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# (±)-1-AZABICYCLO[2.2.1]HEPTAN-3-ONE, O-(3-METHYL-5-ARYL-2-PENTEN-4-YNYL) OXIMES: POTENT MUSCARINIC AGONISTS

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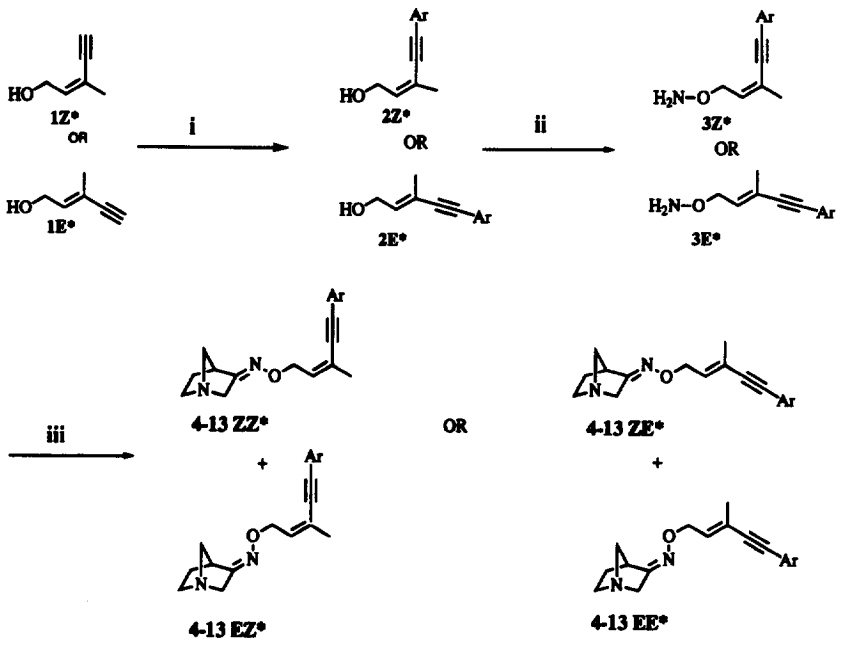
**Abstract.** Synthesis and SAR of a new class of 1-azabicyclo[2.2.1]heptan-3-one, O-(3-methyl-5-aryl-2-penten-4-ynyl) oxime muscarinic agonists are described. Depending on the geometry of the C=N and C=C bonds, subnanomolar potency and/or high efficacy are achieved.

A novel class of bulky 1-azabicyclo[2.2.1]heptan-3-one, O-(3-aryl-2-propynyl) oximes (see preceding article) have shown promising functional m1 muscarinic selectivity (1, 2). To further probe the steric limitations on muscarinic potency, efficacy and selectivity, we have prepared a new class of related O-(3-methyl-5-aryl-2-penten-4-ynyl) oxime analogs. The geometry of the C=N and C=C bonds play a major role in determining the potency and/or efficacy of the oximes. In-vitro radioligand binding assays and the proliferative effects of the compounds on cells transformed with human m1 and m2 receptors were used to determine the efficacy and muscarinic subtype-selectivity of these compounds.

The target oximes were prepared as shown in Scheme 1. Palladium(0)-assisted cross-coupling reaction between commercially available alcohols **1Z*** or **1E*** and an aryl bromide or aryl iodide gave the corresponding alcohols **2Z*** and **2E*** respectively. The alcohols reacted with N-hydroxyphthalimide via the Mitsunobu reaction to give the corresponding O-substituted phthalimides. Hydrolysis of the phthalimides at room temperature with N-methyl hydrazine afforded key hydroxylamines **3Z*** and **3E***. Target oximes, **4-13 ZZ***, **4-13 EZ***, were prepared by condensing appropriately substituted hydroxylamines **3Z*** with 1-azabicyclo[2.2.1]-3-one (3). The **ZZ*** and **EZ*** oximes were separated by medium pressure chromatography on silica eluted with $CH_2Cl_2$ : MeOH (99.5 : 0.5). Oximes **4-13 ZE*** and **4-13 EE*** were prepared similarly from appropriately substituted hydroxylamine **3E*** and the ketone.

Muscarinic receptor binding assays were conducted using [$^3$H]quinuclidinyl benzilate (QNB) to label antagonist sites and [$^3$H]-cis-methyldioxolane (CMD) to label agonist sites in membrane preparations from rat




**Scheme 1**. *Reagents and conditions*; **i**, ArBr, CuI, $(Ph_3P)_4Pd$, $Et_2NH$, THF, rt; **ii**, N-hydroxy phthalimide, $Ph_3P$/THF; **iii**, $H_2NNHCH_3/CH_2Cl_2$, rt; **iv**, 1- azabicyclo[2.2.1]heptan-3-one, MeOH, rt.

neocortex (4, 5). The ratio of QNB/CMD binding affinities has been shown to predict agonist efficacy at muscarinic receptors (6) . Functional potency and selectivity were determined by Receptor Selection and Amplification Technology (R-SAT, Receptor Technologies Inc., patents pending, 7,8). In this assay the human m1 and m2 muscarinic receptors are transiently expressed with the marker gene β-galactosidase in NIH 3T3 cells. Proliferative signals allow ligands to amplify cells that express compatible receptors and the marker, thus allowing assay of receptor activity using colorimetric assays in 96 well format. Proliferative signals for the m2 receptor require co-expression with a chimeric G-protein (7). These results are shown in the table.

Our results indicate that the 1-azabicyclo[2.2.1]heptan-3-one, O-(3-methyl-5-aryl-2-penten-4-ynyl) oximes are potent and efficacious muscarinic agonists. Affinity for the muscarinic agonist in the picomolar range (**13ZZ***, CMD = 0.02 nM; **11ZZ***, CMD= 0.03 nM) and excellent muscarinic agonist efficacy (**12EZ***, QNB/CMD=1858, R-SAT at m1=100% CCh.; **13ZZ***: QNB/CMD =1050, R-SAT at m1=100% CCh) were achieved. Of the four possible geometric isomers, the ZZ oximes tend to be the most potent and the EE the least potent. The ZE and EZ isomers are intermediate in potency. Oximes **4ZZ***, **4EZ***, **10ZE*** and **13ZE*** have slightly higher potency at the m1 receptors (m2/m1=1.96, 1.49, 2.38 and 5.88 respectively). Oximes **6EZ***, **6ZZ***, and **8ZZ*** appear to be equipotent at m1 and m2 receptors (m2/m1=1.25, 1.04 and 1.02 respectively). The rest of the oximes appear to be m2 selective. It is worth noting, however, that in this assay carbachol displays 53 fold selectivity for m2 receptors. Relative to carbachol, most of the oximes described here display

Table. Muscarinic Receptor Binding and Functional Selectivity of (±)-1-Azabicyclo[2.2.1]heptan-3-one, O-(3-methyl-5-aryl-2-penten-4-ynyl) oximes.

| No | Geometry | Ar | $IC_{50}$ nM[a] (% Inh)[b] QNB | CMD | QNB/CMD | R-SAT[c] m1 $ED_{50}$ nM | m1 %CCh | m2 $ED_{50}$ nM | m2 %CCh |
|---|---|---|---|---|---|---|---|---|---|
| 4 | ZZ* | H | 807 | 1.00 | 807 | 12 | 70 | 23.7 | 180 |
| | ZE* | H | 2063 | 4.00 | 516 | 85 | 50 | 11 | 90 |
| | EZ* | H | 4234 | 12.00 | 352 | 30 | 60 | 45 | 100 |
| | EE* | H | 19308 | 21.00 | 160 | - | - | - | - |
| 5 | ZZ* | Ph | 44 | 0.15 | 289 | - | - | - | - |
| | ZE* | Ph | 1587 | 12.40 | 128 | - | - | - | - |
| | EZ* | Ph | 181 | 1.07 | 169 | 120 | 110 | 4.2 | 130 |
| | EE* | Ph | (18%) | (31%) | - | - | - | - | - |
| 6 | ZZ* | Ph (-p-OMe) | 60 | 0.10 | 600 | 8.5 | 70 | 8.9 | 100 |
| | ZE* | Ph (-p-OMe) | 1413 | 12 | 120 | - | - | - | - |
| | EZ* | Ph (-p-OMe) | 1992 | 17.00 | 117 | 8.6 | 40 | 10.7 | 110 |
| | EE* | Ph (-p-OMe) | (19%) | (33%) | - | - | - | - | - |
| 7 | ZZ* | Ph (-m-OMe) | 88 | 0.52 | 169 | 51.1 | 60 | 4.5 | 140 |
| | ZE* | Ph (-m-OMe) | 384 | 4.45 | 86 | 93.2 | 60 | 42.3 | 170 |
| | EZ* | Ph (-m-OMe) | 1514 | 15.31 | 99 | -- | -- | 28.2 | 80 |
| | EE* | Ph (-m-OMe) | 2742 | 13.27 | 207 | 264 | 50 | 12.4 | 110 |

Table ---continued

| No | Geometry | Ar | $IC_{50}$nM (% Inh) QNB | $IC_{50}$nM (% Inh) CMD | QNB/CMD | R-SAT m1 $ED_{50}$nM | R-SAT m1 %CCh | R-SAT m2 $ED_{50}$nM | R-SAT m2 %CCh |
|---|---|---|---|---|---|---|---|---|---|
| 8 | ZZ* | Ph (-p-Me) | 64 | 0.57 | 112 | 10.5 | 70 | 10.7 | 140 |
| | ZE* | Ph (-p-Me) | 681 | 22.16 | - | - | - | - | - |
| | EZ* | Ph (-p-Me) | 383 | 5.02 | 76 | 83.3 | 90 | 4.4 | 90 |
| | EE* | Ph (-p-Me) | (31%) | (29%) | - | - | - | - | - |
| 9 | ZZ* | Ph (-p-Cl) | 81 | 0.45 | 180 | 29.1 | 70 | 2.8 | 110 |
| | ZE* | Ph (-p-Cl) | 439 | 27.12 | 16 | - | - | - | - |
| | EZ* | Ph (-p-Cl) | - | - | - | - | - | - | - |
| | EE* | Ph (-p-Cl) | (52%) | (57%) | - | - | - | - | - |
| 10 | ZZ* | Ph-(3,4-di-Cl) | (77%) | (46%) | - | - | - | - | - |
| | ZE* | Ph-(3,4-di-Cl) | 171 | ~0.8 | >171 | 398 | 100 | 940 | 160 |
| | EZ* | Ph-(3,4-di-Cl) | (51%) | (14%) | - | - | - | - | - |
| | EE* | Ph-(3,4-di-Cl) | (39%) | (49%) | - | - | - | - | - |
| 11 | ZZ* | Ph (-p-F) | 15 | 0.03 | 547 | 20.9 | 80 | 3 | 130 |
| | ZE* | Ph (-p-F) | 1073 | 9.75 | 110 | 31.5 | 50 | 9.1 | 130 |
| | EZ* | Ph (-p-F) | 219 | 1.61 | 136 | 48 | 0.6 | 270 | 150 |
| | EE* | Ph (-p-F) | 3510 | 6.5 | 540 | 156 | 0.4 | 456 | 70 |

Table ---continued

| No | Geometry | Ar | $IC_{50}$nM (% Inh) QNB | $IC_{50}$nM (% Inh) CMD | QNB/CMD | R-SAT m1 $ED_{50}$nM | R-SAT m1 %CCh | R-SAT m2 $ED_{50}$nM | R-SAT m2 %CCh |
|---|---|---|---|---|---|---|---|---|---|
| 12 | ZZ* | Ph-(3,4-di-MeO) | 1245 | 1.38 | 902 | 55 | 90 | -- | 80 |
| | ZE* | Ph-(3,4-di-MeO) | 2380 | 5.37 | 443 | 74 | 60 | 1.8 | 60 |
| | EZ* | Ph-(3,4-di-MeO) | 8547 | 4.60 | 1858 | 162 | 100 | 0.9 | 100 |
| 13 | ZZ* | 2-thienyl | 21 | 0.02 | 1050 | 6.2 | 100 | 2,3 | 160 |
| | ZE* | 2-thienyl | 968 | 4.96 | 195 | 0.4 | 30 | 24.0 | 150 |
| | EZ* | 2-thienyl | 608 | 4.10 | 148 | 11.6 | 60 | 2.5 | 100 |
| | EE* | 2-thienyl | 5593 | 80.47 | 70 | - | - | - | - |
| | Carbachol | | 33000 | 6.7 | 4925 | 1210 | 100 | 22.8 | 100 |

[a]For $IC_{50}$ determination, each drug was tested in triplicate at 5-6 concentrations. [b] Values represent % inhibition of QNB and CMD at drug concentrations of 1μM and 100 nM respectively. [c]$ED_{50}$ values were determined from 6 concentrations tested in duplicate. %CCh = maximal effect obtained for each drug normalized to the effect of carbachol.

a greater level of m1 vs m2 selectivity. The QNB/CMD ratio is obtained from binding assays using rat neocortex membrane. The muscarinic subtype distribution in rat neocortex, though primarily m1, is heterogeneous. Moreover, the ligands QNB and CMD are not subtype selective. R-SAT is a functional assay where cells expressing only one of the muscarinic subtypes is employed. Thus, a lack of direct correlation of efficacy measurements from QNB/CMD ratios and R-SAT is not surprising. Carbacol is generally regarded to be a full agonist at m2 receptors. Several compounds in the series show greater efficacy than carbachol at the m2 receptor. This finding, though not common, is not unprecedented. Saunders and Freedman observed similar results with the potent muscarinic agonist *exo*-3-(3-Amino-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptan (9).

The 1-azabicyclo[2.2.1]heptan-3-one, O-(3-methyl-5-aryl-2-penten-4-ynyl) oximes allow us to extend the size of known muscarinic agonists without loss of affinity and efficacy at the muscarinic receptor. Moreover, the fixed geometry of the oximes may help map the internal topography of the muscarinic receptor. This, in turn, may lead to the discovery of a truly subtype- selective muscarinic agonist.

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