 (2 H, d, *J* = 3 Hz), 4.40 (2 H, t, *J* = 6 Hz), 6.75 (1 H, m). Anal. ($C_{14}H_{23}N_3O_2$, $C_2H_2O_4$, 0.8 H_2O) C, H, N.

Displacement of [^{3}H]-Oxotremorine-M. The procedure has previously been described by us.[14] 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 buffer and centrifuged. The final pellet was homogenized in 20 mM Hepes pH = 7.4 (100 mL per g of original tissue) and used for binding. To 0.5 mL of tissue homogenate was 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 Whatman glass fiber filter with ice-cold buffer, and the filter washed with ice-cold buffer. Radioactivity of the filters was determined by scintillation counting. IC_{50} values were calculated from inhibitory effects of at least four different concentrations in triplicate using the Hill equation.

Displacement of [^{3}H]-Pirenzepine. The hippocampus was removed from male rat brain and homogenized in 10 volumes of 0.32 M sucrose, and the P_2 or synaptosomal fraction was isolated by differential centrifugation. The homogenates were resuspended in 30 volumes of 50 mM Tris–Cl buffer, pH 7.4, and centrifuged at 50000*g*. After resuspension in buffer, the homogenates were preincubated at 4 °C for 0.5 h and centrifuged again. The pellet was resuspended in 6 volumes of buffer and frozen at −70 °C until use.

For the binding assay, aliquots of test compound generally from 1 to 1000 nM were added in triplicate to plastic tubes containing 1 nM ^{3}H-pirenzepine (74.4 Ci/mMol) and hippocampus tissue homogenate containing about 100 μg of protein or 5 mg of tissue wet weight in 1 mL of 20 mM Tris–Cl–1 mM $MnCl_2$ buffer. After incubation for 1 h at 25 °C, the suspensions were filtered with vacuum through Whatman GF/C filters and rinsed three times with 1 mL of cold buffer, and the filters were placed in vials containing PCS scintillation fluid. Radioactivity trapped on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was determined by the addition of 1000 nM atropine.

The concentration required to inhibit binding 50% (IC_{50}) was calculated using the ALLFIT program.

Electrically Stimulated Rabbit Vas Deferens. The methods are a slight modification of the method of Eltze.[20] Briefly, rabbits were anesthetized with pentobarbital sodium (60 mg/kg, i.v.) and the vasa deferentia removed. The organs were carefully dissected free of surrounding tissue and divided into two segments, a prostatic and an epididymal segment. Each tissue segment was suspended between an electrode and a force-displacement transducer in a 10-mL organ bath in modified Krebs–Ringer solution consisting of (in mM): NaCl, 134.0; KCl, 3.4; $CaCl_2$, 2.8; $MgSO_4$, 0.6; KH_2PO_4, 1.3; $NaHCO_3$, 16.0; and glucose, 7.7; 10^{-6} M yohimbine was included to block α-2 adrenoceptors. The solution was maintained at 31 °C and was continuously bubbled with 95% O_2–5% CO_2. The tension of the preparations was set at 0.75 g and left to equilibrate for at least 30 min before continuous field stimulation (0.5 ms, 40 V, 0.05 Hz) was started. In this preparation, M_2 agonists increase twitch height whereas putative M_1 agonists decrease twitch height.

Cumulative concentration–effect curves were determined in four tissues (two prostatic and two epididymal) with dose additions

at 20-min intervals. If the compound inhibited twitch height by at least 50%, then concentration–effect curves were determined in a minimum of six tissues.

Spontaneously Beating Isolated Guinea Pig Atria. Male guinea pigs were killed by cervical dislocation and the hearts rapidly removed and placed in oxygenated Krebs Hensebit solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2–52; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55, bubbled with 5% CO_2 and 95% O_2. The test compounds were added to the bath, and the amplitude of the contractions was measured after 30 s of incubation, after which the compounds were washed off by overflow. In this way dose response curves were constructed for each compound with three to five preparations at each concentration. From these curves IC_{30} values were obtained with extrapolation, and the percentage maximum response obtained noted (acetylcholine and carbacol give a maximum 100% inhibition of the contractions).

Isolated Guinea Pig Ileum. Male Hartley albino guinea pigs (250–600 g) were sacrificed by cervical dislocation. The ileum was removed from each animal and cut into 2–3-cm sections. The segments of ileum were attached with thread to a glass rod and placed in isolated tissue baths containing Krebs' bicarbonate solution aerated with 95% O_2 and 5% CO_2, maintained at 37 °C. The tissues were then attached to a force–displacement transducer (Grass FT 03). All recordings were made with a Beckman dynograph recorder. After an equilibration period, noncumulative concentration–response curves were obtained in each tissue with the agonists.

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Registry No. **2**, 131988-63-1; **2** 2-hydroxy, 17604-74-9; **3**, 131986-28-2; **4a**, 131987-61-6; **4b**, 131987-62-7; **4c**, 131987-63-8; **4d**, 131987-64-9; **4e**, 131987-66-1; **4f**, 131987-69-4; **4g**, 141064-09-7; **4h**, 131987-78-5; **4i**, 141064-10-0; **4j**, 141064-11-1; **4k**, 141064-12-2; **4l**, 141064-13-3; **5a**, 131986-29-3; **5a** oxalate, 141064-18-8; **5b**, 131986-31-7; **5b** oxalate, 141064-19-9; **5c**, 131986-33-9; **5c** oxalate, 141064-20-2; **5d**, 131986-35-1; **5d** oxalate, 141064-21-3; **5e**, 131986-39-5; **5e** oxalate, 141064-22-4; **5f**, 131986-45-3; **5f** oxalate, 141064-23-5; **5g**, 131986-66-8; **5g** oxalate, 141064-24-6; **5h**, 131986-74-8; **5h** oxalate, 141064-25-7; **5i**, 141064-14-4; **5i** maleate, 141064-26-8; **5j**, 141064-15-5; **5j** fumarate, 141064-27-9; **5k**, 141064-16-6; **5k** fumarate, 141064-28-0; **5l**, 141064-17-7; **5l** fumarate, 141064-29-1; **6a**, 131988-00-6; **6b**, 131988-09-5; **6c**, 131987-98-9; **6d**, 131987-99-0; **6e**, 141064-30-4; **6f**, 131988-10-8; **6g**, 141064-31-5; **6h**, 131988-08-4; **7a**, 131987-18-3; **7a** oxalate, 141064-32-6; **7b**, 131987-50-3; **7b** oxalate, 141064-33-7; **7c**, 131987-14-9; **7c** oxalate, 141064-34-8; **7d**, 131987-16-1; **7d** oxalate, 141064-35-9; **7e**, 131987-52-5; **7e** oxalate, 141064-36-0; **7f**, 131987-54-7; **7f** oxalate, 141064-37-1; **7g**, 141064-38-2; **7g** oxalate, 141064-39-3; **7h**, 131987-48-9; **7h** oxalate, 141064-40-6; **8**, 131986-59-9; **8** oxalate, 141064-41-7; **9c**, 131987-46-7; **9c** oxalate, 141064-42-8; **9e**, 131987-38-7; **9e** oxalate, 141064-43-9; **9g**, 131987-34-3; **9g** oxalate, 141064-44-0; **9h**, 131987-40-1; **9h** oxalate, 141064-45-1; **9m**, 141064-46-2; **9m** oxalate, 141064-47-3; **9n**, 141064-48-4; **9n** oxalate, 141064-49-5; **10**, 131987-88-7; **11**, 131986-94-2; **11** oxalate, 141064-50-8; **12f**, 131987-97-8; **13f**, 131987-12-7; **13f** oxalate, 141088-19-9; **14**, 6443-85-2; **15**, 67936-83-8; **15**·Na, 141064-51-9; **16**, 131988-01-7; **17**, 131988-04-0; **18a**, 131988-06-2; **18b**, 131988-05-1; **19a**, 131987-30-9; **19a** oxalate, 141064-52-0; **19b**, 131987-28-5; **19b** oxalate, 141064-53-1; MeOH, 67-56-1; EtOH, 64-17-5; PrOH, 71-23-8; BuOH, 71-36-3; $CH_3(CH_2)_4OH$, 71-41-0; $CH_3(CH_2)_5OH$, 111-27-3; $CH_3(CH_2)_6OH$, 111-70-6; $CH_3(CH_2)_7OH$, 111-87-5; $CH_3CH(CH_3)(CH_2)_3OH$, 626-89-1; $CH_3CH_2CH(CH_3)(CH_2)_2OH$, 589-35-5; $CH_3(CH_2)_2CH(CH_3)CH_2OH$, 105-30-6; $CH_3(CH_2)_3CH(CH_3)OH$, 626-93-7; PrMgBr, 927-77-5; $CH_3(CH_2)_4MgBr$, 693-25-4; $CH_3(CH_2)_6MgBr$, 13125-66-1; $CH_3(CH_2)_7MgBr$, 17049-49-9; $CH_3(CH_2)_5NH_2$, 111-26-2; 3-pyridinecarboxaldehyde, 500-22-1; α-oximido-3-pyridylacetamidoxime, 131988-62-0.

Nucleic Acid Related Compounds. 74. Synthesis and Biological Activity of 2′(and 3′)-Deoxy-2′(and 3′)-methylenenucleoside Analogues That Function as Mechanism-Based Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase and/or Ribonucleotide Reductase[1]

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Treatment of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (**21**) with TBDMS chloride/imidazole/DMF gave a separable mixture of 5′-*O*, 2′,5′-bis-*O* (**22**), 3′,5′-bis-*O* (**23**), and 2′,3′,5′-tris-*O*-TBDMS derivatives. Oxidation of **22** and **23** with CrO_3/pyridine/Ac_2O, treatment of the respective ketonucleosides with methylenetriphenylphosphorane, and deprotection gave 2-amino-6-chloro-9-[3(and 2)-deoxy-3(and 2)-methylene-β-D-*erythro*-pentofuranosyl]purines (**28** and **37**) that were converted into other 2-amino-6-substituted-purine analogues. Tubercidin was converted into 2′-deoxy-2′-methylenetubercidin (**49**) by an analogous route. Inactivation of *S*-adenosyl-L-homocysteine hydrolase by 2′- and 3′-methyleneadenosine analogues was investigated. Mechanism-based inhibition of *S*-adenosyl-L-homocysteine hydrolase and anticancer and antiviral activities of 2′(and 3′)-deoxy-2′(and 3′)-methylenenucleoside analogues are discussed.

Of the plethora of compounds investigated as anticancer and antiviral agents, significant activity has been demonstrated with a remarkable number of analogues and derivatives of nucleic acid components. Recent reviews of

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