Synthesis of *N*-Substituted Piperidine Salts as Potential Muscarinic Ligands for Alzheimer's Applications

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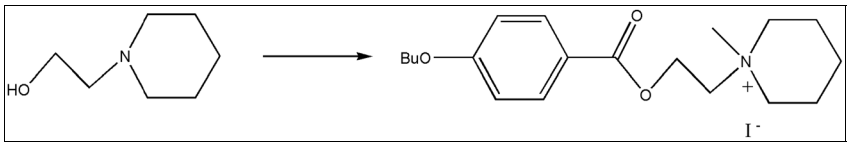

Several heterocyclic *N*-piperidine substituted salts were synthesized that were found to inhibit the specific binding of the antagonist [^{3}H] quinuclidinyl benzilate in radioligand muscarinic binding assays (^{3}H-QNB) in bioassays. One of the heterocyclic salts, compound **7**, met the significance criteria in these assays (>50% inhibition) at 10 μM of the nonselective muscarinic antagonist (^{3}H-QNB) in cells of the Wistar rat cerebral cortex. Furthermore, this compound displayed 61% inhibition at 10 μM of the antagonist (^{3}H-QNB) for the M_5 receptor (IC_{50} 6.34 μM, K_i 3.93 μM, $n_H = 0.996$) in human recombinant CHO cell lines. These data obtained from Ricerca Biosciences suggested that compound **7** was selective for the M_5 receptor. Another study from the Czech Academy of Sciences demonstrated that compound **7** was 3 to 8 times more potent at M_2 than other subtypes of muscarinic receptors in competition with antagonist *N*-methylscopolamine and selective for the M_1 receptors over M_3 and M_5 in antagonizing accumulation of inositol phosphates induced by muscarinic agonist carbachol.

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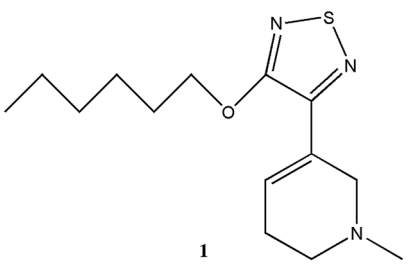
INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory, judgment, and language functions and by the loss of specific neurons that project from the basal forebrain to the hippocampus and cerebral cortex [1]. Degeneration of these nerve cells results in a decrease of neuronal markers such as the universal neurotransmitter acetylcholine and the enzymes choline acetyltransferase and esterase. The cholinergic hypothesis is based on the fact that while basal forebrain neurons that express the M_2 muscarinic receptor are at risk of degenerating, the cortical neurons expressing the M_1 subtype which synapse with them are not altered [2]. In theory, one could design and synthesize muscarinic selective M_1 agonists that would activate the cortical neurons. Such an approach has proven very difficult to achieve because of lack of three-dimensional structural information about the muscarinic binding site(s). To date, three pharmacologically distinct muscarinic receptors and five cloned muscarinic receptors (M_1–M_5) have been identified [3]. These receptors share high amino acid homology, specifically at the transmembrane regions where agonist binding is believed to occur. Therefore, it appears difficult to design selective agonists that can differentiate between these subtypes. Efficacious agonists would have to activate specific neurons expressing the M_1-subtype while having little effect on neurons expressing other types of receptors. To date, only a few compounds have shown sufficient promise to proceed to clinical trials, and these have produced only marginal stimulating responses. Xanomeline, compound **1**, is a functionally selective M_1/M_4 receptor agonist with promising *in vivo* antidementia properties [4]. This drug was initially targeted for the treatment of AD, but clinical trials revealed peripheral side effects that were not consistent with M_1 selectivity. More recent behavioral studies indicate, however, that the compound may decrease psychotic behaviors in patients with AD suggesting that it might be useful in the treatment of psychotic symptoms in patients with schizophrenia. Xanomeline was shown to exhibit a novel mode of interaction with the M_1 receptor different from that used by conventional agonists. There is evidence that the persistent attachment of Xanomeline takes place away from the classical binding site, whereas the active group interacts reversibly with the primary receptor activation site. Other data have shown that Xanomeline binds in a wash-resistant manner as an antagonist at the M_5 receptor [5]. The ability of Xanomeline to activate some subtypes of muscarinic receptors while antagonizing others in a wash-resistant manner represents a unique and complex pharmacological profile. There is an obvious need for more M_1-selective receptor activators because of unwanted side effects with compounds like Xanomeline.



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Our research group is currently working on the synthesis of heterocyclic compounds with structural features similar to Xanomeline that conform to Schulman's [6] model of the muscarinic pharmacophore. This model is based on detailed conformational analyses of acetylcholine and other known muscarinic agonists. In this model, the ammonium head group of muscarinic agonists is shown to interact with an aspartic acid residue, whereas a region of negative electrostatic potential interacts with a positive receptor residue or forms a hydrogen bond at point Q (Fig. 1). Schulman's model is based on the following assumptions:

(1) The distance |PQ| varies by no more than 0.03 nm over a set of agonists. (2) The interaction dihedral angle "PNOQ" and the distance |PQ| define the backbone of the drug, and the range of accessible PNOQ values is 100° to 117°, with positive signs according to the Prelog convention. (3) The distance |PCt| defines the chain length requirements with full agonists having |PCt| values around 0.85 nm.

Compounds **4**, **7**, and **11** were synthesized and all contain the classical NCCO backbone required by Schulman's model for muscarinic binding and similar chemical moieties present in Xanomeline. For example, all three compounds have a piperidinyl group. In addition, compound **7** contains a hydrophobic butoxy group.

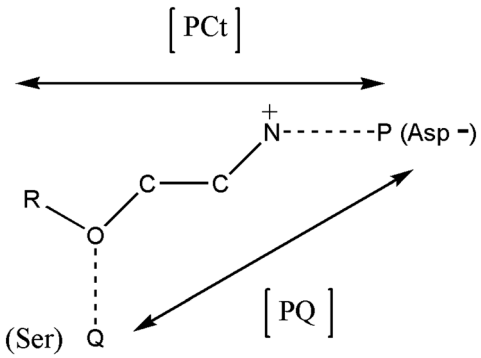


Figure 1. Schulman's model of the muscarinic pharmacophore.

REACTION SCHEMES AND STRATEGIES

The furfuryl ester *N*-substituted piperidinium salt **4** (Scheme 1) was first synthesized by reacting furfuryl chloride with 2-bromoethanol to form **2** that was then coupled with piperidine to yield the piperidine base **3**. The base was then treated with iodomethane to form the salt.

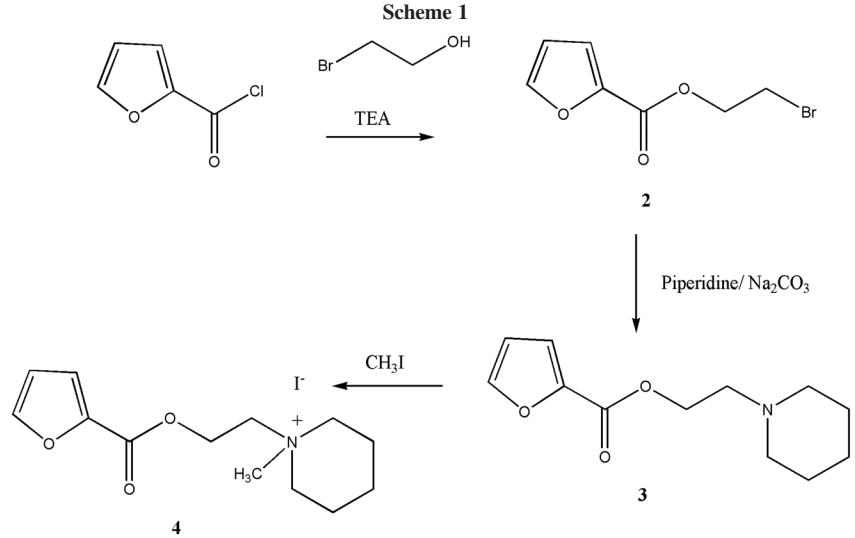
The *p*-butoxy benzoyl ester *N*-substituted piperidinium salt **7** (Scheme 2) was first synthesized by reacting 2-bromoethanol with *p*-butoxybenzoylchloride to form **5** that was then coupled with piperidine to yield the piperidine base **6**. The base was then treated with iodomethane to form the salt.

The thiophene *N*-substituted piperidinium salt **11** (Scheme 3) was synthesized by reducing 5-methyl thiophenecarboxaldehyde with sodium borohydride to form the alcohol **8** that was then converted to the corresponding chloride **9**. The chloride was then coupled with piperidine to form the base **10** that was then converted to the salt **11** by the addition of iodomethane.

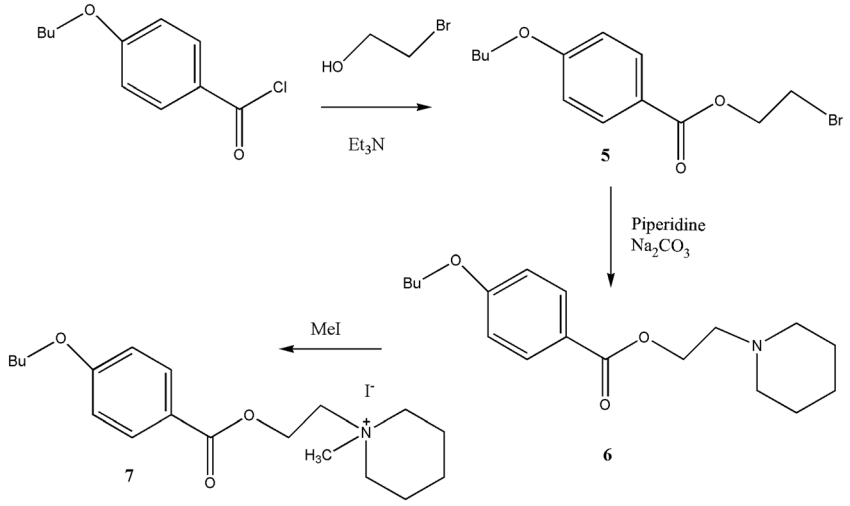
EXPERIMENTAL

Reagents were purchased from Aldrich Chemical Company (St. Louis, MO) unless otherwise noted, and all starting liquid materials were distilled before use. NMR spectra were recorded on a Varian 300 MHz spectrometer (Varian NMR Systems, Palo Alto, CA) housed at Barry University. Mass spectra were recorded on a Clarus 560 S GC/MS system (Perkin Elmer, Shelton, CT). Elemental analyses were carried out by Galbraith Laboratories (Knoxville, TN), and biological assays were conducted at Ricerca Biosciences (Taiwan) and the Institute of Physiology of the Czech Academy of Sciences as described previously [7]. Melting points were recorded on a MEL-TEMP II purchased from Laboratory Devices and are uncorrected.

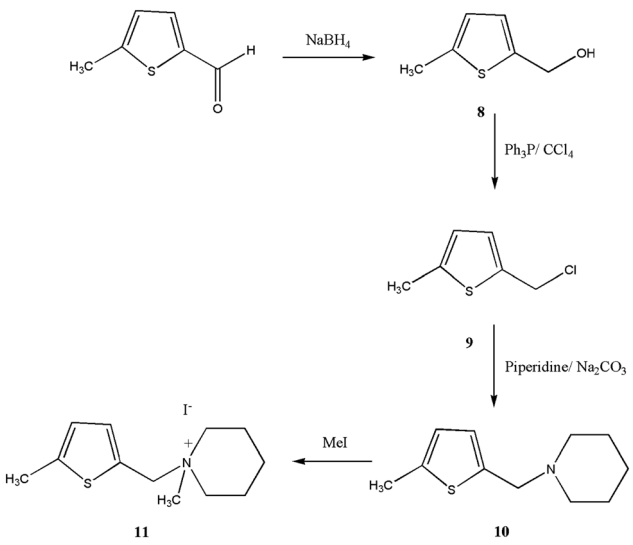
Scheme 1



Scheme 2



Scheme 3



2-(2-Bromoethyl) furfuryl ester (2). To a 100-mL round bottom flask were added 2-bromoethanol (6.25 g, 0.05 mol), triethylamine (5.06 g, 0.05 mol), and 10 mL of anhydrous ether. The flask was cooled in an ice bath, and 2-furfuryl chloride (6.52 g, 0.05 mol) was added dropwise. The mixture was then refluxed for 30 min. The solution was filtered, and 15 mL of 1 M HCl was added to the filtrate. The mixture was extracted several times with H_2O. The ether layer was then dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum to yield compound **2** (3.35 g, 30%). ^{1}H NMR 300 MHz, $CDCl_3$) δ 7.65 (1H), 7.3 (1H), 6.55 (1H), 4.7–4.6 (t, 2H), 3.7–3.6 (t, 2H). MS: *m/z* 219 (M^+), 112, 95 (base peak).

2-(*N*-Piperidine ethyl) furfuryl ester (3). Compound **2** (2 g, 0.009 mol), 20 mL of acetonitrile, 1 g sodium carbonate, and piperidine (0.77 g, 0.009 mol) were added to a 100-mL boiling flask and left to stir overnight. The solution was filtered and concentrated under vacuum to yield 1.05 g of crude compound **3**. The crude material was chromatographed over silica with a 10:1 mixture of CH_2Cl_2/MeOH to yield pure compound **3** (0.70 g, 35%). ^{1}H NMR (300 MHz, CD_3COCD_3) δ7.4 (1H), 7.0 (1H), 6.4 (1H), 4.6 (2H), 4.2 (2H), 3.6 (4H), 2.4 (4H), 1.2 (2H). MS: *m/z* 223 (M^+), 111, 98 (base peak).

2-(*N*-Piperidine ethyl) furfuryl ester *N*-methyl iodide (4). Compound **3** (0.60 g, 0.0027 mol) and 1 mL of iodomethane were added to a 25-mL boiling flask and stirred overnight. The mixture was concentrated, and the crude salt was recrystallized from hexanol to yield compound **4** (0.65 g, 67%), mp 165–167°C. ^{1}H NMR (300 MHz, D_2O) δ 7.7 (1H), 7.3 (1H), 6.6 (1H), 4.9 (2H), 3.8 (2H), 3.5–3.3 (4H), 3.1 (3H), 1.9–1.8 (4H), 1.65-1.55 (2H). *Anal.* Calcd For $C_{13}H_{20}NO_3I$: C 42.75%, H 5.52%, N 3.82%, I 34.75%. Found: C 42.65%, H 5.38%, N 3.78%, I 36.19%.

2-Bromo ethyl *p*-butoxybenzoate (5). To a 100-mL round bottom flask were added 2-bromoethanol (0.625 g, 0.005 mol), triethylamine (0.505 g, 0.005 mol), and 5 mL of dry anhydrous ether. The flask was cooled in an ice bath, and *p*-butoxybenzoylchloride (1.06 g, 0.005 mol) was added dropwise. The mixture was then refluxed for 30 min. The solution was filtered, and 15 mL of 1 M HCl was added to the filtrate. The mixture was extracted several times with H_2O. The ether layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum to yield compound **5** (0.41 g, 27%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.0 (2H), 7.0 (2H), 4.6 (2H), 4.2 (2H), 3.6 (2H), 1.8 (2H),1.6 (2H), 1.0 (3H). MS: *m/z* 300 (M^+), 121, 138 (base peak).

2-(*N*-Piperidine ethyl) *p*-butoxybenzoate (6). Compound **5** (0.41 g, 0.00113 mol), 20 mL of acetonitrile, 1 g sodium carbonate, and piperidine (0.116 g, 0.00113 mol) were added to a 100-mL boiling flask and left to stir overnight. The solution was filtered and concentrated under vacuum to yield 0.39 g of crude compound **6**. The crude material was chromatographed over silica with a 10:1 mixture of CH_2Cl_2/ MeOH to give compound **6** (0.36 g, 88%). ^{1}H NMR (300 MHz, CD_3COCD_3) δ 8.0 (2H), 7.1 (2H), 4.5 (2H), 4.2 (2H), 2.8 (2H), 2.6 (4H), 1.8 (2H), 1.6 (4H), 1.5 (2H), 1.0 (5H). MS: *m/z* 305 (M^+), 165, 98, 138 (base peak).

2-(*N*-Piperidine ethyl) *p*-butoxy benzoylester *N*-methyl iodide (7). Compound **6** (0.36 g, 0.00118 mol) and 1 mL of iodomethane were added to a 25-mL boiling flask and stirred overnight. The mixture was concentrated to yield crude salt **7**

(0.50 g, 63%). The salt was recrystallized from *n*-butanol, mp 111–113°C. ^{1}H NMR (300 MHz, D_2O) δ 7.9 (2H), 7.0 (2H), 4.6 (2H), 4.1(2H), 3.8 (2H), 3.5–3.3 (4H), 3.1 (3H), 1.9–1.8 (4H), 1.7–1.5 (4H), 1.45–1.35 (2H), 0.9–0.8 (3H). *Anal*. Calcd For $C_{19}H_{30}NO_3I$: C 51.03%, H 6.71%, N 3.13%, I 28.38%. Found: C 50.48%, H 6.59%, N 3.19%, I 30.29%.

5-Methyl-2-thiophene methanol (8). A solution containing sodium methylate (3 g, 0.056 mol), sodium borohydride (6 g, 0.16 mol), and 25 mL of methanol was slowly added to a mixture containing 5-methyl thiophene carboxaldehyde (26.39 g, 0.08 mol) and 50 ml of methanol with stirring and cooling. After addition, the reaction mixture was then allowed to warm to room temperature, poured over 200 mL of crushed ice, and acidified with 6 M HCl. The mixture was then extracted with ether, and the combined ether layers was dried over anhydrous magnesium sulfate, filtered, and concentrated to yield **8** (17.90 g, 76.63%), ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d,1H), 6.65 (d,1H), 4.75 (s,2H), 2.5 (s,3H), 2.0 (bs, 1H).

5-Methyl-2-thiophene methyl chloride (9). Compound **8** (8.95 g, 0.0698 mol) was added to a mixture of carbon tetrachloride (50 mL) and triphenyl phosphine (21.0 g, 0.0801 mol) and refluxed for 1 h. The mixture was allowed to cool to room temperature, and 160 mL of anhydrous pentane was added with stirring. The solution was filtered, and filtrate was concentrated to yield **9** (7.13 g, 68.56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.9 (d, 1H), 6.6 (d, 1H), 4.8 (s,2H), 2.5 (s,3H).

1-(5-Methyl-2-thiophene methyl)-1-methyl piperidinium iodide (11). A 4.0 g of anhydrous sodium carbonate was added to a solution of compound **9** (7.13 g, 0.0487 mol) in 40 mL of acetonitrile. Piperidine (4.15 g, 0.0487 mol) was then added with stirring. The reaction mixture was allowed to stir overnight, filtered, and concentrated on a rotary evaporator to yield crude base **10** (9.12 g, 96%). To a solution containing compound **10** (5.0 g, 0.0256 mol) in 20 mL of acetonitrile was added methyl iodide (4 g, 0.028 mol). The solution was allowed to stir overnight and concentrated. The crude salt **11** was recrystallized from 1-butanol (8.16 g 52.85%), mp144–145°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1 (d,1H), 6.8 (d,1H), 4.55 (s, 2H), 3.35–3.2 (m, 4H), 2.9 (s, 3H), 2.4 (s, 3H), 1.95–1.8 (m, 4H), 1.7–1.5 (m, 2H). *Anal*. Calcd For $C_{12}H_{20}SNI$: C 42.73%, H 5.98%, N 4.15%, I 37.62%, S 9.51%. Found: C 42.62%, H 5.77%, N 4.14%, I 38.68%, S 9.92%.

RADIOLIGAND ASSAYS AND DISCUSSIONS

Compounds **4**, **7**, and **11** were assayed at Ricerca Biosciences for muscarinic binding affinity on Wistar rat cerebral cortex at 10 μM in 1% DMSO. Incubation time/temperature: 60 min at 25°C. Incubation buffer: 50 mM phosphate buffer, pH 7.4. All compounds were tested in the presence of 0.15 nM [^{3}H] quinuclidinyl benzilate (a muscarinic antagonist). These compounds were found to significantly displace the antagonist from the muscarinic receptor sites. Compounds **7**, **11**, and **4** displayed 81%, 61%, and 55% inhibition of the antagonist, respectively. Although compounds **4** and **11** were able to bind significantly, neither one had any significant binding selectivity for any particular receptor. Compound **7**, however, was

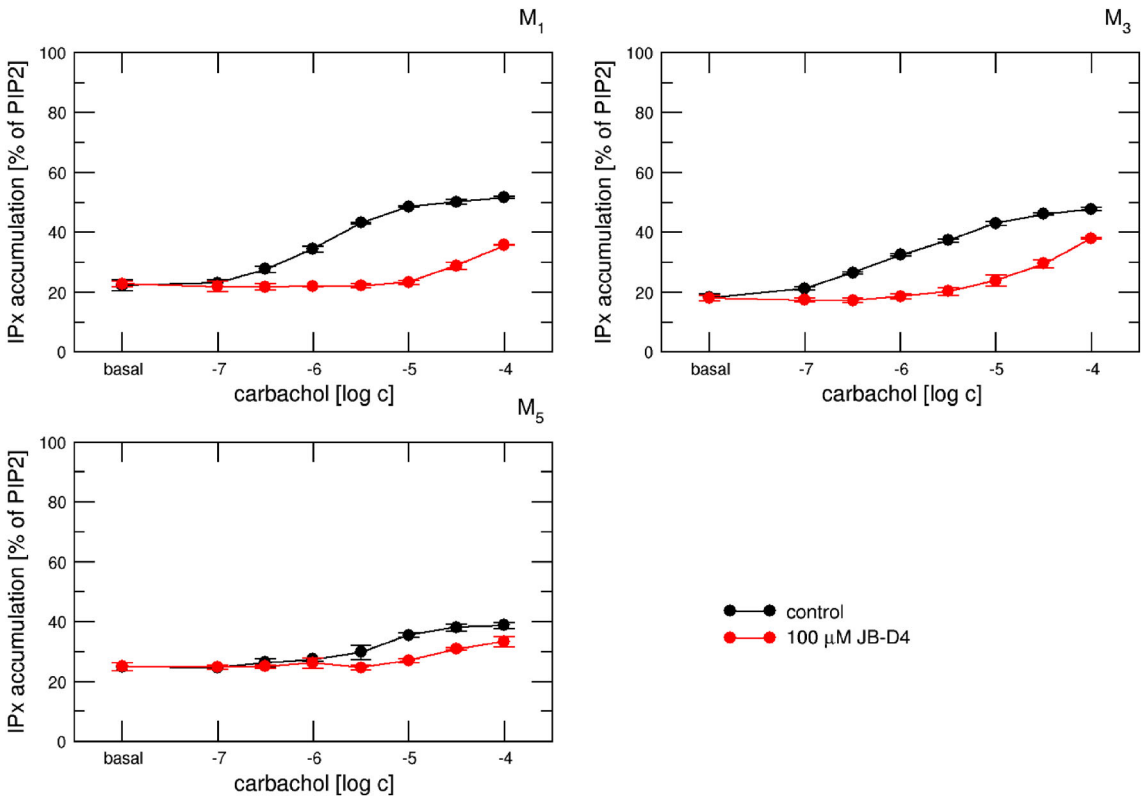


Figure 2. Carbachol-induced accumulation of inositol phosphate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

found to be more selective for the M_5 receptor. This compound displayed 61% inhibition at 10 μM of the antagonist (^{3}H-QNB) for the M_5 receptor (IC_{50} 6.34 μM, K_i 3.93 μM, $n_H = 0.996$) in human recombinant CHO cell lines. Incubation time/temperature: 2 h at 25°C; buffer: 50 mM Tris–HCl, pH 7.4 10 mM $MgCl_2$, 1 nM EDTA; Ligand: 0.8 nM [^{3}H] *N*-methylscopolamine; K_D 1.3 nM, B_{MAX} 5.8 pmol/mg protein. Percent inhibitions at 10 μM for the M_1, M_2, M_3, and M_4 were 47, 36, 23, and 16, respectively. A significant antagonistic response (70% at 20 μM) was also noted with compound **7** in the muscarinic M_1 functional assay (muscarinic M_1, GTPγS binding) with an IC_{50} of 8.9 μM in human CHO-K1 cell lines. Another study was carried on compound **7** at the Institute of Physiology of the Czech Academy of Sciences on similar cell lines and set ups. K_I values were measured: M_1 (2.88 ± 0.13 μM, n_H 1.00); M_2 (0.91 ± 0.02 μM, n_H 0.802); M_3 (3.16 ± 0.06 μM, n_H 0.837); M_4 (7.54 ± 0.31 μM, n_H 1.05); M_5 (2.88 ± 0.29 μM, n_H 1.189) (mean ± SD from three independent experiments performed in quadruplicates). In these assays, compound **7** was found to have highest potency at the M_2 receptor and lowest at the M_4.

Competition of 100 μM on carbachol-induced accumulation of inositol phosphates confirmed greater potency of compound **7** at M_1 and M_3 than at M_5 receptors (Fig. 2). Effects of compound **7** to inhibit carbachol-induced inhibition of cAMP synthesis were insignificant. More analogs of compound **7** are being synthesized to increase both receptor functional selectivity and affinity. Compound 7 is currently being evaluated for its bitopic antagonist properties at all M1–M5 receptors.

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