The Preparation and Human Muscarinic Receptor Profiling of Oxybutynin and *N*-Desethyloxybutynin Enantiomers

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Abstract: Oxybutynin (**1**) is a non-selective muscarinic receptor antagonist that is used clinically for the treatment of urinary incontinence. The major metabolite of oxybutynin in humans is desethyloxybutynin (**2**). We have prepared the enantiomers of **1** and **2** and evaluated their ability to displace *N*-CT[3]-scopolamine chloride ([3]H-NMS) binding on human cloned muscarinic m1-5 receptors. Compounds **1** and **2** potently displaced [3]H-NMS binding at m1, m3 and m4 receptors, but were less potent at the m2 and m5 subtypes. However, metabolite **2** was more potent than the parent compound **1** in the binding assay. In general the *R* enantiomers were more potent than their respective *S* enantiomers. Therefore, we suggest that the cholinergic side effects associated with **2** may be due to its greater apparent potency with m1 and m3 receptors, especially of its *R*-enantiomer, when compared with parent drug **1**.

INTRODUCTION

Muscarinic cholinergic receptors are G-protein coupled receptors that are activated by the neurotransmitter acetylcholine. They are expressed in the central nervous system, smooth muscle, cardiac muscle, and certain glandular tissues [1]. Five muscarinic receptors (m1-5) have been identified, and several of these are drug targets for small-molecule therapy. The search for muscarinic compounds has been particularly focused around m1 agonists and m2 antagonists for the treatment of Alzheimer's disease, and m3 antagonists for urinary urgency, urinary frequency, and urinary urge incontinence. Racemic oxybutynin (Fig. **1**, **1**) is a non-selective muscarinic receptor antagonist that has been prescribed for the treatment of urinary incontinence. Compound **1** undergoes significant first pass metabolism in humans by CYP3A4 to give desethyloxybutynin (Fig. **1**, **2**) [2,3], resulting in higher plasma levels of **2** than **1** [4]. The primary side effect of treatment with **1** is the inhibition of the secretion of saliva (dry mouth syndrome). Diminishing this side effect, as well as a requirement for less frequent dosing, was addressed by the development of the extended release oxybutynin product Ditropan XL® (Oxy-XL), based upon the OROS® extended release technology [3,4]. The levels of **2** were lower in patients given Oxy-XL when compared to immediate release oxybutynin, suggesting reduced first pass metabolism for Oxy-XL [5]. Since the dry mouth syndrome was also lower when Oxy-XL was used, it was suggested that **2** was an important contributor to this side effect [5]. Further, it has been proposed that a greater clinical utility would be expected for the *S* enantiomer of **1** [(*S*)-**1**], because of its favorable therapeutic index [6]. The binding affinities of **1** (but not **2**) have been determined for human m1-5 receptors using Sf9 insect cells produced with a baculovirus infection system [7] and in CHO-K1 cell lines [8,9], and the functional activities of the enantiomers of **1** and **2** have measured in tissue preparations [9,10]. However, the affinities of all the separate enantiomers for m1-m5 receptors has not been reported. In order to understand the role that **1**, **2** and their enantiomers may play in mediating muscarinic receptor pharmacology, we obtained oxybutynin (**1**), desethyloxybutynin (**2**) and their enantiomers, and evaluated their affinities for human m1-m5 receptors in radioligand binding studies.

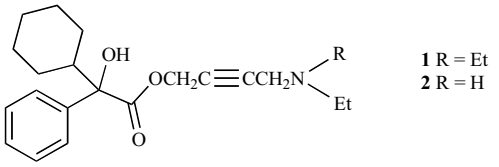

Fig. (1). Oxybutynin (**1**) and desethyloxybutynin (**2**).

CHEMISTRY

We prepared the enantiomers of **1** using the synthetic procedure of Senanayaka *et al.* with minor modifications (Scheme **1**, *R*-isomer shown), starting from either (*R*) or (*S*)-mandelic acid [11,12]. The enantiomers of **2** were prepared by taking the appropriate α-hydroxyacids **3** (*R* enantiomer shown) and alkylating sequentially with 1,4-dibromo-2-butyne followed by ethylamine [13]. The enantiomers of **1** and **2** were fully characterized such as by 300-MHz ^{1}H NMR, and their chemical and stereochemical purity was determined to be >98% by chiral HPLC analysis [14,15].

PHARMACOLOGY

Compounds **1**, **2** and their enantiomers were tested for binding affinity against human m1-5 expressed in CHO cells [16]. Following the manufacturer's protocol, recombinant m1-m5 membranes from CHO cells (Perkin-Elmer) were incubated with various concentrations of **1**, **2** or their respective enantiomers in the presence of [3]H-NMS for 2 hrs at 25 °C. Free radioligand was removed, and membranes were

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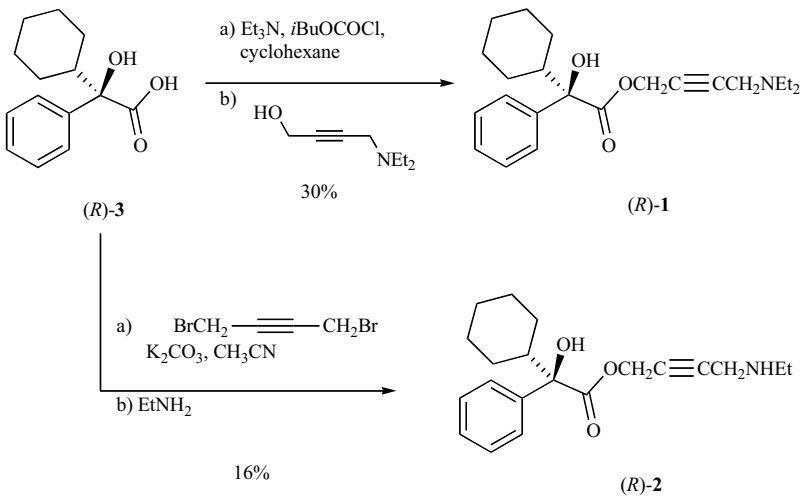


Scheme 1. Synthesis of (*R*)-oxybutynin [(*R*)-**1**] and of (*R*)-desethyloxybutynin [(*R*)-**2**].

collected by filtration over GF/C filters presoaked with polyethylenimine. Binding was measured by counting radioactivity in a scintillation counter. The K_i values were determined using nonlinear curve fit analysis (GraphPad Prism).

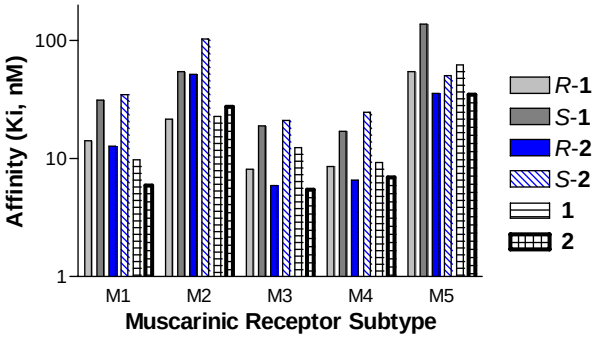
RESULTS AND DISCUSSION

As shown in Table **1**, oxybutynin (**1**), desethyloxybutynin (**2**) and their enantiomers inhibited ^{3}H-NMS binding in a concentration-dependent manner, with K_i's of <100 nM at all five cloned human muscarinic receptors . The highest affinity was observed at the m1, m3 and m4 subtypes. This result is consistent with earlier studies looking at the muscarinic binding profile of **1** alone [8,9]. The data for **2** showed a consistent trend toward greater potency when compared to **1**, at m1 and m3-m5. For example, **2** had K_i values of 6.0, 5.5 and 6.9 nM at the m1, m3 and m4 receptors, respectively, whereas **1** had K_i values of 9.9, 12.3 and 9.3 nM at these sites. The *R* enantiomers of **1** and **2** were more potent than their respective *S* enantiomers, and the relative difference between *R* and *S* appeared to be somewhat greater for **2** than **1**. For example, the ratio (*S*)-**2**/(*R*)-**2** at m3 and m4 was 3.6-3.7 whereas the ratio (*S*)-**1**/(*R*)-**1** at these receptors was 2.0-2.3. The rank order of activities at m1-m5 were the same for **1**, **2** and their enantiomers, suggesting that any therapeutic benefit to be accrued by use of the *S*-enantiomer may result from interactions other than those at the muscarinic receptors. Although the differences between **1**, **2** and their enantiomers were shown to be subtle, the greater potency of **2** may account for the dry mouth syndrome in cases where **2** is formed rapidly, based on two lines of evidence. First, studies with Oxy-XL which have shown that **2** is formed much more gradually when that extended release formulation is used [5]. Second, the pharmacokinetics of the enantiomers of **1** and **2** have been studied after oral administration 6 hrs post-dosing with a single 5 mg tablet of **1** [17]. The levels of **2** greatly exceeded those of **1**, and there was a stereospecific enhancement of the formation of (*R*)-**2** compared to (*S*)-**2,** with mean AUC ratios of 3.3 and 8.9, respectively [17]. Therefore, not only does (*R*)-**2** form preferentially upon oral administration of **1,** but the present study suggests that (*R*)-**2** is more potent than (*S*)-**2** at the muscarinic m1-5 receptors and more potent than **1** at m3-m5. In conclusion, this is the first report of the pharmacological characterization of the enantiomers of **1** and **2** at all five human muscarinic receptors. The characterization of oxybutynin, its metabolites and enantiomers in this study suggest that the formation of (*R*)-desethyloxybutynin [(*R*)-**2**] may contribute to a major cholinergic side effect of oxybutynin therapy, the dry mouth syndrome.

Table 1. Binding Affinities of Oxybutynin (1), Desethyloxybutynin (2) and Their Enantiomers at the Human Muscarinic m1-5 Receptors [K_i and (SEM) in nM, n = 2-3]

	m1	m2	m3	m4	m5
1	9.9 (4.2)	22.7 (8.9)	12.3 (4.3)	9.3 (3.3)	62.4 (16.7)
(*R*)-1	14.1 (2.5)	21.8 (2.2)	8.2 (1.9)	8.5 (2.1)	54.3 (3.3)
(*S*)-1	30.2 (6.0)	54.7 (9.0)	19.0 (0.9)	17.2 (0.8)	137.9 (31.7)
2	6.0 (2.6)	27.7 (5.2)	5.5 (2.2)	6.9 (3.9)	34.7 (0.8)
(*R*)-2	12.9 (2.1)	51.8 (7.4)	5.9 (1.1)	6.6 (1.2)	35.8 (5.5)
(*S*)-2	35.1 (6.0)	104.4 (48.7)	21.0 (0.6)	24.7 (2.0)	51.0 (46.0)

Table 2. Graphic Representation of the Binding Affinities of Oxybutynin (1), Desethyloxybutynin (2) and Their Enantiomers at the Human Muscarinic m1-5 Receptors



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[14] Procedure for the conversion of **1** to **2**. 1,4-Dibromo-2-butyne **6** (0.383 g, 1.8 mmol) was added to a mixture of potassium carbonate (0.341 g, 2.5 mmol) and (*S*)-**3** (0.385 g, 1.6 mmol) in acetonitrile (3.0 mL). The resulting solution was stirred at reflux under argon for 3 hrs. After cooling to ambient temperature, the solution was filtered and the filter cake washed with acetonitrile. Ethylamine (0.4 mL, 70% $EtNH_2/H_2O$, 6.2 mmol) was then added to the solution which was stirred at ambient temperature overnight. The solution was transferred to a Gilson HPLC for purification to give (*S*)-**2** as a colorless oil (trifluoroacetic acid salt, 0.1187 gm, 16%). MS (loop pos.): MH^+ = 330.1. ^1H NMR (300 MHz, CD_3OD) δ: 7.635 (d, 2H), 7.26-7.38 (m, 3H), 3.95 (s, 2H), 3.33 (s, 2H), 3.04 (q, 2H), 2.20 (m, 1H), 1.16-1.84 (m, 10H), 1.27 (t, 3H). $[\alpha]_D^{23}$ = -2.9° (c 1.6, MeOH). (*R*)-**2** was made in a similar manner starting with (*R*)-**1**. The ^1H NMR spectrum for (*R*)-**1** was identical to that of (*S*)-**1**. MS (loop pos.): MH^+ = 330.1. $[\alpha]_D^{23}$ = +2.85° (c 1.79, MeOH).

[15] Each compound was analyzed on a Chiralpak AD-H 25 cm column and found to be stereochemically pure (>98%), with the presence of the enantiomer below detectable limits. (*S*)-**1**, MeOH:H_2O 90:10, retention time 10.4 mins; (*R*)-**1**, MeOH:H_2O 90:10, retention time 7.5 mins; (*S*)-**2**, 100% EtOH, retention time 10.2 mins; (*R*)-**2**, 100% EtOH, retention time 7.9 mins.

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