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Design and Pharmacology of Quinuclidine Derivatives as M_2-Selective Muscarinic Receptor Ligands

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Abstract—In our search for M_2-selective muscarinic receptor antagonists, we synthesized 1,3-disubstituted indenes. The effects of different basic moieties with regard to binding and selectivity towards the five distinct muscarinic receptor subtypes were investigated. The results show that the quinuclidine series afforded the most promising compounds in terms of both receptor affinity and M_2-subtype selectivity.

Five distinct muscarinic receptors have been cloned (M_1–M_5), which has made it possible to identify selective muscarinic receptor agonists and antagonists. Several M_2-selective compounds have been discovered (e.g., tripitramine, himbacine, AF-DX 384) and tested for in vitro and in vivo selectivity.[1–3] Most of these compounds either have a low subtype selectivity, are not very potent, or do not penetrate into the brain.[4] The objective of this investigation was to discover new M_2-selective muscarinic receptor antagonists capable of crossing the blood–brain barrier with the potential for detecting the loss of M_2 receptors with positron emission tomography (PET) in Alzheimer's disease patients.[5]

Due to the lack of crystal structures of the muscarinic receptor proteins, the binding sites for agonists or antagonists are still unknown. Furthermore, the five mammalian muscarinic receptor subtypes display a high degree of sequence identity, which makes it very difficult to identify highly potent and selective compounds.[6] Because several studies have shown that an aspartate residue represents a key element of the ligand binding site, a basic amine appears to be crucial to elicit high potency.[7] It is believed that highly active muscarinic receptor agonists require hydrogen bond acceptor sites,[8] whereas muscarinic receptor antagonists need a bulky lipophilic group for binding into an aromatic cavity of the receptor.[9] Following these rules, benzofulvenes were synthesized that fulfill the requirements mentioned above. The 2,3-disubstituted indene, (*S*)-dimethindene, has been shown to exhibit antagonist activity towards the muscarinic receptors with selectivity for the M_2 subtype.[10] In our efforts to synthesize new M_2-selective muscarinic antagonists, these same building blocks were used.

The affinity profiles for the five human receptor subtypes (M_1–M_5) of this series of compounds have been studied by the use of transfected Chinese hamster ovarian (CHO) cells and the displacement of [^{3}H]-NMS (*N*-methylscopolamine).[1,11] The most promising compounds in terms of both M_2 receptor affinity and selectivity contain a quinuclidine moiety. Exchanging the 2-pyridyl moiety with 3- or 4-pyridine did not significantly alter potency (Table 1).

The synthesis of the benzofulvenes is shown in Scheme 1. Treatment of indene (**I**) with butyllithium followed by addition of (2-chloro-ethyl)-dimethylamine gave **II**. The substituted indene **II** was condensed with pyridine carbaldehydes to give **1**–**2**. Treatment of **I** with *n*-butyllithium and then dry ice yielded acid **III**.[12] 3*H*-Indene-1-carbonyl chloride (**IV**) was prepared by adding thionylchloride to a suspension of 3*H*-indene-1-carboxylic acid (**III**) in benzene.[13] The acid chloride **IV** treated with various amines and alcohols gave the corresponding

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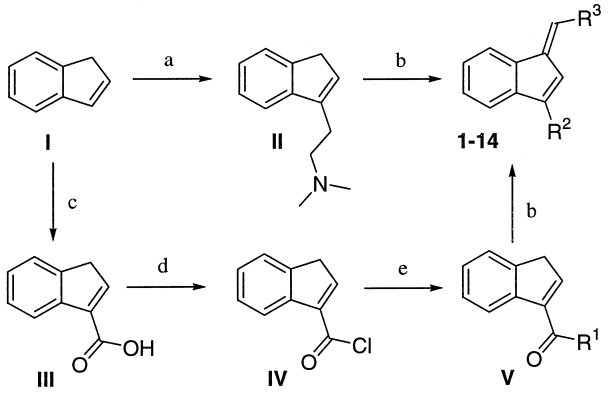
substituted indenes **V**. Treatment of **V** with pyridine carbaldehyde in the presence of potassium hydroxide yielded **9**–**14**. For **3**–**8** no potassium hydroxide was used for catalysis.

A series of benzofulvenes has been synthesized and tested for binding towards the five distinct muscarinic receptor subtypes. Compounds **3**, **4**, and **5** displayed the highest affinities for the muscarinic receptors exhibiting a 19- to 129-fold higher affinity at M_2 receptors compared to the other 1,3-disubstituted indenes. However, compounds **3**–**5** exhibited a decrease in affinity (7- to 9-fold) at M_2 receptors compared to dimethindene.

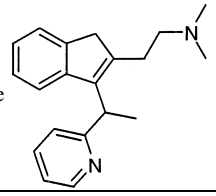
All compounds with basic moieties other than quinuclidine (**1**–**2** and **6**–**14**) show weak binding affinities at the five different muscarinic receptor subtypes. The ester group of compounds **3**–**8** most likely does not form hydrogen bonds with certain amino acids like Asn404 of the M_2 receptor, because the introduction of an ester group did not influence binding.[8] Compounds **3**, **4**, and **5** only show increased selectivity for the M_2 muscarinic receptor subtype (**3**: $M_1/M_2 = 5$-fold; $M_3/M_2 = 5$-fold; $M_4/M_2 = 5$-fold; $M_5/M_2 = 3$-fold). These compounds with a quinuclidine moiety display a 37-fold increase of affinity compared to **6**, which contains the (2-dimethylamino)-ethyl group. The distance between the tertiary amine to the indene-pyridine moiety as well as the position of the nitrogen in the pyridine residue seems to have no significant effects with regard to affinity or selectivity. The compounds **1** and **2**, compared to the closely related structures with ester moieties **6** and **7**, do not display a significant difference in affinity, which led to the assumption made above. The only reason for the relatively high affinity of the compounds **3**, **4**, and **5** is the quinuclidine moiety. According to double mutant cycle analysis on M_2 muscarinic receptors, Asp103 in the third transmembrane region (TM3) is the ligand amine counterion and therefore crucial for both agonist and antagonist binding.[14] Quinuclidine as the basic part

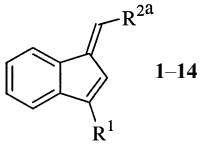


Scheme 1. (a) *n*-BuLi, Cl-$(CH_2)_2N(CH_3)_2$, ether; (b) R^3-CHO, KOH, methanol; (c) *n*-BuLi, CO_2, ether; (d) $SOCl_2$, benzene; (e) X, THF (X = quinuclidinol; *N*,*N*,*N*′-trimethyl-ethane-1,2-diamine; *N*,*N*-dimethyl-ethane-1,2-diamine).

Table 1.

Dimethindene





1–14

Example	R^1	R^2	pK_i[b] M_1	M_2	M_3	M_4	M_5
—	Dimethindene[c]	—	6.58	7.35	6.72	6.39	6.10
1[a]	$(CH_2)_2N(CH_3)_2$	2-Pyridine	5.02	4.90[f]	4.67	4.92	4.72
2	$(CH_2)_2N(CH_3)_2$	3-Pyridine	4.73	4.82	4.56	4.65	4.57
3	**CO_2quinuclidinyl[d]**	**2-Pyridine**	**5.83**	**6.49**	**5.76**	**5.77**	**6.01**
4	**CO_2quinuclidinyl[d]**	**3-Pyridine**	**6.05**	**6.38**	**5.77**	**5.91**	**5.91**
5	**CO_2quinuclidinyl[d]**	**4-Pyridine**	**5.90**	**6.48**	**5.68**	**5.74**	**5.98**
6	$CO_2(CH_2)_2N(CH_3)$	2-Pyridine	5.15	4.92	4.85	4.91	4.75
7	$CO_2(CH_2)_2N(CH_3)_2$	3-Pyridine	4.94	5.13	5.00[f]	4.90[f]	4.52[f]
8	$CO_2(CH_2)_2N(CH_3)_2$	4-Pyridine	4.92[f]	4.98	4.82[f]	4.83[f]	4.57
9	$CONH(CH_2)_2N(CH_3)_2$	2-Pyridine	4.57	4.38	4.21	4.47	4.26
10	$CONH(CH_2)_2N(CH_3)_2$	3-Pyridine	4.55	4.53	4.39	4.67	4.18
11	$COHN(CH_2)_2N(CH_3)_2$	4-Pyridine	4.62	4.80	4.34	4.61	4.27
12	$CON(CH_3)(CH_2)_2N(CH_3)_2$	2-Pyridine	4.66	5.04	4.20	4.33	4.28
13	$CON(CH_3)(CH_2)_2N(CH_3)_2$	3-Pyridine	4.31	4.85	4.14	4.26	4.27
14	COmethylpiperazine[e]	4-Pyridine	4.53	4.41[f]	4.01	4.05	4.26
—	Tripitramine	—	8.80	9.57	7.42	8.19	7.47
—	AF-DX 384	—	7.51	8.22	7.18	8.00	6.27
—	Himbacine	—	6.97	8.00	7.03	7.96	6.31

[a]Compounds **1**–**14** show *E*-configuration determined by nuclear overhauser effect (NOE) experiments.
[b]pK_i values at muscarinic M_1–M_5 receptors were determined in radioligand binding studies at CHO cell membranes. Data are given of at least three experiments performed in duplicate. SD ≤ 0.13 in all cases. Complete protocol is described by Dörje et al.[1] and Buckley et al.[11]
[c]Dimethindene, racemic dimethindene.
[d]Quinuclidinyl, 3-substituted (*R*,*S*)-quinuclidine.
[e]Methylpiperazine, *N*-substituted *N*′-methylpiperazine.
[f]Hill coefficients significantly different from unity.

of the molecules has clearly shown advantages over the other basic moieties. It exhibits not only at least 19-fold higher affinity, but also shows selectivity for the M_2 binding pocket. It is likely that the selectivity between the muscarinic subtypes is based on conformation rather than on single amino acid residues.[15] The rigid quinuclidine is not able to undergo a conformational change and probably shows therefore the observed selectivity. Other basic structures as the ethyl dimethylamine moiety are flexible and show no selectivity for one muscarinic receptor subtype.

In conclusion, the series of fulvenes **1**–**14** demonstrate affinities towards the five muscarinic receptors, and compounds with the quinuclidine moiety clearly show the highest affinities. More importantly, the selectivity for M_2 receptors makes quinuclidine as the ligand amine very valuable in the search for new M_2 selective muscarinic receptor antagonists, especially for related compounds such as dimethindene.

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