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IDENTIFICATION OF SIDE CHAINS ON 1,2,5-THIADIAZOLE-AZACYCLES OPTIMAL FOR MUSCARINIC M1 RECEPTOR ACTIVATION.

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Abstract: Series of analogs to the functional m1 selective agonist, xanomeline (hexyloxy-TZTP), were evaluated for their in vitro m1 efficacy in cell lines transfected with the human m1 receptor. Systematic variation of the side chain and the azacyclic ring led to the discovery of potent muscarinic agonists with robust m1 efficacy, all having the phenylpropargyloxy/thio as the side chain. The most selective compound was the phenylpropargylthio-[3.2.1] endo analog **28**, which is a potent and efficacious m1 agonist with no m2 activity.

Over the last decade many research groups have tried to identify potent m1 selective muscarinic agonists for the treatment of Alzheimer's disease. This research effort was based on the muscarinic cholinergic hypothesis of Alzheimer's disease, suggesting that a muscarinic m1 agonist would substitute for the decreased amount of acetylcholine and activate the m1 receptors involved with learning and memory.[1]

The challenge has been to identify muscarinic agonists with a robust m1 response but lacking the side effects associated with activation of especially m2 and m3 muscarinic receptor subtypes. The effects associated with activation of the remaining two muscarinic receptor subtypes, m4 and m5, is still under debate. The optimal solution to the problem would of cause be to develop agonists with affinity only for the m1 receptor subtype. In spite of many attempts, no affinity selective receptor subtype agonists have, however, been reported. Instead functional m1 selective agonists have been pursued.[2] The problem involved with functional selective agonists has been that the m1 efficacy often has decreased significantly in the attempts to reduce the m2 and m3 efficacy and potency.[3] It was therefore decided to identify a group of compounds with a robust m1 response before optimizing the m1 selectivity.

The present paper describes the m1 Structure Activity-Relationships (SAR) around xanomeline (3-(3-hexyloxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine, hexyloxy-TZTP, **6**)[4] leading to the identification of phenylpropargyloxy/thio as a side chain giving robust m1 efficacy.

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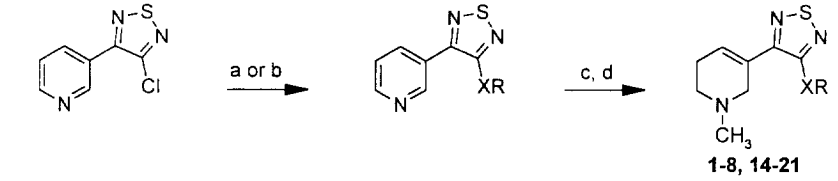
Biological evaluation. Measurement of stimulation of phosphoinositide hydrolysis (PI) in BHK cells transfected with the human m1 receptor was used as the primary efficacy model. This cell line was chosen because it has relatively few spare receptors, and therefore only full agonists will give a full response.[4] Compounds were tested at two relatively high concentrations (30 μM and 100 μM), and the highest % PI relative to 100 μM carbachol was recorded. Affinity for muscarinic receptors of rat brain membranes using the muscarinic agonist oxotremorine-M (Oxo-M) as ligand was also measured.[5] The Oxo-M data was however not used to direct the chemistry, since neither the Oxo-M affinity nor the QNB/Oxo-M ratio predicted the m1 efficacy of the compounds.[6] Selected compounds were further characterized for efficacy and potency in A9L and CHO cell lines transfected with human m1 and m2 receptors, respectively.[4]

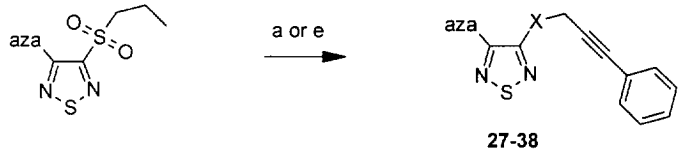
Table 1

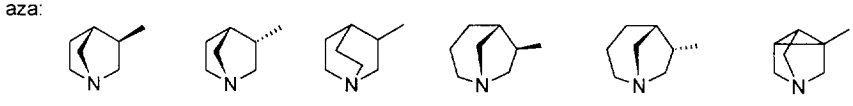
No	R	Oxo-M IC_{50}, nM[a]	% max PI[b]
1	O-Me	22±0.08	7
2	O-Et	5.7±0.24	0
3	O-nPr	1.6±0.06	25
4	O-nBu	1.4±0.12	13
5	O-nPen	2.0±0.25	29
6	O-nHex	9.7±1.1	63
7	O-nHep	6.2±0.02	45
8	O-nOct	30±7.5	20

[a] Data from ref. 5. [b] m1 efficacy data in BHK cells from 1-2 experiments performed in triplicate.

Reaction scheme.



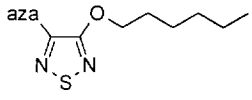




a) NaOR/THF (X=O)[12]; b) NaSH/DMF; R-Halogen, K_2CO_3/DMF (X=S); c) MeI/acetone; d) $NaBH_4$/EtOH; e) NaSH, R-OSO_2Me, K_2CO_3/DMF (X=S)[11]

Chemistry. The TZTP analogs, **1-8**, **14-21**, were synthesized according to earlier published procedures[5] starting with the readily available 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine (see top part of reaction scheme). Displacement of the chlorine by either sodium alkoxide or sodium hydrosulfide followed by alkylation with alkyl halide gave the alkoxy- or the alkylthio-1,2,5-thiadiazole-pyridine analogs, respectively. Quaternization with methyl iodide followed by sodium borohydride reduction gave the desired TZTP products. The azabicyclic analogs were synthesized by a similar procedure starting from the butyl sulfonyl intermediates[7] (see lower part of reaction scheme). The alkyl sulfonyl is a better leaving group than chlorine and the substitution reaction can therefore be conducted under milder conditions. Especially in the reaction with sodium alkoxide, the sulfonyl derivative gives a better yield than the corresponding chlorine analog. The phenylpropargylthio analogs were prepared using the mesylated alkohol instead of phenylpropargyl halide.

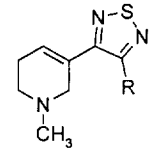
Table 2.



No	aza-ring	Oxo-M IC_{50},nM[a]	% max PI[b] m1
6	THP	10	63
9	[2.2.2]	4.3	55
10	[3.2.1] exo	1.2	40
11	[3.2.1] endo	63	12
12	[2.2.1] exo	0.73	59
13	[2.2.1] endo	16	14

[a]Geometric mean of 2-3 independent experiments performed in triplicate. [b]See table 1.

Table 3.



No	R	Oxo-M IC_{50},nM[a]	% max PI[b] m1
14	O-CH_2Ph	8	12
15	O-$(CH_2)_2$Ph	15	16
16	O-$(CH_2)_3$Ph	13	25
17	O-$(CH_2)_4$Ph	20	47
18	O-$CH_2C{\equiv}CPh$	8.5	90
19	S-CH_2Ph	5.9	10
20	S-$(CH_2)_2$Ph	23	9
21	S-$(CH_2)_3$Ph	1.3	51

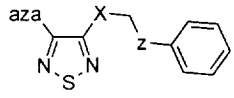
[a]See table 2. [b]See table 1.

Results and discussion. We have earlier published on the SAR of alkoxy-TZTPs using the isolated vas deferens preparation as the M1 model.[5] The SAR in that model identified hexyloxy-TZTP (**6**) as having the highest M1 receptor efficacy and selectivity in the alkoxy-TZTP series. The % stimulation of PI hydrolysis in cloned m1 cell lines gave the same SAR for the alkoxy-TZTP series (table 1), where increasing carbon atom chain lengths gave increasing % PI up to hexyloxy. Increasing the size of the side chain to heptyl/octyloxy (**7**/**8**) decreased the m1 efficacy.

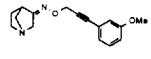
The optimal hexyloxy side chain from the TZTP series was then introduced in azabicyclic analogs for further SAR investigations (table 2). The results showed that exchanging the N-methyltetrahydropyridine (THP, **6**) ring with various azabicyclic ring systems (**9-13**) did not improve the m1 efficacy; especially the endo analogs, **11** and **13**, gave low activation of PI hydrolysis. The THP ring was therefore considered the optimal azacycle. Similar SAR analysis has previous been published on the analogous alkylthio series.[6]

More recently, the SAR on the side chains of TZTP was expanded to include more bulky substituents. This approach was taken in an attempt to improve both the m1 selectivity compared to xanomeline and the oral bioavailability. Xanomeline is first pass metabolized primarily by oxidation of the hexyloxy side chain, and it was speculated that the phenyl group would block that metabolism. Benzyloxy-TZTP (**14**) had no significant effect on PI hydrolysis (table 3), but increasing the number of methylene groups between the phenyl group and the oxygen atom (**15-17**) resulted in an increased m1 efficacy. The same SAR was observed for the bulky alkylthio analogs, where the phenylpropylthio-TZTP analog (**21**) gave 51% PI hydrolysis (table 3). A dramatic effect on the efficacy was further observed when a triple bound was introduced in the side chain, as in phenylpropargyloxy-TZTP (**18**). A similar increase in potency and efficacy, obtained by incorporation of a triple bound in side chains, were reported in the literature for arecoline[8] and for alkoxy-THPO analogs.[9] The phenylpropargyloxy moiety was also used in series of muscarinic agonists of the 3-azabicyclic-oximether type compounds, represented by PD151832 (table 4).[10]

Table 4.



No	aza-ring	X	z	Oxo-M[a] IC_{50},nM	% max PI BHK[b] m1	% max PI A9L[c], m1	EC_{50} nM A9L[c], m1	%max cAMP CHO[c], m2	EC_{50}, nM CHO[c], m2	potency, m2/m1 ratio
21	THP	S	$(CH_2)_2$	1.3	51					
22	[2.2.2]	S	$(CH_2)_2$	14	34					
23	[3.2.1] exo	S	$(CH_2)_2$	1.3	46					
24	[3.2.1] endo	S	$(CH_2)_2$	35	40					
25	[2.2.1] exo	S	$(CH_2)_2$	1.7	63					
26	[2.2.1] endo	S	$(CH_2)_2$	6	65					
27	[3.2.1] exo (R,R)	S	C≡C	1.7	135	105	0.2	109	2	10
28	[3.2.1] endo (R,S)	S	C≡C	363	128	98	1.0	-	>10000	>10000
29	[2.2.1] endo (+)	S	C≡C	42	56	70	>100	49	139	<1
18	THP	O	C≡C	8.5	90	111	110	115	240	2
30	[2.2.2]	O	C≡C	4.7	81	87	15.7	94	62	4
31	[3.2.1] exo (R,R)	O	C≡C	1.2	54	99	0.5	106	9	18
32	[3.2.1] exo (S,S)	O	C≡C	3.4	127	nt	nt	102	29	-
33	[3.2.1] endo (R,S)	O	C≡C	66	84	93	28.3	89	227	8
34	[3.2.1] endo (S,R)	O	C≡C	78	30	nt	nt	41	2253	-
35	[2.2.1] exo	O	C≡C	0.8	156	102	0.99	104	11	11
36	[2.2.1] endo (+)	O	C≡C	41	66	77	159	52	1075	7
37	[2.2.1] endo (-)	O	C≡C	20	69	59	67.3	82	1096	16
38	[2.2.1.0]	O	C≡C	14	49	96	70.5	99	779	11
	Carbachol			23	100	102	3811	100	700	0.2
	Arecoline			24	25	51	5603	119	10000	2
	Pilocarpine			68	27	52	8792	59	63000	7
	PD151832			167	14	27	>1000	100	28000	<28



[a]See table 2. [b]See table 1. [c]Geometric mean of 3 independent experiments performed in triplicate in A9L m1 and in CHO m2 cell lines. Standard error of mean within 15%.

Again the side chains giving robust PI response in the TZTP series were incorporated in the azabicyclic analogs (table 4). The phenylpropylthio azabicyclic analogs (**22-26**) all had some m1 efficacy, although lower than the THP lead structure (**21**). Interestingly the endo compounds **24** and **26** had higher efficacy than the corresponding hexyloxy analogs, **11** and **13**.

The phenylpropargylthio/oxy side chain further increased the m1 response for the azabicyclic and tricyclic analogs (**27-38**). Due to the promising m1 screening results, most of the analogs were prepared as pure enantiomers.[7] In order to further characterize the phenylpropargyl analogs, dose response curves were generated in both an A9L m1 cell line with more spare receptors as well as in a CHO m2 cell line. The data showed that all the prepared analogs, **27-38**, were much more potent agonists than both the structurally related analog PD 151832 and the standard muscarinic agonists, carbachol, arecoline and pilocarpine (table 4). The most potent compounds, **27**, **28**, **31** and **35**, were more than 3000 times more potent than carbachol on PI in m1 cells. Many of the test compounds were also full m2 agonists, but the endo analogs **29**, **34** and **36** were only partial m2 agonists, and the (R,S) [3.2.1] endo analog, **28,** had no m2 efficacy at all.

Further biological evaluations are needed to determine the drug potential of **28**. However, the screening data suggest, at least, that the endo (5R,6S) 6-(3-(3-phenyl-2-propyn-1-ylthio)-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane analog provides an excellent starting point for identifying a functional selective m1 agonist.

Conclusions. The m1 screening data shows that the phenylpropargyloxy/thio side chain gives 1,2,5-thiadiazole-azabicyclic analogs with a more robust m1 response, compared to the corresponding hexyloxy analogs. Based on the extensive SAR of the 1,2,5-thiadiazole-azacyclic muscarinic ligands, it is clear that the m1 receptor subtype has a large lipophilic pocket and occupation of this pocket is essential for m1 activation. The phenylpropargylthio/oxy side chain seems to fit especially well into this cavity. The endo (5R,6S) 6-(3-(3-phenyl-2-propyn-1-ylthio)-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane analog, **28**, is a potent and efficacious m1 agonist with no m2 activity, suggesting that **28** could be a drug candidate.

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11. Reaction conditions for synthesis of phenylpropargylthio azabicyclic analogs exemplified by endo (5R,6S) 6-(3-(3-phenyl-2-propyn-1-ylthio)-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane, L-tartrate (**28**): Endo (5R,6S) 6-(3-propylsulfonyl-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane[7] (0.5 g, 1.66 mmol) and sodium hydrosulfide monohydrate (0.4 g, 5.3 mmol) were dissolved in dry DMF (50 ml) and the reaction mixture was refluxed at 100 °C for 3 hours (or to completion of reaction). After cooling to 0 °C potassium carbonate (0.9 g, 6.6 mmol) and 3-phenyl-2-propyn-1-yl methylsulfonate (1.0 g, 4.76 mmol) were added. The reaction mixture was stirred at room temperature for 4 hours (or to completion of reaction), after which 1N hydrogen chloride was added to pH 2. The aqueous phase was first washed with ether (2x50 ml), then potassium carbonate was added to pH>10 and finely the water phase was extracted with ether (2x100 ml). The combined and dried ether phases were evaporated and the residue (0.4 g) was crystallized with L-tartaric acid (0.2 g, 1.3 mmol) from isopropanol to give the title compound in 0.53 g (65%) yield. ^{1}H-NMR (DMSO) δ: 7.38 (5H, s), 4.45 (2H, s), 4.0-3.87 (4H, m), 3.80 (1H, t), 3.43-3.37 (1H, m), 3.33-3.16 (3H, m), 3.03 (1H, br. s), 1.97-1.82 (1H, m), 1.70-1.60 (1H, m), 1,45-1.37 (1H, m), 1.07-1.00 (1H, m). Mp 176-177 °C. **27**, tartrate: ^{1}H-NMR (DMSO) δ: 7.36 (5H, s), 4.44 (2H, s), 4.00 (2H, s), 3.82-3.70 (2H, m), 3.65 (1H, t), 3.23-3.50 (4H, m), 2.57 (1H, br. s), 2.15-1.90 (1H, m), 1.86-1.65 (3H, m).Mp 192.0-192,5 °C. **29**, tartrate: ^{1}H-NMR (DMSO) δ: 7.36 (5H, s), 7.4-6.8 (2H, br. s), 4.43 (2H, s), 4.08 (2H, s), 3.86-3.74 (1H, m), 3.15-3.39 (2H, m), 3.30-3.10 (3H, m), 3.09-2.90 (2H, m), 1.75-1.55 (1H, m), 1.20-1.05 (1H, m). Mp 170-172 °C.
12. Reaction conditions for synthesis of the phenylpropargyloxy azabicyclic analogs exemplified by exo (5R,6R) 6-(3-(3-phenyl-2-propyn-1-yloxy)-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane, oxalate (**31**): To a mixture of sodium hydride (60% in mineral oil, 0.6 g, 15 mmol) in THF (59 ml) was added 3-phenyl-2-propyn-1-ol (0.62 g, 4.7 mmol) at room temperature and the reaction mixture was stirred for 1 hour. After cooling to 0 °C, exo (5R,6R) 6-(3-propylsulfonyl-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane[7] (0.5 g, 1.66 mmol) was added. The reaction mixture was stirred for 5 hours at room temperature and at 5 °C overnight. Water (50 ml) was added and the reaction mixture was extracted with ether (3x100 ml). The ether phases were evaporated and the residue was dissolved in 1N hydrochloric acid (20 ml). The water phase was washed with ether (100 ml), made basic with 25% aqueous ammonia and then extracted with ether (2x100 ml). The ether phases were dried and evaporated to give crude product. Purification on column chromatography eluting with ethyl acetate:methanol:25%aq.NH_3 (2:1:0.06) gave the desired free base product as an oil. Crystallization with oxalic acid from acetone gave the title compound in 560 mg (78%) yield. ^{1}H-NMR (DMSO) δ: 8.5-7.9 (2H, br. s), 7.55-7.37 (5H, m), 5.36 (2H, s), 3.85-3.73 (2H, m), 3.65 (1H, t), 3.28-3.14 (4H, m), 2.70 (1H, br. s), 2.15-1.90 (1H, m), 1.82-1.66 (3H, m). Mp 120-125 °C. **32**, oxalate: ^{1}H-NMR (DMSO) δ: 10.5-9.0 (2H, br. s), 7.55-7.35 (5H, m), 5.40 (2H, s), 3.85 (2H, d), 3.66 (1H, t), 3.32-3.15 (4H, m), 2.75 (1H, br. s),2.20-1.95 1H, m), 1.85-1.65 (3H, m). Mp 132-135 °C. **33**, oxalate: ^{1}H-NMR (DMSO) δ: 8.3-7.6 (2H, br. s), 5.90 (2H, s), 4.02-3.80 (3H, m), 3.54-3.17 (4H, m), 3.00 (1H, br. s), 1.95-1.4 (3H, m), 1.30-1.05 (1H, m). Tartrate: Mp 163-165 °C. **34**, oxalate: ^{1}H-NMR (DMSO) δ: 8.3-7.6 (2H, br. s), 5.90 (2H, s), 4.02-3.80 (3H, m), 3.54-3.17 (4H, m), 3.00 (1H, br. s), 1.95-1.4 (3H, m), 1.30-1.05 (1H, m). Tartrate: Mp 155-161 °C. **30**, oxalate: ^{1}H-NMR (DMSO) δ: 7.50-7.34 (5H, m), 5.36 (2H, s), 5.7-4.1 (2H, br. s), 3.75-3.58 (3H, m), 3.39-3.10 (4H, m), 2.36 (1H, br. s), 2.10-1.90 (2H, m), 1.75-1.60 (2H, m). Mp 91-94 °C. **35**, tartrate: ^{1}H-NMR (DMSO) δ: 7.51-7.35 (5H, m), 5.56 (2H, s), 4.04 (2H, s), 3.54-3.27 (2H, m), 3.25-2.88 (5H, m), 2.87-2.75 (1H, m), 2.00-1.80 (1H, m), 1.73-1.54 (1H, m). Mp 159-162 °C. **36**, tartrate: ^{1}H-NMR (DMSO) δ: 7.50-7.35 (5H, m), 5.88 (2H, s), 4.02 (2H, s), 3.78-3.69 (1H, m), 3.60-3.50 (1H, m), 3.45-3.36 (1H, m), 3.25-3.07 (3H, m), 3.05-2.92 (2H, m),1.77-1.63 (1H, m), 1.29-1.18 (1H, m). Mp 127-130 °C. **37**,tartrate: ^{1}H-NMR (DMSO) δ: 7.50-7.36 (5H, m), 7.5-6.5 (2H, br. s), 5.88 (2H, s), 4.07 (2H, s), 3.83-3.74 (1H, m), 3.68-3.54 (1H, m), 3.50-3.40 (1H, m), 3.32-2.95 (5H, m), 1.82-1.67 (1H, m), 1.33-1.20 (1H, m). Mp 150-153 °C. **38**, oxalate: ^{1}H-NMR (DMSO) δ: 7.50-7.46 (5H, m), 5.32 (2H, s), 3.33 (2H, s), 3.10 (2H, d), 3.03 (2H, d), 2.52 (2H, s). Mp 182-185 °C.