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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF HETEROCYCLIC ANALOGUES OF THE FUNCTIONAL M_1 SELECTIVE MUSCARINIC AGONIST HEXYLOXY-TZTP

Per Sauerberg[+*], Preben H. Olesen[+], Peter D. Suzdak[+], Malcolm J. Sheardown[+], Charles H. Mitch[#], Steven J. Quimby[#], John S. Ward[#], Frank P. Bymaster[#], Barry D. Sawyer[#], and Harlan E. Shannon[#]

[+]Novo Nordisk CNS Division
Novo Nordisk Park
DK-2760 Måløv, Denmark

[#] The Lilly Research Laboratories
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285, USA

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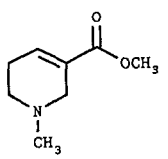
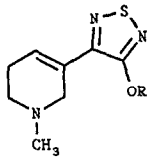
Abstract: Isothiazole (**8a,b**), isoxazole (**14**) and thiophene (**19a,b**) analogues of the potent, M_1 selective muscarinic agonist, hexyloxy-TZTP, **1b**, were synthesized and tested *in vitro* for muscarinic receptor affinity and M_1 efficacy. All the analogues had lower affinity and efficacy at the M_1 muscarinic receptors than **1b**.

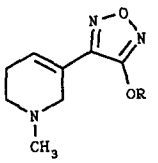
Presynaptic muscarinic cholinergic degeneration together with an unchanged postsynaptic M_1 receptor density in the brain of patients with Alzheimer's disease has led to attempts to develop a cholinomimetic symptomatic treatment for senile cognitive decline[1]. Traditional muscarinic agonists have, however, little or no muscarinic receptor subtype selectivity thus limiting their clinical usefulness due to side effects associated with stimulation of the M_2 and M_3 muscarinic receptors[2]. Consequently, the desire to design an M_1 selective and efficacious muscarinic agonist has attracted increased interest.



Arecoline



Alkoxy-TZTP (1)



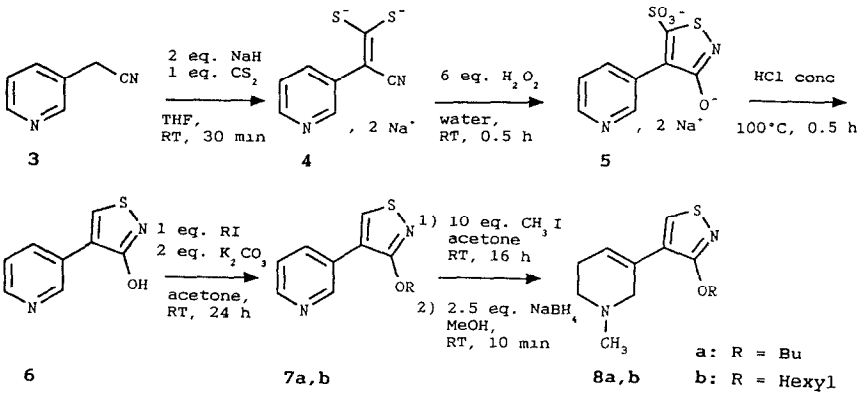
Alkoxy-OTZP (2)

We have recently reported on alkoxy-TZTP (3-(3-Alkoxy-1,2,5-ThiadiaZol-4-yl)-1,2,5,6-Tetrahydro-1-methylPyridine) (**1**), a novel class of potent, functionally M_1 selective muscarinic agonists capable of crossing the blood brain barrier[3]. In this previous paper we concentrated on structure-activity relationships (SAR) of the alkoxy substituent and to a small degree on the heterocyclic effect, as in alkoxy-OTZP (**2**). We now report on a more complete M_1 SAR of the aromatic heterocyclic group.

Based on the finding that C_{4-6} alkoxy-TZTP were the most M_1 selective agonists, butoxy and hexyloxy were chosen as model side chains. By systematic replacement of one or two nitrogens with carbon in the 1,2,5-thiadiazole ring in **1** we designed the corresponding isothiazole (**8a,b**) and thiophene (**19a,b**) analogous, respectively. The isoxazole (**14**) arose from the oxadiazole **2**. This group of compounds would give information on the importance of the nitrogens in the five membered aromatic heterocycle.

The 3-(3-alkoxy-isothiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines (**8a,b**) were synthesized as outlined in Scheme 1[4]. 3-Pyridylacetonitrile (**3**) was treated with carbon disulfide and sodium hydride to give the crude disodium disulfide salt **4**. Oxidation of **4** by hydrogen peroxide gave the isothiazole disodium salt **5**, which was converted to the 3-hydroxy- isothiazole **6** upon treatment with hydrochloric acid. Alkylation of the hydroxy function with alkyl halide in the presence of potassium carbonate gave the alkoxy derivative **7a,b**, which under standard conditions[3] were quaternized with methyl iodide and reduced by sodium borohydride to give the desired tetrahydro-1-methylpyridine analogues (**8a,b**).

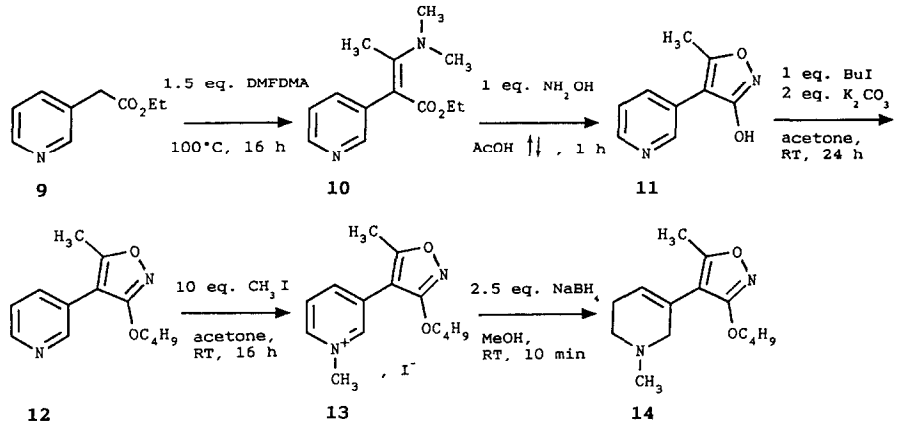
Scheme 1.



The 3-(3-Butoxy-5-methylisoxazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine analogue was synthesized as shown in Scheme 2[5]. Ethyl 3-pyridylacetate (**9**) was treated with N,N-dimethylacetamide dimethyl acetal to give the corresponding enamine **10**. Cyclization of **10** with hydroxylamine gave the 3-hydroxy-5-methylisoxazole, **11**. Alkoxy formation (**12**), quaternization (**13**) and reduction (**14**) was performed as described under Scheme 1.

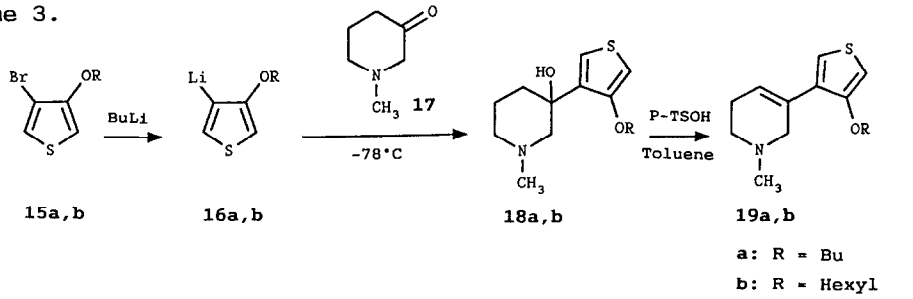
Compound **14** was only synthesized in mg scale and the binding results (Table 1) did not encourage resynthesis.

Scheme 2.



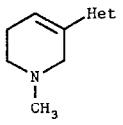
The synthesis of the 3-(3-alkoxy-4-thienyl)-1,2,5,6-tetrahydro-1-methylpyridines (**19a,b**) was accomplished as shown in Scheme 3. 3-Bromo-4-alkoxythiophene[6] was treated with butyl lithium at -78°C to give the bromo/lithium exchanged product (**16a,b**), which upon addition of 1-methyl-3-piperidone (**17**) gave the 3-hydroxy-3-thienyl (**18a,b**) compound. The alcohols were then dehydrated with p-toluenesulfonic acid to yield the desired analogues **19a,b**.

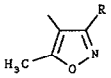
Scheme 3.



The affinity of the compounds for central muscarinic receptor sites was determined by in vitro receptor binding to rat brain membranes. The ability of the compounds to displace tritiated oxotremorine-M (Oxo-M), a potent nonselective muscarinic agonist, was interpreted as the affinity for the agonist conformational state of the muscarinic receptor sites[7]. Displacement of tritiated pirenzepine (Pz), a selective M_1 antagonist, was used to estimate the affinity for M_1 receptor sites in hippocampus[3]. Two in vitro functional M_1 models have in the literature been used to evaluate the M_1 efficacy and potency of muscarinic ligands: the rat superior cervical ganglion[8] and the rabbit vas deferens[9]. We chose to use the inhibition of the twitch response in the electrically stimulated rabbit vas deferens, since this model in our hands had the highest sensitivitify for M_1 agonist activity.

TABLE 1.



Compound	Het	R	Receptor binding to rat brain membranes[a]: IC_{50}, nM [^{3}H]Oxo-M	[^{3}H] Pz	Inhibition of twitch height in rabbit vas deferens[b] IC_{50}, nM	% max
1a		$O(CH_2)_3CH_3$	1.4	5	15	68
1b		$O(CH_2)_5CH_3$	9.7	7	0.01	88
2a		$O(CH_2)_3CH_3$	24	345	-	12
2b		$O(CH_2)_5CH_3$	70	277	(10000)	47
8a		$O(CH_2)_3CH_3$	544	565	-	18
8b		$O(CH_2)_5CH_3$	>900	715	-	43
14		$O(CH_2)_3CH_3$	759	n.t.	n.t.	n.t.
19a		$O(CH_2)_3CH_3$	477	369	-	18
19b		$O(CH_2)_5CH_3$	>900	663	39000	93
	Arecoline		77	1300	545	95
	McN-A-343		355	955	659	91

[a] IC_{50} values were calculated from four concentrations in triplicate. The maximal SEM for the binding IC_{50} values is 15%.

[b] Cumulative concentration effect curves (up to 1 μM) in 2 tissues. If at least 50% inhibition, 4 additional tissues were used. The maximal SEM for the % max values is 20%.

n.t. = not tested

Within each of the aromatic heterocycles tested the butoxy analogue (**1a**, **2a**, **8a** and **19a**) had higher affinity for the Oxo-M labelled binding site than the corresponding hexyloxy analogue (**1b**, **2b**, **8b** and **19b**) (Table 1). A similar tendency was observed for the affinity for the Pz labelled binding site, but the differences in affinity between the butoxy and the hexyloxy analogues were smaller, and for some compounds within the experimental error. However, in the vas deferens assay all the hexyloxy derivatives were more efficacious than the corresponding butoxy analogues.

Replacement of either one or two nitrogens in the 1,2,5-thiadiazole ring, as in **8a,b** or **19a,b**, reduced the affinity for the muscarinic agonist conformational state (Table 1) approximately 350 fold (**1a** -> **8a** or **19a**). The affinity for M_1 receptors was also reduced, but only about 100 fold. Neither of the compounds **8a,b** and **19a** were efficacious enough at the M_1 receptor to inhibit the twitch height in rabbit vas deferens by 50%. And even though **19b** could inhibit the twitch height by 93%, extremely high doses were required. A similar reduction in muscarinic receptor affinity was observed when the N_5-nitrogen in 1,2,5-oxadiazole, **2a**, was replaced by carbon as in **14**. However, the Oxo-M binding ability of **14** was not reduced as dramatically compared to **2a** as **8a** compared to **1a**. Basically because **2a** had much lower affinity for the Oxo-M binding site than **1a**.

These results (Table 1) show that the 1,2,5-thiadiazole moiety is largely responsible for the high M_1 receptor affinity and efficacy. Alteration of the aromatic heterocycle led to compounds with lower affinity. The sulfur atom in the 1,2,5-thiadiazole is apparently important for the receptor interaction, since the 1,2,5-oxadiazoles (**2a,b**) have much lower M_1 receptor affinity. The nitrogens, or at least the N_5-nitrogen is however also very important for optimal receptor recognition. The exchange of the N_5 nitrogen for carbon as in the isothiazoles (**8a,b**) caused an even bigger decrease in muscarinic receptor affinity than the sulfur/oxygen exchange. Exchange of the second nitrogen as in the thiophenes (**19a,b**) did not alter the receptor affinity significantly, indicating that the N_5 nitrogen perhaps is more important for receptor interaction than the N_3 nitrogen. The isomeric isothiazoles could therefore be interesting compounds. For the 1,2,4-oxadiazole muscarinic ligands a correlation between the electrostatic potentials adjacent to the nitrogens and the receptor affinity has been demonstrated[10]. Hydrogen binding to both of the nitrogens in the 1,2,5-thiadiazole ring from two amino acids in the M_1 receptor is therefore likely to exist[11]. Steric properties of the aromatic heterocycle and the side chains can however not be ruled out.

For the 1,2,4-thiadiazole ligands it was concluded that the methyl group was the preferred size of lipophilic substituent for binding to the high affinity state of the receptor[12]. But for the TZTP's we found the C_{4-6} alkoxy to be the optimal size for M_1 receptor affinity and efficacy[3]. This could indicate that the TZTP-substituent is localized in a significantly different position in space when interacting with the receptor than the 1,2,4-thiadiazole substituent, and that the M_1 receptor therefore can accommodate the more bulky substituent. In fact we suggest that the muscarinic receptor subtype efficacy is dependent on the steric interaction between the receptor and the alkoxy substituent, whereas the muscarinic receptor affinity is a result of correctly orientated electrostatic interaction between the five membered aromatic heterocycle and the receptor.

The SAR of the five membered aromatic heterocycles supports the hypothesis that the 1,2,5-thiadiazole moiety is a unique isostere to the arecoline ester functionality.

References and Notes

1. See Introduction to this Symposium-in-Print.
2. Receptor subtype nomenclature according to recommendation in *Trends Pharmacol. Sci. Suppl.* **1989**, VII.
3. Sauerberg, P., Olesen, P.H., Nielsen, S., Treppedahl, S., Sheardown, M.J., Honoré, T., Mitch, C.H., Ward, J.S., Pike, A.J., Bymaster, F.P., Sawyer, B.D., Shannon, H.E., *J. Med. Chem.* **1992**, 35, 2274.
4. Modification of procedure described by Davis, M., Dereani, M.C., McVicars, J.L., Morris, I.J., *Aust. J. Chem.* **1977**, 30.
5. Modification of procedure described by Lin, Y., Lang, S.A., *J. Org. Chem.* **1980**, 4857.
6. Prepared analogous to method described by Corral, C., El-Ashawy, B.M., Lissavetzky, J., Basilo, A., Giraldez, A., *Eur. J. Chem.* **1987**, 22, 251.
7. Sauerberg, P., Kindtler, J.W., Nielsen, L., Sheardown, M.J., Honoré, T., *J. Med. Chem.* **1991**, 34, 687.
8. Shapiro, G., Floersheim, P., Boelsterli, J., Amstutz, R., Bolliger, G., Gammenthaler, H., Gurelin, G., Supavilai, P., Walkinshaw, M., *J. Med. Chem.* **1992**, 35, 15.
9. Eltze, M., *Eur. J. Pharmacol.* **1988**, 151, 205.
10. Saunders, J., Cassidy, M., Freedman, S.B., Harley, E.A., Iversen, L.L., Kneen, C., MacLeod, A.M., Merchant, K.J., Snow, R.J., Baker, R., *J. Med. Chem.* **1990**, 33, 1128.
11. Molecular modeling calculations will be published in near future.
12. MacLeod, A.M., Baker, R., Freedman, S.B., Patel, S., Merchant, K.J., Roe, M., Saunders, J., *J. Med. Chem.* **1990**, 33, 2052.