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Discovery of subtype selective muscarinic receptor antagonists as alternatives to atropine using in silico pharmacophore modeling and virtual screening methods

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ABSTRACT

Muscarinic acetylcholine receptors (mAChRs) have five known subtypes which are widely distributed in both the peripheral and central nervous system for regulation of a variety of cholinergic functions. Atropine is a well known muscarinic subtype non-specific antagonist that competitively inhibits acetylcholine (ACh) at postganglionic muscarinic sites. Atropine is used to treat organophosphate (OP) poisoning and resulting seizures in the warfighter because it competitively inhibits acetylcholine (ACh) at the muscarinic cholinergic receptors. ACh accumulates due to OP inhibition of acetylcholinesterase (AChE), the enzyme that hydrolyzes ACh. However, atropine produces several unwanted side-effects including dilated pupils, blurred vision, light sensitivity, and dry mouth. To overcome these side-effects, our goal was to find an alternative to atropine that emphasizes M1 (seizure prevention) antagonism but has minimum M2 (cardiac) and M3 (e.g., eye) antagonism so that an effective less toxic medical countermeasure may be developed to protect the warfighter against OP and other chemical warfare agents (CWAs). We adopted an in silico pharmacophore modeling strategy to develop features that are characteristics of known M1 subtype-selective compounds and used the model to identify several antagonists by screening an in-house (WRAIR-CIS) compound database. The generated model for the M1 selectivity was found to contain two hydrogen bond acceptors, one aliphatic hydrophobic, and one ring aromatic feature distributed in a 3D space. From an initial identification of about five hundred compounds, 173 compounds were selected through principal component and cluster analyses and in silico ADME/Toxicity evaluations. Next, these selected compounds were evaluated in a subtype-selective in vitro radioligand binding assay. Twenty eight of the compounds showed antimuscarinic activity. Nine compounds showed specificity for M1 receptors and low specificity for M3 receptors. The pK_i values of the compounds range from 4.5 to 8.5 nM in comparison to a value of 8.7 nM for atropine. 2-(diethylamino)ethyl 2,2-diphenylpropanoate (ZW62841) was found have the best desired selectivity. None of the newly found compounds were previously reported to exhibit antimuscarinic specificity. Both theoretical and experimental results are presented.

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1. Introduction

Muscarinic receptors are a family of G-protein coupled receptors (GPCRs) and, like other members of this family, have multiple subtypes.[1] By agonist and antagonist evaluation and molecular dissection, five subtypes of muscarinic receptors have been determined (M1, M2, M3, M4, and M5). All five receptor subtypes have been cloned and pharmacologically characterized.[2,3] Each subtype has a specific role, for example, Ml receptors are found in high density in the central nervous system and peripheral ganglia, which are characterized by high affinity for pirenzepine.[3] M2 receptors are found in cardiac cells and in the lower brain areas, which are characterized by a high affinity for methoctramine, AF-DX 116, and gallamine.[2,3] M3 receptors are located primarily in smooth muscle and exocrine glands, which display high affinity for 1,1-Dimethyl-4-diphenylacetoxypiperidine (4-DAMP), hexahydrosiladifenidol, and *p*-fluorohexahydro-sila-difenidol.[4] Muscarinic receptor antagonists are used as therapeutics; for example, in the treatment of smooth muscle disorders including urinary incontinence, irritable bowel syndrome, and chronic obstructive pulmonary disease.[5–9]

In contrast to subtype selective antagonist, non-selective ligands exhibit many undesirable side-effects and thus limit their

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clinical usefulness. For this reason, despite there being a number of muscarinic receptor agonists and antagonists, only a few have been introduced for therapeutic use, which include glaucoma, gastrointestinal and urinary bladder smooth-muscle disorders, bronchial asthma, peptic ulcers, Sjogren's syndrome, some cardiac arrhythmias, motion sickness, and Parkinson's disease.[8]

The nonselective muscarinic antagonist atropine is used for treatment of organophosphorus (OP) poisoning.[10] OP compounds are widely used in agriculture as pesticides and have been deployed by terrorists as chemical warfare nerve agents.[11] These compounds are highly toxic because OPs inhibit the enzyme acetylcholinesterase (AChE).[12] AChE hydrolyzes the neuron-mediator acetylcholine (ACh) at the synaptic clefts. Inhibition of AChE results in a build-up of ACh, may produce a cholinergic crisis, and can ultimately lead to death.[13] To counter the effects of OP poisoning, anticholinergics (as functional drugs) and AChE reactivators oximes (as causal drugs) are generally used as first aid antidotes.[12–16] Atropine is used during OP poisoning because it competitively inhibits ACh at postganglionic muscarinic sites. Increased parasympathetic stimulation produces miosis, sialorrhea, bronchospasm and bronchorrhea. Treatment with atropine competitively blocks the parasympathetic effects. While oximes reactivate inhibited cholinesterases, atropine combats the effect of excess ACh by competitively binding to muscarinic receptors. Although the present treatment of a combination of atropine and an oxime reactivator for OP intoxication offer protection against their lethality, treatment side-effects are observed. Atropine has several unwanted side effects including dilated pupils, blurred vision, and light sensitivity, and which compromise visual acuity.

With advances in the understanding of function and pharmacological effects of the five muscarinic receptor subtypes, it should be possible, though challenging, to develop muscarinic receptor subtype specific compounds. Although five subtypes of muscarinic receptors have been characterized, lack of small molecule ligands to inhibit muscarinic receptor subtypes selectively remains a major obstacle toward development of novel antimuscarinic therapeutics.[17] We adopted in silico pharmacophore modeling and virtual screening strategies to identify potential subtype selective muscarinic antagonists. Specifically, we are searching for an alternative to atropine that lacks M3 (e.g., eye) antagonism, retains M1 (seizure prevention), and exhibit minimum M2 (cardiac) antagonism, thereby overcoming the side-effects of atropine.

Discovery and development of new therapeutics are expensive and complex processes. It takes over 10 years and an average $500 million to bring a new therapeutic agent from the bench top discovery to the market.[18] Thus, newer technologies that can improve the efficiency of the discovery process are indeed valuable to the pharmaceutical industry.[18,19] We recently found in silico stereo-electronic and three dimensional pharmacophore modeling could be two useful approaches for identification of novel chemotypes as antagonists[20] for muscarinic receptors through virtual screening of compound databases.

The in silico 'three dimensional pharmacophore' may be viewed as an ensemble of steric and electronic properties that are necessary for optimal interaction with a specific receptor to trigger or inhibit its biological response.[21] It is usually represented by a geometric distribution of chemical features such as hydrogen bond acceptors and donors, aliphatic and aromatic hydrophobic sites, ring aromaticity, and ionizable sites in 3D space of a molecule. The advantage of the pharmacophore is that it transcends the structural class and captures those features that are responsible for the intrinsic activity of potential therapeutics as new chemical classes or chemo-types. In support of this approach, we recently published[20] the first in silico pharmacophore model for antimuscarinic activity of α-substituted 2,2-diphenylpropionate antimuscarinics based on published literature affinity values.[14]

Table 1
Training set of compounds[4,23–25]

Compound	Inhibition constant for rat cortex (experimental)[a] (nM)	Inhibition constant for rat cortex (predicted) (nM)	Error
Caramiphen	7.2 ± 4.5	8.4	1.2
Iodocaramiphen	8.6 ± 1.4	11.0	1.2
Nitrocaramiphen	57 ± 21	9.8	−5.8
Atropine	0.12 ± 0.03	0.081	−1.5
Dicyclomine	4.6 ± 7	13.0	2.8
Methoctramine	137 ± 8.0	120.0	−1.2
Oxybutynin	2.0 ± 1	4.0	2.0
Pirenzepine	28.0 ± 2.5	14.0	−2.1
Trihexyphenidyl	1.5 ± 2	5.0	3.3

Experimental and estimated activity (generated by the new pharmacophore model) for subtype specific antagonists taken from published data.

[a] Data taken from published literature (Ref. 4,23–25). Error values for the predicted activity are within an uncertainty of 3.0. An uncertainty function '¢' in the CATALYST[29] paradigm indicates an activity value lying somewhere in the interval from 'activity divided by ¢' to 'activity multiplied by ¢'.

The present study reports the development of a new pharmacophore model from published literature data on relevant subtype specific antimuscarinic agents (Table 1) and use of the model to identify several new antimuscarinic agents including a few subtype selective compounds. The results presented here are from several iterations of virtual screening and selection of compounds obtained from the in-house WRAIR-CIS database.[22] Identification of these antimuscarinic agents, and importantly subtype selective compounds (Table 2), is notable as there are no prior published reports relating to the antimuscarinic activity of these compounds.

2. Results and discussions

Our earlier reported pharmacophore model[20] for antimuscarinic activity was developed from published antimuscarinic activity of α-substituted 2,2-diphenylpropionates and atropine in vitro.[14] Although the reported model contained only two chemical functions, two hydrogen bond acceptors and one aromatic ring feature localized in space, the model proved to be quite predictive. We utilized the model as a template for searching our in-house database[22] and identified ten potent antimuscarinic compounds, eight of which showed inhibition ranging from 2 to 200 nM in a radioligand ([^{3}H]-NMS) binding assay for antimuscarinic inhibition activity (two were about equal in inhibition potency to atropine).[20] More important, none of those compounds was previously reported as antimuscarinics.

However, since our goal was to identify subtype selective antimuscarinic agents that lack M3 antagonism but retain M1 and have minimum M2 antagonism, we embarked on refining the above model to suit the stereo-electronic requirements for the desired subtype selectivity. Accordingly, we selected another training set of compounds (Table 1) from published literature data[4,23–25] having the M2/M1 subtype specificity and developed a new pharmacophore model for subtype selective antimuscarinic activity. This new model was utilized for virtual screening, yielding 28 new antimuscarinic agents including 9 subtype selective compounds (Tables 2–4).

Pharmacophores may be derived in several ways, for example, by analogy to a natural substrate or known ligand, by inference from a series of dissimilar active analogs, or by direct analysis of the structure of a target protein.[21,26–28] A pharmacophore can be used in two ways to identify new compounds that share its features, and thus may exhibit a desired biologic response. In the first approach, de novo design can be performed that link the disjointed parts of the pharmacophore together with fragments in order to