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Mode of interaction of 1,4-dioxane agonists at the M_2 and M_3 muscarinic receptor orthosteric sites

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ABSTRACT

The methyl group in *cis* stereochemical relationship with the basic chain of all pentatomic cyclic analogues of ACh is crucial for the agonist activity at mAChR. Among these only cevimeline (**1**) is employed in the treatment of xerostomia associated with Sjögren's syndrome. Here we demonstrated that, unlike 1,3-dioxolane derivatives, in the 1,4-dioxane series the methyl group is not essential for the activation of mAChR subtypes. Docking studies, using the crystal structures of human M_2 and rat M_3 receptors, demonstrated that the 5-methylene group of the 1,4-dioxane nucleus of compound **10** occupies the same lipophilic pocket as the methyl group of the 1,3-dioxolane **4**.

The endogenous ligand acetylcholine (ACh), interacting with the five G-protein coupled muscarinic ACh receptor (mAChR) subtypes, namely M_1–M_5, plays an important role both in the peripheral and central nervous systems.[1] While muscarinic antagonists are currently used for the treatment of numerous pathologies associated with the hyperactivity of the muscarinic system, the therapeutic use of agonists is limited by several side effects due to the lack of subtype-selectivity. However, cevimeline (**1**), a pentatomic cyclic analogue of ACh, (Fig. 1) and pilocarpine are used in the treatment of xerostomia associated with Sjögren's syndrome.[2] The replacement of the 1,3-oxathiolane nucleus of cevimeline (**1**), a racemic mixture of *cis*-2-methylspiro(1,3-oxathiolane-5,3′)quinuclidine, with the 1,3-dioxolane nucleus led to the agonist **2**, whose desmethyl analogue (compound **3**) was inactive.[3] *cis*-*N*,*N*,*N*-trimethyl-(2-methyl-1,3-dioxolan-4-yl)methanaminium iodide (**4**),[4] another pentatomic cyclic analogue of ACh, also emerged as a potent agonist at mAChRs. Also in this case the presence of a methyl group in *cis* stereochemical relationship with the basic chain is crucial, its desmethyl analogue **5** being practically inactive in the guinea pig ileum.[5]

Hexatomic nuclei are also compatible with mAChR activity and we demonstrated that 1,4-dioxane compounds are effective muscarinic agonists.[6] Among these compounds, **6** (Fig. 1) showed affinity and potency values similar to those of its lower homologue 1,3-dioxolane **4**. In a structure–activity relationship (SAR) study, in which the methyl group of **6** was alternatively or simultaneously inserted in positions 5 and 6 of the 1,4-dioxane nucleus in all combinations, the shift of the methyl group from position 6 to 5 of the 1,4-dioxane nucleus, affording **7**, was detrimental to muscarinic activity.[7] To complete such an SAR study, in the present investigation, the methyl group in position 6 of the 1,4-dioxane nucleus of **6** was moved to positions 2 and 3 (compounds **8** and **9a**,**b**, respectively) (Fig. 1).

As above mentioned, while the methyl group of cyclic pentatomic mAChR agonists, such as the 1,3-dioxolanes **2** and **4**, has been demonstrated to be essential for the activation of the mAChRs, compound **10** (Fig. 1), the desmethyl analogue of the 1,4-dioxane **6**, has been reported to be a potent muscarinic agonist,[8] but so far a complete pharmacological study at all mAChR subtypes has not been performed yet. Therefore, to clarify the role played by the methyl group of 1,4-dioxane **6** in determining its potent mAChR activity, compound **10** was re-prepared and fully pharmacologically characterized.

Finally, docking studies, using the recently published crystal structures of human M_2[9,10] and rat M_3 receptors,[11] were conducted to rationalize the experimental observations and to

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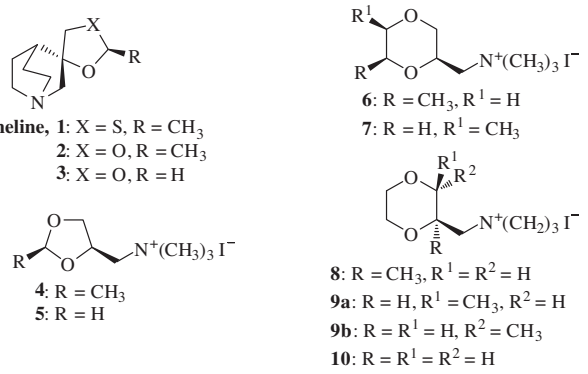

Figure 1. Chemical structures of compounds **1**–**10**.

get information about the mode of interaction of the here reported 1,4-dioxane agonists and their 1,3-dioxolane analogues.

Compounds **8** and **9a**,**b** were prepared following the synthetic procedure reported in Scheme 1. The reaction of 2-(2-methylallyloxy)ethanol (**11**)[12] or 2-(but-3-en-2-yloxy)ethanol (**12**)[13] with mercury(II) acetate followed by treatment with potassium iodide and iodine gave the intermediate iodo-derivatives **13** and **14** (as a *cis*/*trans* mixture), respectively. The *cis* and *trans* diastereomers **14a** and **14b** were separated by flash chromatography. The amination of **13** and **14a**,**b** with dimethylamine, affording **15** and **16a**,**b**, and subsequent treatment with methyl iodide gave compounds **8** and **9a**,**b**, respectively. The structures of diastereomers **9a** and **9b** were assigned by comparing their ^{1}H NMR spectra with those of known 1,4-dioxane 2,3-disubstituted analogues.[14]

Compound **10** was prepared following a synthetic procedure different from that reported in the literature[15] (Scheme 2). The reaction of (1,4-dioxan-2-yl)methanol (**17**)[16] with tosyl chloride, affording **18**, and subsequent amination with dimethylamine yielded **19**, which was treated with methyl iodide to give the methiodide **10**.

The muscarinic pharmacological profile of the novel compounds was evaluated by radioligand binding assays following a previously described protocol.[17] Affinity values, expressed as pK_i, are reported in Table 1 along with those of **4** and **6** for useful comparison.

Compounds **8** and **10** were selected to be evaluated in functional 'in vitro' assays, using carbamyl-β-methylcholine chloride (bethanechol), 4-(m-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) as reference compounds, and their activities are expressed as pD_2 (−logED$_{50}$, agonist potency) and as α (intrinsic activity) (Table 2). Compound **10** was chosen for its interesting affinity profile. Moreover, considering that the introduction of a methyl group at the carbon in position β to the quaternary nitrogen atom of ACh, affording methacholine, is compatible with the muscarinic activity, compound **8**, the β-methyl analogue of **10**, was included in this functional study.

From an analysis of the binding data of compounds **8**–**10** (Table 1) the profile of **10** is particularly interesting, showing affinity values at M_2–M_5 muscarinic subtypes similar to those of **4**, **6**, and carbachol with a slight preference for the M_2 subtype with respect to the other mAChR subtypes. Compared to **4**, the lower affinity value exhibited by **10** for M_1 subtype and its consequent higher selectivity over this subtype can be explained by considering that biophysical and in silico studies emphasized the significant rigidity of the M_1 orthosteric site, which appears to be narrower and more sterically hindered.[18] On these bases, even the simple enlargement of the 1,3-dioxolane to 1,4-dioxane ring is clearly detrimental.

Though it is well known that mAChRs display stereoselective requirements and the biological activity of muscarinic ligands is closely related to their stereochemistry, compound **10** has not been resolved into its two enantiomers as this aspect has already been fully studied for the lead compound **6**.[19] In this case the binding affinities for its four enantiomers demonstrated that the stereogenic center at position 2 of the 1,4-dioxane nucleus does not seem stereochemically important for drug-receptor recognition.

From an analysis of the functional data (Table 2), compound **10** shows potency values quite comparable to those of compound **4** and lead **6** at all mAChRs expressed by the considered tissues. Moreover, its potency at guinea pig ileum is remarkably higher than those of cevimeline (**1**) and compound **2**. To note that the slight preference for the M_2 subtype found in the binding experiments is lost and compounds **4**, **6** and **10** show similar potency values at M_2 and M_3 sites, but higher than those at the mAChRs expressed in rabbit vas deferens and guinea pig lung. Interestingly, unlike what occurs in the case of the 1,3-dioxolane analogues **2** and **4**, the methyl group in position 6 of the 1,4-dioxane nucleus of compound **6** is not a structurally essential requirement to activate mAChRs. The introduction of a methyl group in position 2 of the 1,4-dioxane nucleus of **10** decreases the potency at guinea pig atrium (M_2) and guinea pig ileum (M_3): compound **8** is about 100-fold less active than compound **10**. However, its potency at M_3 mAChR subtype is similar to that of cevimeline. Considering that in this position of the 1,4-dioxane nucleus the 3-methylene group itself might simulate the β-methyl group of methacholine, the further introduction of a methyl group at the carbon in position 2 is detrimental for the muscarinic activity as already demonstrated in the case of β,β-dimethyl-acetylcholine[20] and of the 1,3-dioxolane **4**, whose 4-methyl derivative has been reported to be 300-fold less active.[4] The reduction in activity might be due to unfavorable binding by the 2-methyl substituent and to the hindrance offered by this substituent to the correct orientation of the 2-$CH_2N^+Me_3$ group.

To rationalize the experimental observations and to get information about the mode of interaction of the reported 1,4-dioxane agonists, docking simulations were conducted using the recently published crystal structures of human M_2[9,10] and rat M_3 receptors.[11] Although the conformational analyses of 1,4-dioxane ring showed that the more extended chair conformation is the preferred one,[21] for all considered derivatives docking simulations were performed by considering both chair and twist-chair (and more folded) geometries to evidence how the ring puckering affects the ligand recognition by the simulated muscarinic receptors.

Docking simulations reveal that the M_2 subtype in its open inactive state can suitably recognize both considered ligand conformations stabilizing rather similar interaction patterns. For

11: R = CH$_3$, R^1 = H
12: R = H, R^1 = CH$_3$
13: R = CH$_3$, R^1 = H
14a (*cis*): R = H, R^1 = CH$_3$
14b (*trans*): R = H, R^1 = CH$_3$
15: R = CH$_3$, R^1 = H
16a (*cis*): R = H, R^1 = CH$_3$
16b (*trans*): R = H, R^1 = CH$_3$
8: R = CH$_3$, R^1 = H
9a (*cis*): R = H, R^1 = CH$_3$
9b (*trans*): R = H, R^1 = CH$_3$

Scheme 1. Reagents: (a) $(CH_3COO)_2Hg$; (b) KI, I_2; (c) $(CH_3)_2NH$, benzene; (d) CH_3I, diethyl ether.