Synthesis and Pharmacological Characterization of *O*-Alkynyloximes of Tropinone and *N*-Methylpiperidinone as Muscarinic Agonists

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A number of *O*-alkynyloximes of tropinone and *N*-methyl-4-piperidinone have been synthesized and evaluated for muscarinic activity. The affinities of these oximes were tested in homogenates of cerebral cortex, heart, and submandibulary glands from rats using [^{3}H]pirenzepine and [^{3}H]-*N*-methylscopolamine as radioligands. The oximes bind to the cortical muscarinic receptors with pK_i values varying from 3 to 7. Higher binding affinities were observed for the *O*-alkynyl tropinone oximes than the corresponding piperidinone analogues. Binding to the muscarinic sites in the heart and submandibulary glands was also observed but with lower affinities. Good M_1 subtype selectivity (10-fold or greater) was observed with some oximes (**26a**, **28a**, **32a**) at the cortical sites. These oximes also attenuated scopolamine-induced impairment of the water mask task in mice. Functional assays for M_3 activity on the rat aorta showed that all oximes possessed M_3 agonist action but M_2 agonist activity was not observed at the endothelium-denuded rabbit aorta. Analysis of the quantitative structure−activity relationship (QSAR) indicated that the Connolly surface area is an important determinant of activity, accounting for 70% of the variation in cortical binding affinity among the oximes.

Introduction

Considerable research efforts have been directed to the development of muscarinic agonists for the treatment of Alzheimer's disease (AD).[1] The rationale for this thrust is based on two complementary themes, viz., the cholinergic hypothesis of memory dysfunction[2−4] and the muscarinic regulation of amyloid metabolism.[5] Both hypotheses suggest that there is a role for centrally acting muscarinic agonists in retarding the progression of AD dementia.

Four subtypes of the muscarinic receptor have been identified on the basis of pharmacological studies (designated M_1−M_4), while molecular biological studies have identified five muscarinic receptor subtypes (m1−m5).[6] The muscarinic receptor subtypes are located at different sites in the brain, with high levels of M_1 (m1) receptors in the cortex and hippocampus, areas associated with memory and learning. Development of M_1-selective agonists as a substitution therapy in AD has been pursued by several investigators. However, attaining receptor subtype selectivity among muscarinic agonists, so critical to the development of clinically useful candidates, has proven to be an illusive goal. No agonist is known to discriminate between muscarinic receptor subtypes on an affinity basis, by more than 1 order of magnitude.[7] In addition, screening for selective M_1 receptor agonists is critically dependent on several tissue factors which may vary between tissues and cell lines.[8] As a result, poor correlations between in vitro and in vivo tests are usually observed.

Several investigators have designed oxime ethers as muscarinic agonists with promising results. Bromidge and co-workers[9] proposed the oxime ether as an alternative bioisostere to the ester and 1,2,4-oxadiazole functionalities, based on the broad similarities observed in their electrostatic potentials. The propargyl ether (**1**) of 1-azabicyclo[2.2.1]heptane 3-aldoxime was found to be a high-affinity partial agonist with a good central selectivity (Chart 1). The oxime analogue (**2**) of UH5 (**3**), related to the nonselective muscarinic agonist oxotremorine, demonstrated 5-fold greater m1 selectivity than UH5.[10] The methyl oxime (**4**) of 1-azabicyclo-[2.2.2]octan-3-one has been reported to possess modest M_3 agonistic selectivity.[11] Tecle et al.[12] reported good M_1 selectivity in the phenylpropargyl ether (**5**), thus showing that it is possible to synthesize large and long muscarinic agonists with good potency and efficacy.

In the present study, a series of substituted oximes of *N*-methyl-4-piperidinone and 3-tropinone bearing the alkynyl unsaturation in the side chain have been synthesized by facile methods. Unlike previously synthesized agonists which possess rigid 1-azabicyclic ring systems,[9,11,12] these compounds possess the more flexible piperidine and tropane rings. Variations in the side chain have also been undertaken to reveal the effect of chain length, branching, and location of unsaturation on activity. None of the compounds are chiral, a feature which greatly facilitated their synthesis and separation. The receptor subtype selectivities of these oximes have been evaluated by investigating their ability to displace [^{3}H]pirenzepine from muscarinic receptors in rat cerebral cortex and [^{3}H]-*N*-methylscopolamine from rat heart ventricle and submandibulary glands. In addition, the muscarinic agonist potencies have been evaluated in functional assays on the rat aorta and the endothelium-denuded rabbit aorta for M_3 and M_2 activities, respectively. The effect of selected oximes on the mnemonic responses of mice in the modified Morris water maze was also investigated.

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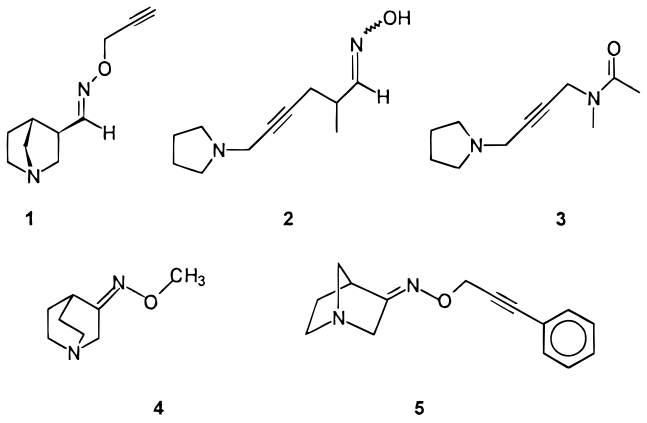
Chart 1. Structures of Compounds **1**−**5**



Chemistry

The syntheses of the O-substituted oximes of tropinone and *N*-methyl-4-piperidinone are outlined in Scheme 1. The intermediate *N*-alkynylphthalimides (**6**−**14**) are synthesized from the reaction of diethyl azodicarboxylate and triphenylphosphine (to form a betaine), an alcohol, and the nucleophilic *N*-hydroxyphthalimide.[13,14] Hydrazinolysis of the intermediate *N*-(alkynyloxy)phthalimides was attempted by various methods such as refluxing/stirring with hydrazine hydrate in ethanol or pyridine.[13,15] It was eventually found that vigorous agitation with neat hydrazine hydrate[16] gave the *O*-alkynylhydroxylamines (**15**−**23**) in satisfactory yields (40−70%). The latter were condensed with the ketone (3-tropinone or *N*-methyl-4-piperidinone) by stirring in methanol at room temperature.[17] The resulting *O*-alkynyloximes of 3-tropinone (**24a**−**32a**) and *N*-methyl-4-piperidinone (**24b**−**32b**), as well as **33a**,**b** and **34a**,**b**, were converted to the water-soluble hydrochloride salts for pharmacological evaluation. The physical data of the *O*-alkynyloximes of 3-tropinone and *N*-methylpiperidin-4-one are given in Table 1. The syntheses of *O*-(1-ethyl-2-propynyl)oximes (**28a**,**b**), *O*-(2-pentynyl)oximes (**29a**,**b**), *O*−(3-pentynyl)-oximes (**30a**,**b**), and *O*−(1-methyl-3-butynyl)oximes (**32a**,**b**) of 3-tropinone and *N*-methyl-4-piperidinone, *N*-methyl-4-piperidin-3-one *O*-methyloxime (**33b**), and *N*-methyl-4-piperidinone oxime (**34b**) have not been previously reported. The remaining oximes (**24a**,**b**−**27a**,**b**, **31a**,**b**, **33a**, **34a**) have been mentioned previously[17] but without supporting physical or pharmacological data.

Results

Receptor Binding Assays. M_1 receptor binding affinity was assessed from the ability of the oximes to displace [^{3}H]pirenzepine from its muscarinic binding sites on the rat cerebral cortex. M_2 and M_3 receptor binding affinities were assessed from the concentration of oxime required to displace [^{3}H]-*N*-methylscopolamine from muscarinic sites in the rat heart and submandibulary glands, respectively. The cerebral cortex in rat expresses a mixture of muscarinic receptor subtypes (M_1 40%, M_2 30%, M_3 5%, and M_4 20%) as found from immunoprecipitation,[18,19] of which the M_1 subtype is predominant. The rat heart expresses a homogeneous population of the M_2 subtype.[20] The rabbit submandibulary gland contains equal proportions of M_3 and M_1,[21] and it is assumed that this also exists in the rat.

Table 2 gives the receptor binding affinities (expressed as pK_i) of the oximes for the various muscarinic receptor subtypes. For comparative purposes, the pK_i and Hill coefficients of some muscarinic ligands were also determined. These include the nonspecific muscarinic antagonists atropine and *N*-methylscopolamine, pirenzepine (M_1 antagonist), McN-A-343 (M_1 agonist), and the nonselective agonist oxotremorine. It was generally observed that the muscarinic agonists had lower binding affinities than the antagonists and Hill coefficients which were less than 1, which may indicate heterogeneity of binding sites. The rank order observed at [^{3}H]pirenzepine-labeled cortical sites was atropine > pirenzepine > oxotremorine > McN-A-343. At the [^{3}H]-*N*-methylscopolamine labeled sites of the heart ventricles and submandibulary glands, the sequence *N*-methylscopolamine > atropine > oxotremorine > McN-A-343 was observed.

As seen from Table 2, the pK_i values of the oximes ranged from 4 to 7 for the M_1 subtype. Lower binding affinities were observed for the M_2 (pK_i = 4−6) and M_3 (pK_i = 3−6) subtypes. Like other muscarinic agonists, the oximes had Hill coefficients which were significantly less than unity, which suggested that binding to multiple affinity states of the muscarinic receptor may have occurred.

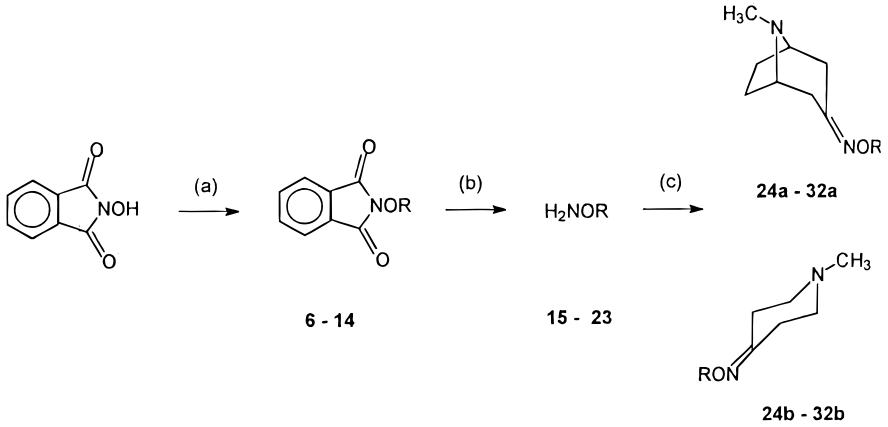
The selectivity of the oximes for the muscarinic subtypes was assessed from the ratios of their K_i values at the various sites (Table 3). A large number of oximes (**25b**, **26a**,**b**, **27b**, **28a**, **31a**, **32a**,**b**) showed at least a 10-fold selectivity for the M_1 over M_3 subtype, but fewer oximes (**26a**, **27a**, **28a**, **32a**) were at least 15-fold more selective for the M_1 over M_2 subtype. Only two oximes (**26b**, **34a**) had a 15-fold or more greater selectivity for M_2 than M_3.

Because of its outstanding M_1 selectivity, the binding affinity of the oxime **26a** which has a but-2-ynyl ($-CH_2C{\equiv}CCH_3$) side chain was investigated in the presence of 30 μM Gpp(NH)p. Ligands with good M_1 selectivity have been noted to possess small Gpp(NH)p-induced shifts.[22] Table 4 gives the binding parameters of **26a** and two other less selective oximes (**24a**, **29a**) in the presence of Gpp(NH)p. The binding affinities of the less selective oximes (**24a**, **29a**) for the cardiac and submandibulary sites were observed to decrease, but little change was observed in their binding affinities for cortical sites. As a result, there was a significant increase in the K_i ratios for M_3/M_1 and M_3/M_2 subtype selectivity in the presence of Gpp(NH)p for **24a** and **29a**.

In the case of oxime **26a**, little change in binding affinity at all three sites (cortical, cardiac, and submandibulary) was noted in the presence of Gpp(NH)p, and no marked incease in subtype selectivity was observed. The small Gpp(NH)p shifts of **26a** in the cortex, heart, and submandibulary glands suggest that it is a ligand with M_1 selectivity.

In Vitro Functional Assays. Functional in vitro assays were carried out on the endothelial intact rat aorta (muscarinic M_3 receptors)[23] and the de-endothelialized rabbit aorta (muscarinic M_2 receptors).[24] ACh induces a dose-dependent relaxation of the phenylephrine-contracted endothelium intact rat aortic ring.

Scheme 1. Synthetic Route to Oximes **24a**,**b**−**32a**,**b**[a]



[a] (a) Alcohol ROH, diethyl azodicarboxylate, triphenylphosphine; (b) hydrazine hydrate; (c) tropinone/*N*-methyl-4-piperidinone, methanol, room temperature.

Relaxation was observed upon cumulative addition of the oximes, indicating that these compounds are agonists. The concentration of the oximes required to produce 50% relaxation of phenylephrine-contracted endothelium intact rat aortic rings (ED_{50}) ranged from 10^{-5} to 10^{-6} M, and they were significantly less potent than oxotremorine (Table 5). There was a reasonable linear correlation between the binding assay pK_i values and the $pK_{ED_{50}}$ values obtained from the M_3 functional assay, as shown in eq 1:

$$pK_{ED_{50}} = 0.34(\pm 0.63)\ pK_i + 3.55(\pm 0.30) \qquad (1)$$

$$n = 22,\ r^2 = 0.59,\ SE = 0.23,\ F = 29.29$$

In endothelium-denuded rabbit aortic rings which have been submaximally precontracted with 10^{-7} M phenylephrine, addition of ACh increased the tone in a dose-dependent manner.[24] The M_1-selective agonist McN-A-343 did not alter the contractile response of the aortic rings up to 10^{-2} M, but the propargyl ester of arecoline (APE), an M_2 and M_3 receptor agonist, increased the contractile tone of the rings at concentrations of 10^{-4} M. However, McN-A-343, but not arecoline, relaxed the precontracted denuded aortic rings with an ED_{50} of 3.53×10^{-7} M. Like McN-A-343, the oximes relaxed the precontracted endothelium-denuded aortic rings, but at much higher ED_{50} values (Table 5). Thus, the oximes do not appear to be agonists at the M_2 receptor sites of the endothelium-denuded rabbit aorta. There was a poor correlation ($r^2 = 0.13$) between the K_i values for M_2 binding and the $pK_{ED_{50}}$ from functional assay.

Behavioral Testing in Mice. Selected oximes (**26a**, **28a**, **32a**) which showed good M_1 binding affinities and selectivities were tested for their ability to attenuate scopolamine-induced impairment of the water escape performance of mice in the modified Morris swim maze. The effects of McN-A-343 and the methyloxime of tropinone (**33a**) which has poor M_1 binding affinity were also investigated for comparison. The variation in latency (time taken for mice to reach platform) over time in mice treated daily with scopolamine, saline (control), or scopolamine + drug (tested at 0.2 mg/kg) was determined. Control mice treated with saline showed a constant performance by day 3 ($F = 15.67$, $p < 0.001$). Scopolamine-treated mice also improved their performance significantly over time ($F = 34.60$, $p < 0.001$), but they still took a significantly longer time to perform the task compared to the control mice ($F = 34.78$, $p < 0.001$). Mice which had received scopolamine and **26a**, **28a**, or **32a** performed significantly better (shorter time) than those receiving scopolamine alone: **26a**, $F = 13.68$, $p < 0.001$; **28a**, $F = 27.35$, $p < 0.001$; **32a**, $F = 14.82$, $p < 0.001$. Similar observations were made with mice treated with McN-A-343 ($F = 15.58$, $p < 0.001$) but not in mice treated with scopolamine + **33a** ($F = 1.00$, $p > 0.05$). No differences in latencies were observed between control mice and mice treated with scopolamine and **26a**, **28a**, and **32a** ($p > 0.05$).

A dose-related study was also carried out in which the latency time in mice that received various doses of oxime **28a** (0.2, 1, 2, or 5 mg/kg) combined with scopolamine (1 mg/kg) was determined. There were no significant differences in the responses of control mice and mice which received **28a** (2 mg/kg) alone ($F = 0.00$, $p > 0.05$). This was also true for mice which received higher doses of **28a** (5, 2 mg/kg) combined with scopolamine ($F = 1.63$, $p > 0.05$). Responses of mice receiving lower doses of **28a** (0.2, 1 mg/kg) did not differ significantly from each other ($F = 0.116$, $p > 0.05$) but were significantly different from responses obtained in scopolamine-treated mice over 4 days (1.0 mg/kg: $F = 11.40$, $p < 0.01$; 0.2 mg/kg: $F = 8.91$, $p < 0.05$).

In another set of experiments, the acute actions of scopolamine in control and oxime-treated mice were investigated. Two groups of mice were treated with saline on day 1, and their performance in the water maze task was monitored daily. There were no significant differences in the responses of these two groups of mice during this period ($F = 0.00$, $p > 0.05$). On day 6 (or day 16 for **26a**), one group of mice was given scopolamine and saline while another group was given scopolamine and drug (**26a**, **28a**, **32a, 33a**). It was observed that the group of mice treated with scopolamine and **26a, 28a**, or **32a** performed significantly better than the control group treated with scopolamine alone ($p < 0.01$, evaluated by two-tailed *t*-test and Mann−Whitney *U*-test). In contrast, no significant difference was detected between control mice and mice treated with **33a**. The results show that the selected tropinone oximes do counteract acute scopolamine-

Table 1. Physical Data of *O*-Alkynyloxime Hydrochlorides of Tropinone (**24a**−**34a**) and *N*-Methyl-4-piperidinone (**24b**−**34b**)

compd	% yield	mp (°C)	IR (KBr cm^{-1})	elemental anal.	accurate mass	^1H NMR (CD_3OD, δ)
24a	67	184−186	3260.30 (υ≡CH) 2122.13 (υC≡C) 1663.15 (υC═N)	C,H,N	192.1271 ($C_{11}H_{18}N_2O$ = 192.1262)	4.66−4.67 (d, 2H, −O−CH_2), 2.87−2.89 (t, 1H, ≡CH), 2.86 (s, 3H, NCH_3), 3.31−2.62 (m, 10H, tropinyl H)
24b	61	147.150	3290.79 (υC≡N) 2122.49 (υC≡C) 1645.80 (υC═N)	C,H,N	166.1107 ($C_9H_{14}N_2O$ = 166.1106)	4.65 (d, 2H, −OCH_2), 3.30−3.39 (m, 4H, piperidinyl H, at C_2, C_6), 2.93 (s, 3H, NCH_3), 2.85−2.87 (t, 1H, ≡CH), 2.67−2.71 (m, 4H, piperidinyl H at C_3, C_5)
25a	37	174−176	3277.30 (υ≡CH) 2112.49 (υC≡C) 1629.52 (υC═N)	C,N H: calcd, 8.09 found, 8.05	206.1421 ($C_{12}H_{18}N_2O$ = 206.1419)	4.68−4.70 (m, 1H, −OCH−), 2.83 (s, 3H, NCH_3), 2.44−2.45 (m, 1H, ≡CH), 2.03−4.31 (m, 10H, tropinyl H) 1.48−1.50 (d, 3H, CH_3)
25b	28	84.86	3292.72 (υ≡CH) 2120.21 (υC≡C) 1657.37 (υC═N)	C H: calcd, 7.91 found, 7.86 N: calcd, 12.93 found, 12.86	180.1262 ($C_{10}H_{16}N_2O$ = 180.1262)	4.82−4.87 (m, 1H, −OCH−), 2.85 (s, 3H, NCH_3), 2.47−2.45 (d, 1H, ≡CH), 2.46−4.32 (m, 8H, piperidinyl H), 1.49−1.51 (d, 3H, −CH_3)
26a	64	172−174	2262.27 (υC≡C) 1641.94 (υC═N)	C,H N: calcd, 10.74 found, 10.87	206.1412 ($C_{12}H_{18}N_2O$ = 206.1419)	1.48−1.50 (d, 3H, −CH_3), 4.59−4.62 (q, 2H, −OCH_2), 2.86 (s, 3H, NCH_3), 1.80−1.82 (t, 3H, ≡CCH_3), 1.86−3.35 (m, 10H, tropinyl H)
26b	51	170−172	2226.27 (υC≡C) 1647.73 (υC═N)	C,H N: calcd, 12.93 found, 12.66	180.1261 ($C_{10}H_{16}N_2O$ = 180.1262)	4.58−4.60 (q, 2H, −O−CH_2), 2.93 (s, 3H, NCH_3), 3.29−3.48 (m, 2H, piperidinyl H at C_2, C_6), 1.80−1.82 (t, 3H, ≡CCH_3), 2.66−2.88 (m, 4H, piperidinyl H at C_3, C_5)
27a	77	157−158	3238.73 (υ≡CH) 2127.92 (υC≡C) 1649.66 (υC═N)	C,N H: calcd, 7.89 found, 7.85	206.1417 ($C_{12}H_{18}N_2O$ = 206.1419)	4.16−4.19 (m, 2H, −OCH_2), 2.83 (s, 3H, NCH_3), 2.51−2.57 (m, 2H, −CH_2−), 2.27−3.32 (m, 10H, tropinyl H), 1.86−1.98 (t, 1H, ≡CH)
27b	54	150−152	3269.58 (υ≡CH) 2127.92 (υC≡C) 1657.37 (υC═N)	C,H N: calcd, 12.93 found, 12.78	179.1193 ($C_{10}H_{16}N_2O$ = 179.1184)	4.12−4.17 (t, 2H, −OCH_2), 2.85 (s, 3H, NCH_3), 2.80−3.77 (m, 8H, piperidinyl H), 2.50−2.58 (d, 2H, −CH_2−C≡), 1.97−1.98 (t, 1H, ≡CH)
28a	77	168−169	3223.30 (υ≡CH) 2120.21 (υC≡C) 1648.66 (υC═N)	C,H N: calcd, 10.91 found, 10.97	220.1568 ($C_{13}H_{20}N_2O$ = 220.1576)	4.66−4.71 (m, 1H, −O−CH), 2.86 (s, 3H, NCH_3), 2.48−2.49 (d, 1H, ≡CH), 1.76−1.98 (m, 2H, CH_2), 1.00−1.04 (t, 3H, CH_3), 2.27−3.32 (m, 10H, tropinyl H)
28b	75	165−167	3246.44 (υ≡CH) 2112.49 (υC≡C) 1665.08 (υC═N)	H,N C: calcd, 57.26 found, 56.77	194.1408 ($C_{11}H_{18}N_2O$ = 194.1419)	4.64−4.69 (m, 1H, −OCH−), 2.89 (s, 3H, NCH_3), 2.60−3.71 (m, 8H, piperidinyl H), 2.48−2.49 (d, 1H, ≡CH), 1.79−1.85 (m, 2H, CH_2), 0.99−1.04 (t, 3H, CH_3)
29a	32	143−146	2235.92 (υC≡C) 1649.66 (υC═N)	C H: calcd, 8.24 found, 8.28 N: calcd, 10.91 found, 10.81	220.1588 ($C_{13}H_{20}N_2O$ = 220.1576)	4.66−4.71 (m, 2H, −OCH_2) 2.83 (s, 3H, NCH_3), 2.21−2.28 (m, 2H−CH_2−), 1.90−3.94 (m, 10H, tropinyl H), 1.12−1.17 (m, 3H, CH_3)
29b	52	not determined (too hydroscopic)	2235.91 (υC≡C) 1645.79 (υC═N)	H C: calcd, 57.26 found, 57.58 N: calcd, 12.14 found, 11.98	194.1426 ($C_{11}H_{18}N_2O$ = 194.1419)	4.63−4.72 (m, 2H, −O−CH_2), 2.86 (s, 3H, NCH_3), 2.00−3.70 (m, 8H, piperidinyl H), 2.21−2.27 (m, 2H, −CH_2−), 1.13−1.18 (t, 3H, CH_3)
30a	77	212−214	2259.05 (υC≡C) 1641.94 (υC═N)	C,H N: calcd, 8.24 found, 8.20	220.1577 ($C_{13}H_{20}N_2O$ = 220.1576)	4.07−4.12 (t, 2H, −OCH_2), 2.85 (s, 3H, NCH_3), 2.44−2.49 (m, 2H, −CH_2−C≡), 1.84−3.97 (m, 10H, tropinyl H), 1.76−1.78 (t, 3H, −CH_3)
30b	31	178−180	2259.05 (υC≡C) 1665.08 (υC═N)	C,H N: calcd, 12.14 found, 12.05	194.1428 ($C_{11}H_{18}N_2O$ = 194.1419)	4.06−4.11 (t, 2H, −OCH_2), 2.88 (s, 3H, NCH_3), 2.44−2.49 (m, 2H, −CH_2−), 2.43−3.69 (m, 8H, piperidinyl H), 1.77−1.78 (t, 3H, CH_3)
31a	32	108−110	3238.73 (υ≡CH) 2120.21 (υC≡C) 1625.52 (υC═N)	C,H N: calcd, 10.91 found, 10.97	220.1575 ($C_{13}H_{20}N_2O$ = 220.1576)	4.12−4.17 (m, 2H, −OCH_2−), 2.83 (s, 3H, NCH_3), 2.23−2.30 (m, 2H, −CH_2−C≡), 2.00−3.97 (m, 10H, tropinyl H), 1.96−1.98 (t, 1H, ≡CH), 1.83−1.90 (m, 2H, −CH_2−)

Table 1 (Continued)

compd	% yield	mp (°C)	IR (KBr cm^{-1})	elemental anal.	accurate mass	^1H NMR (CD_3OD, δ)
31b	37	90−91	3231.01 (υ≡CH) 2120.21 (υC≡C) 1657.31 (υC=N)	C,H,N	194.1420 ($C_{11}H_{18}N_2O$ = 194.1419)	4.12−4.16 (t, 2H, $-OCH_2$), 2.86 (s, 3H, NCH_3), 2.38−3.71 (m, 8H, piperidinyl H), 2.23−2.29 (m, 2H, $-CH_2-C\equiv$), 1.96−1.97 (t, 1H, ≡CH), 1.84−1.90 (m, 2H, $-CH_2-$)
32a	74	142−144	3254.15 (υ≡CH) 2120.21 (υC≡C) 1649.66 (υC=N)	C,H,N	220.1569 ($C_{13}H_{20}N_2O$ = 220.1576)	4.31−4.35 (m, 1H, −OCH−), 2.84 (s, 3H, NCH_3), 2.47−2.51 (m, 2H, $-CH_2-$), 2.26−3.99 (m, 10H, tropinyl H), 1.97−1.99 (t, 1H, ≡CH), 1.30−1.34 (d, 3H, CH_3)
32b	75	147−149	3223.30 (υ≡CH) 2116.34 (υC≡C) 1653.51 (υC=N)	C,H,N	194.1423 ($C_{11}H_{18}N_2O$ = 194.1419)	4.28−4.34 (t, 1H, −O−CH−), 2.86 (s, 3H, NCH_3), 2.44−2.49 (m, 2H, $-CH_2-$), 2.38−3.70 (m, 8H, piperidinyl H), 1.98−2.00 (t, 1H, ≡CH), 1.31−1.34 (d, 3H, CH_3)
33a	73	217−220 (145−147)[17]	1649.65 (υC=N)	C H: calcd, 8.37 found, 8.31 N: calcd, 13.69 found, 13.45	168.1241 ($C_9H_{16}N_2O$ = 168.1263)	3.85 (s, 3H, CH_3), 2.83 (s, 3H, NCH_3), 1.86−3.88 (m, 10H, tropinyl H)
33b	68.7	145−147	1649.86 (υC≡N)	C,H N: calcd, 15.68 found, 15.60	142.1114 ($C_7H_{14}N_2O$ = 142.1106)	3.85 (s, $1H_2$, CH_3), 2.85 (s, 3H, $N-CH_3$), 2.54−3.71 (m, 8H, piperidinyl H)
34a	62	217−220 (217−220)[17]	3254.15 (υOH) 1657.37 (υC=N)	C,H,N		D_2O: 4.75 (s, 1H, OH), 2.86 (s, 3H, NCH_3), 1.84−4.11 (m, 10H, tropinyl H)
34b	57.7	247−248	3192.44 (υOH) 1626.52 (υC=N)	C,H N: calcd, 17.02 found, 17.13		4.75 (s, 1H, OH), 2.85 (s, 3H, NCH_3), 2.65−3.40 (m, 8H, piperidinyl H)

induced learning deficits in mice in a spatial reference memory task such as the water maze performance.

Quantitative Structure−Activity Relationships. Some consideration is paid to the molecular properties of the oximes, such as lipophilic, electronic, and size parameters, as these are likely to influence their affinities for the various muscarinic receptor subtypes.

The lipophilicity of the oximes was assessed from the experimentally determined log k_w values and the theoretical ClogP values. Under the prevailing experimental conditions, the log k_w values are actually distribution coefficients obtained at pH 3.2, where the ionized form of the oxime is predominant. In contrast, the ClogP values have been determined for the neutral molecule. Not withstanding this difference, there was a good correlation (r = 0.91) between the experimental and theoretical hydrophobicity values (Table 6).

Except for the methyloximes (**33a**,**b**), the remaining O-substituted oximes possess an electron-withdrawing alkynyl side chain, the electron-withdrawing effect of which would vary according to chain length and the location of unsaturation along the chain. Quantification of this effect could be done in several ways. One approach would involve determining the Taft's polar substituent constant σ[25] of these side chains. Electron-withdrawing side chains would be associated with large (positive) σ values. The negative charge (O^-) on the oxime oxygen could also be determined, as this would be diminished by the presence of an electron-withdrawing side chain. Another suitable electronic parameter would be the dipole moment of the oxime which reflects the global polarity of the molecule. As seen from Table 6, these variables (σ, O^-, dipole moment) are poorly correlated to each other. In particular, σ and dipole moment are very poorly correlated (r = 0.001) compared to σ and O^- (r = 0.56).

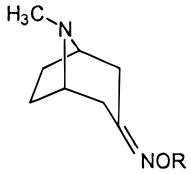
Various size parameters were determined to assess the bulk of the oximes. One such parameter is molar refractivity (MR) which is proportional to the polarizability and volume of a molecule. Since polarizabilities of most organic molecules tend to show limited variation, molar refractivity is widely considered as a steric parameter characterizing the bulk of a substituent. Indeed, Table 6 shows that it is better correlated (r > 0.97) to other size parameters (surface area, volume) than to electronic parameters. In series rich in alkyl substituents, molar refractivity may reflect a hydrophobic effect.[26] The good correlation between MR and the hydrophobic parameter log k_w (r = 0.95) observed in this series may be explained in this manner.

Conventional molecular volume (MV) and solvent-accessible Connolly surfaces (area A and volume V)[27] were determined from the low-energy conformations of the molecules using SYBYL.

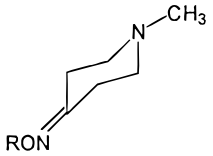
The shape of the alkynyl substituent may also be quantified by the STERIMOL set of parameters proposed by Verloop.[28] These parameters represent length (L) and widths ($B1-B4$) of the substituent, the latter extending perpendicular to the L axis while being perpendicular to each other. Table 6 shows that most but not all STERIMOL parameters are correlated to each other. For example, the width parameter *B1* is well-correlated to *B2* and *B3*, as are the two longest width parameters *B4* and *B5*. As expected, little correlation is observed between length (L) and width ($B1-B5$) parameters. Correlations with other size/electronic parameters are also observed among some STERIMOL parameters, particularly with the length (L) substituent and the longest width parameter *B5*.

Because branching in the alkynyl side chain is an important structural feature in the present series of oximes, it is important to find a steric parameter which

Table 2. Affinities (pK_i) of Oximes (**24a**,**b**−**34a**,**b**) and Other Muscarinic Ligands for M_1, M_2, and M_3 Muscarinic Receptors in the Rat Cortex, Heart, and Submandibulary Glands, Respectively, as Determined from Binding Displacement Studies



(24a - 34a)



(24b - 34b)

		pK_i values[a] for inhibition of		
compd	R	[^{3}H]pirenzepine binding in cortex[b]	[^{3}H]-*N*-methylscopolamine binding in heart[b]	[^{3}H]-*N*-methylscopolamine binding in submandibulary gland[b]
24a	−$CH_2C{\equiv}CH$	5.9 (0.1) (0.57 ± 0.09)	5.2 (0.68 ± 0.06)	5.2 (0.39 ± 0.01)
24b		4.3 (0.47 ± 0.05)	4.6 (0.1) (0.70 ± 0.12)	4.7 (0.1) (0.31 ± 0.03)
25a	−$C(CH_3)HC{\equiv}CH$	5.0 (0.71 ± 0.19)	5.2 (0.1) (0.71 ± 0.14)	5.4 (0.1) (0.67 ± 0.10)
25b		5.3 (0.52 ± 0.07)	4.9 (0.36 ± 0.10)	4.1 (0.28 ± 0.06)
26a	−$CH_2C{\equiv}CCH_3$	6.3 (0.55 ± 0.03)	4.9 (0.1) (0.72 ± 0.06)	4.8 (0.1) (0.19 ± 0.01)
26b		5.4 (0.2) (0.51 ± 0.04)	5.1 (0.1) (0.78 ± 0.06)	3.9 (0.1) (0.24 ± 0.02)
27a	−$CH_2CH_2C{\equiv}CH$	5.8 (0.1) (0.79 ± 0.05)	4.5 (0.85 ± 0.02)	5.0 (0.1) (0.46 ± 0.08)
27b		5.8 (0.1) (0.48 ± 0.06)	5.0 (0.2) (0.54 ± 0.15)	4.4 (0.30 ± 0.09)
28a	−$C(C_2H_5)HC{\equiv}CH$	6.5 (0.1) (0.62 ± 0.16)	5.2 (0.1) (0.58 ± 0.08)	5.2 (0.70 ± 0.09)
28b		5.2 (0.1) (0.49 ± 0.04)	5.0 (0.1) (0.64 ± 0.07)	5.0 (0.80 ± 0.07)
29a	−$CH_2C{\equiv}CC_2H_5$	5.3 (0.1) (0.65 ± 0.03)	4.9 (0.1) (0.74 ± 0.04)	5.5 (0.38 ± 0.03)
29b		5.4 (0.1) (0.51 ± 0.08)	4.8 (0.51 ± 0.03)	4.7 (0.1) (0.25 ± 0.06)
30a	−$CH_2CH_2C{\equiv}CCH_3$	6.1 (0.1) (0.65 ± 0.06)	5.5 (0.1) (0.79 ± 0.08)	5.6 (0.1) (0.59 ± 0.03)
30b		5.7 (0.1) (0.62 ± 0.06)	4.8 (0.1) (0.70 ± 0.06)	4.4 (0.1) (0.65 ± 0.07)
31a	−$CH_2CH_2CH_2C{\equiv}CH$	6.2 (0.1) (0.72 ± 0.03)	5.2 (0.73 ± 0.05)	5.0 (0.80 ± 0.07)
31b		5.1 (0.62 ± 0.06)	5.0 (0.75 ± 0.11)	4.8 (0.1) (0.33 ± 0.02)
32a	−$C(CH_3)HCH_2C{\equiv}CH$	7.0 (0.1) (0.43 ± 0.04)	5.8 (0.1) (0.52 ± 0.04)	5.5 (0.1) (0.51 ± 0.06)
32b		5.5 (0.1) (0.58 ± 0.04)	5.0 (0.1) (0.61 ± 0.04)	4.3 (0.32 ± 0.06)
33a	−CH_3	3.0 (0.66 ± 0.09)	3.9 (0.2) (0.48 ± 0.05)	4.0 (0.1) (0.43 ± 0.04)
33b		4.3 (0.66 ± 0.05)	4.4 (0.1) (0.48 ± 0.18)	4.6 (0.10 ± 0.04)
34a	−H	3.3 (0.45 ± 0.04)	4.4 (0.1) (0.75 ± 0.07)	3.1 (0.27 ± 0.02)
34b		3.3 (0.1) (0.73 ± 0.13)	3.4 (0.54 ± 0.06)	2.5 (0.22 ± 0.02)
atropine		8.4 (0.98 ± 0.08)	8.2 (0.88 ± 0.04)	8.6 (0.40 ± 0.03)
pirenzepine		8.1 (0.91 ± 0.03)		
McN-A-343		5.3 (0.45 ± 0.02)	5.2 (0.59 ± 0.11)	5.6 (0.29 ± 0.10)
oxotremorine		6.2 (0.30 ± 0.09)	6.7 (0.1) (0.55 ± 0.08)	6.4 (0.26 ± 0.07)
N-methylscopolamine			9.8 (0.7) (1.05 ± 0.05)	9.3 (0.93 ± 0.07)

[a] Each value represents the mean (SD) of three independent experiments. Values in parentheses represent the Hill coefficients of the compounds as determined from the Hill plot. [b] The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of the following radiolabeled ligands for the specified tissue are given as follows: [^{3}H]pirenzepine (cortex), 6.8 nM, 63.5 (fmol/mg of wet weight), 1.00; [^{3}H]-*N*-methylscopolamine (heart), 0.22 nM, 36.17 (fmol/mg of wet weight), 1.03; [^{3}H]-*N*-methylscopolamine (submandibulary glands), 0.55 nM, 11.9 (fmol/mg of wet weight), 0.98.

could represent it in QSAR. This appears to be the width parameter *B2* which has a very good correlation (r = 0.88) to the dummy parameter D (D = 1 for branching). *B1* and *B3* are also alternative indicators,

Table 3. Receptor Subtype Selectivities of Oximes **24a**,**b**−**34a**,**b** and Other Muscarinic Ligands as Determined from the Ratio of K_i Values Obtained from Binding Displacement Studies

compd	$K_i(M_2)/K_i(M_1)$	$K_i(M_3)/K_i(M_1)$	$K_i(M_3)/K_i(M_2)$
24a	4.2	4.6	1.1
24b	0.59	0.42	0.72
25a	4.5	0.46	0.72
25b	2.2	14	6.3
26a	24	27	1.1
26b	2.0	35	17
27a	19	6.8	0.34
27b	6.2	25	3.7
28a	20	22	1.1
28b	1.8	1.5	0.85
29a	2.4	0.56	0.23
29b	4.0	5.4	1.4
30a	4.5	3.0	0.66
30b	7.5	2.0	0.27
31a	12	16	1.3
31b	1.1	1.7	1.5
32a	16	29	1.8
32b	2.9	15	5.4
33a	0.14	0.10	0.74
33b	0.74	0.51	0.71
34a	0.74	1.4	18
34b	0.90	7.6	8.5
atropine	1.4	0.60	0.82
McN-A-343	1.4	0.46	0.33
oxotremorine	0.32	0.63	2.0

but with lesser correlations. The Connolly surfaces (log *V*, log *A*) and molecular volume (log MV) determined from the energy-minimized conformations of the oximes are less useful indicators of branching.

Although log *A* is poorly correlated to branching, a casual examination shows that the branched side chains of the oximes **25a**,**b**, **28a**,**b**, and **32a**,**b** generally have smaller surface areas and larger volumes compared to unbranched side chains of the same carbon number (**27a**,**b**, **31a**,**b**).

Discussion

Stepwise multiple linear regression of M_1 binding affinity against the various hydrophobic, size, and electronic parameters indicated that activity was best correlated to either molecular refractivity or surface area (log *A*) of the oximes. However, cross-validation of the data indicated that better predictive ability was obtained with surface area. The regression eq 2 shows that up to 70% of the binding affinity data could be explained by this variable alone.

$$pK_{i(M_1)} = 15.54(\pm 2.27) \log A - 31.27(\pm 5.36) \quad (2)$$

$$n = 22,\ r^2 = 0.70,\ \mathrm{SE} = 0.59,\ F = 46.48$$

$$r^2_{cv} = 0.57,\ \mathrm{SE}_{cv} = 0.69$$

Equation 2 suggests that an increase in surface area enhances binding affinity. Indeed, for the same side chain, greater binding affinity is observed in the larger size tropinone oxime compared to the corresponding piperidinone oxime. In addition, lengthening the alkynyl side chain from three to five carbon atoms (an increase in surface area) generally results in improved binding affinity. For example, pK_i increases for **27b** (4C) > **24b** (3C) and **31a** (5C) > **27a** (4C).

Interestingly, the tropinone oximes with the highest binding affinities have branched side chains: $-CH(CH_2-CH_3)-C{\equiv}CH$ (**28a**) and $-CH(CH_3)-CH_2C{\equiv}CH$ (**32a**). These oximes have larger pK_i values than McN-A-343, determined under similar conditions. However, branching would result in smaller surface areas (as have been observed earlier) which is contrary to the relationship expressed in eq 2. It is also noted that none of the parameters depicting branching (*D*, *B2*, *B1*, *B3*) are present in this equation. A possible reason for this anomaly may be that the percent change in surface area brought about by branching is small compared to the total surface area of the molecule. Moreover, the number of active branched analogues is small, as the desirable effect of branching on affinity is observed only among the tropinone (and not *N*-methylpiperidinone) derivatives.

A comparison of pK_i values of branched and unbranched O-substituted tropinone oximes suggests that for long side chains (viz., pentynyl), branching is favored (**28a** > **29a**, **32a** > **30a**), but this is not so for short side chains (viz., butynyl), as seen in **26a** > **25a**. In addition, for the five-carbon pentynyl side chain, there is a preference for the location of the triple bond at a distal position (as in **31a**) compared to the proximal position (as in **29a**). We compared these results with those obtained earlier[12] from a series of *O*-alkynyloximes of the more rigid 1-azabicyclo[2.2.1]heptane ring. In contrast to the present findings, proximal (rather than distal) unsaturation is favorable in those oximes with side chains of three- to five-carbon side length. Branching was also observed to diminish binding affinity. Further lengthening of the side chain beyond a five-carbon chain length generally resulted in lower activity, an effect which might also be observed in the present series of oximes if longer chain analogues were available. It is clear that the nature of the nitrogen-containing ring system to which the side chain is attached has an important bearing on structure−activity relationships.

Although previously synthesized rigid ring oximes are associated with greater binding affinities,[12] they have poor M_2/M_1 selectivities. The present series of tropinone oximes is good in that despite their moderate M_1 binding affinities, they have markedly greater M_1 (over M_2 and M_3) selectivities. Of the three tropinone oximes (**26a**, **28a**, **32a**) with good M_2/M_1 and M_3/M_1 selectivities (at least 15-fold), two members have branched alkynyl side chains (**28a**, **32a**).

Oximes **26a**, **28a**, and **32a** were able to attenuate scopolamine-induced impairment of the performance of mice in the modified Morris water maze task. In comparison, mice treated with an oxime with low M_1 binding affinity (**33a**) were unable to overcome this effect of scopolamine.

Much discussion has centered on the nature of the muscarinic agonist binding site. Some investigators[29] have proposed that the binding site is located in a very tight pocket with little tolerance for excess volume. Greater steric bulk is thought to lead to loss in agonist potency or a shift toward antagonism. On the other hand, Tecle et al.[12] proposed that longer and larger agonists would ensure maximum contact between the agonist and the internal surface of the binding cavity,

Table 4. Affinities (pK_i) and Receptor Selectivities of Oximes **24a**, **26a**, and **29a** for M_1, M_2, and M_3 Muscarinic Receptors in the Rat Cortex, Heart, and Submandibulary Glands, Respectively, as Determined from Binding Displacement Studies Carried Out in the Presence of Gpp(NH)p

	pK_i[a] for inhibition of			subtype selectivity[b]		
compd	[^{3}H]pirenzepine binding in cortex	[^{3}H]NMS binding in heart	[^{3}H]NMS binding in submandibulary gland	$K_i(M_2)/K_i(M_1)$	$K_i(M_3)/K_i(M_1)$	$K_i(M_3)/K_i(M_2)$
24a	5.9 (0.1)	4.6	3.3	18	320	18
	(0.73 ± 0.05)	(1.02 ± 0.07)	(0.34 ± 0.03)	(4.2)	(4.6)	(1.1)
26a	6.0	4.7 (0.1)	4.7 (0.1)	23	21	0.88
	(0.69 ± 0.12)	(0.55 ± 0.04)	(0.29 ± 0.01)	(25)	(27)	(1.1)
29a	5.1	4.2	4.1	7.7	9.1	1.2
	(0.92 ± 0.18)	(1.05 ± 0.17)	(0.27 ± 0.01)	(2.4)	(0.56)	(0.23)

[a] Each value represents the mean (±SD) of three independent experiments. Values in parentheses represent the Hill coefficients of the compounds as determined from the Hill plot. [b] Values in parentheses represent the ratios of K_i values obtained in the absence of Gpp(NH)p.

Table 5. Concentration of Oximes **24a**,**b**−**34a**,**b** Required to Relax Phenylephrine-Precontracted Aortic Rings by 50% (ED_{50} Values) in Endothelium Intact Rat Aorta and Endothelium-Denuded Rabbit Aorta

	ED_{50} (SEM) for *n* experiments	
compd	endothelium intact rat aorta	endothelium-denuded rabbit aorta
24a	$3.5\ (0.4) \times 10^{-6}$ (8)	$7.1\ (0.4) \times 10^{-6}$ (6)
24b	$6.8\ (0.2) \times 10^{-6}$ (8)	$1.1\ (0.4) \times 10^{-5}$ (6)
25a	$6.9\ (0.2) \times 10^{-6}$ (8)	$2.6\ (0.4) \times 10^{-5}$ (12)
25b	$7.8\ (0.3) \times 10^{-6}$ (8)	$4.1\ (0.4) \times 10^{-5}$ (6)
26a	$3.4\ (0.2) \times 10^{-6}$ (6)	$4.8\ (0.4) \times 10^{-6}$ (8)
26b	$8.7\ (0.2) \times 10^{-6}$ (6)	$8.7\ (0.2) \times 10^{-6}$ (6)
27a	$5.3\ (0.3) \times 10^{-6}$ (8)	$1.5\ (0.4) \times 10^{-5}$ (9)
27b	$7.4\ (0.4) \times 10^{-6}$ (8)	$2.8\ (0.6) \times 10^{-5}$ (8)
28a	$5.5\ (0.2) \times 10^{-6}$ (8)	$4.1\ (0.5) \times 10^{-5}$ (12)
28b	$7.6\ (0.3) \times 10^{-6}$ (8)	$1.8\ (0.4) \times 10^{-5}$ (9)
29a	$1.3\ (0.3) \times 10^{-6}$ (8)	$3.5\ (0.2) \times 10^{-6}$ (8)
29b	$6.2\ (0.3) \times 10^{-6}$ (8)	$1.2\ (0.4) \times 10^{-5}$ (6)
30a	$7.8\ (0.3) \times 10^{-6}$ (8)	$9.8\ (0.3) \times 10^{-6}$ (9)
30b	$6.3\ (0.3) \times 10^{-6}$ (8)	$1.0\ (0.2) \times 10^{-6}$ (8)
31a	$5.9\ (0.4) \times 10^{-6}$ (6)	$6.5\ (0.4) \times 10^{-6}$ (12)
31b	$9.3\ (0.4) \times 10^{-6}$ (6)	$1.3\ (0.3) \times 10^{-5}$ (12)
32a	$5.4\ (0.2) \times 10^{-6}$ (6)	$2.3\ (0.4) \times 10^{-5}$ (10)
32b	$5.0\ (0.3) \times 10^{-6}$ (6)	$2.5\ (0.5) \times 10^{-5}$ (10)
33a	$1.1\ (0.2) \times 10^{-5}$ (8)	$1.1\ (0.2) \times 10^{-5}$ (8)
33b	$1.3\ (0.4) \times 10^{-5}$ (8)	$2.6\ (0.5) \times 10^{-4}$ (6)
34a	$5.3\ (0.3) \times 10^{-5}$ (8)	$3.2\ (0.4) \times 10^{-4}$ (6)
34b	$4.3\ (0.2) \times 10^{-5}$ (8)	$7.8\ (0.3) \times 10^{-5}$ (6)
ACh	$7.3\ (0.5) \times 10^{-8}$ (24)	
carbacol	$2.2\ (0.5) \times 10^{-7}$ (8)	
arecoline	$1.6\ (0.4) \times 10^{-6}$ (12)	
McN-A-343	$9.6\ (0.2) \times 10^{-6}$ (12)	$3.5\ (0.4) \times 10^{-7}$ (4)
oxotremorine	$4.4\ (0.3) \times 10^{-8}$ (12)	

thereby achieving greater subtype selectivity. Our results appear to support the latter hypothesis. The QSAR for the present series of oximes shows that binding affinity increases as the surface area of the side chain is extended from a three- to five-carbon chain length. However, it is likely that an optimum chain length exists beyond which binding affinity would diminish.

Compared to their binding affinities for the M_1 receptor subtype, the oximes generally demonstrated lower binding affinities for the M_2 and M_3 receptor subtypes, with the tropinone oximes showing superior binding affinities than the piperidinone oximes. There was no clear-cut preference for the oximes with branched side chains, although branched analogues such as **32a** and **28a** are still among those with the highest binding affinities at M_2 and M_3. Functional assays revealed that all the oximes demonstrated agonistic activity at the putative M_3 sites on the rat aorta but did not behave as agonists at the putative M_2 sites on the endothelium-denuded rabbit aorta.

Stepwise linear regression of various variables against M_2 binding affinities identified the size parameter (log *V*) as being superior in accounting for activity (eq 3). This was further confirmed by cross-validation. The reliance on size parameters for both M_1 and M_2 binding affinities is not unexpected since binding affinities at these two sites are themselves fairly well-correlated to each other ($r = 0.84$).

$$pK_{i(M_2)} = 6.49(\pm 1.04) \log V - 10.30(\pm 2.44) \quad (3)$$

$$n = 22,\ r^2 = 0.66,\ \mathrm{SE} = 0.31,\ F = 38.66$$

$$r^2_{cv} = 0.56,\ \mathrm{SE}_{cv} = 0.35$$

In a similar manner, ClogP was identified as the best variable accounting for 73% of the observed M_3 binding affinities (eq 4):

$$pK_{i(M_3)} = 0.69(\pm 0.09)\ \mathrm{ClogP} - 3.14(\pm 0.23) \quad (4)$$

$$n = 22,\ r^2 = 0.73,\ \mathrm{SE} = 0.42,\ F = 53.48$$

$$r^2_{cv} = 0.74,\ \mathrm{SE}_{cv} = 0.41$$

When $pK_{ED_{50}}$ values obtained from the M_3 functional assay on the rat aorta endothelium were regressed against the various independent variables, it was found that M_3 agonist activity was best accounted for by a combination of two variables, viz., ClogP and the negative charge on oxygen (O^-), with the latter making a greater contribution to the overall relationship.

$$pK_{ED_{50}(M_3)} = 0.20(\pm 0.06)\ \mathrm{ClogP} + 12.90(\pm 6.08)\ O^- + 8.18(\pm 1.73) \quad (5)$$

$$n = 22,\ r^2 = 0.68,\ \mathrm{SE} = 0.21,\ F = 20.25$$

$$r^2_{cv} = 0.63,\ \mathrm{SE}_{cv} = 0.22$$

Thus, a lower negative charge on oxygen (possibly due to the presence of an electron-withdrawing substituent) enhances activity. It is interesting to note that although it is an electronic parameter, O^- is better correlated to size parameters (*B1*, *B2*, *B5*) ($r > 0.7$) than to the other electronic parameters such as σ or dipole moment. The correlation to width parameters suggests that the shape ("thickness") of the alkynyl side chain plays an impor-

Table 6. Correlation Matrix (*r*) for Electronic, Hydrophobic, and Size Parameters Used in Deriving QSAR Equations[a]

	CLogP	log K_w	σ	O^-	DM[b]	MR[b]	log MV	log *A*	log *V*	*D*[b]	*L*[c]	*B1*[c]	*B2*[c]	*B3*[c]	*B4*[c]	*B5*[c]
CLogP	1.00	0.91	−0.15	0.63 (0.002)	0.57 (0.006)	0.87	0.89	0.88	0.89	0.29	0.70	0.78	0.48	0.59 (0.04)	0.64	0.72
log K_w	0.91	1.00	−0.21 (0.478)	−0.09 (0.733)	0.61 (0.007)	0.95	0.95	0.86	0.96	0.19 (0.445)	0.23 (0.365)	0.21 (0.415)	0.21 (0.397)	0.27 (0.277)	0.20 (0.435)	0.29 (0.245)
σ	−0.15	−0.21 (0.478)	1.00	0.56 (0.016)	−0.00 (0.997)	0.07 (0.771)	−0.08 (0.758)	0.03 (.907)	0.10 (0.708)	0.41 (0.094)	−0.17 (0.500)	0.16 (0.531)	0.43 (0.07)	0.21 (0.616)	−0.05 (0.857)	0.13 (0.611)
O^-	0.63 (0.002)	−0.09 (0.733)	0.56 (0.016)	1.00	0.30 (0.175)	0.56 (0.007)	0.58 (0.005)	0.56 (0.007)	0.60 (003)	0.44 (0.039)	0.23 (0.302)	0.72	0.71	0.46 (0.031)	0.53 (0.011)	0.70
DM[b]	0.57 (0.006)	0.61 (0.007)	−0.00 (0.997)	0.30 (0.175)	1.00	0.72	0.69	0.70	0.69	0.12 (0.592)	0.46 (0.034)	0.32 (0.193)	0.11 (0.627)	0.41 (0.059)	0.67	0.66
MR[b]	0.87	0.95	0.07 (0.771)	0.56 (0.007)	0.72	1.00	0.99	0.98	0.99	0.29 (0.184)	0.73	0.61 (0.003)	0.44 (0.038)	0.49 (0.020)	0.64	0.73
log MV	0.89	0.95	−0.08 (0.758)	0.58 (0.005)	0.69	0.99	1.00	0.97	0.99	0.34 (0.125)	0.72	0.66	0.48 (0.023)	0.54 (0.010)	0.61 (0.003)	0.70
log *A*	0.88	0.86	0.03 (0.907)	0.56 (0.007)	0.70	0.98	0.97	1.00	0.97	0.17 (0.437)	0.81	0.57 (0.006)	0.35 (0.113)	0.46 (0.033)	0.71	0.76
log *V*	0.89	0.96	0.10 (0.708)	0.60 (0.003)	0.69	0.99	0.99	0.97	1.00	0.36 (0.098)	0.71	0.68	0.51 (0.016)	0.55 (0.08)	0.62 (0.002)	0.72
D[b]	0.29	0.19 (0.445)	0.41 (0.094)	0.44 (0.039)	0.12 (0.592)	0.29 (0.184)	0.34 (0.125)	0.17 (0.437)	0.36 (0.098)	1.00	−0.04 (0.863)	0.77	0.88	0.76	−0.06 (0.800)	0.21 (0.349)
L[c]	0.70	0.23 (0.365)	−0.17 (0.500)	0.23 (0.302)	0.46 (0.034)	0.73	0.72	0.81	0.71	−0.04 (0.863)	1.00	0.35 (0.114)	0.08 (0.710)	0.21 (0.348)	0.39 (0.074)	0.38 (0.079)
B1[c]	0.78	0.21 (0.415)	0.16 (0.531)	0.72	0.32 (0.143)	0.61 (0.003)	0.66	0.57 (0.006)	0.68	0.77	0.35 (0.114)	1.00	0.79	0.82	0.46 (0.03)	0.64
B2[c]	0.48	0.21 (0.397)	0.43 (0.07)	0.71	0.11 (0.627)	0.44 (0.038)	0.48 (0.023)	0.35 (0.113)	0.51 (0.016)	0.88	0.08 (0.710)	0.79	1.00	0.53 (0.012)	0.28 (0.206)	0.50 (0.017)
B3[c]	0.59 (0.04)	0.27 (0.277)	0.21 (0.626)	0.46 (0.031)	0.41 (0.059)	0.49 (0.020)	0.54 (0.010)	0.46 (0.033)	0.55 (0.08)	0.76	0.21 (0.348)	0.82	0.53 (0.012)	1.00	0.37 (0.090)	0.51 (0.016)
B4[c]	0.64	0.20 (0.435)	-0.05 (0.857)	0.53 (0.011)	0.67	0.64	0.61 (0.003)	0.71	0.62 (0.002)	−0.06 (0.800)	0.39 (0.074)	0.46 (0.03)	0.28 (0.206)	0.37 (0.090)	1.00	0.95
B5[c]	0.72	0.29 (0.245)	0.13 (0.611)	0.70	0.66	0.73	0.70	0.76	0.72	0.21 (0.349)	0.38 (0.079)	0.64	0.50 (0.017)	0.51 (0.016)	0.95	1.00

[a] Values in parentheses represent two-tailed significance *p* value. If *p* value is not given, $p = 0.001$ or smaller. [b] DM, dipole moment; MR, molar refractivity; *D*, branching in side chain. [c] STERIMOL parameters indicating length (*L*) and width (*B1*−*B5*) of substituent.

tant role in activity, possibly by modulating the charge on the oxygen atom. The contribution of ClogP may reflect the importance of the tropine ring system (which has a larger ClogP value) over the piperidine ring. In the majority of cases, the tropinone oximes have better activity than their piperidinone counterparts.

Equation 6 describes the variables which are best correlated to the ability of the oximes to induce relaxation at the endothelium-denuded rabbit aorta.

$$pK_{ED_{50}(M_2)} = 0.26(\pm 0.06)\ \text{ClogP} + 18.67(\pm 6.51)\ O^- - 0.62(\pm 0.11)\ D + 9.37(\pm 1.85) \quad (6)$$

$$n = 22,\ r^2 = 0.80,\ \text{SE} = 0.21,\ F = 24.35$$

$$r^2_{cv} = 0.62,\ \text{SE}_{cv} = 0.28$$

There are many similarities between the regression eqs 5 and 6. Both equations contain the variables ClogP and O^-, with the latter variable making the greater contribution to activity. The main difference is the presence of the branching parameter *D* in eq 6. The negative coefficient of *D* indicates that branching is not a favorable structural feature among oximes that induce relaxation in the endothelium-denuded rabbit aorta.

The role of branching is evident when the activities of two representative oximes (**25a**, **31a**) are compared. The branched oxime **25a** (1-methylprop-2-ynyl side chain) is as active as the unbranched *O*-pent-4-ynyloxime **31a** at the endothelium intact rat aorta (M_3). However, **25a** is 10 times less active than **31a** at the endothelium-denuded rabbit aorta (M_2).

An attempt was made to determine the variables determining selectivity (pK_{M_1/M_2}, pK_{M_3/M_1}, or pK_{M_2/M_3}) among the different receptor subtypes. As it turned out, all the variables showed poor predictability ($r^2_{cv} < 0.5$). Therefore, no single parameter could be identified from those presently available which could meaningfully account for the selectivities observed among the oximes.

Conclusion

The results of the present study provide information regarding the muscarinic activity and selectivity of *O*-alkynyloximes of tropinone and *N*-methyl-4-piperidinone. Other investigators[11,12] have synthesized oxime derivatives of 1-azabicylo[2.2.2]octane or 1-azabicyclo-[2.2.1]heptane because these rigid ring structures have been associated with good muscarinic agonist activity. The tropinone and *N*-methylpiperidinone rings have generally been bypassed as suitable pharmacophores possibly because of their greater flexibility, arising from the nonbridgehead position of the basic nitrogen atom.[17] Our results show that while the tropinone and *N*-methylpiperidine oximes demonstrate lower binding affinities ($K_i \approx 10^{-7}$ M), selected members demonstrated impressive M_1 selectivities which exceeded those observed with the 1-azabicyclic ring systems.[12] It is seen that oximes **26a**−**28a**, **31a**, and **32a** have at least a 10-fold selectivity for the M_1 subtype over the other receptor (M_2, M_3) subtypes. Oximes **26a**, **28a**, and **32a** were also capable of attenuating scopolamine-induced impairment of the water maze performance in mice. It would appear that rigidity in the basic nitrogen ring system is not an absolute requirement for muscarinic

agonist activity, and acceptable levels of activity and selectivity are attainable in rings of moderate flexibility.

In conclusion, the present results indicate that the tropinone oximes are interesting lead compounds for agonist activity at M_1 receptors. Some members of this class have been shown to bind selectively to M_1 receptors and to exert mnemonic effects in mice. It is particularly interesting to note that functional assays for M_2 activity on the denuded rabbit endothelium reveal that these oximes have no M_2 agonist action.

Experimental Section

Chemistry. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Mass spectra were determined on a VG Micromass 7035 E mass spectrometer (with chemical ionization). IR spectra were recorded on a Philips PU 9624 FTIR instrument in pressed KBr disks or neat. ^{1}H NMR spectra were recorded on a Bruker ACF (300 MHz) spectrometer. Chemical shifts are reported in δ (ppm) relative to Me_4Si as an internal standard. Elemental analyses were performed by the Department of Chemistry, National University of Singapore, and are in agreement with calculated values within ±0.4%, unless indicated.

General Method for the Synthesis of *N*-(Alkynyloxy)-phthalimides 6−14.[13,14] A 0.044-mol (19.15 g) portion of diethyl azodicarboxylate (40% solution in toluene) was added dropwise to a stirred solution of the alcohol (0.04 mol), *N*-hydroxyphthalimide (0.04 mol, 6.52 g), and triphenylphosphine (0.04 mol, 10.50 g) dissolved in tetrahydrofuran (100 mL). An exothermic effect was observed, and the mixture turned red upon addition. After stirring for 24 h at room temperature, the solvent was removed in vacuo. Ether was added to the residue to precipitate triphenylphosphine oxide and diethyl hydrazinedicarboxylate, which were filtered off. The filtrate was evaporated under reduced pressure and the residue applied to a silica gel column which was eluted with benzene to give the *N*-alkoxyphthalimide on recrystallization from ethanol. The following phthalimides were synthesized in this way: *N*-(prop-2-ynyloxy)phthalimide (**6**), *N*-(1-methylprop-2-ynyloxy)phthalimide (**7**), *N*-(but-2-ynyloxy)phthalimide (**8**), *N*-(but-3-ynyloxy)phthalimide (**9**), *N*-(1-ethylprop-2-ynyloxy)phthalimide (**10**), *N*-(pent-2-ynyloxy)phthalimide (**11**), *N*-(pent-3-ynyloxy)phthalimide (**12**), *N*-(pent-4-ynyloxy)phthalimide (**13**), and *N*-(1-methylbut-3-ynyloxy)phthalimide (**14**). Their yields, melting points, and IR and ^{1}H NMR data are given in Table 1.

General Method for the Synthesis of *O*-Alkynylhydroxylamine (15−23) Hydrochlorides.[15] A mixture of *N*-alkynylphthalimide (0.02 mol) and hydrazine monohydrate (0.021 mol, 1 mL) was vigorously shaken at room temperature for 15 min. Diethyl ether (50 mL) was added, and the mixture was shaken for another 15 min before it was filtered. The residue was washed again with 3 × 30-mL portions of ether. Ethereal HCl was added dropwise to the combined ethereal filtrate to precipitate out the O-substituted hydroxylamine hydrochloride which was filtered, washed with ether, and recrystallized from 2-propanol/ether. The following hydroxylamines were synthesized in this way: *O*-prop-2-ynylhydroxylamine (**15**), *O*-(1-methylprop-2-ynyl)hydroxylamine (**16**), *O*-but-2-ynylhydroxylamine (**17**), *O*-but-3-ynylhydroxylamine (**18**), *O*-(1-ethylprop-2-ynyl)hydroxylamine (**19**), *O*-pent-2-ynylhydroxylamine (**20**), *O*-pent-3-ynylhydroxylamine (**21**), *O*-pent-4-ynylhydroxylamine (**22**), and *O*-(1-methylbut-3-ynyl)hydroxylamine (**23**). The yields of the HCl salts, their melting points, and IR and ^{1}H NMR data are given in Table 2. The hydrochlorides of *O*-methylhydroxylamine and hydroxylamine were obtained commercially from Tokyo Kasei Kogyo Pte Ltd. (Tokyo, Japan).

General Method for the Syntheses of Tropinone and *N*-Methylpiperidinone Oxime Ethers 24−34.[16] Equimolar quantities of the ketone (tropin-3-one, *N*-methylpiperidin-4-one) and O-substituted hydroxylamine (or hydroxylamine) HCl were stirred in methanol at room temperature for 18 h. Removal of the solvent in vacuo gave a white solid which was recrystallized from ethanol/benzene. The recrystallized solid was dissolved in water, made alkaline with solid K_2CO_3, and extracted with dichloromethane. After drying over anhydrous Na_2SO_4, the organic layer was removed by evaporation under reduced pressure. A clear yellow oil was obtained which was purified by passing through a silica gel column eluted with hexane/ethyl acetate. The eluted oil was treated with ethereal HCl to give the desired hydrochloride salt of the product. The yields and physical data of the following synthesized oximes of tropinone and *N*-methyl-4-piperidinone are given in Table 1: *O*-prop-2-ynyloxime (**24a**,**b**), *O*-(1-methylprop-2-ynyl)oxime (**25a**,**b**), *O*-but-2-ynyloxime (**26a**,**b**), *O*-but-3-ynyloxime (**27a**,**b**), *O*-(1-ethylprop-2-ynyl)oxime (**28a**,**b**), *O*-pent-2-ynyloxime (**29a**,**b**), *O*-pent-3-ynyloxime (**30a**,**b**), *O*-pent-4-ynyloxime (**31a**,**b**), *O*-(1-methylbut-3-ynyl)oxime (**32a**,**b**), *O*-methyloxime (**33a**,**b**), and unsubstituted oxime (**34a**,**b**).

Pharmacology. Materials. [^{3}H]Pirenzepine (73.9 Ci/mmol) and [^{3}H]-*N*-methylscopolamine (84.8 Ci/mmol) were purchased from NEN (Boston, MA). Acetylcholine (ACh) chloride, phenylephrine HCl, atropine sulfate, *N*-methylscopolamine, and 5′-guanylylimidodiphosphate trisodium salt [Gpp(NH)p] were obtained from Sigma Chemical Co., St. Louis, MO. (±)-3-Quinuclidinyl benzilate (QNB) was purchased from Research Biochemicals Inc., Massachusetts. Tissue solubilizer and scintillation cocktail (Ready Sol-HP) from Beckman were used. The hydrochloride salts of the oximes were prepared in distilled water for pharmacological testing.

Muscarinic Receptor Binding Studies. The binding of the synthesized compounds to muscarinic M_1, M_2, and M_3 receptor subtypes was determined using the cerebral cortex, heart, and submandibulary glands of the rat, respectively. Male SD rats (200−250 g) were purchased from the Laboratory Animal Centre of the National University of Singapore. The animals were given free access to food and water and housed in groups of four under standard conditions of humidity (60%), room temperature (22 °C), and 12-h light−dark cycles. The rats were killed by cervical dislocation. The tissues (cerebral cortex, heart, and submandibulary glands) were removed and cleaned of adhering tissue on an ice-cold surface.

For the submandibulary glands and cerebral cortex, the tissues were homogenized with a Potter-Elvejhem homogenizer in 10 volumes of Na^+/Mg^{2+} HEPES buffer (pH 7.4), containing NaCl 10 mM, $MgCl_2$ 10 mM, and HEPES 20 mM. In the case of the homogenate from submandibulary glands, centrifugation was carried out at 2000*g* for 10 min. The supernatant was decanted and centrifuged at 20000*g* for 20 min. In the case of the cerebral cortex, the homogenate was centrifuged only once at 20000*g* for 20 min. For both tissues, the pellet was resuspended in 10 volumes of Na^+/Mg^{2+} HEPES buffer and resedimented at 20000*g*, 10 min. The washing procedure was repeated once. Finally the pellet was resuspended in 40 volumes of Na^+/Mg^{2+} HEPES buffer and kept in liquid nitrogen till use.

In the case of the rat heart ventricle, homogenization was carried out in 5 volumes of 0.25 M sucrose and 25 mM TRIS buffer (pH 7.5) using a Potter-Elvejhem homogenizer for 30 s. The homogenate was sedimented at 10000*g* (20 min), and the pellet was discarded. The supernatant was decanted and resedimented at 45000*g* (30 min). The second pellet was resuspended in 5 volumes of 0.6 M KCl and 30 mM histidine at pH 7.0 to solubilize actin and myosin filaments and resedimented at 45000*g* (30 min) to yield the crude membrane vesicles. The yield was approximately 1 mg protein/g heart. The pellets were washed three times with 5 volumes of Na^+/Mg^{2+} HEPES buffer (pH 7.4). Finally, the pellet was resuspended in 20 volumes of the same buffer and kept in liquid nitrogen. Protein was determined by the method of Lowry et al.,[30] using bovine serum albumin as standard.

The muscarinic receptor affinities of the oximes were determined from competitive 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]-*N*-methylscopola-

mine were used as the specific radioligands for the cerebral cortex (M_1) and the heart (M_2)/submandibulary gland (M_3), respectively.

In a typical assay, an aliquot (0.2 mL, containing an equivalent of 8.0−1.0 mg of protein/mL) of the resuspended pellets prepared from rat cerebral cortices was incubated at 30 °C with a fixed concentration (0.5 nM) of [^{3}H]pirenzepine and increasing concentrations of the test compound (10 nM−10 mM) for 45 min. Nonspecific binding to rat cerebral cortices was determined from saturation studies in which 0.2-mL aliquots of the tissue homogenate were incubated with increasing concentrations of [^{3}H]pirenzepine (0.1−5.0 nM) in the absence/presence of 1 μM atropine in a total volume of 1 mL for a period of 45 min. The incubation was terminated by centrifugation (8000*g*, 3 min) at room temperature. The tube containing the pellet was washed with 1.5 × 2 mL of saline to remove free radioactivity, and the final pellet was allowed to drain dry. Tissue solubilizer (200 μL) was then added, the mixture was allowed to stand overnight, and radioactivity was counted after addition of 4 mL of liquid scintillator. All binding measurements were determined in triplicate.

A similar procedure was followed for determining M_2 and M_3 binding affinities. For M_2 binding, an aliquot (0.08 mL, containing an equivalent of 0.45−0.50 mg of protein/mL) of the resuspended pellet prepared from rat heart ventricles was incubated at 30 °C with a fixed concentration (0.3 nM) of [^{3}H]-*N*-methylscopolamine and increasing concentrations of the test compound (10 nM−10 mM) for 45 min. An aliquot (0.08 mL containing an equivalent of 10−12 mg of protein/mL) of the resuspended pellet prepared from rat submandibulary gland was used for M_3 binding experiments. For the saturation experiments, 0.08-mL aliquot of the tissue homogenate (heart ventricle or submandibulary glands) was incubated with increasing concentrations of [^{3}H]-*N*-methylscopolamine (0.1−2.5 nM) in the absence or presence of 1 μM (±)-QNB. The M_1, M_2, and M_3 binding affinities of oximes **24a**, **26a**, and **29a** were also determined in the presence of 30 μM Gpp(NH)p.

The displacement data were analyzed using the software Equilibrium Binding Data Analysis (EBDA)[31] to obtain Hill coefficients and IC_{50} values for competing ligands. Affinities, expressed as pK_i, the negative logarithm of the inhibition constants K_i, were calculated from IC_{50} values using the Cheng−Prusoff equation.[32] The binding parameters (K_d, B_{max}) of [^{3}H]pirenzepine and [^{3}H]-*N*-methylscopolamine were also determined from saturation studies described above.

Functional in Vitro Studies. Aortic rings were prepared from rat and rabbit aortas. The animal (male Sprague−Dawley rats, 275 ± 25 g, or male rabbits, 1.75 ± 0.25 kg) was paralyzed by cervical dislocation and sacrificed immediately by decapitation, and the aortic rings were isolated as described.[24,25] The endothelium of the rabbit aorta was removed by gently rubbing the intima with a cotton bud for 30−60 s. Each aorta was dissected free of adhering tissue, cut into 2-mm segments, and mounted in an organ bath containing Krebs-Ringer bicarbonate solution of composition (mM) NaCl 118, KCl 5, $NaHCO_3$ 25, glucose 10, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, and EDTA 0.026, under a resting tension of 1 g (1.5 g for rabbit aorta) under conditions previously described.[24]

After an equilibration period of 60 min (at 37 °C, 95% O_2−5% CO_2 aeration), phenylephrine (10^{-7} M) was added to the bath to produce a contraction of the rat aortic ring which was sustainable for 15 min. ACh (10^{-6} M) was then added to produce a relaxation response which confirmed the intactness of the endothelium in the aortic ring. After 30 min of re-equilibration with two washings, phenylephrine (10^{-7} M) was added to produce a contraction. Cumulative concentrations of the test compound (10^{-6}−10^{-4} M) were then added at 2-min intervals to produce a relaxation response. For the rabbit aortic ring, phenylepherine (10^{-7} M) was added to the bath after the equilibration period to produce a submaximal increase in the tone of the ring. This was followed by the cumulative additions (10^{-6}−10^{-4} M) of test compound at 2-min intervals. Each rat or rabbit aortic ring was used only once for testing against a test compound. Each test compound was evaluated using at least three concentrations. The EC_{50} of each test compound was evaluated from a plot of the percent contractile response of phenylephrine versus concentration of test compound using a commercial software program, Pharmacological Calculation System Version 4.2.[33]

Behavioral Testing in Mice. Swiss albino mice (male, 20 ± 2 g) were required to perform a water escape task which was modified from the standard version of the Morris water maze.[34] The task consisted of locating a circular, transparent platform, measuring 8 cm in diameter, which was located at the center of a temperature-regulated (25 ± 1 °C) pool measuring 60 × 60 × 10 cm^3. The platform was submerged 1 cm below the water surface, and the water was colored black to make the platform invisible. The mouse was always introduced at the same corner of the pool and the time taken for it to locate and mount the platform (the latency period) was used as the dependent measure.

In the first experimental setup, mice were divided into three groups of 9. The control group received saline, another group received scopolamine (1 mg/kg, ip), and the third group received drug (**26a**, **28a**, **32a**, or **33a** at 2 mg/kg, sc) + scopolamine (1 mg/kg, ip). The drugs were freshly prepared each day and administered daily to the mice 60 min before starting the experiment. Each mouse was then subjected to the water maze task. In addition, one drug (**28a**) was investigated at different dose levels (5−0.2 mg/kg) by the same procedure.

In the second experimental setup, the mice were divided into two groups of 10 mice. Each mouse was tested individually over a period of 5 days or longer. On day 6 (or day 16 in mice tested for a longer period), one group received saline and the other group received the drug (**26a**, **28a**, **32a**, **33a**, or McN-A-343) at 2 mg/kg, sc. After 30 min, scopolamine (1 mg/kg, ip) was administered to mice in both groups, and they were subjected to the water escape task 30 min later. The responses of the two groups of mice to the same task were evaluated again the following day.

Statistical Analysis. The effects of drugs on the daily performance (latency period) were calculated by analysis of variance (ANOVA) with a split-plot design (between-within subjects). The pairwise comparisons were performed by two-tailed *t*-test and Mann−Whitney *U*-test.

Determination of Capacity Factors (*k*′) of Oximes from Reversed-Phase HPLC. The hydrophobicity of the oximes was assessed from their capacity factors (*k*′) determined by reverse-phase HPLC on a LiChrosorb R RP-18 (10 μm) stationary phase, using as mobile phase varying concentrations of methanol and a buffer (pH 3.2) prepared from 0.175 M acetic acid and 0.014 M triethylamine in deionized water. Determinations were carried out at 30 °C with a flow rate of 1.5 mL/min and diode-array detector wavelengths set at 254 and 240 nm. A stock solution (0.1 g/mL) of the oxime was prepared in methanol. An aliquot (20 μL) of a solution (1 mL) prepared from the stock solution (0.1 mL), acetone (0.05 mL), and the mobile phase was injected into the column, and the retention time was determined (in triplicate). Five different mobile phase compositions, varying from 50% to 75% (w/w) methanol content, were used for each compound. The capacity factor (*k*′) was determined from $\log k' = \log[(V_s - V_o)/V_o]$, where V_s and V_o are the retention volumes (retention time × flow rate) of the oxime and acetone, respectively. Linear regression of log *k*′ of each compound against mobile phase composition and extrapolation to 100% aqueous phase gave log k_w of each compound at pH 3.2.

Molecular Modeling Methods. The following parameters were determined from the energy-minimized conformations of the oximes (as free bases) obtained by minimization using the SYBYL 6.2 (Tripos Associates, St. Louis, MO) Tripos force field (MAXIMIN2), with calculations continued until the rms gradient was less than 0.001 kcal mol^{-1} Å: ClogP, molecular volume (MV), molecular refractivity (MR), total dipole moment (DM), Connolly surfaces (volume and surface area, calculated from MOLCAD in SYBYL), and negative charge on the oxime O using the Gasteiger Huckel method. Total dipole moment is calculated from the protonated oximes. The ClogP of the oximes could not be calculated directly. Modified ClogP values were obtained by addition of log *P* values of the tropinone/*N*-

methylpiperidinone fragment and the O-substituted side chain for each oxime. Verloop's STERIMOL substituent parameters (*L, B1*−*B5*) were calculated using the computational chemistry program Physical Properties! Pro Revision 2.3 (CSW, Fairfield, CA). *L* refers to the substituent length measured along the axis formed by the bond between the substituent and the atom to which it is attached. *B1*−*B5* are radii extending perpendicular to *L1* and are also perpendicular to each other. *B1* and *B5* give the minimum and maximum widths of the substituents, respectively. As defined in the program, the following pairs (*B1, B4*) and (*B2, B3*) are in opposite directions.

Statistical Methods. Multiple linear regression analyses were carried out using SPSS for Windows (SPSS Inc., Chicago, IL). The following statistical parameters were determined for each regression equation: 95% confidence interval variables, correlation coefficient *r*, measure of explained variance r^2, Fisher significance ratio *F*, and standard error SE. Cross-validated r^2 and SE were determined using the QSAR module of SYBYL 6.2.

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Supporting Information Available: Tables containing physical data of **6**−**14** and **15**−**23** and QSAR parameters of **24a**,**b**−**34a**,**b** (4 pages). Ordering information is given on any current masthead page.

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