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# Synthesis and Muscarinic Activities of 3-(Pyrazolyl)-1,2,5,6-tetrahydropyridine Derivatives

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**Abstract**—A series of 3-(pyrazolyl)-1,2,5,6-tetrahydropyridine derivatives (**B**) was synthesized and tested for muscarinic activity in receptor binding assays using [$^3$H]-oxotremorine-M ($^3$H-OXO-M) and [$^3$H]-pirenzepine ($^3$H-PZ) as ligands. Potential muscarinic agonistic or antagonistic properties of the compounds were determined using binding studies measuring their potencies to inhibit the binding of $^3$H-OXO-M and $^3$H-PZ. Preferential inhibition of $^3$H-OXO-M binding was used as an indicator for potential muscarinic agonistic properties; this potential was confirmed in functional studies on isolated organs. All compounds with agonistic properties showed $^3$H-PZ/$^3$H-OXO-M potency ratios in excess of 20. In contrast, for antagonists this ratio was found to be close to unity. Mono-halogenation resulted in compounds (**4b** and **4d**) with $M_3$ agonistic properties as shown by their atropine sensitive stimulant properties in the guinea pig ileum, but with very little or no $M_1$ activity. Some minor in vivo effects were observed for both these compounds, with the iodinated compound **4d** inducing salivation. Compound **4d** also showed some positive mnemonic properties in rats where spatial short-term memory had been compromised by temporary cholinergic depletion. These data indicate that some $M_3$ agonism may be desired in therapeutic agents aimed at the treatment of the cognitive deficits of Alzheimer's disease patients.

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive loss of memory, judgment, language, and motor functions. The observation that the degeneration of cholinergic neurons in the forebrain of patients suffering from Alzheimer's disease correlated with the reduction in cognitive function led to the formulation of the cholinergic deficit hypothesis.[1,2] The loss of cholinergic neurons is reflected in the greater loss of presynaptic in comparison to postsynaptic muscarinic cholinergic receptors.[3,4] Thus, current strategies to treat the cognitive symptoms of Alzheimer's disease have concentrated on stimulation of the postsynaptic receptors either by increasing the availability of acetylcholine via cholinesterase inhibitors such as tacrine,[5] or by direct stimulation with a cholinergic agonist. To date the cholinesterase inhibitors have been shown to improve some aspects of cognitive performance, however efficacy has been poor and side effects problematic.[5–7] Similarly, studies involving cholinergic agonists have been marred by the side effect profile of the agonists (e.g., oxotremorine; RS 86),[8,9] although a recent report has suggested that careful regulation of the dose can lead to significant improvements in function without the appearance of severe side effects.[10] There are at least five known muscarinic cholinergic receptors subserving a variety of functions apart from cognition. These receptors are distributed throughout both the peripheral and central nervous systems,[11] and therefore it is not surprising that studies involving agonists acting at all subtypes produce many undesirable effects. Interest has now focused on the effects of subtype selective muscarinic agonists as a means to improve the therapeutic potential of cholinergic agents.[12] Indeed, recent animal studies with subtype selective muscarinic cholinergic agonists have shown positive results in tests of learning and memory,[13] however the results of clinical trials are awaited.

Most research has focused on the development of $M_1$ agonists because of their distribution in the brain and their apparent survival in patients with Alzheimer's disease. However, more information on the function of all subtypes is required in order to assess their ability to influence learning and memory as well as their impact on peripheral systems. The aim is to develop compounds which preferentially affect the central nervous system and show less peripheral side effects than agonists such as RS 86 and oxotremorine.

In these experiments we demonstrate that it is possible to synthesize selective $M_3$ agonists which may have beneficial cognitive effects at doses lower than required to elicit substantial peripheral activity.

A useful starting point for the design of the title series was provided by the consideration of the muscarinic activity of arecoline (**A**). The therapeutical benefit of this compound is rather limited because of its short duration of action due to fast hydrolysis of the ester function (see Discussion).[10] In this paper we describe the bioisosteric replacement of the susceptible ester function of arecoline (**A**) by a hydrolysis resistant pyrazole function (**B**, Fig. 1; Table 1), to overcome this disadvantage.

## Chemistry

Efficient synthetic routes for the preparation of the desired novel muscarinic agonists (Table 1) were devised by using commercially available pyridine derivatives **1** and **9** (Schemes 1–4) as starting material, as described previously.[14]

A building block for many of the compounds was 3-acetylpyridine (**1**), which was converted into the enamine **2** using N,N-dimethylformamide dimethylacetal (DMF-DMA) as reagent (Scheme 1). Cyclization of **2** with hydrazine in 2-methoxyethanol gave the desired 3-(1*H*-pyrazole-3-yl)pyridine (**3a**). From this key intermediate the mono-halogen derivatives **3b–d** were prepared by using one equivalent of the appropriate N-halosuccinimide as reagent. A second equivalent of the N-halosuccinimide gave the disubstituted pyrazoles **3e,f**. The desired N-methyl 1,2,5,6-tetrahydro-1-methylpyridine derivatives **4a–f** were obtained from **3a–f** by quaternizing the pyridine moiety with methyl iodide and reducing the corresponding pyridinium salts with sodium borohydride.[15]

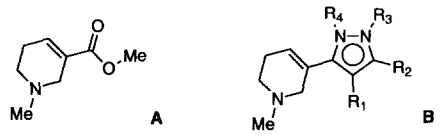
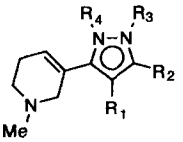



**Figure 1.**

The N-methyl pyrazole derivatives **6a,b** and **8a,b** were synthesized as outlined in Scheme 2 and 3. Methylation of the pyrazole nitrogen of **3a** using dimethylsulfate (Scheme 2) gave 3-(1-methylpyrazole-3-yl)pyridine (**5a**) and the isomeric 3-(1-methylpyrazole-5-yl)pyridine (**7a**) (Scheme 3) in a ratio of 8:1. Unequivocal evidence for the structures of **5a** and **7a** was derived from NOE-NMR spectroscopy. Treatment of 3-(1-methylpyrazole-3-yl)pyridine (**5a**) with one equivalent N-bromosuccinimide gave the 4-bromo-1-methylpyrazole derivative **5b**. The desired 1,2,5,6-tetrahydro-1-methylpyridine derivatives **6a** and **6b** were prepared using the two step procedure as described for **4a–f**. Treatment of the enamine **2** with methylhydrazine (Scheme 3) gave 3-(1-methylpyrazole-5-yl)pyridine (**7a**) and a small amount of the isomeric compound **5a**, *vide supra*. Treatment of 3-(1-methylpyrazole-5-yl)pyridine (**7a**) with one equivalent N-bromosuccinimide gave the 4-bromo-pyrazole derivative **7b**. According to the methods described above 3-(1-methylpyrazole-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine (**8a**) and the bromo derivative **8b** were prepared from **7a** and **7b**, respectively.

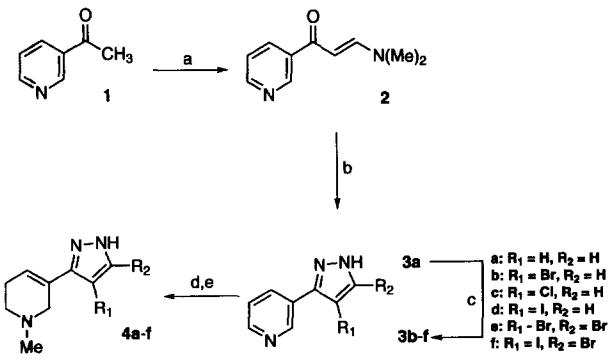
**Table 1.** Physical properties

3-(1*H*-Pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridines **4a–g**
3-(1-Methylpyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridines **6a,b**
3-(1-Methylpyrazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridines **8a,b**




| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Salt | mp (°C) | Molecular Formula[c] |
|---|---|---|---|---|---|---|---|
| **4a** | H | H | H | — | mal[a] | 147 | $C_9H_{13}N_3$; $C_4H_4O_4$ |
| **4b** | Br | H | H | — | $\frac{1}{2}$ fum[b] | 228 | $C_9H_{12}BrN_3$; $\frac{1}{2}C_4H_4O_4$ |
| **4c** | Cl | H | H | — | fum[a] | 212 | $C_9H_{12}ClN_3$; $C_4H_4O_4$ |
| **4d** | I | H | H | — | mal[a] | 178 | $C_9H_{12}IN_3$; $C_4H_4O_4$ |
| **4e** | Br | Br | H | — | mal[a] | 208 | $C_9H_{11}Br_2N_3$; $C_4H_4O_4$ |
| **4f** | I | Br | H | — | mal[a] | 133 | $C_9H_{11}BrIN_3$; $C_4H_4O_4$ |
| **4g** | $CO_2Me$ | H | H | — | $\frac{1}{2}$ fum[b] | 202 | $C_{11}H_{15}N_3O_2$; $\frac{1}{2}C_4H_4O_4$ |
| **6a** | H | H | Me | — | mal[a] | 141 | $C_{10}H_{15}N_3$; $C_4H_4O_4$ |
| **6b** | Br | H | Me | — | mal[a] | 153 | $C_{10}H_{14}BrN_3$; $C_4H_4O_4$ |
| **8a** | H | H | — | Me | mal[a] | 128 | $C_{10}H_{15}N_3$; $C_4H_4O_4$ |
| **8b** | Br | H | — | Me | mal[a] | 160 | $C_{10}H_{14}BrN_3$; $C_4H_4O_4$ |

[a]mal = Maleic acid.
[b]fum = Fumaric acid.
[c]Compounds gave satisfactory microanalyses for C, H, and N and exact *m/z* data.

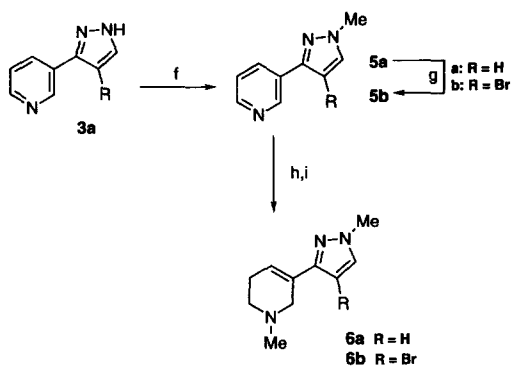


**Scheme 1.** (a) $Me_2NC(OMe)_2$, DMF; (b) $H_2NNH_2 \cdot H_2O$, $MeOCH_2CH_2OH$; (c) N-halogen succinimide, DMF; (d) MeI, $CH_3CN$; (e) $NaBH_4$, MeOH.

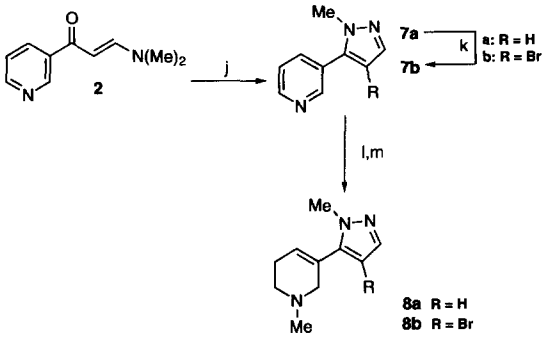
3-(4-Carbomethoxy-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (**4g**) was prepared from methyl nicotinoate **9** (Scheme 4). Thus, treatment of **9** with methylacetate–NaOMe gave the β-ketoester **10**. Subsequently, condensation of **10** with DMF-DMA gave the enamine **11**. Cyclization of **11** with hydrazine in 2-methoxyethanol gave the 4-carbomethoxy-1*H*-pyrazole derivative **12**. Finally, the desired derivative **4g** was obtained from **12** by quarternizing the pyridine moiety with methyliodide and reducing the products with sodium borohydride.

## Pharmacology

N-Methylation in the pyrazole ring (position $R_3$ and $R_4$, see Table 3) showed varying results depending on the presence or absence of a halogen (at $R_1$) and the position of the methyl substituent (at $R_3$ or $R_4$) involved. In all cases the affinity in $^3$H-PZ binding did not decrease (Table 2). Methylation (**6a**, $R_3 = Me$ compared to **4a**; $R_3 = H$, Table 3) in the unsubstituted pyrazole caused a 33-fold increase of the affinity in $^3$H-PZ binding. In contrast, methylation in the halogenated ring (**6b**, $R_3 = Me$; Table 3) hardly affected the affinity in $^3$H-PZ binding. Methylation at $R_4$ failed to affect the affinity in $^3$H-PZ binding both in the unsubstituted and the halogenated compounds (**8a** and **8b**, Table 2). The effects on $^3$H-OXO-M binding were



**Scheme 2.** (f) $Me_2SO_4$, $K_2CO_3$, MeOH; (g) N-halogen succinimide, DMF; (h) MeI, $CH_3CN$; (i) $NaBH_4$, MeOH.

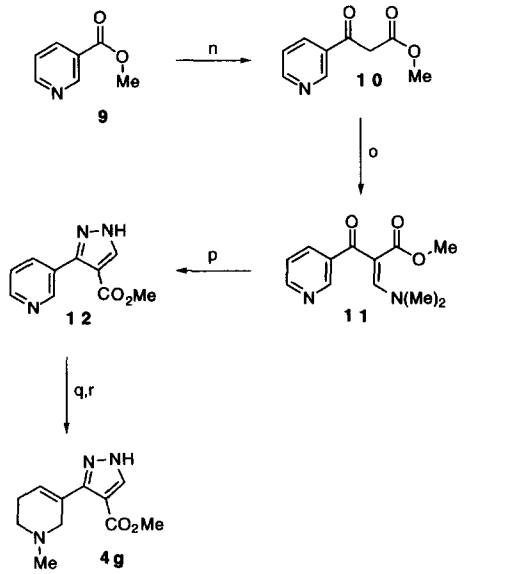


**Scheme 3.** (j) $H_2NNHCH_3$, $MeOCH_2CH_2OH$; (k) N-halogen succinimide, DMF; (l) MeI, $CH_3CN$; (m) $NaBH_4$, MeOH.

most striking for methylation (Table 3: **8b**; $R_4 = Me$ compared to **4b** no substituent at $R_4$) in the bromo-substituted pyrazole, which resulted in a 30-fold decrease in $^3$H-OXO-M affinity. In all cases of the N-methylation of the pyrazole the $^3$H-PZ:$^3$H-OXO-M ratio decreased indicating a shift towards more antagonistic properties.

Halogenation (Br-, Cl- and I-substitution) at the $R_1$-position of the unsubstituted pyrazole ring resulted in a 10- to 30-fold increase in affinity in the $^3$H-OXO-M and $^3$H-PZ binding tests without much effect on the ratio (compounds **4b**, **4c**, and **4d** compared with **4a**, $R_1 = H$; Table 4). In the presence of a methyl-substituent in the ring at $R_3$ (**6b**), bromination had no effect on the profile. In contrast, bromination in the presence of a methyl-substituent in the ring at $R_4$ (**8b**) caused a preferential increase in $^3$H-PZ affinity.

Bromination at the $R_2$-position in the presence of a halogen substituent at $R_1$ ($R_1 = Br$ **4e**; $R_1 = I$ **4f**) preferentially decreases the affinity in the $^3$H-OXO-M



**Scheme 4.** (n) $CH_3CO_2Me$, NaOMe, toluene; (o) $(CH_3)_2NCH(OMe)_2$; (p) $H_2NNH_2 \cdot H_2O$, $HOCH_2CH_2OMe$; (q) MeI, $CH_3CN$; (r) $NaBH_4$, MeOH.

**Table 2.**[a] In vitro muscarinic effects

| Compd | $^3$H-OXO-M $K_i$ (μM) ± SEM | No. | $^3$H-PZ $K_i$ (μM) ± SEM | No. | $^3$H-PZ/$^3$H-OXO-M Ratio[b] |
|---|---|---|---|---|---|
| Carbachol | 0.0042 ± 0.0001 | (4) | 21.4 ± 5.5 | (3) | 5095 |
| **4a** | 0.765 ± 0.144 | (3) | 42.7 ± 11.6 | (3) | 56 |
| **4b** | 0.058 ± 0.022 | (4) | 2.56 ± 0.34 | (3) | 44 |
| **4c** | 0.134 ± 0.008 | (4) | 3.71 ± 0.27 | (3) | 28 |
| **4d** | 0.029 ± 0.002 | (5) | 1.30 ± 0.30 | (4) | 45 |
| **4e** | 0.522 ± 0.24 | (3) | 2.56 ± 0.34 | (3) | 5 |
| **4f** | 0.295 ± 0.022 | (3) | 0.99 ± 0.44 | (3) | 3.4 |
| **4g** | 1.37 ± 0.11 | (3) | 10.9 ± 0.9 | (3) | 8 |
| **6a** | 0.231 ± 0.056 | (3) | 1.28 ± 0.17 | (3) | 6 |
| **6b** | 0.186 ± 0.014 | (3) | 1.05 ± 0.21 | (3) | 6 |
| **8a** | 2.57 ± 0.26 | (4) | 37.1 ± 2.7 | (3) | 14 |
| **8b** | 1.71 ± 0.18 | (4) | 4.65 ± 1.04 | (4) | 2.7 |

[a]Data refer to means ± SEM for the number of experiments indicated.
[b]The ratio $^3$H-PZ:$^3$H-OXO-M represents the ratio of affinities of $^3$H-PZ and $^3$H-OXO-M binding; see Experimental.

binding, decreases the $^3$H-PZ:$^3$H-OXO-M ratio and the resulting compounds lost their agonistic profile.

## Functional Studies

Compounds **4a–4d** were tested in a number of in vivo and in vitro functional tests. Compounds **4b** and **4d** gave full agonist responses in tissue preparations indicating $M_3$ mediated effects, whereas effects in other in vitro preparations were predominantly antagonistic (Tables 5 and 6).

Some mydriasis was observed after application of each compound, but the effect was always less than 50% of the maximal response shown by oxotremorine and appeared to plateau. In this test the full agonist oxotremorine has an $ED_{50}$ of approximately 0.3 μg.

Salivation was induced to a minor extent by **4c** and **4d** at the highest dose tested. In this procedure the full agonist oxotremorine has a minimal effective dose of approximately 0.05 mg/kg.

The most active compound from the series **4d** (Fig. 2) was tested further in models of learning and memory. Activity was noted in a number of experiments. In a test of short-term spatial memory, **4d** partially reversed the disruptive effects of central cholinergic depletion on performance at doses which did not alter other aspects of performance, such as response time or ability to complete trials. Higher doses led to a generalized disruption of performance.

## Discussion

Previous studies have indicated that muscarinic cholinergic agonists show preferential inhibition of the radiolabelled agonist binding as compared to antagonist binding.[17] Potential agonist can be putatively identified by their preferential inhibition of agonist over antagonist binding with ratios between 20 and 10,000. In these studies pirenzepine was used as the antagonist as, unlike other antagonist such as 3-quinuclidinyl benzilate, it has been reported not to label the high affinity state of the cholinergic receptor,

**Table 3.**[a] Effect of N-methylation on muscarinic cholinergic properties

| Compound | Comparison | | | | Increase in potency | | Change in potency ratio |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $^3$H-OXO-M | $^3$H-PZ | $^3$H-PZ:$^3$H-OXO-M |
| **6a** | H | | Me | — | | | |
| vs | | | | | 3.3 | 33 | 0.1 |
| **4a** | H | | | — | | | |
| **6b** | Br | H | Me | — | | | |
| vs | | | | | 0.31 | 2.4 | 0.13 |
| **4b** | Br | H | | — | | | |
| **8a** | | | — | Me | | | |
| vs | | | | | 0.30 | 1.15 | 0.26 |
| **4a** | H | | | — | | | |
| **8b** | Br | H | — | Me | | | |
| vs | | | | | 0.034 | 0.55 | 0.062 |
| **4b** | Br | H | | — | | | |

[a]Data refer to the change in binding potency to $^3$H-OXO-M and $^3$H-PZ binding and the effect of the methylation in the potency ration of $^3$H-OXO-M and $^3$H-PZ binding.

**Table 4.**[a] Effect of halogenation on muscarinic cholinergic properties

| Compound | Comparison | | | | Increase in potency | | Change in potency ratio |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $^3$H-OXO-M | $^3$H-PZ | $^3$H-PZ:$^3$H-OXO-M |
| **4b** | Br | H | | — | | | |
| vs | | | | | 13 | 17 | 0.76 |
| **4a** | H | | | — | | | |
| **4c** | Cl | H | | — | | | |
| vs | | | | | 5.7 | 11.5 | 0.50 |
| **4a** | H | | | — | | | |
| **4d** | I | H | | — | | | |
| vs | | | | | 26 | 33 | 0.79 |
| **4a** | H | | | — | | | |
| **6b** | Br | H | Me | — | | | |
| vs | | | | | 1.2 | 1.2 | 1 |
| **6a** | H | H | Me | — | | | |
| **8b** | Br | H | — | Me | | | |
| vs | | | | | 1.5 | 8 | 0.19 |
| **8a** | H | H | — | Me | | | |
| **4e** | Br | Br | H | — | | | |
| vs | | | | | 0.11 | 1 | 0.11 |
| **4b** | Br | H | H | — | | | |
| **4f** | I | Br | H | — | | | |
| vs | | | | | 0.10 | 1.3 | 0.08 |
| **4d** | I | H | H | — | | | |

[a]For explanation of the data, see legend Table 3.

**Table 5.**[a] Muscarinic cholinergic activity in guinea pig ileum (MUGI), rat left atrium (M2LA), and rabbit vas deferens (RVD)

| Compound | MUGI | M2LA | RVD |
|---|---|---|---|
| **4b** | $pD_2=5.2$ $\alpha=1$ | $pD_2=4.6$ $\alpha=0.3$<br>$pA_2=5.3$ | $pD_2=3.5$ |
| **4d** | $pD_2=5.4$ $\alpha=0.9$ | $pD_2<3.5$<br>$pA_2=5.8$ | $pD_2=3.8$<br>$pA_2=6.2$ |

[a]Muscarinic cholinergic activity mediated via $M_3$, $M_2$, and $M_1$ receptors was measured in the guinea pig ileum, rat left atrium and rabbit vas deferens, respectively (for details see Experimental). Antagonistic activity was measured by shifting a dose–response curve for the cholinergic agonists (acetylcholine (MUGI), carbachol (M2LA), and McN A 343 (RVD)) to the right and calculating the $pA_2$-value as described previously,[16] while compounds contracting the organs were tested for agonistic activity. The cholinergic character of the response was evaluated by measuring the sensitivity to atropine. Data refer to means of four or more determinations and $\alpha$ refers to intrinsic activity where $\alpha=1$ is full agonist and $\alpha<1$ represents partial agonist properties.

**Table 6.**[a] In vivo studies

| Compound | Antagonism of mydriasis ED50, mg/rat | Induction of salivation MED, mg/kg |
|---|---|---|
| **4a** | >0.3 | >10 |
| **4b** | >0.2 | >10 |
| **4c** | >0.3 | ≥10 |
| **4d** | >0 | 10 |

thus agonists inhibit the binding in a monophasic manner, potentially allowing a more accurate measurement of agonist affinity in the antagonist binding test. Pirenzepine was also chosen as it is a known $M_1$ selective compound and the original aim of the synthesis was to identify $M_1$ selective compounds. Using this method to evaluate the predictivity of $^3$H-OXO-M:$^3$H-PZ binding ratios as an indicator of muscarinic agonistic potential four of the synthesized compounds classify as potential agonists. The unsubstituted pyrazolyl-1,2,5,6-tetrahydro-1-methylpyridine **4a** was weakly active in $^3$H-OXO-M binding, but showed a high $^3$H-PZ:$^3$H-OXO-M ratio. Mono-halogenation (compounds **4b–4d**) increased the affinity as compared to the parent compound **4a** in the $^3$H-OXO-M binding about 5- to 25-fold and the affinity in $^3$H-PZ binding about 10- to 35-fold. However, none of the compounds synthesized would be classified as strong agonists on the basis of these results, a fact confirmed in vitro and in vivo testing. The use of such a ratio has been criticized in recent years as not being accurate. Given that the major aim of research in the muscarinic field has been for an $M_1$ selective agonist, it is not surprising that selective compounds show up less clearly in tests using agonist and antagonists possessing affinity for all subtypes. Nevertheless, the information provided by the ratio can still be considered as a guideline for further pharmacological testing.

No other substitutions produced changes strongly indicative of agonist activity. Additional bromo substituents caused a 10-fold decrease of the affinity of the compounds in $^3$H-OXO-M binding without effecting the affinity in $^3$H-PZ binding. Bromination of

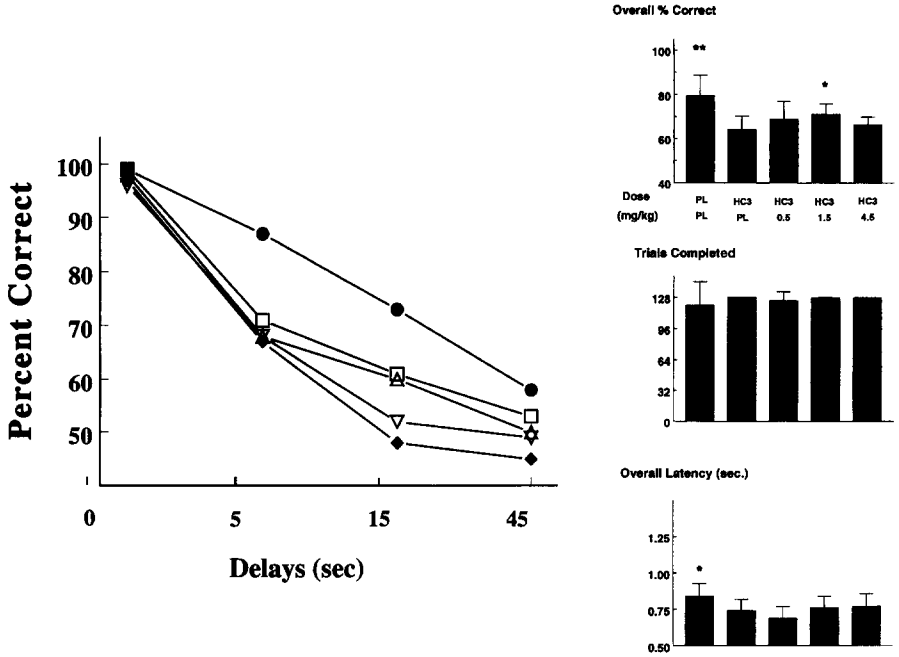



**Figure 2.** Reversal of hemicholinium-3-induced deficit in a delayed matching to position task (DMTP). The data show accuracy as measured by percentage correct responding at four different time points. Each symbol represents a separate performance-matched group of rats receiving either placebo (●), the cholinergic depleting agent hemicholinium-3 (HC-3, ■) or HC3, and three doses of compound **4d** (po; 0.5 mg/kg, □; 1.5 mg/kg, △; 4.5 mg/kg ◆). HC3 causes a delay-related disruption in short-term spatial memory which is partly reversed by 1.5 mg/kg of **4d** (Tukey test $P<0.05$ following significant ANOVA, $F(4,41)=7.04$, $P<0.01$). Improvements in performance occurred at a dose which did not change noncognitive parameters, such as speed or frequency of responding. Higher doses of **4d** were tested in a separate experiment but produced more behavioral disruption.

the methyl analogue **8a**→**8b** caused an 8-fold increase in affinity in $^3$H-PZ binding without affecting the $^3$H-OXO-M binding. Surprisingly, bromination of **6a** caused no changes in binding. Methyl substitution on the pyrazole nitrogens of **4a** and **4b** caused an increase in the potency ratio as the result of a relative increase of $^3$H-PZ binding affinity as compared to $^3$H-OXO-M binding affinity.

The putative agonistic properties of some of these compounds were investigated using a variety of in vitro and in vivo tests (Tables 5 and 6). The weak inhibition of the twitch in the RVD model by compounds **4b** and **4d** is of little significance and indicates that these compounds possess few $M_1$ or $M_4$ agonistic properties. In contrast, the two compounds **4b** and **4d** showed $M_3$ agonistic properties in the guinea pig ileum with $pD_2$-values of 5.2 or 5.4, respectively. Intrinsic activity was almost unity indicating full agonistic properties for both compounds. Differing results were obtained in the stimulated left atrium. Compound **4b** showed weak partial agonistic properties with a $pD_2$ of 4.6 and intrinsic activity $\alpha = 0.3$. Compound **4d** was devoid of any agonistic properties, but instead showed muscarinic antagonistic properties in this model with a $pA_2$ of 5.8. Thus, systematic modification to the pyrazole ring tends to lead to more selective $M_3$ agonists; **4b** differing from **4d** in the change from a weak partial $M_2$ agonist to a full antagonist.

Regulation of salivation and pupil diameter are thought to be under the control of $M_3$ receptors.[12,18] It was surprising therefore not to observe much effect from any of the compounds in these tests. Halogenation did increase the likelihood of activity (Table 6), however the response was not that typically seen after agonists such as pilocarpine: salivation was evident only at high doses, and antagonism of mydriasis although present, was incomplete even with high doses of **4d** the most potent compound.

Sufficient activity was present in this series to partially reverse a cholinergic-related deficit in a spatial memory task (Fig. 2). This effect occurred at a lower dose than the direct effects on other peripheral systems in vivo. $M_1$ antagonists have been reported to exert detrimental effects on memory and so the $M_1$ activity is unlikely to have contributed to the small beneficial effects on performance. Due to the presynaptic location of $M_2$ receptors,[19] and the fact that presynaptic blockade of cholinergic receptors can enhance acetylcholine release,[19] $M_2$ antagonists are under consideration as therapeutic agents in Alzheimer's disease in their own right.[20] Therefore this property would be desirable in compounds possessing activity at $M_1$ or $M_3$ receptors such as **4b** and **4d**, and hopefully lead to an additive and therefore overall greater effect on the cholinergic system. However, it must be noted that although positive effects of $M_2$ antagonists have been reported in the swim maze,[20] beneficial effects have not been observed in other models of cognitive function such as the delayed matching to position task,[21,22] visual discrimination procedures[23] or the T-maze.[24] Therefore, it is not clear whether the $M_2$ antagonism seen here

contributed significantly to the beneficial effects on memory. The RVD identifies compounds with both $M_1$- and $M_4$-like agonist effects,[25,26] as no significant agonist effects of **4d** could be demonstrated in the RVD experiments, neither $M_1$ or $M_4$ agonistic effects are likely to contribute significantly to the response in the DMTP. Of the remaining cholinergic receptors, little is known concerning the putative effects of $M_5$ in the brain and $M_3$ receptors represent only a small proportion of the muscarinic receptors in the brain.[11] Nevertheless, $M_3$ activation may well be responsible for the small improvements in memory seen in the DMTP. Compounds with strong $M_3$ and $M_1$ antagonistic properties have a much greater disruptive effect on DMTP performance than compounds with strong $M_1$ and weaker $M_3$ antagonistic effects.[21] This suggests that $M_3$ receptors may play an additional, or synergistic role in regulating memory. Similar beneficial effects on mnemonic performance has been observed in several unrelated series of compounds possessing similar pharmacological profiles to that reported here (weak $M_3$ agonist, $M_2$ antagonist, $M_1$ inactive or antagonist; Plate, R. et al. unpublished observation[21]). Taken together these data would suggest a strong role for $M_3$ activation in partially reversing compromised performance in the DMTP. However, some caution is warranted: a potent $M_3$ agonist failed to improve performance in a different delayed matching task.[28] The reasons for such a discrepancy are unknown at the present time. It may be that potent $M_3$ agonists induce strong side effects which overshadow any small beneficial effects on memory. In this respect it is noteworthy that higher doses of **4d** led to a disruption of performance in the memory task, which may also indicate that the appearance of peripheral side effects interferes with putative effects on cognition.

Therefore, even though $M_3$ receptors represent only some 10% of central muscarinic receptors,[11] they may offer an additional target for therapeutic agents, especially if as suggested by these data, central and peripheral effects may be separated by dose (see data in Table 6 and Fig. 2). These data are therefore the first to indicate that cholinergic ligands without significant $M_1$ agonistic properties are capable of attenuating cognitive deficits in the DMTP task. The current data do not indicate that $M_3$ agonist effects on cognition are sufficiently large for selective $M_3$ agonists to be developed for Alzheimer's disease. However, these results add further arguments for the development of compounds with both $M_1$ and $M_3$ agonist activity for the treatment of Alzheimer's disease by emphasizing the potential for cognitive improvements with $M_3$ stimulation additional to those expected with $M_1$ agonists.

## Experimental

Melting points were taken on a Buechi capillary melting point apparatus and are uncorrected. The elemental analyses were within 0.45 of the theoretical values. Proton magnetic resonance spectra were measured on a Bruker WP200, AC200 or AM360 instrument (using standard conditions). Chemical shifts are reported as δ-values (parts per million) relative to $Me_4Si$ as an internal standard. Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates. Spots were visualized with a UV handlamp and $Cl_2$/tetramethylbenzidine. For column chromatography Merck silica gel 60 was used. Fast atom bombardment (FAB) mass spectra were recorded with a Finnigan MAT 90 mass spectrometer (Finnigan MAT, Bremen, FRG). Samples were dissolved in methanol and mixed with the matrix compounds on standard stainless steel targets. Exact masses of the protonated molecular ions were determined with the peak matching technique at a mass resolution of >8900 (10% valley definition) in the positive ion mode using two reference masses either from poly(ethylene glycol), average MW 400, or poly(propylene glycol), average MW 425. Average exact masses were calculated from at least 10 computer-controlled measurements using the bracketing method.

### 3-(1*H*-Pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4a)

**3-(Dimethylamino)-1-pyridine-3-ylprop2-en-1-one (2).** A solution of 3-acetylpyridine (**1**) (125 g, 1.03 mol) and DMF-DMA (138 g, 1.14 mol) in dry dimethylformamide (DMF) was refluxed for 3 h. The reaction mixture was evaporated to dryness under reduced pressure. Trituration of the residue with ether gave **2** in 72% yield (yellow crystals).

**3-(1*H*-pyrazol-3-yl)pyridine (3a).** Hydrazine monohydrate (3.2 g, 64 mmol) was added to a solution of **2** (10.2 g, 58 mmol) in 2-methoxyethanol under $N_2$ and heated for 2.5 h. The solvent was removed under reduced pressure to give **3a** as a gum in quantitative yield. $^1$H NMR (200 MHz, $CDCl_3$): δ 9.05 (s, 1H), 8.55 (d, 1H), 8.10 (d, 1H), 7.60 (s, 1H), 7.30 (q, 1H), 6.60 (d, 1H).

**3-(1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4a).** Iodomethane (11.6 g, 82 mmol) was added to a solution of **3a** (9.2 g, 50 mmol) in acetonitrile under $N_2$. After heating to reflux for 3 h, the reaction mixture was cooled and the precipitated crystals were filtered (yield 91%). To a cooled (5 °C) suspension of this pyridinium iodide derivative (13.1 g, 46 mmol) in methanol, sodium borohydride (6.5 g, 170 mmol) was added in small portions while the temperature was kept at 5 °C. The reaction mixture was stirred for 1 h, then acidified using glacial acetic acid and adjusted at pH 6. The solvent was removed under reduced pressure. The residue was suspended in brine and basified with concentrated $Na_2CO_3$ solution and extracted ethyl acetate. The combined organic layers were dried ($Na_2SO_4$) and evaporated to dryness to give **4a** as an oil (54% yield). Maleic acid salt: mp 147 °C (MeOH/$Et_2O$). $^1$H NMR (200 MHz, $D_2O$): δ 7.75 (s, 1H), 6.60 (s, 1H), 6.35 (s, 2H, mal), 4.35 (d, 1H), 4.00

(d, 1H), 3.65 (m, 1H), 3.30 (m, 1H), 3.10 (s, 3H, N-Me), 2.70 (m, 2H). Exact mass calcd for $C_9H_{14}N_3$ $[M+H]^+$ 164.1188, found: 164.1177.

### 3-(4-Bromo-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4b)

**3-(4-Bromo-1*H*-pyrazol-3-yl)pyridine (3b).** N-Bromosuccinimide (22.8 g, 128 mmol) was added in small portions over a period of 30 min to a stirred solution of **3a** (116 mmol) in DMF while the temperature was kept at 5 °C. The mixture was stirred at this temperature for a further 3 h. The solution was poured into water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($Na_2SO_4$), and evaporated to dryness under reduced pressure. The product **3b** (100% crude) was obtained as an oil, which crystallized on standing.

In a similar manner as described above **3b** was converted into **4b** as a fumarate salt (66% yield); mp 228 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.70 (s, 1H), 6.70 (s, 1H), 6.50 (s, 1H, 1/2 fum), 4.40–3.90 (m, 2H), 3.70–3.20 (m, 2H), 3.05 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_9H_{13}BrN_3$ $[M+H]^+$ 242.0293, found: 242,0285.

### 3-(4-Chloro-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4c)

**3-(4-Chloro-1*H*-pyrazol-3-yl)pyridine (3c).** N-Chlorosuccinimide (0.51 g, 3.6 mmol) was added in small portions, over a period of 30 min, to a stirred solution of **3a** (3.5 mmol) in DMF (5 mL) while the temperature was kept at 5 °C. After stirring at this temperature for 3 h the solution was poured into water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The product (100% crude) was obtained as an oil. Ether was added to the solution of this oil in dichloromethane and the precipitate was discarded. Evaporated and crystallization gave the pyrazole **3c** (0.53 g; 83%); mp 136 °C.

In a similar manner as described above **3c** was converted into **4c** as a fumarate salt (20% yield); mp 212 °C (decomp). $^1$H NMR (200 MHz, DMSO-$d_6$): δ 7.83 (s, 1H), 6.6 (s, 1/2H, fum), 6.46 (m, 1H) 3.34 (s, 2H), 2.6 (m, 2H), 2.4 (s, 3H, NMe), 2.32 (m, 2H). Exact mass calcd for $C_9H_{13}ClN_3$ $[M+H]^+$ 198.0798, found: 198.0779.

### 3-(4-Iodo-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4d)

**3-(4-Iodo-1*H*-pyrazol-3-yl)pyridine (3d).** N-Iodosuccinimide (5.0 g, 22 mmol) was added in small portions over a period of 30 min to a stirred solution of **3a** (20 mmol) in DMF while the temperature was kept at 5 °C. The mixture was stirred at this temperature for 3 h. The solution was poured into water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The product **3d** (100% crude) was obtained as an oil.

In a similar manner as described above **3d** was converted into **4d** as a maleate salt (37% yield); mp 178 °C. $^1$H NMR (200 MHz, MeOD): δ 7.70 (s, 1H), 6.90 (s, 1H), 6.25 (s, 2H, mal), 4.20 (s, 2H), 3.45 (t, 2H), 3.05 (s, 3H, NMe), 2.70 (M, 2H). Exact mass calcd for $C_9H_{13}IN_3$ $[M+H]^+$ 290.0154, found: 290.0142.

### 3-(4,5-Dibromo-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4e)

**3-(4,5-Dibromo-1*H*-pyrazol-3-yl) pyridine (3e).** N-Bromosuccinimide (2.6 g, 14.8 mmol) was added in small portions over a period of 30 min to a stirred solution of **3b** (13.4 mmol) in dry DMF. The mixture was stirred at rt for 3 h. The solution was poured into water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The product **3e** (100% crude) was obtained as an oil.

In a similar manner as described above **3e** was converted into **4e** as a maleate salt (42% yield); mp 208 °C. $^1$H NMR (200 MHz, $D_2O$): δ 6.70 (s, 1H,), 6.25 (s, 2H, mal), 4.20 (s, 2H), 3.50 (t, 2H), 3.10 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_9H_{12}Br_2N_3$ $[M+H]^+$ 319.9398, found: 319.9381.

### 3-(4-Iodo-5-bromo-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4f)

**3-(5-Iodo-4-bromo-1*H*-pyrazol-3-yl)pyridine (3f).** N-Bromosuccinimide (2.6 g, 14.7 mmol) was added in small portions over a period of 30 min to a stirred solution of **3d** (14.7 mmol) in dry DMF while the temperature was kept at 5 °C. The mixture was stirred at rt for 20 h. The solution was poured into water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($Na_2SO_4$) and evaporated to dryness under reduced pressure. After purification with column chromatography (silica gel 60; eluent: ethylacetate:cyclohexane 1:1) the product **3f** was obtained as a solid (39% yield).

In a similar manner as described above **3f** was converted into **4f** as a maleate salt (15% yield); mp 133 °C. $^1$H NMR (200 MHz, MeOD): δ 6.90 (s, 1H), 6.25 (s, 2H, mal), 4.15 (s, 2H), 3.45 (t, 2H), 3.05 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_9H_{12}BrIN_3$ $[M+H]^+$ 367.9259, found: 337.9249.

### 3-(1-Methylpyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (6a)

**3-(1-Methylpyrazol-3-yl)pyridine (5a).** To a suspension of **3a** (16.82 g, 116 mmol) and potassium carbonate (16 g, 116 mmol) in 350 mL dry methanol dimethylsulfate (290 mmol) was added under $N_2$. After

stirring for 6 h at rt, the reaction mixture was filtered and the solvent was removed under reduced pressure. The residue (a mixture of **5a** and the isomeric derivative **7a**, in a ratio 8:1, gas chromatography) was subjected to column chromatography (silica gel 60; eluent EtAc:MeOH, 6:1) and gave 18 gram of crude **5a**, which on treatment with ethereal HCl and recrystallization gave **5a** as HCl salt in 28% yield. $^1$H NMR (200 MHz, $D_2O$): δ 8.90 (S, 1H), 8.60 (2d, 2H), 7.90 (t, 1H), 7.70 (s, 1H), 6.70 (s, 1H), 3.95 (s, 3H, NMe).

In a similar manner as described above **5a** was converted into **6a** as a maleate salt (20% yield); mp 141 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.60 (s, 1H), 6.50 (s, 1H), 6.35 (s, 2H), 4.30 (d, 1H), 4.00 (d, 1H), 3.90 (s, 3H, NNMe), 3.65 (m, 1H), 3.10 (s, 3H, NMe), 2.65 (m, 2H). Exact mass calcd for $C_{10}H_{16}N_3$ $[M+H]^+$ 178.1344, found: 178.1336.

### 3-(4-Bromo-1-methylpyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (6b)

**3-(4-Bromo-1-methylpyrazol-3-yl) pyridine (5b).** N-Bromosuccinimide (6.5 g, 36 mmol) was added in small portions over a period of 30 min to a stirred solution of **5a** (free base, 5.1 g, 32 mmol) in dry DMF while the temperature was kept at 5 °C. After 2 h the solution was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($Na_2SO_4$) and evaporated to dryness under reduced pressure. The product **5b** (100% crude) was obtained as an oil which crystallized on standing.

In a similar manner as described above **5b** was converted into **6b** as a maleate salt (43% yield); mp 153 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.70 (s, 1H), 6.75 (s, 1H), 6.20 (s, 2H, mal), 4.25 (d, 1H), 3.90 (d, 1H), 3.85 (s, 3H, NNMe), 3.70–3.20 (m, 2H), 3.10 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_{10}H_{15}BrN_3$ $[M+H]^+$ 256.0449, found: 225.0431.

### 3-(1-Methylpyrazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine (8a)

**3-(1-Methylpyrazol-5-yl)pyridine (7a).** Methylhydrazine (2.9 g, 63 mmol) was added to a solution of **2** (10.0 g, 57 mmol) in 2-methoxyethanol under $N_2$. After heating to reflux for 3 h the solvent was removed under reduced pressure to give a gum (a mixture of **7a** and the isomeric derivative **5a**; in a ratio 97:3, gas chromatography). Treatment with HCl in ethanol and recrystallization gave **7a** in 53% yield.

In a similar manner, as described above, **7a** was converted into **8a** as a fumarate salt (39% yield); mp 128 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.50 (s, 1H), 6.70 (s, 2H, fum), 6.35 (s, 1H), 6.20 (s, 1H), 4.20–3.90 (m, 2H), 3.85 (s, 3H, NNMe), 3.65 (m, 1H), 3.35 (m, 1H), 3.05 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_{10}H_{16}N_3$ $[M+H]^+$ 178.1344, found: 178.1333.

### 3-(4-Bromo-1-methylpyrazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine (8b)

**3-(4-Bromo-1-methylpyrazol-5-yl)pyridine (7b).** From **9** (3.2 g, 20 mmol) and N-Bromosuccinimide (4.8 g, 22 mmol), **7b** was prepared by using the procedure described for compound **5b** (quantitative yield).

Compound **8b** was prepared from **7b** by using the procedure described for **3a** as a fumarate salt (46% yield). Mp 160 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.55 (s, 1H), 6.65 (s, 1H, 1/2 fum), 6.25 (s, 1H), 4.20–3.90 (m, 2H), 3.85 (s, 3H, NNMe), 3.70 (m, 1H), 3.40 (m, 1H), 3.05 (s, 3H, NMe), 2.75 (m, 2H). Exact mass calcd for $C_{10}H_{15}BrN_3$ $[M+H]^+$ 256.0449, found: 256.0422.

### 3-(4-Carbomethoxy-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4g)

**Methyl 3-oxo-3-(3-pyridinyl)propionate (10).** Sodium metal (12.6 g, 0.55 mol) was cut into small pieces and carefully added to methanol under an atmosphere of $N_2$. After 1 h, the methanol was removed by distillation. Toluene was added and the mixture was distilled again, after which a solution of methyl nicotinate **9** (50 g, 0.36 mol) in methyl acetate (53.6 g, 0.72 mol) was added dropwise at 90 °C. After 20 h of reflux the reaction mixture was concentrated and methyl acetate (27 g, 0.36 mol) was added. After 6 h of reflux the reaction mixture was cooled and the product was removed by filtration. This sodium enolate was dissolved in diluted acefic acid and extracted with ether. The combined organic layers were dried ($Na_2SO_4$) and evaporated to dryness under reduced pressure to give **10** as a orange oil (78% yield).

**Methyl 3-(dimethylamino)-2-(pyridine-3-carbonyl) acrylate (11).** A mixture of **10** (5.0 g, 28 mmol) and DMF–DMA (3.7 g, 31 mmol) was stirred at rt for 2 h. Then the excess of reagent was removed under reduced pressure to give **11** in quantitative yield.

**3-(4-Carbomethoxy-1*H*-pyrazol-3-yl) pyridine (12).** Hydrazine monohydrate (30 mmol) was added to a solution of **11** (28 mmol) in 2-methoxyethanol under $N_2$ and heated to reflux for 3 h. Removal of the solvent under reduced pressure gave **12** as a white solid after crystallization from methanol (49% yield). $^1$H NMR (200 MHz, MeOD): δ 8.90 (s, 1H), 8.55 (d, 1H), 8.20 (m, 2H), 7.50 (m, 1H), 3.75 (s, 3H, $CO_2Me$).

**3-(4-Carbomethoxy-1*H*-pyrazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine (4g).** In a similar manner as described above **12** was converted into **4g** as a fumarate salt (67% yield); mp 128 °C. $^1$H NMR (200 MHz, $D_2O$): δ 7.50 (s, 1H), 6.70 (s, 2H, fum), 6.35 (s, 1H), 6.20 (s, 1H), 4.20–3.90 (m, 2H), 3.85 (s, 3H, $CO_2Me$), 3.65 (m, 1H), 3.35 (m, 1H), 3.05 (s, 3H, NMe), 2.70 (m, 2H). Exact mass calcd for $C_{11}H_{16}N_3O_2$ $[M+H]^+$ 222.1242, found: 222.1224.

## In vitro studies

**Binding of [methyl-$^3$H]-oxotremorine-M acetate ($^3$H-OXO-M) in homogenates of frontal cortex.** The rapid filtration method of Freedman et al.[17] was used to measure the agonist character of muscarinic cholinergic drugs in rat cerebral cortex homogenates. For routine measurements the concentration of [$^3$H]-OXO-M was 0.5 nM, tissue concentration was about 1 mg/mL original tissue and incubation was for 40 min at 30 °C. Nonspecific binding was defined as the amount of binding of [$^3$H]-OXO-M in the presence of 2 μM atropine sulphate and represented about 10% of total binding.

**Binding of [N-methyl-$^3$H]-pirenzepine [$^3$H-PZ] in homogenates of rat forebrain.** The rapid filtration method of Freedman et al.[17] was used to characterize $M_1$-muscarinic cholinergic properties of drugs in rat forebrain membranes. For routine measurements the concentration of [$^3$H]-PZ was 1 nM, tissue concentration was about 10 mg/mL original tissue and incubation was for 60 min at 25 °C. Nonspecific binding was defined as the amount of binding of [$^3$H]-PZ in the presence of 1 μM atropine sulphate and represented about 20% of total binding.

**Evaluation of data.** Displacement curves were obtained for the various compounds by measuring the specific binding in the present of at least four different concentrations and $IC_{50}$ values were obtained using a four parameter fitting procedure. $K_i$ values were obtained from the $IC_{50}$ values by using the Chang–Prusoff equation $K_i = IC_{50}/1 + C/K_d$ in which equals the radiolabelled ligand concentration and $K_d$ equals the dissociation constant for the radiolabelled ligand. $K_d$ values used for these calculations were as follows: [$^3$H]-OXO-M binding: $K_d = 0.7$ nm; [$^3$H]-PZ binding: $K_d = 8.3$ nM.

**Interactions with peripheral receptors in isolated organs.** Interactions with $M_1$- and $M_4$-like muscarinic cholinergic receptors were studied in the stimulated rabbit vas deferens.[25,26] An automated assay was used as described previously for the rat vas deferens.[16] The stimulation-induced twitches were inhibited by the $M_1$ and $M_4$ selective cholinergic agonist McN A 343 with a $pD_2$ of about six. Agonists were characterized by the induction of contractions in an atropine-sensitive manner. The results for antagonists (expressed as $pA_2$-values) were quantified by measuring the shift to the right of the S-shaped curve characterizing the McN A 343 induced contractions.

Interactions with $M_2$ muscarinic cholinergic receptors were studied in the isolated left atrium of the rat. An automated assay was used similar to the $\beta_1$-adrenoreceptor described previously,[16] but with the following adaptation. The inhibition by cholinergic agonists of the electrical stimulation evoked switch of the atrium was measured using carbachol as a reference. Cholinergic agonistic activity was compared to carbachol. Potential $M_2$ activity was verified via antagonism in the presence of the $M_2$ selective antagonist AF-DX 116. Antagonistic activity was measured as a shift to the right of the dose–response curve to carbachol in the presence of the compound as described above.

Interactions with $M_3$ muscarinic cholinergic receptors were measured in the isolated guinea pig ilieum. A fully automated method was used as described previously.[16] Contractions induced with acetylcholine as an agonist ($pD_2$-values between six and seven) were used to evaluate the potency of cholinergic antagonist. Antagonistic activity was measured as described above.

## In vivo studies

Miosis. Antagonism of clonidine-induced mydriasis was studied using the method described by Hagan et al.[18] Male Wistar rats (250–300 g, Harlan Zeist, the Netherlands) were anesthetized with 60 mg/kg of Nembutal, placed on a heated blanket and the pupil diameter measured. Clonidine (0.3 mg/kg) or placebo is then administered sc and 20 min later the pupil diameter measured. Immediately following this measurement separate groups of rats (n = 6) are administered placebo or one of the drug doses in a 20 microlitre drop applied directly onto the eye. Thereafter the pupil is measured at 10, 30, and 60 min after application. Changes in pupil diameter are calculated with respect to levels induced by clonidine.

**Salivation.** The induction of salivation was measured using mice. Male CD-1 mice (30 g, Charles Rivers, Germany) were anesthetized using Avertine. After 10 min separate groups of mice (n = 6) were injected sc with several doses of the test drug in a saline/mulgofen vehicle (1–10 mg/kg). The mice were then placed on a filter paper for 20 min. After this time the filter paper was removed and the extent of any stain due to salivation measured. A dose for which the resulting stain equaled 1 cm$^2$ was recorded as the minimal effective dose.

**Reversal of hemicholinium-3-induced memory deficit.** Drug effects on rat short-term spatial memory were assessed using a minor variation of the delayed matching to position procedure reported by Dunnett[29] and described in detail elsewhere.[21] Male Long Evans rats (350 g Harlan Zeist, NL) were trained on the spatial matching task in standard operant chambers (Coulbourn Instruments Inc., PA, U.S.A.) connected to a MEDLab interface (Med Associates Inc., VT, U.S.A.) and controlled by an IBM PS2 PC. After stabilization training the rats were implanted with guide cannula into the lateral ventricle as described previously.[21] At 1 h before testing, five separate groups of rats (n = 9 or 10) were infused with 5 μL of merlys, or 5 μL of merlys containing 1 μg of the cholinergic depleting agent hemicholinium-3. Hemicholinium-3 leads to a central depletion of acetylcholine and leaves both central and peripheral cholinoreceptors open for stimulation and may therefore allow a more relevant

test of the cognitive effects of cholinergic agonist; Alzheimer's patients suffer from loss of transmitter not blockade of receptors. Thirty minutes before the session rats were administered the test drug po in a saline vehicle. The five treatment groups were: placebo icv and po; hemicholinium-3 icv and placebo po; hemicholinium-3 icv and 0.5 mg/kg of **4d**; hemicholinium-3 and 1.5 mg/kg of **4d**; hemicholinium-3 and 4.5 mg/kg of **4d**. Several parameters were recorded apart from accuracy as measured by percent correct responding at each delay; these included response latencies, trials completed (from a maximum of 128) and head entries into the food tray. Data were analyzed using ANOVA and, where significance was attained, by post hoc testing using the Tukey multiple comparisons test.

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