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Brief Article

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1,4-Dioxane, a Suitable Scaffold for the Development of Novel M_3 Muscarinic Receptor Antagonists†

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Supporting Information

ABSTRACT: In this study the modulation of the pharmacological profile from agonist to antagonist was successfully obtained by replacing the methyl group in position 6 of the 1,4-dioxane scaffold of the potent M_2/M_3 muscarinic agonist **1** with bulkier groups. In particular, the 6,6-diphenyl substitution provided the potent M_3 preferring antagonist (±)-**17**, which in in vivo study proved to be effective in reducing the volume-induced contractions of rat urinary bladder and was devoid of cardiovascular effects.

INTRODUCTION

Muscarinic acetylcholine receptors (mAChRs) are subdivided into five different subtypes, M_1−M_5, and are involved in the regulation of several physiological functions.[1] Though muscarinic agonists have been reported to be useful in cognitive disorders, muscarinic antagonists are therapeutically more interesting, being currently used for the treatment of numerous pathologies associated with the hyperactivity of the muscarinic system. In particular, M_3 antagonists are useful for the treatment of smooth muscle disorders, such as chronic obstructive pulmonary disease (COPD),[2] overactive bladder (OAB),[3] and irritable bowel syndrome (IBS).[4] In the symptomatic treatment of OAB, mAChR antagonists block M_2 and M_3 receptors localized on detrusor smooth muscle cells and on urothelium and suburothelium within the urinary bladder wall. In this tissue, the M_3 subtype has been demonstrated to mediate the direct contractile responses necessary for the normal bladder function.[3] At present, combination therapies of β_2-agonists with muscarinic antagonists are given for severe COPD and antimuscarinic drugs are currently marketed for the treatment of OAB and IBS. However, because of the lack of M_3 subtype selectivity, these drugs present numerous side effects, especially those cognitive and cardiovascular owing to their interaction with M_1 and M_2 subtypes, respectively.[2−5] Therefore, potent and selective M_3 antagonists would maximize therapeutic efficacy and minimize unpleasant side effects. We have recently demonstrated that the 1,4-dioxane ring is a suitable scaffold for building ligands targeting mAChRs. In particular, **1** (Figure 1) has emerged as a preferential M_2/M_3 subtype agonist.[6] Since the replacement of the methyl group in muscarinic agonists, such as ACh or muscarine and its 1,3-dioxolane analogue (**2**), with bulkier groups modulates the pharmacological profile from agonist to antagonist,[7] in this study, the methyl group in position 6 of the 1,4-dioxane nucleus of **1** has been replaced by ethyl, phenyl, two phenyl, or diphenylmethane moieties (**3**−**6**, respectively) to obtain novel muscarinic antagonists, possibly endowed with subtype selectivity. Moreover, since the interaction of a ligand with mAChRs is usually extremely specific, the role of stereochemistry has been investigated through the cis and trans diastereomers of the 6-monosubstituted derivatives and the enantiomers of the 6,6-diphenyl-substituted **5**. Finally, considering that ligands with a tertiary basic function can act as antagonists at mAChRs,[7] probably by binding in the cationic form, the racemic tertiary amine **17** and its enantiomers have also been pharmacologically evaluated.

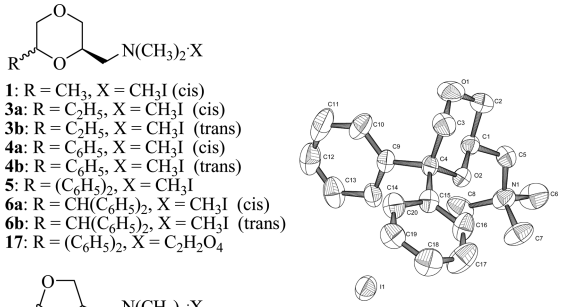

Figure 1. Structures of **1**−**6**, **17**, **22**, and **23** and X-ray structure of (*R*)-(+)-**5**.

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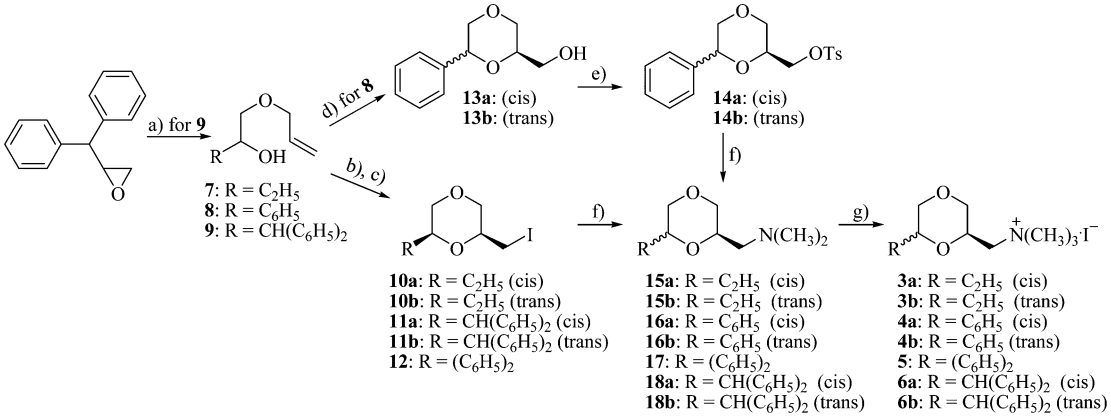
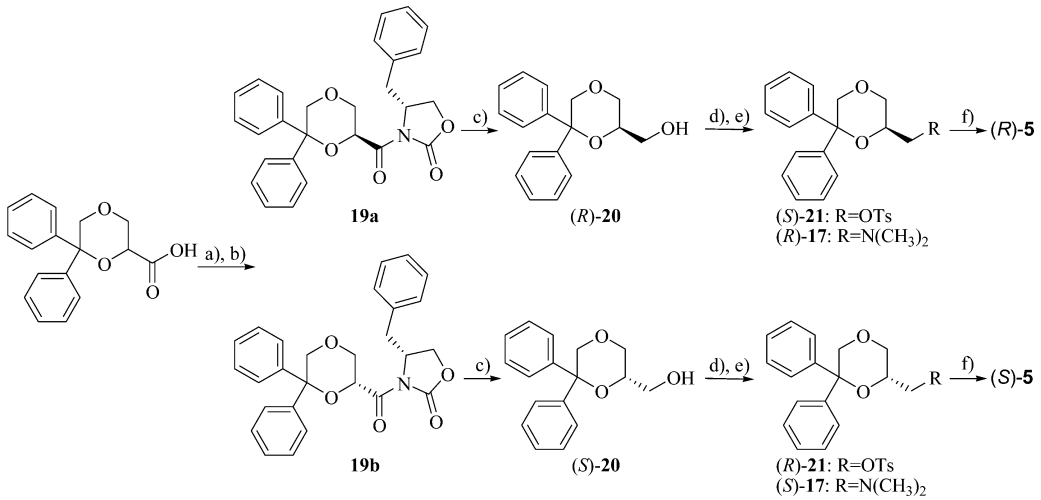
Scheme 1[a]



[a]Reagents: (a) $CH_2{=}CHCH_2OH$, Na; (b) $(CH_3COO)_2Hg$, H_2O; (c) KI, I_2; (d) *m*-CPBA, CH_2Cl_2; (1*S*)-(+)-10-CSA, CH_2Cl_2; (e) *p*-TsCl, pyridine; (f) Me_2NH, benzene; (g) CH_3I, diethyl ether.

Scheme 2[a]



[a]Reagents: (a) $(COCl)_2$, CH_2Cl_2, cat. DMF; (b) *n*BuLi, (*R*)-(+)-4-benzyl-2-oxazolidinone, THF, −78 °C; (c) $LiBH_4$, THF, H_2O; (d) *p*-TsCl, pyridine; (e) Me_2NH, benzene; (f) CH_3I, diethyl ether.

CHEMISTRY

Compounds **7** and **8** were obtained as previously reported in the literature,[8,9] whereas the novel compound **9** was prepared by treating 2-benzhydryloxirane[10] with metallic sodium and allyl alcohol (Scheme 1). The allyloxyalkanols **7** and **9** were treated with mercury(II) acetate followed by an aqueous solution of iodine and potassium iodide, affording a mixture of the diastereomers **10a**/**10b** and **11a**/**11b**, respectively, which were separated by column chromatography. Iodo derivative **12** was obtained as described in the literature.[9] Alcohols **13a** and **13b**, prepared starting from **8**,[9] were treated with tosyl chloride in pyridine to yield **14a** and **14b**, respectively. The amination of iodo derivatives **10**−**12** and tosyl derivatives **14** with dimethylamine afforded amines **15**−**18**, which were transformed into the methiodides **3**−**6**. The stereochemical relationship between the substituents in positions 2 and 6 of 1,4-dioxane nucleus of **3a**/**3b** and **6a**/**6b** was assigned by comparing the ^{1}H NMR spectra of the corresponding free amines **15a**/**15b** and **18a**/**18b** with those of the 6-methyl and 6-phenyl analogues, whose structures had previously been determined by X-ray crystallography or the NOE effect, respectively.[6,9] In particular, in the ^{1}H NMR spectra the protons of the 2-methylene group of **15b** and **18b** show a deshielding effect with respect to the same protons in the diastereomers **15a** and **18a**. Therefore, the stereochemical relationship between the substituents in positions 2 and 6 is cis in **15a** and **18a** and trans in **15b** and **18b**. For the preparation of (*R*)-(+)- and (*S*)-(−)-**5** the 6,6-diphenyl-1,4-dioxane-2-carboxylic acid[9] was converted into the corresponding acyl chloride which, treated with the lithium salt of the (*R*)-(+)-4-benzyl-2-oxazolidinone, yielded the diastereomers **19a** and **19b**, easily separable by column chromatography (Scheme 2). These derivatives were reduced with lithium borohydride to yield the enantiomeric alcohols (*R*)-(+)- and (*S*)-(−)-**20**, respectively, which were treated with tosyl chloride and subsequently with dimethylamine to afford the free amines (*R*)-(+)- and (*S*)-(−)-**17**. These were transformed into the corresponding methiodides (*R*)-(+)- and (*S*)-(−)-**5**. Enantiomeric purity of amines (*R*)-(+)- and (*S*)-(−)-**17**, determined by ^{1}H NMR spectroscopy on addition of the chiral shift reagent (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)-MTPA] and in comparison with the spectrum of racemic compound (±)-**17**, was found to be >98% (detection limit) for both of them. In fact, the spectrum of racemic (±)-**17** in the presence of (+)-MTPA showed two double doublets at δ 3.35

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Table 1. Affinity Constants (pK_i)[a] of 1, 3−6, 17, Enantiomers of 5 and 17, and NMS for Human Cloned Muscarinic Receptors, Expressed in CHO Cells; Potency ($pD_2 = -\log EC_{50}$)[b] and Intrinsic Activity (α)[c] for 1 and 3, and Dissociation Constants (pK_B)[d] for 4, (±)-5, (S)-(−)-5, (R)-(+)-5, (±)-17, (S)-(−)-17, (R)-(+)-17, 22, 23, Oxybutynin, Methoctramine, and 4-DAMP in the Isolated Guinea Pig Left Atrium (M_2), Longitudinal Ileum (M_3), and Lung (M_4) Muscarinic Receptors[e]

	binding data, pK_i					functional data				
						M_2		M_3		M_4
compd	hM_1	hM_2	hM_3	hM_4	hM_5	pD_2 (pK_B)	α	pD_2 (pK_B)	α	pD_2 (pK_B)
1 (cis)	4.52	5.80	5.12	4.94	5.11	7.57 ± 0.11	1	7.34 ± 0.13	1	
3a (cis)	4.81	5.20	4.75	4.60	4.58	5.62 ± 0.20	1	4.82 ± 0.05	1	*f*
3b (trans)	<4	4.74	4.09	<4	<4	5.45 ± 0.11	0.7	5.19 ± 0.08	1	*f*
4a (cis)	5.17	5.19	5.01	4.91	4.99	(5.56 ± 0.11)	0	(5.20 ± 0.08)	0	(5.24 ± 0.20)
4b (trans)	4.58	4.40	4.50	4.53	4.48	(5.14 ± 0.17)	0	(<5)	0	(<5)
(±)-**5**	9.10	8.24	8.44	8.58	8.36	(8.21 ± 0.16)	0	(8.34 ± 0.03)	0	(7.77 ± 0.22)
(*S*)-(−)-**5**	9.30	8.55	8.83	8.83	8.77	(7.31 ± 0.08)	0	(8.52 ± 0.10)	0	(6.70 ± 0.19)
(*R*)-(+)-**5**	7.79	7.48	7.21	6.82	6.97	(6.67 ± 0.01)	0	(7.22 ± 0.03)	0	(6.62 ± 0.04)
6a (cis)	5.42	4.55	5.05	4.95	5.12					
6b (trans)	4.80	4.10	4.67	4.77	4.50					
(±)-**17**	8.34	7.78	8.66	8.31	8.27	(6.95 ± 0.19)	0	(8.24 ± 0.05)	0	(7.02 ± 0.22)
(*S*)-(−)-**17**	8.90	8.01	8.82	8.63	8.74	(7.63 ± 0.01)	0	(8.34 ± 0.03)	0	(6.94 ± 0.14)
(*R*)-(+)-**17**	7.45	7.02	7.14	7.15	7.09	(7.00 ± 0.25)	0	(7.45 ± 0.12)	0	(7.24 ± 0.01)
NMS	9.49	9.75	9.87	9.85	9.68					
22						(8.29)[g]		(7.91)[g]		
23						(7.11)[g]		(6.38)[g]		
oxybutynin	8.62	7.93	8.82	8.44	7.85	(7.45 ± 0.28)	0	(8.47 ± 0.08)	0	(7.19 ± 0.26)
methoctramine						(7.8−8.3)[h]		(6.3−6.9)[h]		(7.6)[h]
4-DAMP						(8.0−8.4)[h]		(9.02 ± 0.06)		(9.4)[h]

[a]Data are the mean ± SEM of three experiments performed in duplicate. K_i values were from two to three experiments which agreed ±10%. [b]pD_2 values are the −log of the agonist concentration that caused 50% of the maximum response attainable in that tissue. [c]Intrinsic activity was measured by the ratio between the maximum response of the agonist and the maximum response of bethanechol at guinea pig atrium and ileum receptors. [d]Dissociation constants were calculated according to Furchgott.[20] [e]The results are the mean ± SEM of four to six independent experiments. [f]This compound showed no agonist activity and, when tested as antagonist, proved inactive up to 10 μM. [g]Data from ref 7. [h]Data from ref 21.

and 3.55 ppm for the 2-methylene protons, whereas only one double doublet was observed for (+)-**17** and (−)-**17** at δ 3.35 and 3.55 ppm, respectively. The absolute configuration *R* was assigned to the dextrorotatory enantiomer (+)-**5** through X-ray diffraction analysis (Figure 1).

RESULTS AND DISCUSSION

The muscarinic binding profile of the novel compounds was evaluated using [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) as radioligand to label cloned human muscarinic hM_1−hM_5 receptors, expressed in Chinese hamster ovary (CHO) cells (Table 1).[11] The functional activities of the tested compounds were determined on guinea pig left atrium (M_2),[12] ileum (M_3),[13] and lung strips (putative M_4)[14] and are reported as pD_2 or pK_B values for agonist or antagonist compounds, respectively (Table 1). The antagonist potencies of (±)-**17** and (*S*)-(−)-**5** on rabbit vas deferens (putative M_1)[15] were also determined. Moreover, the affinity values, functionally determined by us or collected from the literature, of reference muscarinic antagonists methoctramine and 4-DAMP, purportedly selective toward M_2 and M_3 receptor subtypes, respectively, dioxolane derivatives **22** and **23**[7] (Figure 1), lower homologues of **5** and **17**, respectively, and oxybutynin are also reported for the sake of comparison. Some discrepancies observed between pK_i and pK_B values can be explained by strain/species differences[16] and by the different organization of the native and cloned receptor populations.[17] Those between functional activity on rabbit vas deferens and guinea pig lung preparations and the binding data on hM_1 and hM_4, respectively, are expected and would reinforce the doubts regarding the prediction of these functional models.[17,18] Binding data show that the replacement of the methyl group in position 6 of **1** with an ethyl or only one phenyl group (**3** or **4**, respectively) does not significantly alter the muscarinic affinities of the lead **1** (Table 1). Interestingly, the introduction of two phenyl groups in the same position (**5**) markedly increases the affinity for all muscarinic subtypes. In contrast, the increase of the distance between the diphenyl hydrophobic portion and the basic function of **5**, affording the diastereomers **6a** and **6b**, lowers the binding affinities at all the muscarinic receptor subtypes to values similar to those of **1**. This effect is independent of the stereochemical relationship between the substituents in positions 2 and 6. The high muscarinic affinity displayed by **5** is enhanced by its eutomer (*S*)-(−)-**5**, while the enantiomer (*R*)-(+)-**5** shows significantly lower values at the five mAChR subtypes (20-, 6-, 17-, 58-, and 25-fold, respectively), suggesting that in this series of compounds the stereocenter in position 2 plays a critical role in the interaction of the antagonist with mAChRs. Interestingly, the tertiary amine (±)-**17** and its (*S*)-eutomer are preferring M_3 over M_2 subtype. However, they are also endowed with similar high affinity for all the other muscarinic subtypes. In particular, considering that they might cross the brain−blood barrier, the blockade of central M_1 receptors would produce cognitive side effects.[5] The functional data reported in Table 1 show that the 6-ethyl diastereomers **3a** and **3b**, inactive at the M_4 subtype, behave as agonists at M_2 and M_3 receptor subtypes but are endowed with about 100-fold lower potency with respect to the lead **1**, confirming that the increase of the distance between the terminal methyl group and the basic nitrogen atom (Ing's rule) is a critical requirement for the agonist potency. The 6-

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monophenyl analogues **4a** and **4b** behave as weak antagonists, and their activity appears to be scarcely affected by the stereochemical relationship between the substituents in positions 2 and 6 of the 1,4-dioxane nucleus. The introduction of two phenyl groups in position 6 of the 1,4-dioxane nucleus produces the potent nonselective antagonist **5**, which shows affinity values similar to those of its dioxolane analogue **22**. Therefore, as expected, the modulation of the biological muscarinic profile from a full potent M_2/M_3 agonist (**1**) to a potent antagonist (**5**) has successfully been obtained. The important role played by the configuration of the stereocenter in position 2 on the affinity for M_2 and M_3 receptor subtypes, emerged from the binding assays, is confirmed by the functional studies, with the eutomer being the (*S*)-enantiomer. Interestingly, unlike the methiodide **5**, the tertiary amine **17** and to a lesser extent its (*S*)-eutomer preferentially block the M_3 receptor subtype with respect to the M_2, with an M_3/M_2 selectivity ratio slightly higher than those of oxybutynin and the conventional M_3 selective antagonist 4-DAMP. The observation that a reversal of the selectivity ratio is observed with respect to its dioxolane lower homologue **23**, which shows a pK_B value at M_2 higher than that at M_3, is also noteworthy. Surprisingly, preferring M_3 antagonist activity, missing in the racemic methiodide (±)-**5**, is also displayed by its (*S*)-eutomer. On the basis of their favorable preferring M_3 antagonism unmasked by in vitro assays, the oxalate (±)-**17** and the methiodide (*S*)-(−)-**5** were also evaluated on rabbit vas deferens preparation (putative M_1). On this preparation, the tertiary amine (±)-**17** shows a pK_B of 7.86 ± 0.03, which is lower than that at M_3 (pK_B = 8.24), whereas the pK_B found for (*S*)-(−)-**5** (pK_B = 8.86 ± 0.01) is higher than that at M_3 subtype (pK_B = 8.52). Moreover, these two compounds were evaluated in vivo in the anesthetized rat for their ability to affect the volume-induced contractions of urinary bladder and the cardiovascular parameters (Table 2). Compared to the reference compound oxybutynin, an antagonist used for the treatment of OAB,[5] the newly synthesized 1,4-dioxane derivatives exhibit a higher potency. Moreover, at 1 μg/kg iv, they display a comparable or enhanced efficacy in reducing the area of the voiding contractions. Similar to oxybutynin and unlike methoctramine,[19] a selective M_2 antagonist, they are devoid of significant effects on mean arterial pressure (MAP) and on heart rate (HR) (Table 2), confirming that they are preferring M_3 over M_2 subtype as shown in functional in vitro assays. In conclusion, this study has demonstrated that a 1,4-dioxane nucleus might be a suitable scaffold for building muscarinic antagonists. The potent and preferential M_3 antagonism of the tertiary amine (±)-**17** might open a promising strategy for designing novel muscarinic subtype-selective antagonists.

Table 2. Potency, Expressed as ID_{50}[a] (μg/kg iv) and Efficacy, Expressed as I_{max} (%)[b] in the Micturition Reflex and Percentage of Changes in Mean Arterial Pressure (MAP) and Heart Rate (HR) of (*S*)-(−)-5, (±)-17, and Oxybutynin in the Anaesthetized Rat

	cystometric		cardiovascular	
compd	ID_{50}	I_{max} (%)	MAP (%)	HR (%)
(*S*)-(−)-**5**	201.4	96	−12	+5
(±)-**17**	134.0	72	−18	+4
oxybutynin	347.4	75	+20	+7

[a] ID_{50} is the dose required to produce 50% inhibition of AUC peaks from saline baseline. [b] I_{max} is the % maximal inhibition of VIBC amplitude compared to saline baseline.

EXPERIMENTAL SECTION

The purity of the novel tested compounds was determined by combustion analysis and was ≥95%.

(6,6-Diphenyl-1,4-dioxan-2-yl)-*N*,*N*-dimethylmethanamine Oxalate (17). A solution of **12**[9] (1.1 g, 2.9 mmol) and dimethylamine (5 mL) in dry benzene (15 mL) was heated in a sealed tube at 120 °C for 60 h. The residue was dissolved in $CHCl_3$, which was washed with 2 N NaOH and dried over Na_2SO_4. The removal of the solvent gave a residue, which was purified by column chromatography. Eluting with $CHCl_3/CH_3OH$ (97:3) afforded **17** as the free base: 0.7 g (80% yield). ^1H NMR ($CDCl_3$): δ 2.22 (s, 6, $N(CH_3)_2$), 2.42 (m, 2, CH_2N), 3.38−3.62 (m, 2, cycle), 3.76−3.84 (m, 2, cycle), 4.64 (d, 1, cycle), 7.19−7.57 (m, 10, ArH). The free amine was transformed into the oxalate salt, which was recrystallized from 2-PrOH (mp 152−153 °C). Anal. ($C_{19}H_{23}NO_2 \cdot C_2H_2O_4$) C, H, N.

(6,6-Diphenyl-1,4-dioxan-2-yl)-*N*,*N*,*N*-trimethylmethanaminium Iodide (5). A solution of **17** (0.4 g, 1.4 mmol) in Et_2O (10 mL) was treated with an excess of methyl iodide. After 24 h at room temperature the solid was filtered and recrystallized from 2-PrOH (mp 232−234 °C). ^1H NMR (DMSO): δ 3.05 (s, 9, $N(CH_3)_3$), 3.25−4.01 (m, 6, CH_2N, cycle), 4.82 (d, 1, cycle), 7.21−7.37 (m, 10, ArH). Anal. ($C_{20}H_{26}INO_2$) C, H, N.

(*R*)-4-Benzyl-3-((*S*)-6,6-diphenyl-1,4-dioxane-2-carbonyl)-oxazolidin-2-one (19a) and (*R*)-4-Benzyl-3-((*R*)-6,6-diphenyl-1,4-dioxane-2-carbonyl)oxazolidin-2-one (19b). Oxalyl chloride (0.7 mL) and DMF (0.2 mL) were added to a solution of 6,6-diphenyl-1,4-dioxane-2-carboxylic acid[9] (1.6 g, 5.6 mmol) in CH_2Cl_2 (15 mL). After 1 h at 25 °C the solvent was removed and the residue was dissolved in dry toluene (15 mL) and added at −78 °C to the lithium anion of (*R*)-(+)-4-benzyl-2-oxazolidinone prepared by adding *n*-BuLi (2.25 mL) to a solution of (*R*)-(+)-4-benzyl-2-oxazolidinone (1.0 g, 5.6 mmol) in THF (30 mL) at −78 °C and stirring for 1 h. After 45 min at −78 °C Et_2O (50 mL) was added. The organic phase was washed with NH_4Cl (20 mL) and dried over Na_2SO_4. The removal of the solvent afforded a mixture of diastereomers, which were separated by column chromatography, eluting with cyclohexane/EtOAc (9:1). **19a** eluted first: 1.3 g; 52% yield; mp 85−87 °C. ^1H NMR ($CDCl_3$): δ 2.82 (dd, 1, CHAr), 3.25 (dd, 1, CHAr), 3.64 (m, 2, cycle), 4.23 (m, 3, CH_2OCO, CHN, cycle), 4.61 (m, 2, CH_2OCO, cycle), 5.27 (dd, 1, cycle), 7.18−7.56 (m, 15, ArH). The second fraction was **19b**: 0.77 g; 31% yield; 93−94 °C. ^1H NMR ($CDCl_3$) δ 2.83 (dd, 1, CHAr), 3.39 (dd, 1, CHAr), 3.69 (m, 2, cycle), 4.19 (m, 3, CH_2OCO, CHN, cycle), 4.62 (m, 2, CH_2OCO, cycle), 5.38 (dd, 1, cycle), 7.09−7.61 (m, 15, ArH).

(*R*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methanol [(*R*)-(+)-20]. H_2O (0.035 mL) and then $LiBH_4$ (0.036 g) in Et_2O (10 mL) were added to a solution of **19a** (0.75 g, 1.7 mmol) in Et_2O (50 mL) at 0 °C. After 2 h at 0 °C, 1 N NaOH (3 mL) was added. The mixture was extracted with EtOAc, which was dried over Na_2SO_4. The removal of the solvent afforded a residue which was purified by column chromatography, eluting with cyclohexane/EtOAc (8:2) to give (*R*)-(+)-**20** as a solid: 0.28 g; 60% yield; mp 114−115 °C; $[\alpha]^{20}_D$ +222.32 (*c* 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound.[9]

(*S*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methanol [(*S*)-(−)-20]. This was obtained as a solid following the procedure described for (*R*)-(+)-**20**: 60% yield; mp 114−115 °C; $[\alpha]^{20}_D$ −225.58 (*c* 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound.[9]

(*S*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methyl 4-Methylbenzenesulfonate [(*S*)-(+)-21]. Tosyl chloride (0.4 g, 2.1 mmol) was added to a stirred solution of (*R*)-(+)-**20** (0.4 g, 1.5 mmol) in pyridine (5 mL) at 0 °C over 30 min. After 3 h at 0 °C, the mixture was left for 20 h at 4 °C in the freezer. Then it was poured into ice and concentrated HCl (5 mL) and extracted with $CHCl_3$. The organic layers were washed with 2 N HCl (15 mL), $NaHCO_3$ saturated solution (15 mL), and

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H_2O (15 mL) and then dried over Na_2SO_4. The evaporation of the solvent afforded (*S*)-(+)-**21** as a solid (mp 130−131 °C): 0.44 g; 69% yield; $[\alpha]^{20}_D$ +123.48 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 2.41 (s, 3, CH_3), 3.41 (m, 2, CH_2O), 3.70 (m, 2, cycle), 4.01 (m, 2, cycle), 4.48 (d, 1, cycle), 7.08−7.78 (m, 14, ArH).

(*R*)-(6,6-Diphenyl-1,4-dioxan-2-yl)methyl 4-Methylbenzenesulfonate [(*R*)-(−)-21]. This was obtained as a solid following the procedure described for (*S*)-(+)-**21** (mp 130−131 °C): 70% yield; $[\alpha]^{20}_D$ −121.56 (*c* 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of (*S*)-(+)-**21**.

(*R*)- and (*S*)-(6,6-Diphenyl-1,4-dioxan-2-yl)-*N*,*N*-dimethylmethanamine Oxalate [(*R*)-(+)-17 and (*S*)-(−)-17]. These were prepared as described for **17**: 81% yield. (*R*)-(+)-**17**: $[\alpha]^{20}_D$ +221.34 (*c* 1, $CHCl_3$). (*S*)-(−)-**17**: $[\alpha]^{20}_D$ −219.97 (*c* 1, $CHCl_3$). Their ^1H NMR spectra were identical to that of the racemic compound **17**. The free amines were transformed into the oxalate salts, which were recrystallized from 2-PrOH (mp 152−153 °C). Anal. ($C_{19}H_{23}NO_2 \cdot C_2H_2O_4$) C, H, N.

(*R*)- and (*S*)-(6,6-Diphenyl-1,4-dioxan-2-yl)-*N*,*N*,*N*-trimethylmethanaminium Iodide [(*R*)-(+)-5 and (*S*)-(−)-5]. These were prepared as described for **5** and were recrystallized from 2-PrOH: 86% yield; mp 232−234 °C. (*R*)-(+)-**5**: $[\alpha]^{20}_D$ +208.14 (*c* 1, MeOH). (*S*)-(−)-**5**: $[\alpha]^{20}_D$ −207.29 (*c* 1, MeOH). The ^1H NMR spectrum was identical to that of the racemic compound **5**. Anal. ($C_{20}H_{26}INO_2$) C, H, N.

■ ASSOCIATED CONTENT

Supporting Information

General chemistry; details for the syntheses of **3**, **4**, and **6**; elemental analysis results for **3**−**6**, **17**, (*S*)-(−)-**5**, (*R*)-(+)-**5**, (*S*)-(−)-**17**, and (*R*)-(+)-**17**; X-ray crystallographic data for (*R*)-(+)-**5**; experimental details of binding, functional, and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

†The X-ray coordinates of compound (*R*)-(+)-**5** have been deposited with Cambridge Crystallographic Data Centre with accession number CCDC 820353.

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■ ABBREVIATIONS USED

mAChR, muscarinic acetylcholine receptor; COPD, chronic obstructive pulmonary disease; OAB, overactive bladder; IBS, irritable bowel syndrome; [^{3}H]NMS, [^{3}H]*N*-methylscopolamine; CHO, Chinese hamster ovary; TMS, tetramethylsilane; MAP, mean arterial pressure; HR, heart rate; AUC, area under the curve; VIBC, volume-induced bladder contraction

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