Selective muscarinic M_1 antagonists: drug design and discovery

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Years of clinical research to treat disorders such as peptic ulcers and obstructive lung disease with the first generation of modestly selective M_1 antagonists (e.g. pirenzepine, telenzepine) have been disappointing. For some indications (chronic obstructive pulmonary disease), nonselective antagonists (ipratropium bromide) have exhibited better clinical efficacy. The advent of a new generation of centrally active and highly selective M_1 antagonists, such as PD150714, spirotramine FC1594, (–)-*S*-ET126 and *R*-4-(pyrrolidino)-1-(4-fluorophenylcarbamoyloxy)-1-phenyl-2-butyne (4-F-PhPyMcN), offers new opportunities for using selective muscarinic agents as therapeutic agents or research tools.

Muscarinic antagonists, such as the belladonna alkaloids atropine and scopolamine, have long been used for treatment of a variety of human diseases, including disorders of the nervous system (e.g. Parkinson's disease and motion sickness), digestive tract system (e.g. peptic ulcer and irritable bowel syndrome) and respiratory system [e.g. asthma and chronic obstructive pulmonary disease (COPD)][1]. However, while these compounds have modest clinical benefit, their use is limited by side effects such as dry mouth, blurred vision, dizziness and tachycardia[1]. Some newer, synthetic antimuscarinics such as trihexyphenidyl (for tremor in Parkinson's disease) and ipratropium bromide (for respiratory indications) have demonstrated improved efficacy and reduced side effects compared with the belladonna alkaloids, but they are not free of side effects[1]. Therefore, use of muscarinic antagonists has been superseded by newer generations of therapeutic agents, such as dopaminergic agonists and amantadine for parkinsonism[2], proton pump inhibitors, antibiotics and histaminergic H_2 antagonists for peptic ulcer[3,4], and β-adrenergic agonists and anti-inflammatory agents for respiratory diseases[5,6]. In general, these newer generations of agents have had better clinical efficacy and safety, and have relegated muscarinic antagonists to second or third line therapies.

However, since all of the antimuscarinics discussed above are nonselective across muscarinic receptor subtypes (described below), it is possible that many of the troublesome side effects of these agents result from interactions with certain receptor subtypes. It has been hypothesized that, by developing more subtype-selective muscarinic antagonists, better therapeutic agents might be developed. Much research over the past decade or more has focused on developing antagonists selective for the M_1 receptor subtype, and this approach has been reviewed previously[7–9]. The purpose of this review is to examine recent efforts in the

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 1359-6446/97/$17 00. PII. S1359-6446(97)01076-3

discovery and development of selective muscarinic M_1 antagonists. Although the focus is on antagonists for M_1 receptor subtypes, other receptor subtypes will be included in discussions where relevant, because many of the M_1 antagonists developed have significant affinity for other muscarinic subtypes, such as M_3 and M_4.

Muscarinic receptor

Muscarinic receptors constitute one of the two major receptor classes for acetylcholine (ACh). Pharmacological studies have demonstrated the existence of at least four different subtypes of muscarinic receptors, denoted M_1–M_4, which are distinguished on the basis of binding of selective agents and second messenger systems activated. For example, M_1 and M_3 stimulation activates phospholipase C activity, while M_2 and M_4 stimulation leads to inhibition of adenylate cyclase[10,11] . M_2 and M_4 receptor subtypes are also thought to be the two major subtypes of muscarinic autoreceptors found throughout the nervous system and target tissues. Cloning studies have identified five different subtypes of muscarinic receptors (termed m_1–m_5), which generally correspond to their respective pharmacological subtypes, although a distinctive M_5 pharmacological species has not been clearly identified[11]. M_1 receptor sites are typically defined by high-affinity binding with the antagonist pirenzepine (Figure 1), although it has long been recognized that pirenzepine also exhibits reasonably high affinity for cloned m_4 receptors and M_4 sites[12,13]. While no full agonists are completely selective for the M_1 receptor subtype, a partial agonist, xanomeline (an arecoline derivative from Lilly/Novo Nordisk), does show selectivity for M_1 (Ref. 14).

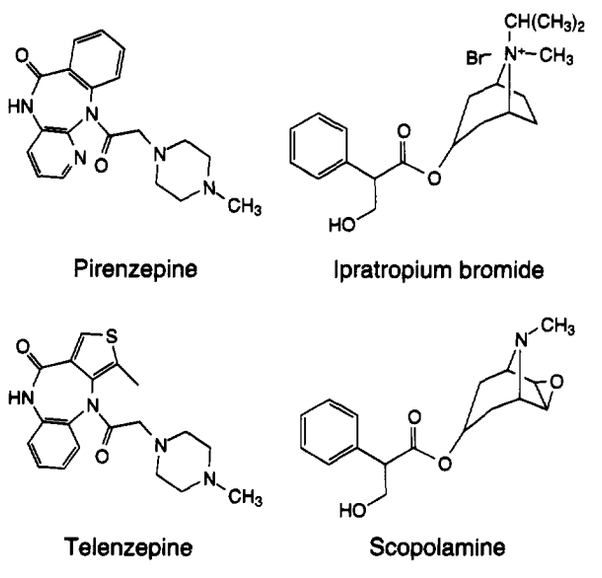


Figure 1. *Some early muscarinic antagonists.*

The anatomical distribution of muscarinic receptors generally follows innervation patterns by cholinergic neurons in the central and peripheral nervous systems (CNS and PNS). Mapping studies for M_1/m_1 receptors have been carried out using radiolabeled pirenzepine[15], oligonucleotide probes and riboprobes for m_1 mRNA (Refs 16,17), and subtype-selective antisera[18–21].

M_1 receptors in the digestive tract

Background and rationale

In the digestive tract, cholinergic innervation is from the parasympathetic nervous system through the vagus nerve to various structures, including the salivary glands, esophagus, stomach and colon. Preganglionic, vagal efferents innervate cholinergic postganglionic cells, such as the myenteric nerve plexus, which in turn innervates smooth muscle and glandular tissue, leading to smooth muscle contraction and secretory responses, respectively. Within the stomach, several specific cell types exhibit muscarinic secretory responses, including parietal cells (producing hydrochloric acid), zymogen cells (pepsinogen), G cells (gastrin) and mucous cells. Additionally, two types of histamine-secreting cells are found in the stomach, enterochromaffin-like (ECL) cells and mast cells, of which only the ECL cells show ACh-dependent histamine release[22]. Several recent reviews have extensively examined the anatomy, physiology and pharmacology of gastric acid secretion[23,24].

While most muscarinic receptors in the digestive tract are of the m_2 and m_3 subtype, M_1/m_1 receptors are also found in lower abundance[21,25]. In rat gastric mucosa, m_1 immunoreactivity is abundant in superficial layers of the gastric glands, and m_1 mRNA is found in surface mucous cells and zymogen cells but not parietal cells[17], suggesting a possible role for M_1 receptors in modulating gastric secretion[25]. Pirenzepine, acting as an M_1 antagonist, inhibits acid secretion from the parietal cells. However, in the absence of demonstrable M_1 receptors on the parietal cells, it is suggested that pirenzepine instead acts on the ECL cells and reduces their release of histamine, resulting in inhibition of hydrochloric acid secretion.

Various studies have also presented evidence for muscarinic presynpatic modulation of ACh release in the gut.

For example, while the nonselective antagonist atropine increased ACh release from superfused rat gastric mucosa, pirenzepine reduced it, suggesting that presynaptic M_1 receptors facilitate ACh release[26]. Another study found evidence for presynaptic M_3 receptors that inhibit ACh release in rat stomach[27]. Therefore, M_1 antagonists may be able to reduce (directly or indirectly) secretion of various substances (e.g. hydrochloric acid and gastrin) and thereby have therapeutic use in the treatment of gastric ulcer.

Clinical studies

Clinical studies of muscarinic M_1 antagonists have been ongoing since the 1980s and have generally demonstrated that, while these compounds are moderately effective in the acute treatment of duodenal and gastric ulcers, the healing rates are lower than those of proton pump inhibitors or H_2 antagonists[28]. In a recent study on intraoperative stress-induced ulcer[29], the effects of pirenzepine and ranitidine on gastric juice pH and viscosity in patients under general anesthesia were compared. Pirenzepine increased pH and maintained viscosity in gastric juice, while ranitidine raised pH higher but decreased gastric juice viscosity, suggesting a possible advantage for pirenzepine in the prevention of stress ulcers. Some studies have also examined the ability of pirenzepine to complement ulcer therapy with the proton pump inhibitor omeprazole. One study[30] found that the 24 h pH 3 holding time for omeprazole (70%) was modestly increased (to 89%) by co-administration of pirenzepine, and that the increase in serum gastrin level (2.5-fold over baseline) observed with omeprazole treatment was decreased by co-administration of pirenzepine. Another study found similar effects on serum gastrin levels using co-administration of pirenzepine with omeprazole over a 6-week period in ulcer patients[31]. It is doubtful that M_1 antagonists with a limited selectivity, such as pirenzepine, will have significant therapeutic benefits for gastrointestinal indications as stand-alone therapies compared with H_2 blockers or proton pump inhibitors, but they may have a niche in adjunctive therapy.

Compounds in development

Recent preclinical studies have examined more novel M_1, M_1/M_3 and M_3 antagonists. For example, a novel pirenzepine analog, nuvenzepine (DF545, Dompe) has been shown to block putative M_3-related contractile responses in the guinea-pig ileum more potently than pirenzepine, but does not antagonize M_1-related (McNA343 stimulated) ileal contractions as potently as pirenzepine, suggesting that the compound has preferential M_3 effects in this preparation[32]. Another study showed that nuvenzepine not only inhibited ACh-induced ileal contractions, but also weakly inhibited histamine-induced ileal motor activity, suggesting the compound may be a weak H_1 antagonist[33].

M_1 receptors in the respiratory system

Background and rationale

Like the digestive tract, structures in the respiratory system (e.g. smooth muscle in bronchioles and some glandular tissue) are innervated by parasympathetic cholinergic efferents via the vagus nerve. Cholinergic vagal stimulation provides the major bronchoconstrictor stimulus to the respiratory system and may contribute to airway narrowing in both COPD and chronic asthma[8]. In the respiratory system, M_1 receptors are not the major muscarinic suptype; M_2 and M_3 receptor subtypes predominate in the bronchioles and trachea[21,25]. M_2 receptors in the airways act as autoreceptors on postganglionic neurons, inhibiting ACh release, and may also act in smooth muscle to counteract the bronchodilator actions of adrenergic stimulation[8]. M_3 receptors appear to mediate smooth muscle constriction and secretion from submucosal glands[8].

Importantly, in the respiratory system, species and tissue specific distributions of M_1/m_1 and M_4/m_4 receptor subtypes have made mapping studies more difficult. For instance, in rabbit peripheral lung, there are dense pirenzepine binding sites, but these sites also have high affinity for himbacine (preferential M_2, M_4 antagonist) and pK_i values for four other antagonists similar to those of NG108-15 cells (which express only m_4 receptors) and thus appear to be M_4 receptors[13]. This was confirmed by northern blot analysis[13]. A recent study showed that pirenzepine, rispenzepine (an M_1/M_3 antagonist) and 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP; a modestly selective M_3 antagonist) blocked electrically evoked tracheal contraction, whereas methoctramine (an M_2 antagonist) augmented the contractile response, suggesting the presence of inhibitory M_2 autoreceptors[34]. Based on the information described above, a strategy for therapeutic use of muscarinic antagonists has evolved focusing on M_1 and M_3 antagonists or possibly combined M_1/M_3 antagonists[8,9]. M_1 antagonists may reduce the bronchoconstriction in obstructive lung diseases such as COPD and asthma, while M_3 antagonists may also act as bronchodilators as well as possibly reducing mucus secretion[8].

Clinical studies

Nonselective muscarinic antagonists, such as ipratropium bromide (Figure 1), have been used extensively in respiratory medicine, principally as bronchodilators. Numerous studies have documented the robust clinical efficacy of ipratropium bromide as a bronchodilator in COPD (Refs 35,36). Ipratropium bromide is now considered as a first-line agent for COPD, as well as a second-line adjunctive therapy in asthma[37].

Although there is therapeutic potential for selective M_1 antagonists in respiratory diseases, clinical trials of M_1 antagonists (e.g. pirenzepine) have been disappointing. A small clinical study comparing the bronchodilator effects of ipratropium bromide and pirenzepine in patients with reversible or irreversible obstructive airway disease demonstrated that ipratropium bromide improved respiratory function measures [e.g. forced vital capacity (FVC) and forced expiratory flow rate (FEF_{25-75})] in both sets of patients[38], whereas pirenzepine was unremarkable. Another study showed that nebulized pirenzepine could modestly improve a measure of lung function [peak expiratory flow (PEF_{25})] by about 20%, while ipratropium bromide improved the same measure by 66% (Ref. 39). The authors interpreted these data as suggesting that the role of M_1 receptors in vagally mediated bronchomotor tone may be small compared with that of M_3 receptors. Ukena and coworkers found that administration of telenzepine (Figure 1) (3 mg, orally, four times a day for five days) did not improve respiratory function [FVC and forced expiratory volume in one second (FEV_1)] in COPD patients[40]. However, it is possible that the dose and route of administration used made the bioavailability of telenzepine too low to observe bronchodilatory effects.

The fact that the nonselective antagonist ipratropium bromide has significant clinical benefit in respiratory indications while modestly selective M_1 antagonists (e.g. pirenzepine and telenzepine) do not gives little support to the hypothesis that M_1 receptors are important in respiratory disease or that they provide a suitable target. The therapeutic benefits of nonselective muscarinic antagonists might be the result of interactions at particular receptor subtypes (e.g. M_3) or a combination thereof. To reiterate, it has been suggested that other subtype-selective antagonists (e.g. M_3) might be better therapeutic agents[8,9].

M_1 receptors in the CNS

Background and rationale

In the CNS, m_1 receptor immunoreactivity is found in relatively high density in forebrain areas such as the hippocampus (especially CA1), cerebral cortex and striatum, thereby constituting a major proportion (> 30%) of the total muscarinic receptors[18,20]. Muscarinic m_4 receptor proteins are found in high abundance in the striatum (about 50% of total muscarinic receptor protein), moderate levels (15–20%) are present in the cerebral cortex, hippocampus and midbrain, and the lowest levels (10% or less) in hindbrain areas[20,41]. Finally, m_2 receptors are found in moderate abundance (10–40%) in forebrain areas, but are the predominant muscarinic subtype in the brainstem (pons and cerebellum), constituting 70–84% of muscarinic proteins[20,25,41].

Functional studies of muscarinic receptors in the CNS have suggested roles for these receptors in limbic system and basal ganglia function. For example, muscarinic M_1 receptors in hippocampus and cerebral cortex appear to be involved in cognitive function (e.g. memory) because intracerebral injection of pirenzepine disrupts representational (working) memory[42]. In the basal ganglia, studies of dopamine release in the striatum using *in vivo* microdialysis in freely moving rats have shown that nonselective muscarinic agonists and M_2 antagonists increase dopamine release and that pirenzepine dose-dependently decreases dopamine release[43,44]. The function of m_1 and m_4 receptors on striatal neurons is not well understood.

Clinical studies

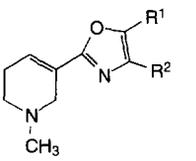
The major CNS indication for muscarinic antagonists has been for idiopathic and drug-induced Parkinson's disease[1], particularly in the treatment of tremor and rigidity[2]. Parkinson's disease is a hypokinetic movement disorder characterized by loss of dopaminergic cells in the substantia nigra with disruption of normal neural activity in basal ganglia circuits[45]. While antimuscarinics such as trihexyphenidyl are effective in Parkinson's disease[1], their mechanism is unclear. Trihexyphenidyl appears to be modestly selective (i.e. no more than tenfold) for M_1/m_1 and m_4 over M_2 and M_3 (Refs 46,47) (cf. trihexyphenidyl analogs in the SAR section below). Its antiparkinsonian effects have been hypothesized to be mediated through M_4 receptors, but not M_1 receptors. The rationale for use of a selective m_4 antagonist in Parkinson's disease[47] is based on the predominance of the m_4 subtype in the striatum relative to the m_1 subtype[41], although the functional role of the m_4 receptor subtype in the striatum and basal ganglia circuitry remains unclear. Recently, researchers at Parke-Davis have discovered a lead compound PD102807, which is a benzoxazine isoquinoline exhibiting m_4 selectivity against m_1, m_2, m_3 and m_5 (Refs 47,48).

Memory studies

Selective muscarinic m_1 antagonists might be valuable as pharmacological tools both for recreating in the laboratory some of the cognitive defects associated with Alzheimer's disease and in the development of selective m_1 agonists for the treatment of this disease; they might also be useful tools for characterizing the different receptor subpopulations. Muscarinic cholinergic antagonists such as scopolamine (Figure 1) have been reported to impair memory in animals[49,50]. However, it was found later that scopolamine may impair performance in memory tasks by affecting both memory and nonmemory variables such as motivation, attention and sensory perception[51,52]. Scopolamine, a nonselective antagonist, has approximately equal affinity for all five subtypes of cloned muscarinic receptors[53]. It is possible, therefore, that this affinity profile can explain the poor selectivity of scopolamine for memory and nonmemory variables by inactivation of different subtypes simultaneously.

To address this question, Bymaster and coworkers[46] demonstrated that the relatively selective muscarinic M_1 antagonist trihexyphenidyl disrupted memory at doses lower than required to decrease rates of responding in a spatial alternation delayed response task, whereas scopolamine decreased rates of responding at doses lower than those required to disrupt memory. This indicates that trihexyphenidyl is more selective in impairing memory over performance of nonmemory components.

A series of alkoxy-oxadiazolyltetrahydropyridines (**1a**, A-OXTPs: R^1 = C_1 to C_4 alkoxy group; R^2 = H, or C_1 to C_4 alkyl group; Figure 2) were shown to have higher affinity (K_i = 0.8–90 nM) for [^{3}H]pirenzepine than [^{3}H]quinuclidinyl benzylate or [^{3}H]oxotremorine-M labeled receptors and to be equipotent and equieffective in antagonizing both salivation and tremor[54]. They are not as selective as pirenzepine. In an operant paradigm using rats, they produced a dose-related decrease in percent correct responding at doses three- to tenfold lower than those that decreased rates of responding. However, only one compound, MB-OXTP (**1b**, R^1 = CH_3O, R^2 = C_4H_9), produced effects on percent correct responding consistent with a selective effect on memory as opposed to nonmemory variables. This study provides evidence that MB-OXTP, a modestly M_1-selective muscarinic receptor antagonist, appears to be more selective in its effects on memory than previously studied muscarinic antagonists. This compound could prove useful for further elucidating the role of the cholinergic system in memory processes.



1a A-OXTP

1b MB-OXTP: $R^1 = CH_3O$, $R^2 = CH_3(CH_2)_3$

Figure 2. *Alkoxy-oxadiazolyltetrahydropyridines.*

Structure–activity relationships

Although pirenzepine binds with high affinity and modest selectivity at M_1 sites in the brain or in the periphery *in vitro*, it does not cross the blood–brain barrier because of its hydrophilic nature, and produces behavioral effects only at high doses[55]. Certain limitations in using pirenzepine to study the muscarinic receptor in the CNS *in vitro* or *in vivo* may be related to the hydrophilic nature of the drug. There is a need for potent, lipophilic and more selective muscarinic ligands with which to study M_1 receptors *in vitro* and *in vivo*. In this section, recent progress in the design of selective M_1 antagonists will be covered.

Caramiphen/aprophen and related derivatives

An examination of para-substitution of caramiphen (**2a**; Figure 3) reveals that compounds with electron-withdrawing ($+\sigma$) substituents, such as nitro, cyano, tetrazolyl and iodo, showed M_1 selectivity, while the derivatives with electron-donating groups ($-\sigma$) groups such as amino and pyrrolidinyl were nonselective in the binding assays[56]. Caramiphen and its analogs were shown to be M_1 selective antagonists (four- to tenfold greater preference for M_1 than for M_3). M_1 binding affinities ([^{3}H]pirenzepine in rat cortex) as well as M_1 over M_2 selectivity of nitrocaramiphen (**2b**, K_i =2.1 nM; ratio = 71) and iodocaramiphen (**2c**, K_i = 5.5 nM; ratio = 59) are shown to be better than or comparable with those of pirenzepine (K_i = 5.2 nM; ratio = 52). Additionally, nitrocaramiphen demonstrates a tenfold selectivity for the M_1 over the M_3 site[56,57].

A conformationally restricted compound (**4**; Figure 3), derived from the muscarinic antagonists caramiphen and aprophen (**3a**), displays approximately the same affinity and selectivity as caramiphen for M_1 (K_i = 2.1 nM) over M_2 (K_i = 44 nM) and M_1 over M_3 (M_3/M_1 = 9). Methyl-aprophen (**3b**) shows good M_1 affinity (K_i = 7 nM) and moderate M_1 selectivity over M_2 and M_3 receptor subtypes (ratio ≈ 15)[58].

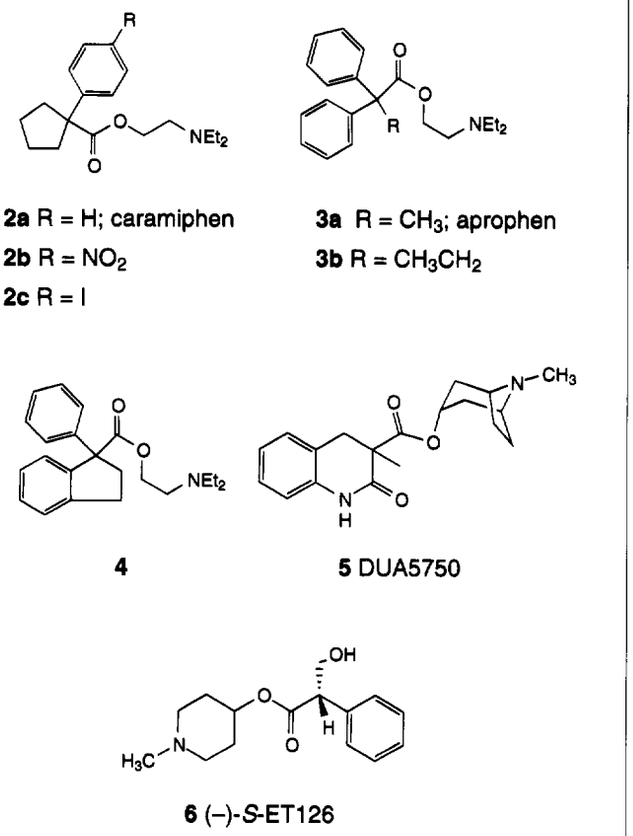


Figure 3. *Caramiphen, aprophen and related derivatives.*

DAU5750 (**5**; Figure 3), a new tropanyl tetrahydroquinoline ester[59], which has been shown to be a mixed M_1–M_3 antagonist (M_2/M_1 = 19), is indicated as an effective bronchospasmolytic, possibly free of anticholinergic side effects. This compound is more potent in M_1 binding assay than in M_3 binding assay (M_3/M_1 = 2). Interestingly, the selectivity profiles of both the eutomer and the distomer, as measured by the affinity ratio (≈ 28) for the M_1, M_2 and M_3 receptor subtypes, were almost identical.

(–)-*S*-ET126 (**6**; Figure 3), was evaluated on M_1 (rabbit vas deferens; pA_2 = 8.99), M_2 (rat left atrium; pA_2 = 8.21) and M_3 (rat ileum; pA_2 = 6.84) muscarinic receptors. The drug shows a surprisingly high M_1 over M_3 subtype selectivity (M_1/M_3 = 178; M_1/M_2 = 8). Like pirenzepine, this compound prevents the antinociception induced by M_1 agonists. Unlike pirenzepine and spirotramine[60], (–)-*S*-ET126 is able to cross the blood–brain barrier, which makes it useful for *in vivo* investigations[61].

DAMP analogs

The constrained tetramine, spirotramine FC15-94 (**7a**; Figure 4), derived from spiro-DAMP (**7b**), displayed a highly selective M_1 affinity over the M_2, M_3 and M_4 subtypes (M_1 >> M_4 = M_2 = M_3; ratio ≈ 75)[60]. Because of its high affinity for muscarinic M_1 receptors and significantly lower affinity for the M_2, M_3 and M_4 subtypes, the spirotramine has an affinity profile better than that of pirenzepine. In contrast, the corresponding nonspiro analogs 4-DAMP (**8a**) and hydroxy-DAMP (**8b**) were only highly selective over M_3 (M_3/M_1 = 27 for 4-DAMP; M_3/M_1 = 60 for hydroxy-DAMP) with equal affinity for the rest of the receptor subtypes. Tumiatti and coworkers also reported[62] that two conformationally rigid stereoisomers, **9a** and **9b**, which are

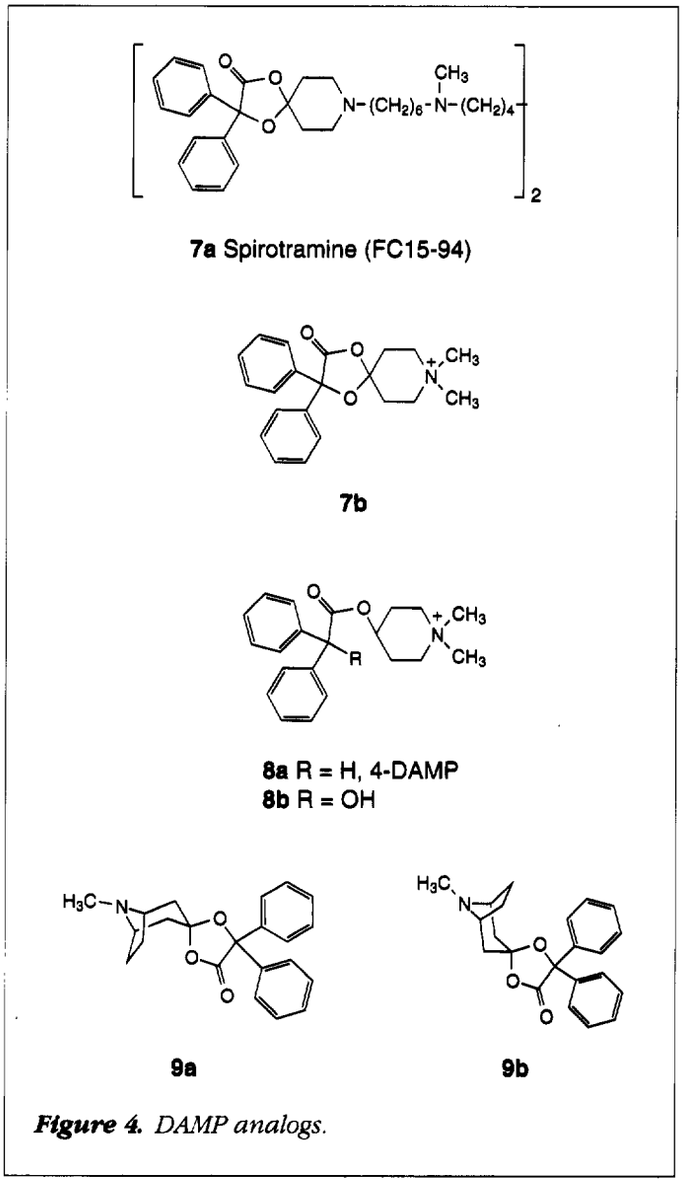
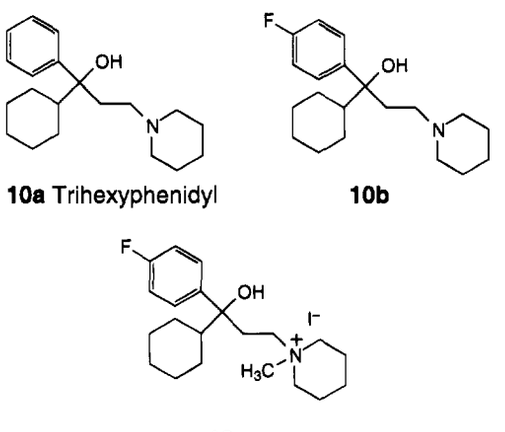


Figure 4. *DAMP analogs.*

analogs of 4-DAMP (**8a**; a relatively selective muscarinic M_1/M_3 receptor antagonist) and spiro-DAMP (**7b**), are highly selective for muscarinic M_1 receptors and displayed only a modest, if any, M_2/M_3 selectivity ratio in both affinity and functional assays. Their affinity profiles[62] ($M_1 > M_4 = M_3 \geq M_2$ for **9a**; $M_1 > M_4 = M_2 \geq M_3$ for **9b**) are comparable with that of pirenzepine and resemble that of spirotramine FC1594 (**7a**). These conformationally rigid templates might be useful for the identification of spatial characteristics of the M_1 ligand binding site.

Trihexyphenidyl analogs

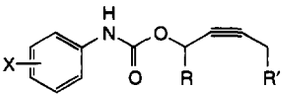
The enantiomers of trihexyphenidyl (THP) (**10a**; Figure 5) exhibit high stereoselectivity (up to 400-fold) at native muscarinic receptors, the *R*-isomer being the eutomer with a selectivity profile of M_1 (pK_i = 9.0) = M_4 (8.8) > M_3 (8.1) > M_2 (7.7)[63]. Several tertiary and quaternary chiral THP analogs, differing in the structure of the basic amino (ammonium) group and the structure of the chain connecting the carbinol carbon atom and the cationic head, as well as four analogs with a *p*-fluorophenyl instead of a phenyl group, exhibited an affinity profile similar to that of THP (Ref. 63). The same trend was observed in enantiomers of *p*-fluorotrihexyphenidyl (**10b**) and its methiodide (**10c**), which were reported to act as pure competitive antagonists in both functional and binding assays[64]. While *R*-**10b** retained the same affinity profile as THP, the methiodide, *R*-**10c**, showed $M_1(m_1)$ selectivity over the other three receptors: $M_1(m_1) > M_2 = M_3(m_3) = m_4$; K_i = 10.3 for M_1. The difference in selectivity profile appears to be a result of methylation, which potentiated affinity for $M_1(m_1)$, M_2 and $M_3(m_3)$



10a Trihexyphenidyl **10b**

10c

Figure 5. *Trihexyphenidyl analogs.*



11 X = 3-Cl, R = H, R′ = $(CH_3)_3N^+$; McNA343
12a X = 4-F, R = CH_3, R′ = pyrrolidino
12b X = 4-F, R = Ph, R′ = pyrrolidino
12c X = 4-F, R = Ph, R′ = $(CH_3)_2N$

Figure 6. *McNA343 analogs.*

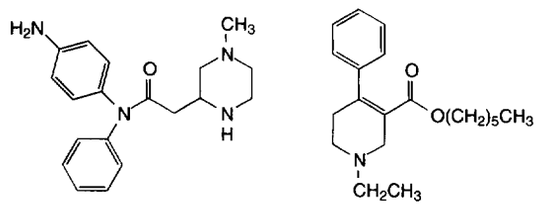
receptors up to 16-fold while not affecting the affinity for m_4 receptors[64].

McNA343 analogs

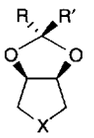
Enantiomers of McNA343 (**11**) analogs **12a**, **12b** and **12c** (Figure 6) were evaluated for antimuscarinic activity in rabbit vas deferens (M_1), guinea-pig atria (M_2) and smooth muscle (M_3). The *S*-isomers in this series were eutomers[65]. Although *S*-**12a** is known as a partial agonist[66], replacement of the methyl group (R = CH_3) with a phenyl produced a eutomer *S*-**12b**, which displayed high potency at M_1 receptors (pA_2 = 9.23) but small discriminative ability (M_1/M_2 = 12; M_1/M_3 = 4). The corresponding *S*-isomer of the dimethylamino derivative **12c** was a weak nonselective antimuscarinic ligand. Contrary to the nonselectivity of the eutomer *S*-**12b**, the distomer, *R*-**12b**, was 251-fold selective for M_1 versus the M_3 receptor subtype (M_1/M_3 selectivity of pirenzepine is 22). This compound displays the best M_1/M_3 selectivity ever reported in the literature. To achieve an M_1/M_3 subtype selectivity either in agonists or antagonists has posed a formidable challenge to medicinal chemists; the remarkable M_1/M_3 selectivity of *R*-**12b** and (–)-*S*-ET126 (**6**) may offer a new opportunity for future design of a selective M_1 muscarinic ligand.

Other SARs

A series of new 3-tropanol and 3-quinuclidinol esters of phenyl-substituted pyrrolidin-, piperidin- and azepin-2-oxo-carboxylic acid were synthesized. The 3-tropanol esters were less active than quinuclidinol esters, which were shown to have a preferential *in vitro* activity at M_1 and M_3 receptor subtypes. Generally, they are more selective at M_1 (ranging from 13 to 51 nM) over M_2 (413–1865 nM) than M_1 over M_3 (86–51 nM). The selectivity is also observed *in vivo*[67].



13 MDL74019DG **14** PD150714



15 R = H, R′ = H, X = CH_3N or $(CH_3)_2N^+$
16 R = H, R′ = CH_2CHPh_2, X = CH_3N
17 R = CH_2CHPh_2, R′ = H, X = CH_3N

Figure 7. *Bicyclodioxolane analogs and benzhydryl derivatives.*

MDL74019DG (*N*-4-aminophenyl-*N*-phenyl-2-[1-(4-methylpiperazino)]-acetamide dimaleate, **13**; Figure 7) is a novel centrally acting muscarinic antagonist with a selectivity profile in affinity assays of m_1 (K_i = 218 nM) > m_3 (1,005 nM), m_4 (1,400 nM), m_5 (1,650 nM) > m_2 (11,250 nM), as well as in functional assays for m_1 versus m_2. The compound antagonized physostigmine-induced yawning in rats (a model for central M_1 activation). On the other hand, the compound had no effect on pilocarpine-induced chewing (model for central M_2 activation). At subcutaneous doses of up to 30 mg/kg, MDL74019DG did not cause memory impairment in rats[68].

PD150714 (**14**; Figure 7) was one of the most potent and selective compounds from a series of 1,4-disubstituted tetrahydropyridine carboxylic acids, with an IC_{50} value of 27.3 nM at m_1 human cloned receptors, and 100-fold (m_2), 48-fold (m_3), 74-fold (m_4), and 19-fold (m_5) selectivities versus the other receptors. The compound appeared to be more selective *in vitro* than the prototypical antagonist pirenzepine. The selectivity was also observed in functional assays (phosphatidyl inositol hydrolysis and cAMP accumulation)[69,70].

A series of bicyclodioxolane analogs (**15**; Figure 7) were shown to be either marginally selective M_1 or non-selective antimuscarinic ligands[71]. Two benzhydryl derivatives (**16**, **17**) exhibited an affinity profile $M_1 \cong M_4 > M_2 >> M_3$ (Ref. 72).

A 64-amino acid peptide isolated from the venom of an African snake is a potent and selective m_1 antagonist. This newly isolated toxin (m_1 toxin) is the ligand that is known to be capable of fully blocking m_1 receptors without affecting m_2–m_5 receptors. Bound toxin can either prevent the binding and action of agonists or antagonists, or prevent the dissociation of antagonists. The toxin is useful for identifying m_1 receptors during anatomical and functional studies, for recognizing and stabilizing receptor complexes, and for occluding m_1 receptors so that other receptors are more readily studied[73].

Imaging agents

Compound **18** (Figure 8), a close analog of the nonselective muscarinic antagonist QNB (**19**), displays about sixfold selectivity for m_1 versus m_2 receptors. Its lipophilicity makes it a good chemical lead for the design of a single photon emission computerized tomography (SPECT) imaging agent for m_1 receptors *in vivo*[74]. Another halogenated quinuclidinyl ester derivative (**20**) was found to be a potent but nonselective muscarinic antagonist, also with the potential to become a SPECT imaging agent for cerebral muscarinic receptors[75]. One of the phenyl rings in QNB can be replaced with an alkoxyalkyl group (e.g. **21**) without noticeable loss of binding affinity[76].

The *N*-methyl groups of pirenzepine and telenzepine were modified to produce chemically functionalized *N*-alkyl analogs using a 'functionalized congener' approach. The potency and selectivity of the derivatives were highly dependent on substitutions of the *N*-methyl group. The affinity in a series of n-alkyl amino derivatives progressively increased with the number of methylene groups. The most

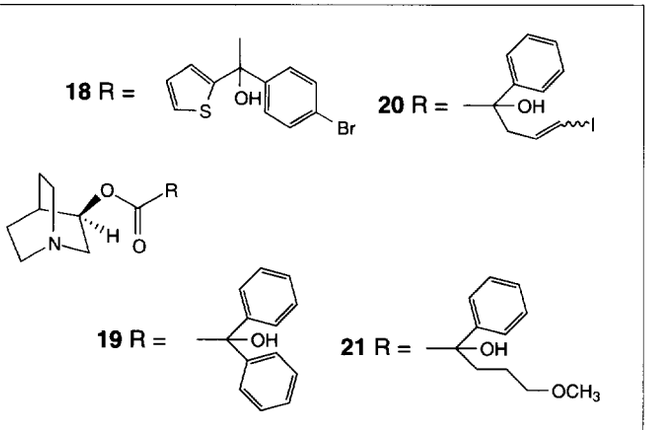
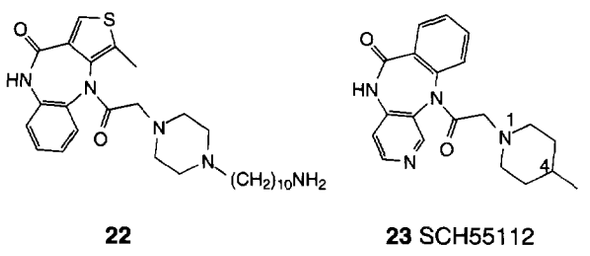


Figure 8. *Quinuclidinyl esters.*



22 **23** SCH55112

Figure 9. *Pirenzepine analogs.*

potent member of the series, containing an n-decylamino group, has been designated TAC (telenzepine amine congener; **22**; Figure 9). The amines were acylated with various reporter groups, resulting in molecular probes of nanomolar affinity[77,78]. A novel fluorescent conjugate of TAC with the fluorescent trisulfonated pyrene dye Cascade Blue may be useful for assaying the receptor as an alternative to radiotracers[77].

Modeling

A new modeling study indicated that the N4 piperazyl nitrogen of pirenzepine was responsible for a hydrogen bond to m_1 Thr32 (m_2 Ala30). When this N4 nitrogen was replaced with carbon, the resulting SCH55112 (**23**; Figure 9) lost the 55-fold M_1/M_2 selectivity of pirenzepine resulting from the specific decrease in M_1 affinity (m_1 K_i = 82 nM versus 4.6 nM for pirenzepine)[79].

Among a series of antimuscarinic achiral 3-heteroaryl substituted quinuclidin-2-ene derivatives, 3-(2-benzofuranyl)-quinuclidin-2-ene displayed the highest M_1 receptor affinity (K_i = 9.6 nM)[80,81]. Modeling studies revealed that these derivatives preferentially adopt conformations in which the heteroaryl substituent is coplanar with the double bond of the quinuclidine ring system, and that such conformers are likely to correspond to those interacting with the muscarinic receptors[80,81]. The docking experiments[80] of this compound in the previously defined m_1 receptor model[82] indicate that the quinuclidin-2-ene ring is located in an area of the receptor defined by Val102, Ala160 and Val385, i.e. in the same location as the quinuclidine ring of the potent agonists such as L670548. The aromatic part of the molecule is directed away from the agonist binding site with the indole ring of Trp400 (TM7) participating in an edge-to-face interaction with the benzenoid part of the benzofuranyl moiety. Using bacteriorhodopsin as a template, conformational analysis and receptor modeling of m_1 and m_2 selective antagonists indicated that the additional negatively charged amino acid in the m_2 receptor is responsible for the subtype specificity of m_1 and m_2 receptors[83].

Conclusions

In the last decade, great progress has been made in designing new generations of M_1-selective antagonists and in improving the selectivity over the first generation of M_1 antagonists such as pirenzepine and trihexyphenidyl. The latter were shown to be modestly M_1/m_1 or M_4/m_4 selective over M_2/M_3 receptor subtypes. Among the new generation, two centrally active M_1 antagonists, PD150714 (**14**) and the spirotramine FC1594 (**7**), have demonstrated remarkable selectivity profiles of $m_1 >> m_2 >> m_4 >> m_3 >> m_5$ and $M_1 >> M_4 = M_2 = M_3$, respectively, although their affinity can be further improved. Two outstanding M_1 antagonists, (–)-*S*-ET126 (**6**) and *R*-**12b**, exhibited very high M_1 over M_3 selectivity (ratio of M_3/M_1 = 178 and 251, respectively). Selective muscarinic M_1 antagonists could have therapeutic potential for both gastrointestinal (e.g. peptic ulcer) and respiratory (e.g. COPD) reflex indications, and may be useful in some CNS applications, such as brain imaging, and as cognition impairing tools for research. While there has been some evidence for therapeutic effects of modestly selective M_1 antagonists in the treatment of peptic ulcer, most clinical studies for other indications have been disappointing. Given the anatomical distribution and physiological function of M_1 and M_3 receptors, it seems that combined M_1/M_3 antagonists may have a better chance for therapeutic use than selective M_1 antagonists. However, it is unclear if the failure in clinical trials is due to the modest selectivity of the M_1 antagonists used or inherent limitations of M_1 antagonists. The new generation of selective M_1 antagonists should be able to address this question.

ACKNOWLEDGEMENTS

We are indebted to Dr Eugene Palmer for helpful discussions and suggestions.

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