Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 207–213

BIOORGANIC & MEDICINAL CHEMISTRY

# Quinuclidinone *O*-Alkynyloximes with Muscarinic Agonist Activity

Brinda Somanadhan,[a] Weng-Keong Loke,[b] Meng-Kwoon Sim[c] and Mei-Lin Go[a,*]

[a]*Department of Pharmacy, National University of Singapore, Republic of Singapore*
[b]*Defence Science National Laboratories, Republic of Singapore*
[c]*Department of Pharmacology, National University of Singapore, Republic of Singapore*

Received 26 April 2001; accepted 21 July 2001

**Abstract**—A series of quinuclidinone *O*-alkynyloximes (**14**–**19**) were synthesized and evaluated in radioligand displacement assays for binding affinities to $M_1$–$M_3$ muscarinic receptors. Radioligand displacement assays were carried out using [$^3$H] oxotremorine-M and [$^3$H] pirenzepine on rat cortical tissue and [$^3$H] *N*-methylscopolamine on rat heart and submandibulary glands. Two alkynyloximes **15** and **18** had pirenzepine/oxotremorine M ratios which were indicative of muscarinic agonist and partial agonist activity, respectively. They were tested for their mnemonic effects in mice using the swimming escape task and found to attenuate scopolamine induced impairment of the task in mice at 2 mg/kg. The results show that the *O*-alkynyloxime moiety linked to azacycles of appropriate size and rigidity (for example quinuclidine and tropane) is a potentially useful muscarinic pharmacophore that can be exploited for the design of muscarinic agonists. 

## Introduction

Among various neurotransmitter systems, cholinergic neurons in the brain are reduced early in the course of Alzheimer's disease (AD) and the dysfunction of cholinergic neurons is widely held to be an important factor responsible for cognitive deficits encountered in the disease.[1] Muscarinic agonists represent a rational treatment strategy in AD and several members have entered phase III clinical trials. Unfortunately, disappointing clinical results have been reported for most muscarinic agonists due to a lack of selectivity, low intrinsic activity, poor bioavailability and a narrow safety margin.[2] Recent reports on the potential of $M_1$ agonists as disease modifying agents acting via muscarinic regulation of P-amyloid metabolism and tau phosphorylation suggest that continued interest in the clinical value of these drugs will remain in the near future.[2,3]

Our previous studies have shown that the tropinone oxime (**1**) has a 20-fold selectivity for $M_1$ receptors over $M_2$ receptors, does not have functional agonism in an $M_2$ assay and attenuated scopolamine-induced impairment of the water escape task in mice.[4] The corresponding piperidinone oxime of **1** had no such effect, suggesting a significant role for the azacycle in these actions. The oxime functionality shares common electrostatic properties with the ester linkage and has been employed successfully but less commonly as a bioisostere for the ester group in muscarinic ligands.[4–7] The oxime moiety is stable, readily introduced in synthesis and offers a convenient handle for structural and physicochemical modification. Its introduction in a series of fluoroquinolones was observed to improve the pharmacokinetic parameters of the target compounds.[8]

In the present study, a series of quinuclidine oximes have been synthesized with the objective of further defining the role of the azacycle. The quinuclidine ring is a common pharmacophore present in several muscarinic agonists like talsaclidine (WAL 2014), sabcomeline and L-689 660 (Fig. 1).[9] The purpose of this study is to explore the muscarinic activity and selectivity of a series of quinuclidine oximes with a view of exploring the utility of combining the quinuclidine ring with selected oxime side chains noted earlier to be associated with $M_1$ selectivity.

## Results and Discussion

Figure 2 illustrates the synthesis of the quinuclidinone oximes (**14**–**19**). The intermediate *N*-alkynyloxyphthalimides (**2**–**7**) were obtained by the Mitsunobu

*Corresponding author. Tel.: +65-874-2654; fax: +65-874-1554; e-mail: phagoml@nus.edu.sq

0968-0896/02/$ - see front matter 
PII: S0968-0896(01)00267-X

reaction in which diethyl azodicarboxylate and triphenylphosphine were reacted to give a betaine intermediate, and subsequently reacted with the appropriate alcohol and *N*-hydroxyphthalimide.[10] Hydrazinolysis of the product in hydrazine hydrate gave the candidate *O*-alkynylhydroxylamine (**8**–**13**), which was then condensed with quinuclidin-3-one in methanol to give the final compound (**14**–**19**). The stereochemistry of these compounds were not investigated but they are likely to exist as a mixture of *E*/*Z* isomers. Table 1 summarises the physical data and yields of the quinuclidinone oximes.

Competitive radioligand-binding assays using [$^3$H] oxotremorine-M or [$^3$H] pirenzepine in rat cortical membranes, where $M_1$ receptors predominate, were used to determine the affinities of the quinuclidinone oximes for muscarinic receptors. The affinities of the compounds for the receptors labelled by the muscarinic antagonist pirenzepine (PZ) indicate their affinity for $M_1$ receptors, while affinities for the receptors labelled by the muscarinic agonist oxotremorine-M (Oxo-M) were considered to indicate affinity to the 'agonist conformational state' of the muscarinic receptor sites. The ratio of $K_i$ values for the displacement of the tritiated ligands PZ to Oxo-M from cortical sites has been used to predict agonist efficacy at muscarinic receptors.[11] Full agonists would have ratios greater than 100, antagonists give ratios close to unity and intermediate values are indicative of partial agonism.[6,12] The $M_2$ and $M_3$ receptor binding affinities of the oximes were also assessed from their ability to displace [$^3$H] methylscopolamine from muscarinic sites in the rat heart and submandibulary glands respectively.[13,14]

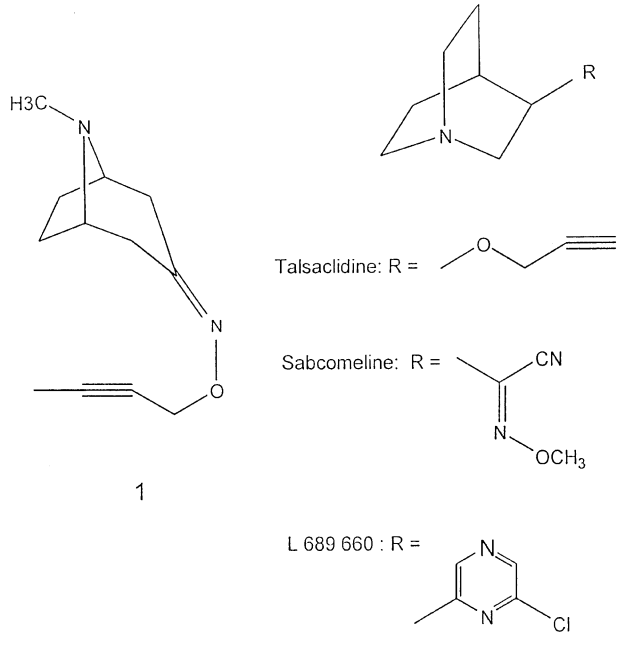



**Figure 1.** Structural formulae of some $M_1$ agonists.

Table 2 summarises the receptor binding affinities of the oximes for the various muscarinic receptor subtypes ($M_1$–$M_3$). As seen from the $K_i$ values of the oximes for the various tissues, no subtype selectivity is evident from this series of quinuclidinone oximes. This is in contrast to what was observed earlier for the tropinone oximes bearing the same alkynyl side chains as the present series.[4] For example, both **1** and **15** share the same (1-but-2-ynyl) side chain but tropinonone **1** has a 20-fold selectivity for $M_1$ over $M_2$, while a negligible difference is observed for the quinuclidinone **15**. However, a wide range of pharmacological profiles is seen with the quinuclidinone oximes. Based on its PZ/Oxo-M ratio, **15** is identified as an agonist. Partial agonism is predicted for **14,16,18,19** while the oxime **17**, like quinuclidinyl benzilate and atropine, is an antagonist (Table 2).

The *O*-propargyl oxime of quinuclidinone (**20**) has been reported earlier to have a PZ/Oxo-M ratio which indicates presence of partial agonist activity.[5]

The binding affinities of **20** were concurrently determined along with those of the present series of oximes (**14**–**19**) and as shown in Table 2, **20** has a PZ/Oxo-M ratio (581) which is reflective of an agonist. In contrast to the other oximes (**14**–**19**), **20** has a slight binding preference (4–18×) for the $M_2$ receptors of the heart. It is interesting to note that in that study by Plate et al.,[5] only **20** and not the saturated *O*-propyl or unsaturated *O*-propenyl oximes demonstrated PZ/Oxo-M ratios which were in the partial agonist range. This gives good support to the value of the *O*-alkynyl oxime moiety as a group associated with agonist activity. Our present study further shows that lengthening the alkynyl side chain beyond a three-carbon chain length still retains agonist activity.

The PZ/Oxo-M ratios were not determined for the earlier series of tropinone oximes, although the $M_1$ selective

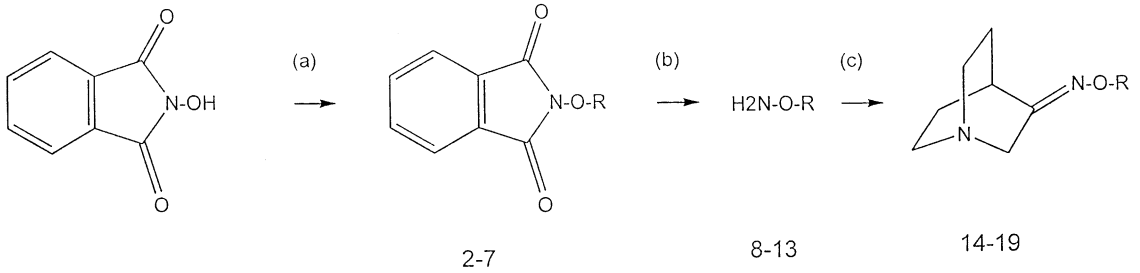



**Figure 2.** Synthetic route to oximes **14**–**19**. (a) Alcohol ROH, diethyl azodicarboxylate, triphenylphosphine; (b) hydrazine hydrate; (c) quinuclidin-3-one, methanol, room temperature. **2**,**8**,**14**: R = –CH(CH$_3$)C≡CH; **3**,**9**,**15**: R = –CH$_2$C≡C–CH$_3$; **4**,**10**,**16**: R = –CH$_2$CH$_2$C≡CH; **5**,**11**,**17**: R = –CH(C$_2$H$_5$)C≡CH **6**,**12**,**18**: R = –CH$_2$C≡C–CH$_2$CH$_3$; **7**,**13**,**19**: R = –CH$_2$CH$_2$C≡C–CH$_3$.

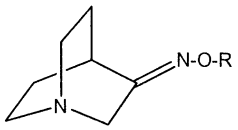
**Table 1.** Physical data of quinuclidinone *O*-alkynyloximes (**14**–**19**)

| Compound | % Yield | Mp (°C) | IR (KBr $cm^{-1}$) | Elemental analysis | Accurate mass | $^1H$ NMR ($CD_3OD$, δ) |
|---|---|---|---|---|---|---|
| **14** | 70 | 182–183 | 3250.43 (υ≡CH)<br>2104.92 (υC≡C)<br>1664.27 (υC=N) | ($C_{11}H_{17}N_2OCl$)<br>C: calcd, 57.77; found, 57.48<br>H: calcd, 7.44; found, 7.43<br>N: calcd, 12.25; found, 12.19 | 192.1252<br>($C_{11}H_{16}N_2O$ = 192.1263) | 4.91–4.83 (dq, 1H, –OCH–)<br>4.25–4.24 (t, 2H, ring C2-H)<br>3.56–3.34, 2.27–2.16, 2.14–1.99 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>2.94–2.89 (m, 2H, ring C4-H, ≡CH)<br>1.49–1.47 (d, 3H, –CH—$\underline{CH_3}$) |
| **15** | 51 | 218–220 | 2231.24 (υC≡C)<br>1657.52 (υC=N) | ($C_{11}H_{17}N_2OCl$)<br>C: calcd, 57.77; found, 57.68<br>H: calcd, 7.44; found, 7.41<br>N: calcd, 12.25; found, 12.05 | 192.1266<br>($C_{11}H_{16}N_2O$ = 192.1263) | 4.63–4.61 (q, 2H, –$OCH_2$–<br>4.24–4.23 (t, 2H, ring C2-H<br>3.58–3.33, 2.27–1.99 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>2.92–2.87 (m, 1H, ring C4-H)<br>1.82–1.80 (t, 3H, ≡C–$CH_3$) |
| **16** | 49 | 218–219 | 3228.25 (υ≡CH)<br>2111.67 (υC≡C) | ($C_{11}H_{17}N_2OCl$)<br>C: calcd, 57.77; found, 57.76<br>H: calcd, 7.44; found, 7.42<br>N: calcd, 12.25; found, 12.38 | 192.1280<br>($C_{11}H_{16}N_2O$ = 192.1263) | ($CDC1_3$): 4.21–4.16 (t, 2H, –$\overline{O}CH_2$–)<br>4.10 (s, 2H, ring C2-H)<br>3.61–3.50, 3.83–3.27, 2.25–2.04 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>2.97–2.92 (t, 1H, ring C4-H)<br>2.57–2.51 (dt, 2H, –$CH_2$–$\underline{CH_2}$-C≡)<br>2.01–1.99 (t, 1H, ≡$\overline{CH}$) |
| **17** | 43 | 100–102 | 3271.64 (υ≡CH)<br>2121.31 (υC≡C)<br>1645.95 (υC=N) | ($C_{12}H_{19}N_2OCl$)<br>C: calcd, 59.38; found, 59.95<br>H: calcd, 7.84; found, 7.72<br>N: calcd, 11.54; found, 11.17 | 206.1421<br>($C_{12}H_{18}N_2O$ = 206.1419) | $CDCl_3$: 4.71–4.65 (dt, 1H, –OCH–)<br>4.08 (s, 2H, ring C2)<br>3.53–3.24, 2.21–2.10 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>3.04–2.99 (m, 1H, ring C4-H)<br>2.51–2.49 (d, 1H, ≡CH)<br>1.88–1.77 (m, 2H, $CH_3$–$CH_2$–CH–)<br>1.06–0.99 (t, 3H, $CH_3$–$CH_2$–) |
| **18** | 52 | 168–170 | 2236.06 (υC≡C)<br>1666.20 (υC=N) | ($C_{12}H_{19}N_2OCl$)<br>C: calcd, 59.38; found, 59.01<br>H: calcd, 7.84; found, 7.77<br>N: calcd, 11.54; found, 11.53 | 206.1394<br>($C_{12}H_{18}N_2O$ = 206.1419) | 4.86 (s, 2H, –$OCH_2$–)<br>4.24 (s, 2H, ring C2-H)<br>3.56–3.33, 2.27–2.00 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>2.92–2.89 (t, 1H, ring C4-H)<br>2.27–2.15 (m, 2H, ≡C-$CH_2$–$CH_3$)<br>1.13–1.09 (t, 3H, –$CH_2$–$CH_3$) |
| **19** | 49 | 178–180 | 2200.38 (υC≡C)<br>1662.34 (υC = N) | ($C_{12}H_{19}N_2OCl$ 1/10 $H_2O$)<br>C: calcd, 58.94; found 58.93<br>H: calcd, 7.86; found, 7.91<br>N: calcd, 11.54; found 11.46 | 206.1424<br>($C_{12}H_{18}N_2O$ = 206.1419) | 4.13–4.07 (t, 2H, –$OCH_2$–)<br>4.24–4.21 (t, 2H, ring C2-H)<br>3.55–3.33, 2.27–2.15, 2.12–2.00 (m, 8H, ring C5-H, C6-H, C7-H, C8-H)<br>2.91–2.86 (m, 1H, ring C4-H)<br>2.49–2.42 (m, 2H, –$CH_2\underline{CH_2}$– C≡)<br>1.74–1.72 (t, 3H, ≡C –$\overline{CH_3}$) |

members did demonstrate the ability to attenuate scopolamine-induced impairment of the water escape task in mice, in the same way as muscarinic agonists.[4] In addition, they also demonstrated agonist like activity on the rabbit vas deferens, an in vitro model commonly used to assess $M_1$ activity.[15] In this study, the binding affinities of **1** were redetermined (Table 2) and the surprising observation was made of its PZ/Oxo-M ratio which was found to be near 1. No reasonable explanation can be given for this observation except to suggest that the PZ/Oxo-M ratio may have exceptions when it comes to its predictive utility.

Compared to the tropane ring, the quinuclidine ring is small and rigid. Bromidge and co-workers[16] have proposed that the nature of the azabicyclic ring has little influence on affinity, but affects the ratio predicting efficacy. Small, rigid rings like 1-azabicyclo[2.2.1] heptane

**Table 2.** Affinities ($K_i$) of quinuclidin-3-one alkynyloximes and related compounds for $M_1$, $M_2$ and $M_3$ receptors in the rat cortex, heart and submandibulary glands, respectively, as determined from binding displacement studies




| Compound | R | $K_i$[a] Values for inhibition of | | | | PZ/Oxo-M ratio[c] |
|---|---|---|---|---|---|---|
| | | [$^3$H]Pirenzepine binding in cortex[b] | [$^3$H]Oxotremorine-M binding in cortex[b] | [$^3$H]-*N*-Methylscopolamine binding in heart[b] | [$^3$H]-*N*-Methylscopolamine binding in submandibulary glands[b] | |
| **14** | $-CH(CH_3)C\equiv CH$ | $8.39(\pm5.83)\times10^{-7}$ | $5.96(\pm2.30)\times10^{-8}$ | $2.62(\pm0.76)\times10^{-7}$ | $2.85(\pm0.64)\times10^{-7}$ | 14.08 |
| **15** | $-CH_2C\equiv C\text{-}CH_3$ | $8.94(\pm8.88)\times10^{-7}$ | $7.08(\pm0.99)\times10^{-9}$ | $2.18(\pm0.85)\times10^{-7}$ | $4.64(\pm1.70)\times10^{-7}$ | 126.27 |
| **16** | $-CH_2CH_2C\equiv CH$ | $11.57(\pm13.34)\times10^{-7}$ | $7.99(\pm2.31)\times10^{-8}$ | $6.28(\pm2.27)\times10^{-7}$ | $7.26(\pm1.46)\times10^{-7}$ | 14.48 |
| **17** | $-CH(C_2H_5)C\equiv CH$ | $2.33(\pm2.32)\times10^{-7}$ | $4.40(\pm2.86)\times10^{-7}$ | $3.13(\pm1.21)\times10^{-7}$ | $4.07(\pm0.73)\times10^{-7}$ | 0.53 |
| **18** | $-CH_2C\equiv C\text{-}CH_2CH_3$ | $9.69(\pm11.18)\times10^{-7}$ | $1.51(\pm1.59)\times10^{-8}$ | $3.21(\pm0.28)\times10^{-7}$ | $7.00(\pm2.72)\times10^{-7}$ | 64.17 |
| **19** | $-CH_2CH_2C\equiv C\text{-}CH_3$ | $3.74(\pm2.51\times10^{-7}$ | $7.16(\pm2.00)\times10^{-8}$ | $3.45(\pm0.81)\times10^{-7}$ | $2.95(\pm0.87)\times10^{-7}$ | 5.22 |
| **20**[d] | $-CH_2C\equiv CH$ | $1.76(\pm0.40)\times10^{-6}$ | $3.02(\pm2.40)\times10^{-9}$ | $4.10(\pm0.46)\times10^{-7}$ | $7.74(\pm3.07)\times10^{-7}$ | 582.78 |
| **1**[d] | | $5.01(\pm1.40)\times10^{-7}$ | $5.63(\pm4.50)\times10^{-7}$ | $8.04(\pm0.56)\times10^{-6}$ | $5.42(\pm0.68)\times10^{-6}$ | 0.89 |
| Carbachol | | $9.12(\pm0.19)\times10^{-6}$ | $8.13(\pm1.50)\times10^{-9}$ | $8.32(\pm0.77)\times10^{-7}$ | $8.32(\pm0.38)\times10^{-6}$ | 1121.77 |
| QNB[e] | | $1.20(\pm0.61)\times10^{-9}$ | $1.30(\pm1.35)\times10^{-9}$ | $3.62(\pm6.09)\times10^{-9}$ | $1.43(\pm1.41)\times10^{-9}$ | 0.92 |
| Atropine | | $2.71(\pm3.21)\times10^{-9}$ | $3.40(\pm3.53)\times10^{-9}$ | $5.05(\pm6.74)\times10^{-9}$ | $3.29(\pm4.26)\times10^{-9}$ | 0.79 |

[a]$K_i$ values are means ± SD of at least four independent experiments.
[b]The $K_d$ (dissociation constant) and $B_{max}$ (maximal number of binding sites) of the following radiolabelled ligands for the specified tissue are as follows: [$^3$H]pirenzepine (cortex) 15.83 nM, 1600 fmol/mg protein; [$^3$H]oxotremorine (cortex) 1.20 nM, 520 fmol/mg protein; [$^3$H]-*N*-methylscopolamine (heart) 0.30 nM, 1856 fmol/mg protein; [$^3$H]-*N*-methylscopolamine (submandibulary glands) 0.10 nM (563 fmol/mg protein).
[c]Ratios are calculated from $K_i$ for the displacement of [$^3$H]pirenzepine (PZ, rat cortex) divided by $K_i$ for displacement of [$^3$H]oxotremorine (Oxo-M, rat cortex).
[d]Compounds **1** and **20** were synthesized and purified according to methods described in refs 5 and 4, respectively.
[e]Quinuclidinyl benzilate.

are more likely to give high ratios, predictive of full agonism, in contrast to sterically bigger and more flexible azacycles. The quinuclidine ring is not as rigid or small as 1-azabicyclo[2.2.1] heptane, but the presence of an appropriate side chain can lead to agonist or partial agonist activity in the resulting ligand. This can be seen from the present series of quinuclidinone oximes where the change from antagonist to agonist activity, as predicted from the PZ/Oxo-M ratios, can be attributed to the nature of the *O*-substituted oxime side chain.

The side chains of **15** (agonist) and **18** (highest PZ/Oxo-M ratio among the partial agonists) are characterised by non-branching and non-terminal alkynyl functions, with a two carbon distance between the electron rich triple bond and the oxime oxygen. The non-terminal location of the triple bond would result in a linkage of greater rigidity and the absence of an acidic alkynyl hydrogen for hydrogen bonding. It is likely that steric and electronic factors may a role in defining structure–activity.

The oximes **15**, **17** and **18** were tested for their ability to attenuate scopolamine-induced impairment of the water escape task in mice. These compounds are representative of an agonist, antagonist and partial agonist (as predicted from the PZ/Oxo-M ratios), respectively. In this test, the time taken by the animal to locate and mount the platform was monitored for five consecutive days. On the sixth day, the control group of mice received saline followed by scopolamine before they are subjected to the swimming task. The drug-treated groups received **15, 17, 18** or McN-A-343 at a dose of 2 mg/kg (ip), followed by scopolamine (3.5 mg/kg). Swimming times were again monitored (without drug treatment) on the seventh day.

Figure 3a shows the swimming times of two groups of mice over a period of seven days. The mice received **15** (2 mg/kg) and saline on the sixth day, respectively. The swimming times of both groups showed an improvement over the first five days and reached an asymptote by the third to fourth day. No significant difference was detected in the performance of the two groups of mice from days 1 to 5 ($F = 2.04$, $p > 0.05$). On the sixth day, the control group of mice which received saline followed by scopolamine showed an increase in its swimming time. In contrast, the other group of mice treated with **15** had significantly shorter swimming times than the control group ($p = 0.001$, Mann–Whitney U-test). The swimming times of the two groups of mice were again not significantly different on day 7.

A similar trend is observed in mice treated with **18** (Fig. 3b). Swimming times improved over the first five days for the two groups of mice and there was no significant difference in their performance over this time period. Upon administration of drug on the sixth day, a significant difference in swimming times was noted for the control and drug (**18**) treated group of mice ($p = 0.015$). In the case of mice treated with **17** (antagonist), no significant difference was detected between the drug treated and control groups on day 6 ($p = 0.34$) (Fig. 3c). Mice treated with the $M_1$ agonist McN-A-343 showed

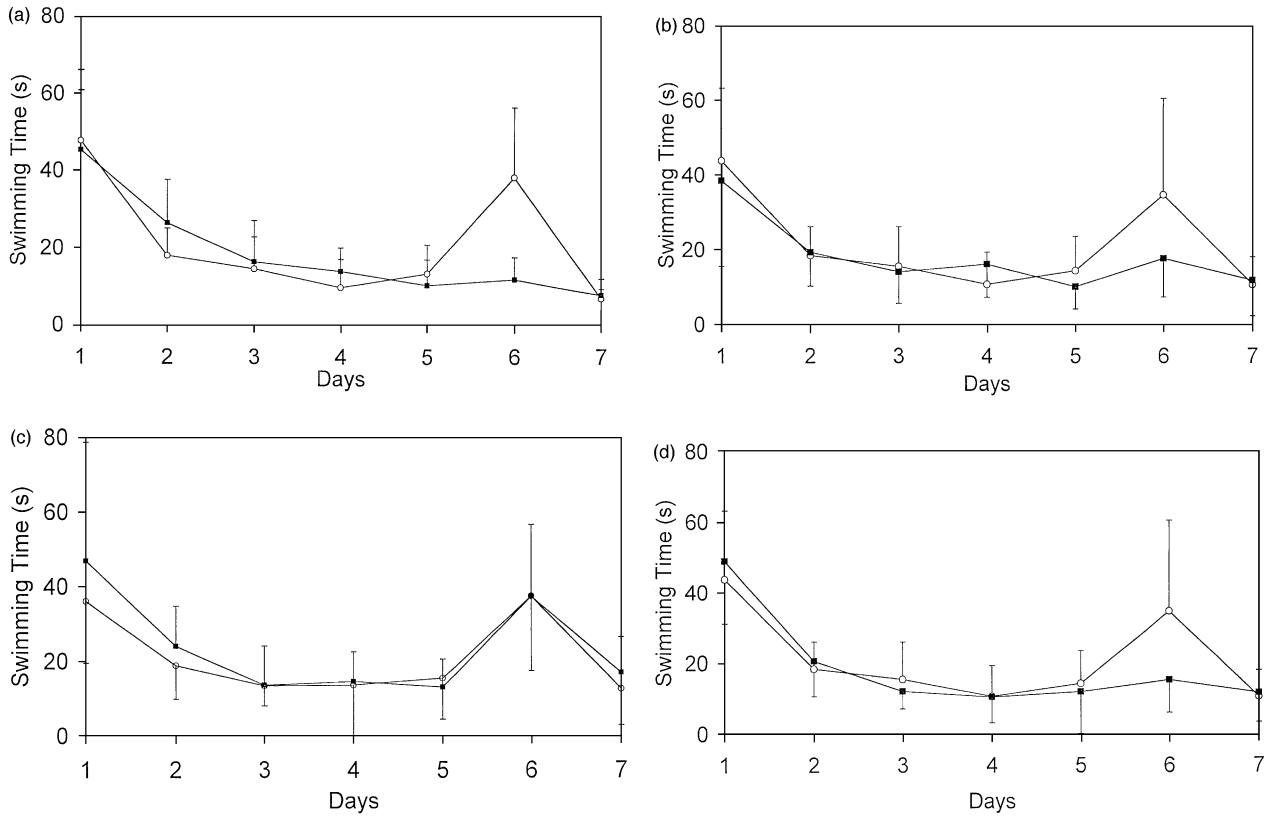



**Figure 3.** Time taken to find the platform (swimming time) in mice (days 1–7) which were given drug (2 mg/kg) and scopolamine (3.5 mg/kg) (■) or saline and scopolamine (3.5 mg/kg) (○) on day 6. Each point represents the mean group performance in successive daily blocks of trials and vertical bars indicate SD. Drugs used are (a) **15** (b) **18** (c) **17** (d) McN-A-343. given at a dose of 2 mg/kg, ip. $N = 20$ animals, except for **17** ($N = 10$).

significantly lower swimming times compared to control mice ($p = 0.002$, Fig. 3d).

The effect of **15** on the swimming task was further investigated using two other concentrations of the drug (5 and 1 mg /kg). The results are shown in Figure 4. The lower dose of **15** (1 mg/kg) was not able to overcome scopolamine induced impairment of the swimming escape and swimming times for this group of mice were not significantly different from those of the control mice ($p = 0.28$). Higher doses of **15** (5 and 2 mg/kg) were more effective and significantly shorter swimming times were noted for these groups when compared to the control ($p < 0.05$). However, there was no significant difference in the swimming times of mice receiving 5 and 2 mg/kg ($p = 0.54$) but a significant difference was detected in mice receiving 5 and 1 mg/kg ($p = 0.05$). Therefore, a dose related response (5, 1 mg/kg) could be demonstrated for **15** with respect to its effects on swimming times of mice receiving scopolamine. Another control group of mice received only **15** (2 mg/kg, without scopolamine) on day 6. The results showed that **15** had no effect on swimming performance per se.

## Conclusion

Two quinuclidinone *O*-alkynyloximes (**15, 18**) have been shown to protect mice from scopolamine-induced impairment of the swimming escape task. Both oximes have PZ/Oxo-M ratios which indicate presence of agonist/partial agonist activity but lack subtype selectivity. Another oxime that had a PZ/Oxo-M ratio of less than 1 (antagonist) failed to exert mnemonic effects on mice in the swimming escape task. Both **15** and **18** have short *O*-alkynyloxime side chains (4–5 carbon chain length) with non-terminal triple bonds. Steric and electronic characteristics of the side chain are likely to be important in defining agonist activity. The results show that the *O*-alkynyl oxime moiety is a potentially useful muscarinic pharmacophore when coupled with azacycles of appropriate size and rigidity.

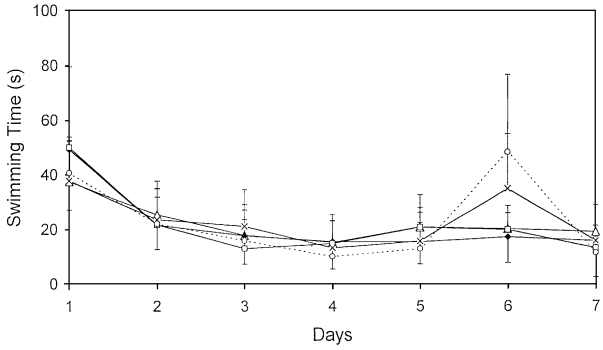



**Figure 4.** Time taken to find the platform (swimming time) in mice (days 1–7) given different doses of **15** on day 6. Each point represents the mean group performance in successive daily blocks of trials and vertical bars indicate SD. (○) saline + scopolamine; (◆) 5 mg/kg **15** + scopolamine; (□) 2 mg/kg **15** + scopolamine; (X) 1 mg/kg **15** + scopolamine; (△) **15** only. Dose of scopolamine was 3.5 mg/kg, ip. $N = 10$ animals for each group.

## Experimental

### Chemistry

Melting points were determined on a Gallenkamp apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Bruker ACF (300 MHz) spectrometer. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane as an internal standard. Mass spectra were determined on a VG Micromass 7035 E mass spectrometer (with chemical ionization) by the Chemical and Molecular Analysis Centre of the National University of Singapore. IR spectra were recorded on a Jasco FTIR-430 instrument in pressed KBr disks. Combustion analyses are indicated by symbols (C,H,N) if they are within ±0.4% of the theoretical values. Thin layer chromatography of intermediates and final compounds were done on silica gel sheets using $CH_3Cl$–MeOH (4:0.1) as eluting solvents and the compounds were visualized under $I_2$ vapour.

### General method for the syntheses of *N*-(alkynyloxy) phthalimides (2–7)

The syntheses of *N*-[1-(1-methylprop-2-ynyl)oxy] phthalimide (**2**), *N*-[1-(but-2-ynyl)oxy] phthalimide (**3**), *N*-[1-(but-3-ynyl)oxy] phthalimide (**4**), *N*-[1-(1-ethylprop-2-ynyl)oxy] phthalimide (**5**), *N*-[1-(pent-2-ynyl)oxy] phthalimide (**6**) and *N*-[1-(pent-3-nyl)oxy] phthalimide (**7**) have been reported earlier following literature methods.[4]

### General method for the syntheses of *O*-alkynyl hydroxylamine hydrochlorides (8–13)

The syntheses of *O*-(1-methylprop-2-ynyl)hydroxylamine (**8**), *O*-(but-2-ynyl)hydroxylamine (**9**), *O*-(but-3-ynyl)hydroxylamine (**10**), *O*-(1-ethylprop-2-ynyl)hydroxylamine (**11**), *O*-(pent-2-ynyl)hydroxylamine (**12**) and *O*-(pent-3-ynyl)hydroxylamine (**13**) have also been reported previously following literature methods.[4]

### General method for the syntheses of quinuclidin-3-one oxime ethers (14–19)

Equimolar (12 mmol) quantitites of quinuclidin-3-one HCl and the *O*-alkynylhydroxylamine were stirred in methanol at room temperature for 18 h. Removal of the solvent in vacuo gave a white solid that was dissolved in water, made alkaline with solid $K_2CO_3$ and extracted with chloroform. After drying over anhydrous $Na_2SO_4$, the organic layer was removed by evaporation under reduced pressure. A clear pale yellow oil was obtained which was purified by passing through a silica gel column eluted with $CHCl_3$/MeOH (4:0.1). The eluted oil was treated with freshly prepared ethereal HCl to give the desired HCl salt of the final compound. The yields and physical data of quinuclidin-3-one *O*-(1-methylprop-2-ynyl)oxime (**14**), quinuclidin-3-one *O*-(but-2-ynyl)oxime (**15**), quinuclidin-3-one *O*-(but-3-ynyl)oxime (**16**), quinuclidin-3-one *O*-(1-ethylprop-2-ynyl)oxime (**17**), quinuclidin-3-one *O*-(pent-2-ynyl)oxime (**18**) and quinuclidin-3-one *O*-(pent-3-ynyl)oxime (**19**) are summarised in Table 1.

### Pharmacology

**Materials.** [$^3$H] Pirenzepine (79.3 Ci/mmol), [$^3$H]-*N*-methylscopolamine (82.0 Ci/mmol), [$^3$H]-oxotremorine-M acetate (84.8 Ci/mmol) were purchased from NEN (Boston, MA, USA). Atropine sulphate and (±)-3-quinuclidinyl benzilate (QNB) were purchased from Sigma Chemical Company (St Louis, MO, USA) and Research Biochemicals International (Natick, MA, USA) respectively. Tissue solubilizer and scintillation cocktail (Ready-Sol-HP) from Beckman were used for preparing the samples for counting. The oxime hydrochlorides were dissolved in distilled water for pharmacological testing.

Tissues of the rat (male, Sprague–Dawley, 200–250 g) were isolated for the radioligand displacement assays and Swiss albino mice (male, 20±2 g) were used for the swimming escape task. Animals were purchased from the Laboratory Animal Centre of the National University of Singapore and were handled according to international guidelines for animal research.[17]

### Muscarinic receptor binding studies

The binding of the oximes to muscarinic $M_1$, $M_2$ and $M_3$ receptor subtypes were determined using the cerebral cortex, heart, submandibulary glands of the rat, respectively. The preparation of the tissues for binding studies was carried out according to previously described methods.[4] The receptor affinities of the oximes were determined from experiments in which the ability of varying concentrations of the test compound to displace a fixed concentration of a receptor specific radiolabeled ligand was monitored. [$^3$H] Pirenzepine and [$^3$H] oxotremorine-M were used as the specific radioligands for the cerebral cortex. [$^3$H] *N*-methylscopolamine was used as the specific radioligand for the muscarinic receptors of the heart and submandibulary glands. Radioligand binding assays in the rat cerebral cortex, heart and submandibulary glands were carried out as previously described.[4]

**Behavorial testing in mice.** Swiss albino mice were required to perform a water escape task which was modified from the standard version of the Morris water maze.[18] The task required mice to locate a circular platform (8 cm diameter) placed at the left hand corner of a water bath (60×60×10 cm), filled with water at 28 °C (±1). The platform was submerged 1 cm below the water surface, and the water was colored brown to match the brown plasticine covering the surface of the platform. The sides of the bath are covered with brown paper and the experiment was carried out in a quiet room with the lights dimmed. The mouse was introduced at the same corner of the bath and the time taken to locate and mount the platform (swimming time) was monitored. Mice were divided into groups of 10 and for each group, the mice were tested individually over a period of 5 days. On the sixth day, the control group of mice received saline (ip), other groups of mice received the test drug (**15**,**17**,**18**) at 2 mg/kg (ip) and another group of mice received the $M_1$ agonist McN-A-343

(Sigma Chemical Co., St Louis, Mo, USA) at 2 mg/kg (ip). After 30 min, the mice in all groups received scopolamine (3.5 mg/kg, ip). After another 30 min, the mice were subjected to the water escape task. On the seventh day, the mice were again evaluated, but this time, without any drug treatment. In another test, the entire 7-day experiment was repeated with **15**, given at different doses of 1, 2 and 5 mg/kg, followed 1/2 h later with scopolamine (3.5 mg/kg) on the sixth day. The drug solutions were freshly prepared in saline on the day of the test. The effects of drugs on the swimming times were calculated by analysis of variance (ANOVA) with a split-plot design (between-within subjects). The pairwise comparisons were performed by Mann–Whitney U-test on SPSS for Windows (Version 10, SPSS Inc., Chicago, IL, USA).

## Acknowledgements

This work has been supported by a grant RP-148-000-014-112 from the National University of Singapore.

## References and Notes

1. Court, J. A.; Perry, E. K. *Pharmac. Ther.* **1991**, *52*, 423.
2. Fisher, A. *CNS Drugs* **1999**, *12*, 197.
3. Haring, R.; Fisher, A.; Marciano, D.; Pittel, Z.; Kloog, Y.; Zuckerman, A.; Eshhar, N.; Heldman, E. *J. Neurochem.* **1998**, *71*, 2094.
4. Xu, R.; Sim, M. K.; Go, M. L. *J. Med. Chem.* **1998**, *41*, 3220.
5. Plate, R.; Plaum, M. J. M.; de Boer, T.; Andrews, J. S. *Bioorg. Med. Chem.* **1996**, *4*, 239.
6. Tecle, H.; Barrett, S. D.; Lauffer, D. J.; Augelli-Szafran, C.; Brann, M. R.; Callahan, M. J.; Caprathe, B. W.; Davis, R. E.; Doyle, P. D.; Eubanks, D.; Lipiniski, W.; Mirzadegan, T.; Moos, W. H.; Moreland, D. W.; Nelson, C. B.; Pavia, M. R.; Raby, C.; Schwarz, R. D.; Spencer, C. J.; Thomas, A. J.; Jaen, J. C. *J. Med. Chem.* **1998**, *41*, 2524.
7. Tecle, H.; Mirzadegan, T.; Moos, W. H.; Moreland, D. W.; Pavia, M. R.; Schwarz, R. D.; Davis, R. E. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 821.
8. Hong, C. Y.; Kim, Y. K.; Chang, J. H.; Kim, S. H.; Nam, D. H.; Kim, Y. Z.; Kwak, J. H. *J. Med. Chem.* **1997**, *40*, 3584.
9. Felder, C. C.; Bymaster, F. P.; Ward, J.; DeLapp, N. *J. Med. Chem.* **2000**, *43*, 4333.
10. Mitsunobu, O. *Synthesis* **1981**, *1*, 1981.
11. Freedman, S. B.; Harley, E. A.; Iversen, L. L. *Br. J. Pharmacol.* **1988**, *93*, 437.
12. Bromidge, S. M.; Brown, F.; Cassidy, F.; Clark, M. S. G.; Dabbs, S.; Hadley, M. S.; Hawkins, J.; Loudon, J. M.; Naylor, C. B.; Orlek, B. S.; Riley, G. J. *J. Med. Chem.* **1997**, *40*, 4265.
13. Peralta, E. G.; Ashkenazi, A.; Winslow, J. W.; Smith, D. H.; Ramachandran, J.; Capon, D. J. *EMBO J.* **1987**, *6*, 3923.
14. Dorje, F.; Levey, A. I.; Brann, M. R. *Mol. Pharmacol.* **1991**, *40*, 459.
15. Xu, R. Synthesis and Pharmacology of Derivatives of Tropine and related compounds. PhD Thesis, National University of Singapore, 1999; p 190.
16. Bromidge, S. M.; Brown, F.; Cassidy, F.; Clark, M. S. G.; Dabbs, S.; Hawkins, J.; Loudon, J. M.; Orlek, B. S.; Riley, G. J. *Bioorg. Med. Chem. Lett.* **1992**, *8*, 791.
17. Howard-Jones, N. *WHO Chron.* **1985**, *39*, 51.
18. Morris, R. G. M. *Learn. Motiv.* **1981**, *12*, 239.