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DESIGN, SYNTHESIS AND ANTIMUSCARINIC ACTIVITY OF SOME IMIDAZOLIUM DERIVATIVES

Hiroyuki Miyachi,* Hiromi Kiyota, Mitsuru Segawa

Central Research Laboratories, Kyorin Pharmaceutical Co., Ltd., 2399-1 Mitarai, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan.

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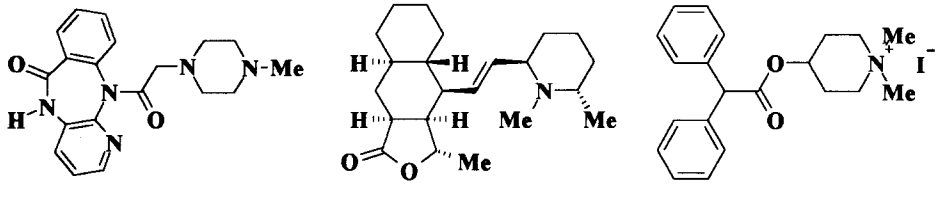
Abstract: A series of imidazolium salt derivatives was prepared as part of a search for subtype-selective antimuscarinic agents. On the basis of measurements of the antimuscarinic activity and subtype-selectivity for M_2 and M_3 muscarinic receptors, the structure-activity relationships of these compounds are discussed.

INTRODUCTION

Muscarinic acetylcholine receptors, members of the huge superfamily of G protein-coupled receptors,[1-3] are heterogeneous, and have been classified into at least three pharmacologically distinct receptor subtypes (M_1-M_3).[4] Receptors having high affinity for terenzepine[5] or pirenzepine[6] are designated as M_1, and are found at high density in neuronal tissues.[6] Those having high affinity for AF-DX 116[7] or himbacine[8] are designated as M_2, and are mainly present in cardiac cells.[9] The M_3 receptor is located specifically in glandular and smooth muscle,[9] and exhibits high affinity for HHSiD (hexahydrosiladifenidol) [10] or 4-DAMP (4-diphenylacetoxy-*N*,*N*-dimethylpiperidinium iodide) [9] (Figure 1).



Pirenzepine **Himbacine** **4-DAMP**

Figure 1. Representative subtype-selective muscarinic acetylcholine receptor antagonists

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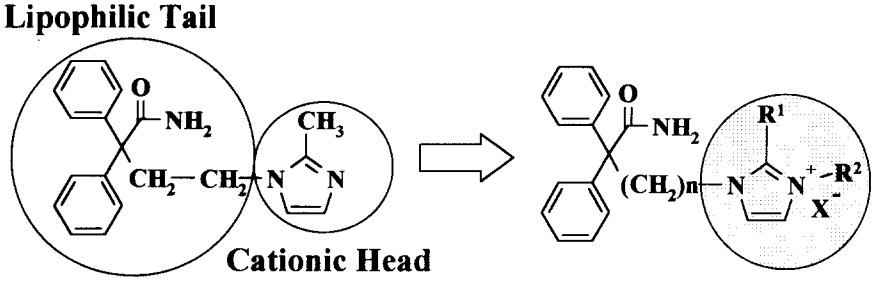
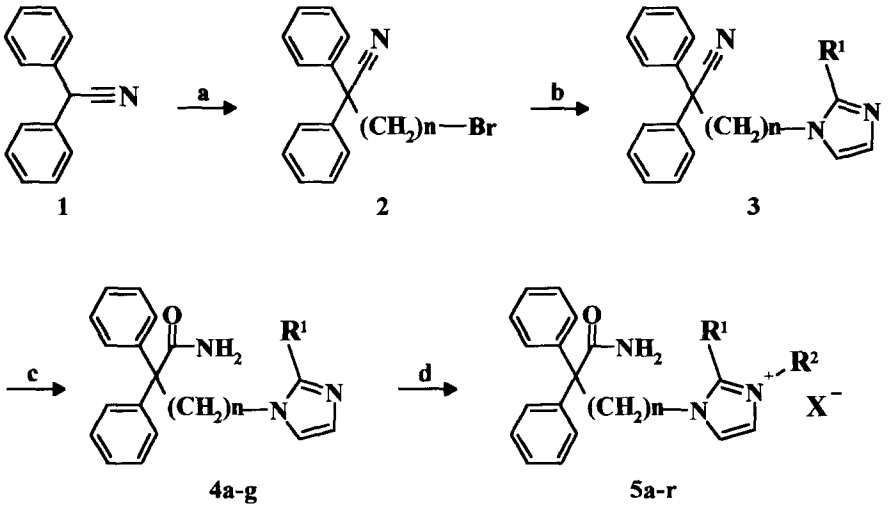
On the other hand, a molecular cloning study indicated that muscarinic acetylcholine receptors are composed of at least five molecularly distinct receptor proteins (m_1-m_5),[11] and the signal transduction cascade of each receptor has been defined.[12] The pharmacologically defined M_1-M_3 receptors are thought to correspond to the cloned m_1-m_3 subtypes. Despite the fruitful results obtained by molecular pharmacological studies, much remains to be learned about the structural requirements for muscarinic ligands (agonist and antagonist) that discriminate one receptor subtype from others, and about the interactions between muscarinic receptors and muscarinic ligands that exhibit subtype-selectivity, although several so-called selective muscarinic receptor antagonists are available (as described above).

Recently, we have reported the design, synthesis and antimuscarinic activity of some novel 4-(imidazol-1-yl)-2,2-diphenylbutyramide derivatives as subtype-selective antimuscarinic agents, and selected 4-(2-methylimidazol-1-yl)-2,2-diphenylbutyramide (KRP-197, **4b**) as a candidate drug for the treatment of urinary incontinence associated with bladder muscle instability.[13] Clinically, antimuscarinic agents such as oxybutynin[14] and terodiline[15] were reported to aggravate the symptoms of dementia patients, although the occurrence rate of this side effect was low. The adverse effect might be due to the penetration of these drugs through blood-brain barrier (BBB), and subsequent inhibition of signal transduction by the neurotransmitter acetylcholine. Tertiary amine class antimuscarinic agents are able to penetrate the BBB to some extent, depending on their p*K*a values.[16] Quaternization of the tertiary amino function is a commonplace chemical manipulation in this class of compounds, widely used to control unwanted effects especially in the central nervous system. Therefore, as a part of our continuing research directed toward the development of subtype-selective antimuscarinic agents, we synthesized some quaternized derivatives of KRP-197 and evaluated their antimuscarinic activity. We also discuss the structure-activity relationships and subtype selectivity of these compounds.



The compounds prepared in this study were synthesized by means of standard procedures as outlined in chart 1 and characterized by ^{1}H-NMR, mass spectral, and elemental analyses (details and physicochemical data of the compounds will be published elsewhere).



[a]*Reagents*: (a) Br-(CH_2)n-Br (n = 2-4), $NaNH_2$, toluene; (b) imidazole derivative, Et_3N, *N,N*-dimethylformamide; (c) aq H_2SO_4; (d) alkyl iodide or benzyl bromide, acetone.

Chart 1[a]. Schematic procedure for the synthesis of the imidazolium derivatives.

Functional activity at receptor subtypes was studied in cardiac and smooth muscle preparations. Potencies are expressed as affinity constants (K_b), i.e., the calculated molar concentration of the compound (antagonist) required to cause a 2-fold increase in the effective concentration (EC_{50}) of the muscarinic agonist carbachol.[17]

Results and discussion

The antimuscarinic activity of the present series of imidazolium salts is summarized in Table 1, together with the results for precursor imidazole derivatives. As already mentioned,[13] introduction of an alkyl substituent at the 2 position of the imidazole ring greatly influenced the antimuscarinic activity and subtype-selectivity in a series of imidazole derivatives (**4a-4e**). Introduction of a methyl group increased both anti-M_3 and anti-M_2 activities. On the other hand, introduction of bulkier substituents generally decreased the antimuscarinic activity; for example, the 2-*n*-propylimidazole derivative (**4d**) exhibited 500- and 60-fold less potent anti-M_3 and anti-M_2 activities as compared to those of the 2-methyl derivative (**4b**), and it showed no marked M_3-selectivity. These results prompted us to speculate that both the width and the length of the substituents introduced at the imidazole ring play a critical role in binding to the receptors, and the shape of the cavity of the anionic site cavity is different in each muscarinic receptor subtype. As for spacer methylene chain length in the imidazole series, the maximum antimuscarinic activity was obtained when the spacer was the ethylene chain (**4b**), for both M_2- and M_3-subtypes, and elongation of the spacer (**4c**, **4d**) decreased the activity.

These data indicate that the distance between the cationic head (imidazole moiety) and the lipophilic tail (diphenylacetamide moiety) of these molecules is important for potent antimuscarinic activity.

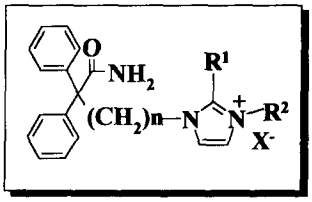
Methyl quaternization of compounds **4b-4d** afforded compounds **5a-5c**, which exhibited almost the same anti-M_3 and anti-M_2 activity and showed no remarkable subtype-selectivity. It is important to note that the effect of quaternization of each imidazole precursor (**4b-4d**) on the antimuscarinic activity is quite different, i.e., methyl quaternization of **4b** did not affect the anti-M_2 activity, while it considerably decreased the anti-M_3 activity. In contrast, methyl quaternization of **4c** and **4d** considerably increased both the anti-M_3 and anti-M_2 activity. This discrepancy is interesting but the reasons remain unknown.

Alkyl quaternization of **4b,** affording compounds **5d-5f**, did not affect the anti-M_2 activity, while it slightly decreased the anti-M_3 activity. Therefore, alkyl quaternization products (**5d-5f**) exhibited equipotent anti-M_2 and anti-M_3 activity and decreased subtype-selectivity, but the benzyl quaternary salt **5g** was unique in that it was much more effective at the M_3 receptor and less potent at the M_2 receptor, though overall it showed less potent activity. The result that **5g** exhibited comparable M_3-selectivity to that of 4-DAMP prompted us to synthesize substituted benzyl imidazolium derivatives (**5h-5r**), and the results for these compounds are also listed in Table 1.

In a series of chloro- and methyl-substituted compounds, both anti-M_3 and anti-M_2 activities increased in the order of *ortho*< *meta*< *para*, indicating that the position of the substituents plays a critical role in the antimuscarinic activity. On the other hand, the electronic factor of the substituent had little or no effect: the chloro- and methyl-substituted compounds show almost the same activity. The 4-bromo-substituted compound (**5n**) showed the strongest inhibitory activity among the substituted benzyl imidazolium analogs listed in Table 1. These data support our previously reported hypothesis that the three-dimensional interactions between the cationic head moiety of the imidazolylbutyramide derivatives and anionic sites of functional muscarinic acetylcholine receptor subtypes play a critical role in subtype-selectivity.[13]

Interestingly, in a series of unsubstituted benzyl imidazolium derivatives (**5g**, **5q**, and **5r**), the maximum antimuscarinic activity was obtained when the spacer was the propylene chain (**5q**), for both M_2- and M_3-subtypes. Therefore, the optimum methylene chain length is shifted from n = 2 to n = 3 by quaternization of the present series of imidazole derivatives. These results might be explained in terms of the minor change of the distance between the cationic head and the hydrophobic tail. In imidazole derivatives, the positive charge is localized to the distal N3 nitrogen, while in the series of imidazolium derivatives, the positive charge is delocalized on the imidazolium ring and the distance between the cationic head and the hydrophobic tail is a little shorter than that in the imidazole series.

TABLE 1 Antimuscarinic Activity of Imidazolium Derivatives of KRP-197 in Guinea-Pig Atria (M_2-Receptor) and Ileum (M_3-Receptor)



						*K*b (nM)		
No.	n	R^1	R^2	X	mp (℃)	M_3	M_2	M_2/M_3 [a]
4a	2	H	-	-	172.0-175.0	5.07	68.4	13.5
4b[b]	2	Me	-	-	189.0-190.0	0.317	4.13	13.0
4c	2	Et	-	-	144.0-146.0	78.1	258	3.30
4d	2	*n*-Pr	-	-	150.0-152.0	177	254	1.44
4e	2	*t*-Bu	-	-	136.0-138.0	30.2	73.9	2.45
4f	3	Me	-	-	128.0-129.0	1.14	21.1	17.6
4g	4	Me	-	-	154.0-156.0	43.7	148	3.39
5a	2	Me	Me	I	234.0-236.0	3.78	6.34	1.68
5b	2	Et	Me	I	229.0-230.5	1.14	4.68	4.11
5c	2	*n*-Pr	Me	I	215.0-216.0	12.5	21.8	1.74
5d	2	Me	Et	I	189.0-192.0	7.11	9.24	1.30
5e	2	Me	*n*-Pr	I	173.0-175.0	4.07	12.3	3.02
5f	2	Me	*n*-Bu	I	164.0-166.0	3.12	18.2	5.83
5g	2	Me	$CH_2C_6H_5$	Br	230.0-232.0	4.96	66.8	13.5
5h	2	Me	CH_2Ph-2-Me	Br	224.0-226.0	21.1	47.3	2.24
5i	2	Me	CH_2Ph-3-Me	Br	210.0-212.0	2.52	11.6	4.60
5j	2	Me	CH_2Ph-4-Me	Br	240.0-242.0	1.02	7.29	7.15
5k	2	Me	CH_2Ph-2-Cl	Br	198.0-199.0	11.8	66.9	5.67
5l	2	Me	CH_2Ph-3-Cl	Br	221.0-222.0	5.62	24.5	4.36
5m	2	Me	CH_2Ph-4-Cl	Br	133.0-135.0	0.647	6.03	9.32
5n	2	Me	CH_2Ph-4-Br	Br	219.0-221.0	0.117	2.58	22.1
5o	2	Me	CH_2Ph-4-NO_2	Br	215.0-217.0	0.858	4.53	5.28
5p	2	Me	CH_2Ph-4-Ph	Br	248.0-249.0	1.24	30.8	24.8
5q	3	Me	$CH_2C_6H_5$	Br	171.0-173.0	0.639	6.35	9.94
5r	4	Me	$CH_2C_6H_5$	Br	155.0-157.0	0.756	19.8	26.2
	4-DAMP					0.794	8.51	10.7

a) The selectivity ratio is the difference between the K_b values at M_2 (atrium) and M_3(ilium) muscarinic receptors. b) KRP-197.

In conclusion, this structure activity relationship study has led to the identification of the M_3-selective imidazolium derivative **5n**, which exhibited more potent and M_3-selective activity than the typical M_3-selective antimuscarinic agent, 4-DAMP. Compound **5n** might be useful not only as a pharmacological tool to study muscarinic acetylcholine receptor localization and internalization, but also as peripherally selective antimuscarinic agent.

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