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Design and synthesis of *N*-[6-(Substituted Aminoethylideneamino)-2-Hydroxyindan-1-yl]arylamides as selective and potent muscarinic M_1 agonists

Bin Liu [a,*,†], Carrie H. Croy [a,†], Stephen A. Hitchcock [a], Jennifer R. Allen [a,#], Zhigang Rao [a,#], David Evans [b], Mark G. Bures [a], David L. McKinzie [a], Marla Leigh Watt [a,#], G. Stuart Gregory [a], Marvin M. Hansen [a], Paul J. Hoogestraat [a], James A. Jamison [a], Fese M. Okha-Mokube [a], Robert E. Stratford [a,#], William Turner [a,#], Frank Bymaster [a,#], Christian C. Felder [a,*]

[a] *Lilly Research Laboratories, Indianapolis, IN 46285, United States*
[b] *Eli Lilly and Company, Windlesham, Surrey, UK*

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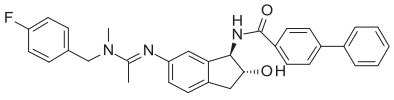
ABSTRACT



LY593093

The observation that cholinergic deafferentation of circuits projecting from forebrain basal nuclei to frontal and hippocampal circuits occurs in Alzheimer's disease has led to drug-targeting of muscarinic M_1 receptors to alleviate cognitive symptoms. The high homology within the acetylcholine binding domain of this family however has made receptor-selective ligand development challenging. This work presents the synthesis scheme, pharmacokinetic and structure–activity-relationship study findings for M_1-selective ligand, LY593093. Pharmacologically the compound acts as an orthosteric ligand. The homology modeling work presented however will illustrate that compound binding spans from the acetylcholine pocket to the extracellular loops of the receptor, a common allosteric vestibule for the muscarinic protein family. Altogether LY593093 represents a growing class of multi-topic ligands which interact with the receptors in both the ortho- and allosteric binding sites, but which exert their activation mechanism as an orthosteric ligand.

Abbreviations: GPCR, G protein-coupled receptors; CHO, Chinese hamster ovary; GTPγ^{35}S, [^{35}S]-guanosine-5′-*O*-(3-thio)triphosphate; BOC, butoxycarbony; EC_{50}, half maximal effective concentration of drug; pK_a, log of acid dissociation constant (K_a); Log*D*, log of the partition coefficient of molecule; AUC, area under the curve; SAR, structure–activity relationship; VDW, van der Waals.

* Corresponding authors.
E-mail addresses: bfliu@lilly.com (B. Liu), felder_christan_c@lilly.com (C.C. Felder).
† These authors contributed to the creation of the work equally.
# These authors currently work at: Merck Research Labs, Kenilworth, NJ 07033, United States (MLW); Amgen, Thousand Oaks, CA 91320, United States (JRA); Koch Industries, Wichita, KS 67220, United States (ZR); Department of Basic Pharmaceutical Sciences, College of Pharmacy, Xavier University of Louisiana , New Orleans, Louisiana 70125, United States (RES); Assembly Biosciences, Bloomington, IN 47401, United States (WT); Euthymics Biosciences, Cambridge MA 02141, United States (FB).

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Muscarinic acetylcholine receptors belong to the super family of G protein-coupled receptors and are widely distributed in both the brain and periphery. Five distinct subtypes (M_1–M_5) have been identified and characterized using a variety of pharmacological and molecular biological techniques.[1] Muscarinic receptors play critical roles in the homeostatic regulation of the parasympathetic and central nervous system functions. Advances in understanding the physiological roles of each muscarinic receptor subtype have resulted in renewed interest in developing drugs that target selected members of this receptor family. In particular, the M_1 receptor remains as a target for the treatment of cognitive deficits associated with Alzheimer's disease, schizophrenia, and other neurological and psychiatric disorders for the following reasons: (1) neuronal localization of the M_1 receptor is abundant in hippocampus and cortex, areas of the brain that mediate cognition and attention, (2) M_1 receptor activation by compounds such as Xanomeline improve memory in animals and humans; however, because of poor selectivity over M_2 and M_3 receptor subtypes, they are associated with dose-limiting parasympathomimetic side effects including, for example, cardiovascular effects, hypothermia, tremor, salivation, diarrhea, and profuse sweating,[2] and (3) transgenic M_1 receptor knockout mice show deficits in biochemical measures of memory related signal transduction and in behavioral learning tasks.[3] The combined evidence strongly suggests that a highly selective and potent M_1 agonist or positive modulator might be useful in treating memory and cognitive deficits associated with Alzheimer's disease without producing the unwanted side-effects associated with activation of M_2 and M_3 receptors.

The heterogeneity of muscarinic signaling pathways and the variety of assay technologies employed to quantify their activation have served to complicate the interpretation of subtype selectivity. We have described an anti-G protein antibody assay for measuring agonist-stimulated [^{35}S]-guanosine-5′-*O*-(3-thio)triphosphate (GTPγ^{35}S) binding against M_1–M_5 receptors expressed in CHO cells.[4] Amongst the attractive qualities of this assay format is the ability to measure agonist signal transduction proximal to the ligand GPCR binding event. Thus the potential to overestimate agonist efficacy is reduced and a common assay format is possible across all five subtypes. Implementation of this assay has enabled re-examination of several muscarinic agonists reported to possess M_1 selectivity and advanced into clinical trials in recent years only to be subsequently terminated.[5] Alvameline (LU25-109), Sabcomeline, Talsaclidine, and Xanomeline are examples of such purported M_1 receptor agonists that have subsequently been found lacking M_1 selectivity. These reference compounds were designed from natural products that resemble constrained versions of acetylcholine including muscarine, arecoline, and pilocarpine (Fig. 1). Not surprisingly, in our hands using the aforementioned GTPγ^{35}S assay, none of these clinical candidates displayed M_1 selectivity over M_2 and M_3 receptor subtypes which are believed to mediate the majority of muscarinic agonist-induced side-effects (Table 1). Furthermore, maximal activity for these compounds at the M_1 receptor reveals partial agonist activity relative to acetylcholine. This pharmacological profile, along with considerable pharmacokinetic issues that plague this platform, comprises a rationale for why these compounds have all failed as medications for the treatment of cognitive disorders.

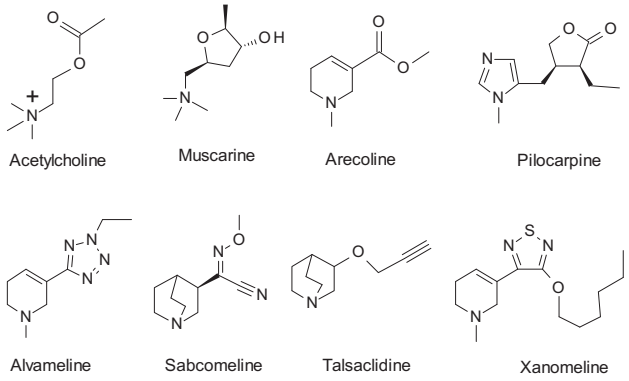

Figure 1. Muscarinic agonists.

The targeted analogs were synthesized as outlined in Scheme 1. In short, targets were synthesized from Boc-protected *trans*-1-amino-6-nitro-indan-2-ol (**1**) by amidine formation followed by removing the BOC group with TFA and then amide bond formation. They can be also synthesized through amide **2** synthesis followed by amidine group formation.[6] The Boc-protected *trans*-1-amino-6-nitro-indan-2-ol (**1**) was synthesized via the sequence of (1) reduction of the nitroindanone (**4**), (2) dehydration of the hyrdroxynitroindane (**5**), (3) epoxidation of the nitroindene, (4) epoxide-ring opening of (**6**) with ammonium hydroxide, (5) isolation of the needed isomer (**8**), (6) protection of the primary amine of (**8**) with a BOC group, and (7) reduction of the nitro group of (**9**) to aniline (Scheme 2).[7]

As a part of the drug research and development efforts at Lilly, a high throughput screen of a library resulted in the discovery of M_1 receptor agonist hit **10** (Fig. 2) that possessed the undesired M_2 receptor activity with an E_{max} of 80% at 280 nM. Besides the undesirable activity favoring the M_2 receptor, it was chemically unstable in rat plasma. Plasma stability studies indicated that about half of the parent compound decomposed through formamidine hydrolysis in 4 h. Thus, the initial effort was to find analogs that possessed good rat plasma stability. We demonstrated that conversion of the formamidine to acetamidine rendered a marked improvement in plasma stability. The compound **11** (Fig. 2) showed 5% decomposition in rat plasma in 4 h. Further SAR delivered a compound **12** (Fig. 2), stable in rat plasma, with good metabolic stability and oral bioavailability (68%, rat), but which still lacked M_1 receptor selectivity over M2 and M_4 receptor subtypes [M_1 EC_{50} = 80 nM (64%), M_2 EC_{50} = 24 nM (28%), and M_4 EC_{50} = 113 nM (34%)]. Interestingly, **12** demonstrated moderate efficacy in a rat spatial learning task following an oral dose of

Table 1
In vitro activity of first generation muscarinic agonists from GTPγ^{35}S assay

	M_1 receptor		M_2 receptor		M_3 receptor		M_4 receptor		M_5 receptor	
	EC_{50} (nM)	% eff	EC_{50} (nM)	% eff	EC_{50} (nM)	% eff	EC_{50} (nM)	% eff	EC_{50} (nM)	% eff
Acetylcholine	25.9 ± 2	102 ± 1.9	7 ± 0.49	102 ± 1.6	205 ± 62	117 ± 4.1	48.8 ± 9.7	93.4 ± 2.4	19.1 ± 2.3	110 ± 1.9
Alvameline	n.a.	—	296 ± 5.7	49.2 ± 1.6	n.a.	—	n.a.	—	n.a.	—
Sabcomeline	63.6 ± 10.5	39.6 ± 1.4	62.1 ± 7.4	87.7 ± 1.7	117 ± 17	28.3 ± 1.9	214 ± 138	21.3 ± 1.9	123 ± 23	38.9 ± 1.3
Talsaclidine	820 ± 91	68.2 ± 2.5	849 ± 69	94.4 ± 2.8	2696 ± 202	26.1 ± 6.6	844 ± 101	22.2 ± 1.3	422 ± 136	23.7 ± 4.1
Xanomeline	67.3 ± 13	59.2 ± 3.5	121 ± 11	83.5 ± 2.3	n.a.	—	229 ± 155	48.7 ± 6.7	142 ± 26	25.9 ± 4.5

Values are averaged from minimum of two independent experiments % eff = maximal agonist efficacy response relative to acetylcholine. n.a. = agonist efficacy <20% at highest concentration tested.