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DISCOVERY OF A HIGHLY POTENT, FUNCTIONALLY-SELECTIVE MUSCARINIC M_1 AGONIST,WAY-132983 USING RATIONAL DRUG DESIGN AND RECEPTOR MODELLING

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Abstract: Rational drug design utilizing a receptor homology model of the human muscarinic M_1 receptor led to the discovery of the highly potent (K_i = 2 nM), efficacious, and in vivo functionally-selective M_1 agonist, WAY-132983.

Introduction

The "cholinergic hypothesis"[1] states that memory loss due to decreased levels of acetylcholine, characteristic of Alzheimer's Disease (AD), can be ameliorated by correcting the levels of this neurotransmitter in the brain. The only approved cholinergic drugs for this disease are acetylcholinesterase inhibitors, which rely on the activity of viable pre-synaptic acetylcholine neurons that continue to degenerate in AD. Not surprisingly, the clinical efficacy of cholinesterase inhibitors in AD is not robust. In contrast, direct stimulation of muscarinic M_1 cholinergic receptors (mAChR) is expected to provide greater benefit than acetylcholinesterase inhibitors, since M_1 receptors reside on post-synaptic neurons that are not as seriously impacted by neurodegeneration processes in AD.[2] Selectivity of the M_1 agonist effect in vivo is necessary to avoid undesirable side effects mediated by other muscarinic receptor subtypes, primarily M_2 (heart) and M_3 (salivary glands). Of the many drugs that have entered clinical trials claiming to be M_1 agonists, xanomeline (Lilly) is the most potent. Although it has shown efficacy in AD patients on clinical measures of cognition, daily living and behavior when given as an oral formulation (Phase II),[3,4] extensive metabolism in humans (i.e., short half-life) led to its reformulation as a transdermal patch (Phase III).[5] In this paper we discuss the use of a receptor homology model of the genetically defined human M_1 receptor to convert an M_2 selective M_1/M_2 muscarinic agonist **1** (M_1 K_i = 2,500 nM, PI turnover 82%; M_2 K_i = 40 nM) to a potent M_1 agonist **3** (M_1 K_i = 30 nM, PI turnover = 88%, ED_{50} = 1.4 nM; M_2 K_i = 20 nM, cAMP = 47% of carbachol, ED_{50} = 600 nM). Lead compound **3** was optimized using an SAR study to give WAY-132983 (**7**), an orally active, functionally-selective M_1 agonist with better potency than xanomeline.

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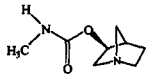
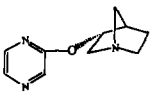
Results

A model of the M_1 receptor active site was built according to published methods and coordinates.[6] Docking of carbachol, a standard M_1/M_2 agonist, to the M_1 receptor active site model using the docking program within Sybyl from Tripos, Inc. (St. Louis, MO) showed three point ligand binding, which included two hydrogen bonds from the carbachol ester to threonine 192 and asparagine 382 and a salt bridge from the carbachol quaternary amine to aspartic acid 105. Using this nine amino acid model of the active site, novel M_1 agonists were discovered that were derivatives of carbamic acid. Although these compounds were very efficacious, they had only modest M_1 affinity. WAY-131256 represents the best compound in the series (K_i = 26,000 nM, PI turnover = 100% of the carbachol response).[7] Another strategy was to use a pyrazine ether and an azabicycle, respectively, to mimic the ester and quaternary ammonium portions of acetylcholine. These compounds were very efficacious M_1/M_2 agonists with micromolar affinities. In contrast to WAY-131256, which was active in vivo as a functionally-selective M_1 agonist (Minimum Effective Dose (MED): 1 mg/kg in the rat radial arm maze model),[7] pyrazine ether **1** was an M_2 selective agonist in vivo with an LD_{50} of 30 mg/kg in rats. Docking of xanomeline and **1** into the M_1 receptor active site model did not give any insight into how xanomeline's structure related to its high M_1 affinity and efficacy.

Next, an M_1 receptor homology model was built based on the X-ray crystal structure of bacteriorhodopsin and the amino acid sequence of the human M_1 muscarinic receptor. The protein module (Biopolymer) and the docking module within Sybyl were used.



WAY-131256



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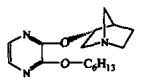

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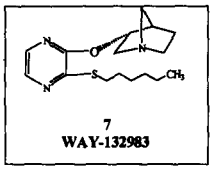

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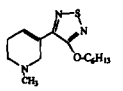
WAY-132983



xanomeline

The amino acid sequence of the human M_1 receptor was built and laid over the X-ray crystal structure. The amino acid side chains were manually manipulated to avoid unfavorable energetic interactions. Keeping the X-ray structure fixed, the amino acid sequence was forced to assume the shape of bacteriorhodopsin. The resulting 7-membered transmenbrane receptor model was then minimized. Standard M_1/M_2 agonists (e.g., carbachol and acetylcholine) were placed near the active site using the published coordinates.[6] Docking was completed by the Docking module within Sybyl and the protein ligand complex was minimized. Minimized structures of novel compounds were then docked in the same way. The muscarinic receptors are members of the G protein coupled receptor family, which contain seven transmembrane loops. A minimized structure of

xanomeline was allowed to dock in the M_1 receptor homology model. The thiadiazole ring of xanomeline docked into a pocket between transmembranes 3, 5, and 6 and the hexyloxy side chain slipped between the fifth and sixth transmembrane loops of the receptor model. The thiadiazole ring of xanomeline formed hydrogen bonds with threonine 192 and asparagine 382 and the tertiary nitrogen of its piperidine ring formed a salt bridge with aspartic acid 105. Binding of xanomeline to the human M_1 receptor expressed in Chinese Hamster Ovary (CHO) cells confirmed potent M_1 affinity (K_i = 42 nM).

It was reasoned that the *hexyloxy* side chain of xanomeline was responsible for its high M_1 affinity while maintaining its functional agonism. A minimized structure of pyrazine ether **3** was built which placed a *hexyloxy* group in position 3. When the structure of 3-hexyloxypyrazine ether **3** was allowed to dock in the M_1 receptor model, **3** oriented itself such that one hydrogen bond was formed between a pyrazine nitrogen and threonine 192, and a second hydrogen bond was formed between the azabicyclic ether oxygen and asparagine 382 . A third interaction was a salt bridge that formed between the azabicyclic nitrogen and aspartic acid 105. The hexyloxy side chain extended between the fifth and sixth transmembrane loops of the receptor model as it had done in the case of xanomeline. Compound **3** was synthesized (Scheme 1) and found to be equipotent to xanomeline and equally efficacious. Resolution of **3** gave its active enantiomer **6**, which was a potent (K_i = 14 nM), efficacious M_1 agonist. A focused SAR study to optimize **3** revealed that the pyrazine ether analogs had higher affinity and efficacy than the corresponding pyrazine thioether analogs. Within the pyrazine ether series, both 3-hexyloxy- and 3-hexylthio-substituted analogs were potent and efficacious. *Endo* analogs were more potent and efficacious than *exo* analogs. The most potent and efficacious analog was **7** (K_i = 2 nM, PI turnover = 80% of the carbachol response). No binding selectivity was observed for **7** in human M_1-M_5 receptors in CHO cells. However, functional selectivity was observed both in vitro and in vivo.

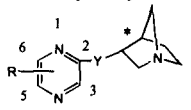
Side-effect assessment of WAY-132983 and xanomeline in rats showed significant salivation at 10 mg/kg for both compounds following either ip or po administration.[8] However, cognitive assessment using pre-trained rats in a forced choice, 1h delayed nonmatched-to-sample radial arm maze task showed that WAY-132983 (0.3 mg/kg, ip) was more potent than xanomeline (1 mg/kg, ip) in reducing scopolamine (0.3 mg/kg, sc) induced increased errors.[14] In addition, cognitive assessment after oral administration of WAY-132983 in primates (Rhesus and cynomolgus monkeys) using pre-trained aged subjects demonstrated a significant increase in correct choices during both short and long delay cycles. [15] (Table 2)

Discussion

A receptor homology model of the human M_1 receptor proved to be a more useful tool for the discovery of potent muscarinic M_1 agonists than a simple nine amino acid active site model. Using the receptor model it was possible to evaluate energy minimized structural analogs of the M_2 selective muscarinic agonist **1**, which led to the proposal of **3** as a potential M_1 agonist target. Synthesis and biological evaluation of **3** validated the

utility of the receptor homology model to predict M_1 activity. Using the model it was also possible to predict which analogs would not be agonists. For example, the energy minimized structure of compound **5** did not align itself well in the homology model when docked by the docking program within Sybyl. Thus it was predicted to be an antagonist. The compound was synthesized in order to test the accuracy of the prediction. In spite of its high M_1 affinity (K_i = 22 nM), **5** lacked significant agonist activity (PI turnover = 2% of the carbachol response). With the exception of the dystomer **12**, all other analogs in Table 1 exhibited 42-107% of the maximum carbachol response in the PI turnover assay.

Table 1. hm_1 Affinity and Efficacy of Pyrazine Ether and Thioether Derivatives



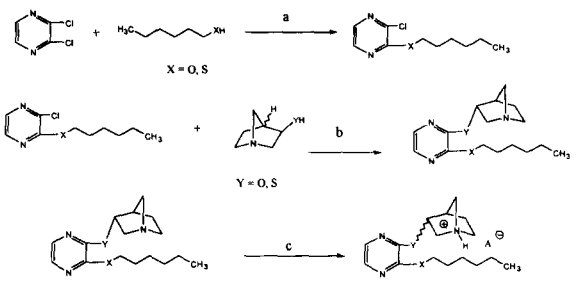
Compound[a]	Y	Stereochemistry		R	hm_1 in CHO cells Ki (nM) [^{3}H]-QNB	PI (% carbachol)
1	O	*endo*	(+/-)	6-H	5600	96
2	O	*endo*	(+)	6-H	2500	83
3	O	*endo*	(+/-)	3-O-hexyl	30	88
4	O	*endo*	(+/-)	3-S-hexyl	7	85
5	O	*endo*	(+/-)	6-S-hexyl	22	2
6	O	*endo*	(+)	3-O-hexyl	14	75
7 *	O	*endo*	(-)	3-S-hexyl	2	80
8	O	*exo*	(+/-)	3-S-hexyl	123	104
9	O	*exo*	(+)	3-S-hexyl	75	107
10	O	*exo*	(-)	3-S-hexyl	124	44
11	O	*exo*	(-)	3-O-hexyl	690	56
12	O	*exo*	(+)	3-O-hexyl	465	9
13	S	*exo*	(+/-)	3-O-hexyl	292	42
14	S	*exo*	(+/-)	3-S-hexyl	30	62
xanomeline					42	79

*WAY-132983

[a]All compounds are novel and gave satisfactory spectral analysis.

WAY-132983 was profiled in additional preclinical tests, which are summarized in Table 2. The compound was found to be a very potent agonist at human M_1 receptors in CHO cells (EC_{50} = 1 nM). An assessment of metabolic stability in an in vitro human liver microsome assay showed WAY-132983 to have a $t_{1/2}$ of 113 min whereas a $t_{1/2}$ of 6 min was measured for xanomeline in the same assay . Taken together, these data indicate that WAY-132983 is a potent, functionally-selective M_1 agonist which may be beneficial in the treatment of Alzheimer's Disease.

Scheme 1



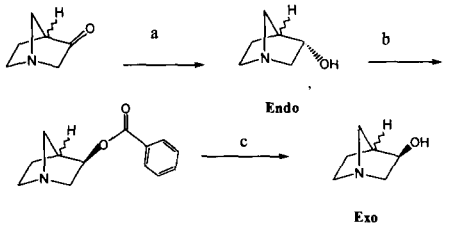
(a) NaH, DMF/THF, -5 ° C; (b) NaH, THF; (c) HCl, EtOH or to resolve racemates *d*- or -*l* tartaric acid, EtOH.

Experimental

The compounds in Table 1 were prepared according to Schemes 1 and 2.

Racemic *endo* azabicyclo[2.2.1]heptan-3-ol was resolved using *d* and *l* tartaric acids in ethanol. Racemic *exo* azabicyclo[2.2.1]heptan-3-ol was resolved according to a literature procedure.[9] Thioalcohols were prepared by reacting *endo*-azabicyclo[2.2.1]heptan-3-ol with thioacetic acid under *Mitsunobu* conditions (triphenylphosphine, di-isopropylazidodicarboxylate, THF, 5 °C, thiolacetic acid, 25 °C, 20 h) followed by hydrolysis of the thioacetate ester (NaOMe/MeOH) to give racemic *exo*-azabicyclo[2.2.1]heptan-3-thiol. A second *Mitsunobu* reaction was carried out on the *exo* thiol to give racemic *endo*-azabicyclo[2.2.1]heptan-3-thiol.

Scheme 2

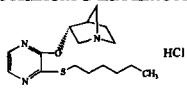


(a) H_2, PtO_2 EtOH, NaOH; (b) di-isopropylazadicarboxylate, triphenylphosphine, benzoic acid; (c) $NaOCH_3$.

The binding affinities of standards (carbachol, arecoline, oxotremorine, pilocarpine) and novel compounds at the individual human muscarinic receptor subtypes were determined in samples of homogenized Chinese Hamster Ovary (CHO) cells that had been transfected with CMV vector containing cDNA for the expression of human muscarinic receptors (M_1- M_5). [K_d (pm) and B_{max} (fmol/mg protein): M_1 26.1, 204; M_2 20.6, 338; M_3 55.5, 1272; M_4 88.0, 2995; and M_5 95.8, 276]. The procedure used was based on the displacement of [^{3}H]-QNB binding as described in a literature procedure.[10] The affinity of **7** for the high affinity agonist state of the M_1 receptor was determined using displacement of [^{3}H]Oxo-M in rat cortical homogenate as described by Freedman et al.[11]

The ability of test compounds to stimulate phosphoinositide (PI) turnover in Chinese Hamster Ovary (CHO) cells, tranfected with CMV vector containing cDNA expressing human M_1 (M_3 or M_5) muscarinic receptors was determined according to a literature procedure.[12] The PI turnover of novel compounds is reported as a percentage of the maximal response observed for the standard muscarinic full agonist, carbachol (300 μM), which was considered to be 100%. The range of concentations used to assess PI metabolism was 30×10^{-10} μM to 1×10^{-7} μM. Compounds exhibiting muscarinic affinity in the CHO human M_2 or M_4 assay were tested in the forskolin-stimulated cAMP assay. [13] This assay measures agonism at M_2 or M_4 receptors.

Table 2. Preclinical Pharmacology of WAY-132983



In Vitro			In Vivo	
Affinity (K_i, nM)			Cognitive impairment reduction, MED	
M_1 -antagonist ligand, human	2.1	+/- 0.13,3 (1.86-2.89)	AF64A-impaired RAM, rat (mini-osmotic pump, sc)	0.03 mg/kg/day
-agonist ligand, rat brain	0.4			
M_2 -antagonist ligand, human	3.4	+/- 0.39,3 (2.76-4.30)	DMTS[τ] task, aged monkeys, po	0.03 mg/kg (short delay) 0.01 mg/kg (long delay)
M_3 -antagonist ligand, human	5.9	+/- 0.33,3 (5.29-6.60)		
Functional Agonism EC_{50} (nM)		Maximum % carbachol	Rate of metabolism	
M_1 (PI)	1.1	+/- 0.04,7 (0.00-1.16) 80	-human liver microsomes ($T_{1/2}$ min)	113
M_2 (cAMP)	8	+/- 1.0,3 6.0-11.0)	[τ]DMTS = delayed match to sample.	
M_3 (PI)	3.7	+/- 0.26,5 (3.26-4.27) 53		

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