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Synthesis, and In Vitro and In Vivo Muscarinic Pharmacological Properties of a Series of 1,6-Dihydro-5-(4*H*)-pyrimidinone Oximes

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Abstract—A series of 1,6-dihydro-5-(4*H*)-pyrimidinone oxime derivatives I was synthesized (Scheme 1, Tables 1 and 2) and tested for muscarinic activity (Table 3) in receptor binding assays using [^{3}H]-oxotremorine-M (Oxo-M) and [^{3}H]-pirenzepine (Pz) as ligands. Potential muscarinic agonistic or antagonistic properties of the compounds were determined using binding studies that measured their potencies to inhibit the binding of Oxo-M and Pz. Preferential inhibition of Oxo-M binding was used as an indicator for potential muscarinic agonistic properties; this potential was confirmed in functional studies on isolated organs. The series produced a wide range of active compounds with differing degrees of selectivity in M_1, M_2, and M_3 functional models. Several compounds that have mixed agonist/antagonist profiles were able to reduce cholinergic-related cognitive impairments in models of mnemonic function. Substitutions (**I**, e.g. R_2 or R_3 = Me) at the 1,6-dihydro-5-(4*H*)pyrimidine ring disrupted binding and efficacy, whereas systematic variation of the oximes substituent R1 resulted in various degrees of potency and selectivity dependent on the nature of the substitution.

Introduction

The finding 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] Evidence suggesting a greater loss of presynaptic rather than postsynaptic receptors[3,4] has led to the current treatment strategy concentrating 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 cholinergic agonists. To date, the cholinesterase inhibitors have been shown to improve some aspects of cognitive performance; however, efficacy has been poor and side effects problematic.[5–8] The recent introduction of donepezil has led to a reduction in some but not all side effects, although efficacy is still less than optimal.[9] Similarly, studies involving cholinergic agonists have been marred by the side effect profile of the agonists (e.g. oxotremorine **IV**[10] and RS 86 **V**[11]), although some studies have indicated that careful titration of the dose may lead to significant improvements in function without compromising side effects.[12,13]

There are at least five known cholinergic receptors and it is apparent that these receptors regulate a wide range of peripheral and central functions apart from cognition.[14] Therefore, it is not surprising that studies involving agonists acting at all subtypes produce many undesirable effects. Thus, interest has focused on the effects of selective muscarinic agonists as a means to improve the therapeutic potential of cholinergic agents, and especially for compounds with M_1 and M_1/M_3 agonist properties;[15] (see ref. 16 for a discussion of some potential problems with this approach). Recent animal studies have shown positive results in tests of learning

Key words: Muscarinic agonist; muscarinic antagonist; 1,6-dihydro-5-(4*H*)-pyrimidinone oximes; memory.
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and memory with some of these compounds;[17–19] however, the full results of clinical trials are still awaited for the majority of these compounds. The putative M_1 selective compound xanomeline has been reported to have some beneficial effects in patients, however efficacy, and side effects are still problematic.[20]

The aim of the experiments was to design and test a series of compounds for selective effects at specific muscarinic receptor subtypes in vitro, and then to investigate the central and peripheral effects in vivo, including effects on memory. Previous studies had indicated that compounds with agonist properties at M_1, and to a lesser extent M_3, receptors may have an acceptable pharmacological profile; no effects or antagonist effects at M_2 receptors may also be desirable. The ultimate object of these studies being to develop compounds that preferentially affect the central nervous system and show lesser peripheral side effects than the agonists reported to date.

A useful starting point for the design of the title series was provided by the consideration of the muscarinic activity of arecoline (**II**), however, the therapeutic benefit of this compound is limited because of short duration of action. Recently, we described the synthesis and testing of a series of quinuclidine oxime derivatives **III**[21] having an oxime function as a bioisosteric mimic of arecoline's ester function. Compounds in this series produced weak agonists selective for the M_3 receptor.[21]

Our ongoing search for more potent and selective muscarinic agonists, starting from quinuclidine oxime derivatives **III**,[21] led to the bioisosteric replacement of the quinuclidine heterocyclic moiety by a 1,6-dihydro-(4*H*)-pyrimidine[22] based on molecular modelling considerations. Analogous molecular modelling considerations have been described by Messer et al.[23] Thus, in this paper we describe the synthesis of 1,6-dihydro-(4*H*)pyrimidine derivatives leading to a series of compounds I, which are potent and efficacious muscarinic agonists (Fig. 1, Table 1).

Chemistry

Synthesis of 1,6-dihydro-5-(4*H*)-pyrimidinone oxime derivatives and 1,4,5,6-tetrahydro-(*N*)-alkoxy-5-pyrimidinamine derivatives[22] Condensation of the *O*-alkylhydroxylamine derivatives with diaminoacetone gave the oxime derivatives **1** (Scheme 1). Alternatively, we prepared these oximes **1** in a three step procedure commencing with 1,3-dichloroacetone. *O*-Alkylhydroxylamines were, if necessary, prepared according to methods described in the literature.[24,25] The intermediate 1,3-dichloroacetone oxime derivatives **2** were treated with potassium phthalimide and gave the 1,3-diphthaloyl acetone oxime derivatives **3**. Deprotection of the amino groups using the sodium salt of hydroxylamine as reagent gave the diamino acetone oxime derivatives **1**.

Ring closure of the diamino acetone oxime derivatives **1** using trimethyl orthoformate as the reagent gave the desired 1,6-dihydro-5-(4*H*)-pyrimidinone oxime derivatives **4**–**34**, which were converted into their hydrochloride or maleic acid salts (overall yields and some analytical data are given in Table 1). Treatment of **2**

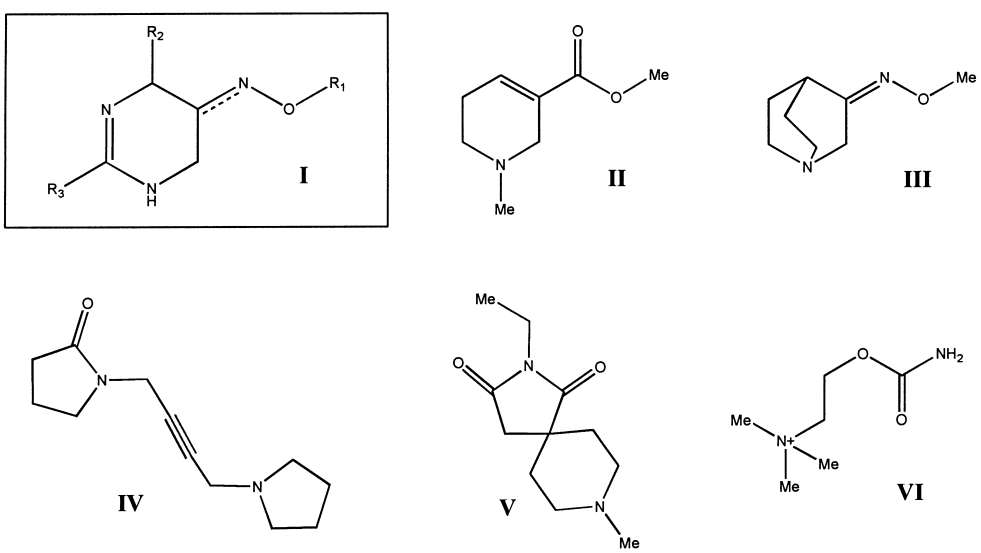


Figure 1.

with trimethyl orthoacetate as reagent gave the corresponding 2-methyl 1,6-dihydro-5-(4*H*)-pyrimidinone oxime derivative **35**.

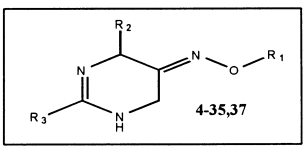
In an analogous fashion 1,3-diamino-l-methylacetone[26] was treated with *O*-ethyl-hydroxylamine to give **36**. Finally, the 4-methyl-1,6-dihydro-5(4*H*)-pyrimidinone oxime **37** was obtained by cyclisation of the 1,3-diamino-1-methyl oxime derivative **36** using trimethylorthoformate as reagent.

Reduction of the oxime function of **4**, **5**, and **6** using trimethylamine borohydride complex (TMA BH_3) in methanolic hydrochloride as the reagent gave the desired 3,4,5,6-tetrahydro-*N*-hydroxy-5-pyrimidinamine derivatives **38**, **39**, and **40**, respectively (Scheme 2, Table 1).

Results

The physical properties of the derivatives are shown in Tables 1–3. These show the receptor binding properties of the series. The ratio of antagonist/agonist binding has been suggested[15] to give an indication of agonist potential: a large ratio indicates an agonist (viz. Table 3 ratio Pz/Oxo-M). This was explored further in various functional in vitro models (Table 4). It appears that the

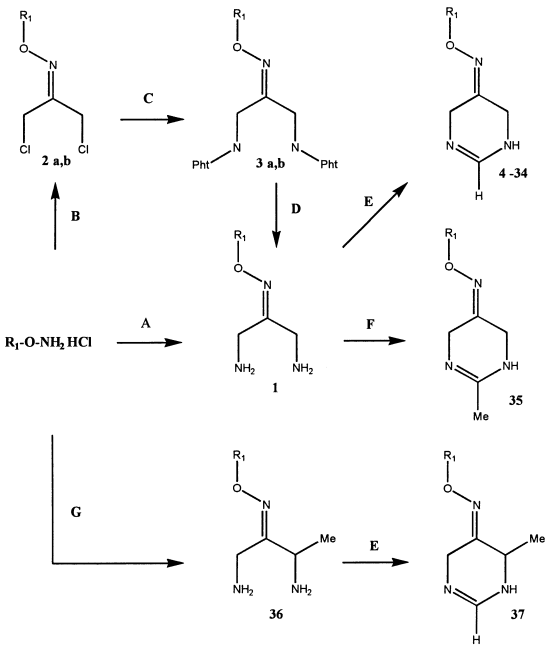
Table 1. 1,6-Dihydro-5-(4*H*)-pyrimidinone oximes



Compd	R_1	R_2	R_3	mp (°C)	Yield (%)	Molecular Formula
4	H	H	H	168	54	$C_4H_7N_3O$·HCl
5	methyl	H	H	170	48	$C_5H_9N_3O$·HC1
6	ethyl	H	H	164	58	$C_6H_{11}N_3O$·HCl
7	propyl	H	H	147	45	$C_7H_{13}N_3O$·HCl
8	butyl	H	H	145	49	$C_9H_{15}N_3O$·HCl
9	*sec*-propyl	H	H	172	58	$C_7H_{13}N_3O$·HCl
10	*tert*-butyl	H	H	199	49	$C_8H_{15}N_3O$·HCl
11	1-methyl-1-propyl	H	H	178	40	$C_8H_{15}N_3O$·HCl
12	methylcyclopropyl	H	H	158	57	$C_8H_{13}N_3O$·HCl
13	propenyl	H	H	165	57	$C_7H_{11}N_3O$·HCl
14	(*E*)-2-butenyl	H	H	136	16	$C_8H_{13}N_3O$·HCl
15	(*Z*)-2-butenyl	H	H	129	32	$C_8H_{13}N_3O$·HCl
16	1-methyl-2-propenyl	H	H	176	8	$C_8H_{13}N_3O$·HCl
17	2-methyl-2-propenyl	H	H	151	9	$C_8H_{13}N_3O$·HCl
18	3-methyl-2-butenyl	H	H	140	44	$C_9H_{15}N_3O$·HCl
19	(*E,E*)-2,4-hexendienyl	H	H	144	37	$C_{10}H_{15}N_3O$·HCl
20	2-propynyl	H	H	205	51	$C_7H_9N_3O$·HCl
21	2-butynyl	H	H	176	35	$C_8H_{11}N_3O$·HCl
22	2-pentynyl	H	H	167	40	$C_9H_{13}N_3O$·HCl
23	2-hexynyl	H	H	146	52	$C_{10}H_{15}N_3O$·HCl
24	(*E*)-2-penten-4-ynyl	H	H	188	58	$C_9H_{11}N_3O$·HCl
25	(*E*)-3-methyl-2-penten-4-ynyl	H	H	186	32	$C_{10}H_{13}N_3O$·HCl
26	(*Z*)-3-methyl-2-penten-4-ynyl	H	H	146	42	$C_{10}H_{13}N_3O$·HCl
27	1-methyl-2-propynyl	H	H	189	10	$C_8H_{11}N_3O$·HCl
28	benzyl	H	H	168	60	$C_{11}H_{13}N_3O$·HCl
29	(2-chlorophenyl)methyl	H	H	187	26	$C_{11}H_{12}C_1N_3O$·HCl
30	(2-fluorophenyl)methyl	H	H	176	50	$C_{11}H_{12}FN_3O$·HCl
31	(2-trifluoromethylphenyl)methyl	H	H	176	7	$C_{12}H_{12}F_3N_3O$·HCl
32	3-phenyl-2-propynyl	H	H	168	55	$C_{13}H_{13}N_3O$·HCl
33	3-(3-methoxyphcnyl)-2-propynyl	H	H	147	44	$C_{14}H_{15}N_3O_2$·HCl
34	(*E*)-3-phenyl-2-propenyl	H	H	137	39	$C_{13}H_{15}N_3O$·$C_4H_4O_4$
35	2-propenyl	H	H	135	32	$C_8H_{13}N_3O$·HCl
37	ethyl	H	H	153	45	$C_7H_{13}N_3O$·HCl

compounds with the highest ratios tend to have full agonist properties in each of the subtype selective preparations (Table 4). However, compounds with lower ratios may also be agonists but this is then expressed as partial agonistic activity or, alternatively, muscarinic-subtype selective agonist effects in different tissue preparations.

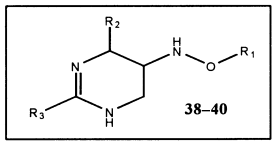
There is a significant correlation between the Pz/Oxo-M binding ratios and potencies as agonists in M_1 ($r=0.69$, $p<0.05$), M_2 ($r=0.823$, $p<0.01$) and M_3 ($r=0.731$, $p<0.01$) mediated responses in isolated organ preparations (data from Tables 3 and 4). For the M_1 and M_2 preparations this correlation was also evident for



Scheme 1. Synthesis of dihydropyrimidine oxime derivatives. (A) $O=C(CH_2NH_2)_2$, MeOH, 20–96 h reflux; (B) $O=(CH_2Cl_2)_2$, MeOH, 2 h, 40 °C; (C) PhtNK, DMF, 4 h, 100 °C; (D) $NaONH_2$, MeOH, 2 h, rt; (E) $HC(OCH_3)_3$, MeOH, 20 h, reflux; (F) $CH_3C(OCH_3)_3$, MeOH, 20 h reflux; (G) $H_2NH_2C(C=O)CH(CH_3)NH_2$MeOH, 20 h reflux.

Scheme 2. Synthesis of tetrahydro-*N*-alkoxy-5-pyrimidinamine derivatives. (H) Trimethylamine–borohydride complex, HCl/MeOH, 2 h, rt.

Table 2. Tetrahydro-*N*-hydroxy-5-pyrimidinamines



Compd	R_1	R_2	R_3	MP (°C)	Yield	Molecular Formula
38	H	H	H	221	18	$C_4H_9N_3O{\cdot}2HCl$
39	methyl	H	H	214	46	$C_5H_{11}N_3O{\cdot}2HCl$
40	ethyl	H	H	178	76	$C_6H_{13}N_3O{\cdot}2HCl$

Table 3. Receptor binding affinity and affinity ratios for assays using agonist, oxotremorine-M (Oxo-M), and antagonist pirenzepine (Pz) ligands (for further details see Experimental)

Compd	Oxo-M K_I (μM)	Pz K_I (μM)	Ratio Pz/Oxo-M
4	5.855	126	21.52
5	0.0698	91.8667	1,315.51
6	0.0122	17.0667	1,397.38
7	0.162	1.14	7.04
8	0.0631	7.94	125.83
9	0.115	11.795	102.6
10	0.5205	6.493	12.47
11	0.895	5.52	6.17
12	0.1807	9.85	54.52
13	0.0465	20.6	443.01
14	0.0396	9.3	234.85
15	0.0389	14.69	378.12
16	0.199	12.6	63.32
17	0.33	11.9	36.06
18	0.0202	4.07	201.49
19	0.992	1.86	1.88
20	0.0226	15.95	705.75
21	0.0096	13.6	1,413.72
22	0.0097	8.38	863.92
23	0.173	7.18	41.50
24	0.0631	7.94	125.83
25	0.0074	4.77	643.44
26	0.0019	1.742	894.86
27	0.112	16.7	149.11
28	0.107	5.95	55.61
29	0.159	1.65	10.38
30	0.0579	1.87	32.30
31	0.388	1.67	4.30
32	0.8905	5.2845	5.93
33	1.285	4.135	3.22
34	5.16	15.215	2.95
35	2.125	4.4795	2.11
37	3.085	71.425	23.15
38	11.1	11.6	1.05
39	6.4	500	78.13
40	0.338	101	298.82
Arecoline **II**	0.0079	1.58	200
Oxotremorine **IV**	0.00056	0.25	446
Carbachol **VI**	0.0148	38.2	2581

measures of efficacy (M_1, $r = 0.902$, $p < 0.01$; M_2, $r = 0.833$, $p < 0.01$) as well as for potency, for M_3, this is true only for potency.

The series contain compounds with a wide range of properties: full, partial and selective agonists are all represented. M_3 activity is the most common property, and all chemical modifications tend to reduce M_1 and M_2 agonist properties before removing M_3 activity in vitro. Similar effects are evident in vivo. Tables 5 and 6 illustrate the activity in vivo in various models of peripheral and central (cognitive or EEG) cholinergic-related activity.

Although compounds that are strong agonists at the M_1, M_2, and M_3 receptors are clearly identified in the models indicative of acetylcholine-related side effects (e.g. salivation, mydriasis) these compounds are not as uniformly active in the cognitive models (Table 5, Figs 2 and 3). Generally speaking, full agonists (high efficacy and potency at the M_1, M_2, and M_3 receptors) are generally capable of reversing cholinergic-induced deficits in the

Table 4. Effects of series members on isolated central and peripheral organs exhibiting receptor selective effects (for further details see Experimental)

	M_1 Hippocampal slice		M_2 Rat left atrium			M_3 Guinea Pig ileum		
Compd	pD_2	α	pD_2	α	pA_2	pD_2	α	pA_2
4	nt							
5	4.8	0.84	5.61 (0.07)	0.93		5.47 (0.1)	1.25	
6	5.5	0.89	6.15 (0.07)	0.95		6.73 (0.03)	1.27	
7	< 4	0			4.86 (0.04)	5.2 (**)	1.0	
8	< 4	0			5.12 (0.05)	4.61 (0.02)	0.73	
9	4.8	0.49	5.1(0.003)	0.71	4.74 (0.07)	5.5 (0.01)	1.22	
10	< 4	0			4.63			< 4.5
11					5.03 (0.05)			4.5
12	< 4	0			5.03 (0.05)	4.66 (0.07)	1.04	
13	5.0	0.86	5.33 (0.07)	0.65		5.45 (0.02)	1.4	
14	< 4.2	0.7	4.84 (0.05)	0.63		5.6 (0.07)	1.27	
15	4.6	0.54	5.27 (0.01)	0.77		5.51 (0.04)	1.29	
16	4.5	0.24			4.93 (0.04)	5.24 (0.04)	1.2	
17	5.0	0.08			4.39	4.8	0.7	
15	< 4.3	0.5	5.07 (0.02)	0.52		6.04 (0.08)	1.32	
19					4.76	< 4		
20	4.8	0.85	6.43 (0.04)	0.89		5.98 (0.03)	1.41	
21	5.1	0.82	5.95 (0.01)	0.91		5.89 (0.02)	0.95	
22	4.9	0.96	6.02 (0.16)	0.95		5.74 (0.03)	1.51	
23	< 4	0			4.9			4.14
24	4.5	0.75	5.0	0.8	4.5	5.1	1.1	
25	< 4.3	0.6	5.55 (0.17)	0.8	5.58 (0.04)	5.83 (0.02)	1.05	
26	5.3	0.9	6.03 (0.01)	0.87		6.75 (0.02)	1.15	
27	4.5	0.48	5.19 (0.002)	0.7	4.8 (0.05)	5.31 (0.05)	1.44	
28	< 4	0			4.96 (0.1)			4.2
29	< 3.5	0			5.46 (0.11)			5.64 (0.15)
30	< 4	0			5.36 (0.09)			< 4.5
31	nt							
32	< 4	0						
33	< 3.5	0			4.58 (0.13)			5.06 (0.13)
34	< 3.5	0			4.54 (0.13)	4.64	0.2	
35	nt							
37	< 4	0						
38	nt							
39	nt							
40	4.6	0.64	5.35 (0.03)	0.82		5.08 (0.02)	1.57	
Arecoline **II**	5.4	0.72	6.9	(1)		6.5	(1)	
Oxotremorine **IV**	6.1	0.56	7	(1)		7.4	(1)	
Carbachol **VI**	5.7	1	6.86	(1)		6.6	(1.2)	

Agonist values pD_2, antagonist values pA_2; α intrinsic activity; figures represent mean (SEM) where appropriate; nt—not tested in any preparation.

swim maze, but are less effective in the DMTP model. In contrast partial agonists, or compounds with a mixed profile (e.g. M_1, M_3 agonist, M_2 partial antagonist **24**) tend to have similar positive effects in both models (Table 5, Figs 2 and 3). There are also differential effects of full agonists and compounds with a mixed agonist/antagonist profile on sleep–waking behavior as measured by the EEG (Table 6). For example, the full agonist **6** increases quiet waking and decreases REM sleep in a manner similar to that observed with oxotremorine; **24** (M_1 and M_3 agonist, M_2 antagonist) has similar depressant effects on REM sleep but in contrast to the full agonists increases active waking rather than quiet waking. The effects on active waking may play a role in the more positive effects of the mixed agonist/antagonists on cognitive performance (Table 5, Figs 2 and 3).

Table 5. Effects of series members on peripherally and centrally mediated behaviours

Compd	Mydriasis[a]	Salivation[b]	DMTP[c]	SWIM[d]
4	> 1	> 10		
5	0.03	1	> 10	2.2
6	0.003	0.1	> 1	1.5
7	> 0.3	10	> 25	
8	> 1	10		
9	0.3	1	15	15
10	> 1	> 10		
11	> 1	≥10		
12	> 1	(10)		
13	0.03	1	> 5	2.2
14	0.1	3	> 1	
15	0.03	3	> 5	
16	0.3	3		
17	0.3	> 10		
18	0.1	3	> 10	
19	> 1	> 10		
20	0.03	1	> 2	(10)
21	0.03	0.3	> 1	
22	0.03	3		
23	> 0.3	> 10		
24	0.3	3	1	0
25	0.3		> 5	1.0
26	< 0.1			
27	0.1	3		
28	0.3	≥10	> 22	
29		> 10		
30	> 1	> 10		
32	> 1	> 10		
33		> 10		
35	> 1	> 10		
37	0.3			
38	0.3			
40	0.3			
Arecoline **I**	0.01		> 0.5	1
Carbachol **VI**[*]	0.0001			
Oxotremorine **IV**	0.0003	0.05	> 0.05	0.03

[a]Mydriasis (MYD): reversal of clonidine-induced mydriasis.
[b]Salivation: induction of salivation in anaesthetised mice.
[c]DMTP: reversal of hemicholinium-3-induced memory deficit in the delayed matching to position task in rats.
[d]SWIM: reversal of hemicholinium-3-induced performance deficit in the two choice island task of spatial memory.
All values represent the minimal effective dose (MED) in mg/kg, or in the case of mydriasis mg per rat; $> x$ represents the highest dose tested at which no significant effect was observed; see experimental section for further details.
[*]Not tested further due to strong convulsant properties in vivo.

The ability of compounds **6** (top panel) and **24** (bottom panel) to reverse mnemonic impairments in the two-island-swim-maze task induced by central acetylcholine depletion following icv administration of hemicholinium-3 (HC3). Both the full agonist (**6**) and the mixed M_1/M_3 agonist, M_2 antagonist (**24**) can reverse the HC3-induced deficit. (See also Table 5, Fig. 3).

Discussion

Cholinergic properties in binding studies

The present series of muscarinic cholinergic ligands was evaluated for potential muscarinic agonistic or antagonistic activity by measuring Oxo-M and Pz binding and by assessing the Pz/Oxo-M potency ratio (Table 3). In general modifications of the substituent RI had a greater effect on agonist (Oxo-M) than on antagonist (Pz) binding.

The role of chain length and branching of substituent R_1

Extending the side chain substituent R_1 in a linear manner from hydrogen **4** ($R_1 = H$) via methyl **5** ($R_1 =$ methyl) to ethyl **6** ($R_1 =$ ethyl), increased the agonist binding and decreased the antagonist binding resulting in a large increase in the Pz/Oxo-M potency ratio (compounds **4**–**6**; Tables 1–3). Further extension of the chain length to **7** ($R_1 =$ propyl) and **8** ($R_1 =$ butyl) however led to a decrease in the agonist binding as well as in the Pz/Oxo-M potency ratio, indicating a bell-shaped relationship with **6** ($R_1 =$ ethyl) as most potent agonist. Addition of a cyclopropyl group to the methyl side chain of **5**, viz. **12** ($R_1 =$ methylcyclopropyl) lowered the agonist binding as well as the Pz/Oxo-M potency ratio. Moreover, addition of methyl substituents to **6** ($R_1 =$ ethyl) led to **9** ($R_1 =$ sec-propyl), **10** ($R_1 =$ *tert*-butyl) and **11** ($R_1 =$ 1-methyl-l-propyl), compounds with decreased Oxo-M binding and Pz/Oxo-M binding ratios.

The role of unsaturation

The effect of unsaturation in the side chain of **7** ($R_1 =$ *n*-propyl) was studied. An increase in potency in the

Table 6. Effects of series members on rat sleep–waking behaviour as measured by EEG

Compd	Dose (mg/kg)	Active waking	Quiet waking	Quiet sleep	Deep sleep	Pre-REM sleep	REM sleep
Oxotremorine **IV**	0.01	−2	4	−16	37	−2	−46
	0.1	−34	73	−49	−1	−51	−100
6	2.2	−25	75	−30	−44	−52	−100
24	3.2	96	−46	−14	−1	−31	−48
	10	100	−13	−21	−22	−30	−74

Compounds showing full agonist properties at M_1, M_2, and M_3 muscarinic subtypes show similar effects to oxotremorine (e.g. **6**); compounds with a mixed M_1/M_3 agonist and M2 partial antagonist profile (e.g. **24**) show a different pattern of changes in sleep EEG. Both types of compounds suppress REM sleep, but differ considerably in their effects on active and quiet waking. Values represent percent change from placebo control. For further details see Experimental.

Oxo-M binding and subsequent Pz/Oxo-M potency ratio was found for the propenyl derivative **13** and the 2-propynyl derivative **20** as compared with the saturated propyl **7**. Addition of substituents, at the propenyl's 3-position of **13**, further increased affinity in agonist binding in **14** (R_1 = (*E*)-2-butenyl), **15** (R_1 = (*Z*)-2-butenyl), **18** (R_1 = 3-methyl-2-butenyl), **25** (R_1 = (*E*)-3-methyl-2-penten-4-ynyl), and with **26** (R_1 = (*Z*)-3-methyl-2-penten-4-ynyl) having the highest affinity in the agonist binding in this series of muscarinics. In contrast, the affinity of the 1-methyl and 2-methyl derivatives **16** (R_1 = 1-methyl-2-propenyl) and **17** (R_1 = 2-methyl-2-propenyl), in agonist binding, as well as the affinity of the 3-alkenyl derivative **19** (R_1 = (*E,E*)-2,4-hexendienyl), were found to be lower than the parent propenyl derivative **13**, whereas the agonist binding of **24** (R_1 = (*E*)-2-penten-4-ynyl) was found again to be comparable to that of **13**. Modification of the side chain of **20** (R_1 = propynyl) to **21** (R_1 = butynyl) or **22** (R_1 = pentynyl) led to an increase in agonist binding affinity and hence increased the Pz/Oxo-M potency ratio. Further extension of the side chain with one methylene group more (**23** R_1 = hexynyl) resulted in a significantly lowered agonist binding affinity, indicating a bell-shaped relationship. In agreement with this observation, addition of a methyl group to the propenyl side chain **13**, gave **27** (R_1 = 1-methyl-2-propynyl) a compound with decreased agonist affinity. As compared with **13** (R_1 = propenyl) the benzyl derivatives **28** (R_1 = benzyl) **29** (R_1 = (2-chlorophenyl)methyl) **30** (R_1 = (2-fluorophenyl)methyl) **31** (R_1 = (2-trifluoromethylphenyl)methyl) did not show an increase in agonist binding affinity or in the PZ/Oxo-M ratio.

The separate series of tetrahydro-*N*-hydroxy-5-pyrimidinamine derivatives **38** (R_1 = H), **39** (R_1 = methyl) and **40** (R_1 = ethyl) that were derived from the oximes by selective reduction of the oxime double bond showed significantly lower agonist binding affinity compared to the parent oxime derivatives **4**, **5**, and **6**, respectively. Additional methyl substituents to the 1,6-dihydro-(4*H*)-pyrimidine ring (viz. **13** versus **35**, R_3 = Me and **6** versus **37**, R_2 = Me) decreases agonist binding affinity and results in the loss of potential agonistic properties as reflected in the Pz/Oxo-M binding affinity ratio.

Cholinergic properties in isolated organs

Many of the 1,6-dihydro-5-(4*H*)-pyrimidinone oximes described are agonists on all three muscarinic cholinergic receptor subtypes M_1, M_2, and M_3 tested (viz. **5**, **6**, **13**, **15**, **20**, **21**, **22**, **26**, Tables 3 and 4) of these compounds **5**, **6**, **13**, **20**, **21**, **22**, and **26** are carbachol-like in having high efficacy at all three subtypes, the rest are oxotremorine-like in having a much lower efficacy at M_1, receptors. Three compounds are M_2 and M^3 selective agonists (viz. **14**, **18**, **25**), two compounds are M_3 selective agonists (viz. **7**, **8**) and several compounds have combined M_1, and M_3 agonist with M_2 partial antagonist properties (viz. **9**, **24**, **27**). All the agonist profiles identified using functional assays in isolated organs are characterized by a Pz/Oxo-M binding ratio >100. However, this ratio showed no apparent relationship to the observed subtype selectivity.

Within the series several compounds approached the desired pharmacological profile (**9**, **16**, **24**, **26**, **27**; Tables 3 and 4). This profile included mixed M_1/M_3 agonistic and M_2 partial antagonist properties, activity in several cholinergic-related models of CNS activity, and a reduced side-effect liability. Changing **6** (R_1 = ethyl) to **7** (R_1 = propyl) resulted in a loss of both M_1 (hippocampal slice) and M_2 (left atrium) agonistic properties. However, **9** (R_1 = isopropyl) produced the desired profile with a retention of M_1 and M_3 agonist properties and a reduction in M_2 agonist effects; a similar profile is obtained with **24** (R_1 = (*E*)-2-penten-4-ynyl; Tables 4 and 5).

Cholinergic properties in behavioural models

The effects of the compounds in vivo support but do not exactly mirror the in vitro effects. In general, compounds with the highest affinity and overall potency in vitro have the greatest and most potent effects in the mydriasis and salivation tests (e.g. **5**, **6**, **20**; Tables 4

and 5). However, effects in the mydriasis test appear to be more closely correlated with in vitro activity than effects on salivation. Thus, within some subseries (e.g. **4**–**8**) the in vivo effects accurately reflect the in vitro properties, whereas some particular compounds do not produce the muscarinic profile in vivo which would be predicted from the in vitro data (e.g. **28** and **38**). This may reflect interference from activity at other receptors known to mediate drug effects in these models, for example alpha adrenergic receptors in the eye.

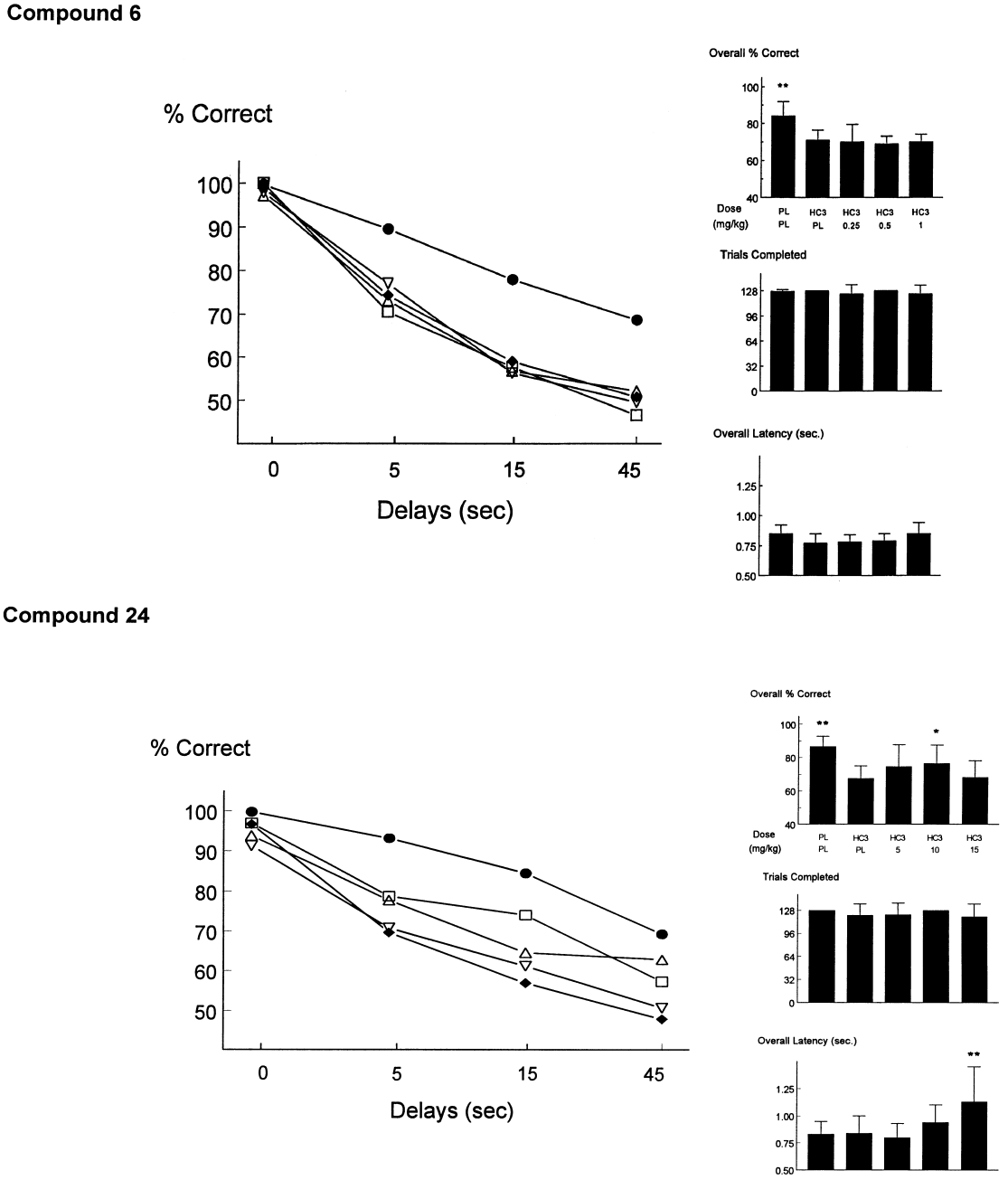


Figure 2. Left: accuracy of responding, as measured by % correct responding, after different delays: ● placebo; ◆ HC3 replacement; △ low dose; □ mid dose; ▽ high dose. Right, top: accuracy of responding collapsed across delays per dose per session. Middle: trials completed per dose per session. Bottom: speed of responding per dose per session. All values mean and standard deviation; * significant difference from HC3. For further details see Experimental.

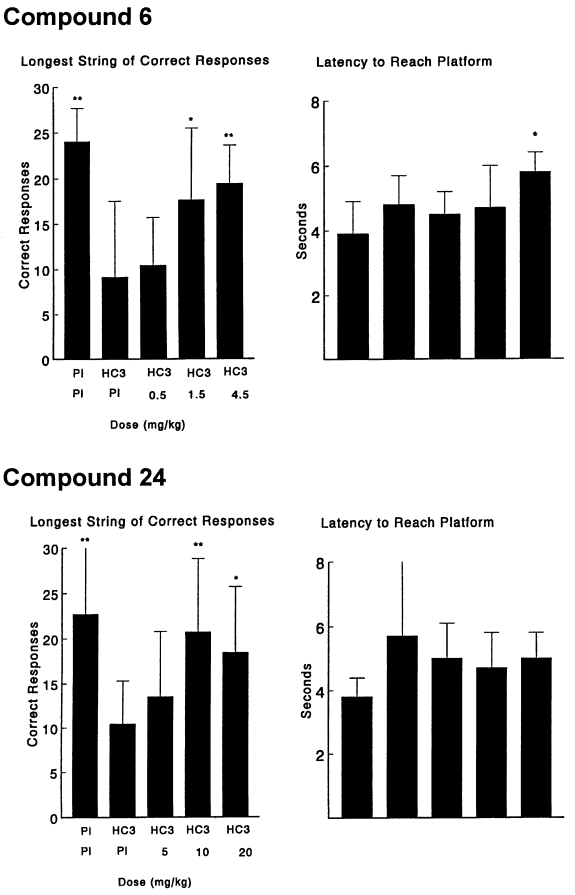


Figure 3. Left: accuracy as measured by the mean number of consecutive correct responses in a session of 30 trials. Right: mean time taken to swim to platform. All data mean and standard deviation: *significant difference from HC3. For further details see Experimental.

Cholinergic properties in mnemonic models

The effects of the compounds tested in the two mnemonic models are particularly interesting. Both models test ostensibly spatial memory, and both use animals where performance has been impaired by the depletion of acetylcholine in the brain by prior administration of hemicholinium-3 (HC3, see Experimental for further details). Compounds that have beneficial effects in one model do not automatically show beneficial effects in the other (Table 5, Figs 1 and 2). General agonists (i.e., compounds with high efficacy at all receptors tested such as **5**, **6**, **13**, **20**, oxotremorine, and arecoline) were able to reverse the HC3-induced deficit in the swim maze, but were unable to significantly reverse the deficit in the DMTP. In contrast two compounds (**9** and **24**) were able to improve performance in both models. These latter two entities lack the strong M_2 agonist effects apparent in the other compounds (Table 4).

Although designed to measure the same area of memory, the nature of the two tasks differs considerably. The swim maze can be seen as a stressful, forced response task: rats have to swim and thus make a response. The DMTP task is positively as opposed to aversely motivated, the only consequence of not responding is less food in the session. Detrimental side effects are thus more readily able to interfere with performance in the DMTP than in the swim maze, where lack of responding may be life-threatening. The most likely cause for this difference in efficacy in the two tests is probably related to the M_2-activity. The results of the salivation experiments shown in Table 5 are from anaesthetized animals. Anaesthetized rats appear to be more sensitive to M_3-mediated peripheral effects than conscious animals: at the doses used in the mnemonic models significant salivation is seen only with the most potent compounds. General full agonists (e.g. **6**) cause dramatic changes in the cardiovascular system in conscious rats at doses slightly above those used in the DMTP and similar to the doses that are active in the swim maze (unpublished observations). It may be that the stress of being immersed in the water negates some of the adverse effects of full agonists on M_2-mediated cardiac responses, a protective effect not evident in the DMTP. Recent studies with cholinergic agonists suggest that sympathetic activation can alleviate some peripheral effects of muscarinic stimulation.[28]

General aspects

In general, there exists a good correlation between the Pz/Oxo-M binding ratio and agonistic properties. Compounds showing the highest ratios have the highest intrinsic activity at all receptors (e.g. carbachol (**VI**), **6**, **21**, **22**). Indeed, the binding ratios of **6** and **21** are only twofold lower than carbachol and three to seven times higher than that of arecoline or oxotremorine which are known partial agonists at some receptors. These compounds show no antagonistic properties in any model. For M_1- and M_2-mediated effects, the correlation exists for both potency, which might be expected, but also for efficacy that may actually be more useful. However, in contrast to previous reports that agonist/antagonist ratios may be used to predict selectivity,[28] in this study the ratio could not be used to predict properties other than agonist-like or not agonist-like. For example, although for compounds **22** and **26** the ratios and the pharmacological profile are similar (both full agonist in all preparations, Table 4), this is not true for compounds **8** and **24**, which also have similar binding ratios. In the latter case, **8** is a weak partial M_3 agonist, whereas **24** is an agonist in both M_1 and M_3 preparations. Thus, a high ratio may indicate a full agonist, lower ratios may indicate selectivity for one receptor subtype (e.g. **8**), a partial agonist or a mixed agonist/antagonist profile (e.g. compounds **9** and **24**). The use of

binding ratios to predict activity is also limited by the tissue preparation used. For example, in tissues with a high receptor reserve, such as the guinea pig ileum, compounds with slight agonist properties will be readily identified as agonists. This probably accounts for the lack of correlation between efficacy and binding ratio and highlights the importance of choosing the relevant tissue preparation for pharmacological investigation of novel chemical entities. Other research has indicated functional assays to be a more reliable indicator of selectivity than binding affinities.[29] These studies indicate the difficulties faced in designing selective compounds. Although much work has concentrated on the production of selective M_1 agonists, the few compounds that have emerged have not yet been shown to be unequivocally beneficial in the treatment of mnemonic loss in Alzheimer's disease. Indeed, some have argued that M_1 agonists may be less effective than initially expected due to a fault in M_1 receptor function in Alzheimer's disease.[16] The addition of some M_3 agonism and M_2 antagonism to this profile has thus been desired; both M_2 antagonists and M_3 agonists have shown positive effects in animal models.[21,30,31] However, definitive data from the clinic is still awaited, and the unwanted activity of at least the current M_3 agonists in the periphery limits their therapeutic potential.

Conclusion

A series of muscarinic agonists that differed considerably in their affinity and pharmacological profile were synthesized based around arecoline. Systematic variation of R_1 yielded compounds, which were full agonists at the M_1, M_2, and M_3 receptors, selective agonists at the M_3 receptors, or M_1/M_3 agonists with partial agonist or no agonistic effects at the M_2 receptors; substitutions into the heterocyclic ring generally disrupted the affinity and pharmacology. Some compounds with mixed agonist/antagonist profiles were able to reverse some cholinergic-related performance deficits. However, no compound had a profile free of peripheral side effects, accordingly beneficial effects in the mnemonic models were limited.

Experimental

Chemistry

Melting points (Ep) 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. 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 M.M. 400, or poly(propylene glycol), average M.M. 425. Average exact masses were calculated from at least 10 computer-controlled measurements using the bracketing method. All new compounds gave satisfactory m/z data. Thin-layer chromatography (TLC) was carried out by using Merck precoated silicagel F_{254} plates (thickness 0.25 mm). Spots were visualized with a UV handlamp and Cl_2/tetramethylbenzidine. For column chromatography Merck silica gel 60 was used. No particular attempts were made to optimize the reaction conditions for the reactions described.

The *O*-alkyl hydroxylamines used were commercially available, prepared from the appropriate bromine derivative[19] or prepared from the corresponding alcohol derivative[20] according to general literature procedures.

General procedure 1: 1,6-dihydro-5-(4*H*)-pyrimidinone derivatives from diamino-acetone 1,6-dihydro-5-(4*H*)-pyrimidinone *O*-methyloxime (5). 1,3-Diamino acetone dihydrochloride monohydrate (2.6 g, 14.6 mmol) and *O*-methyl hydroxylamine hydrochloride (1.5 g, 18 mmol) were dissolved in methanol. The solution was heated to reflux for 48 h. The progress of reaction was monitored by ^{1}H NMR spectroscopy. ^{1}H NMR: (MeOD, 200 MHz) δ 4.04 (s, 3H, OCH_3), 3.95 (s, 2fl, CH_2), 3.7 8 (s, 2H, CH_2). The crude product was dissolved in methanol and 50 mmol trimethyl-orthoformate was added. The mixture was heated to reflux for 20 h the solvent was removes in vacuo. After recrystallisation from methanol/ethylacetate the desired 1,6-dihydro-5-(4*H*)-pyrimidinone *O*-methyloxime **5**. was obtained in 36% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.30 (s, 2H, CH_2), 4.1 0 (s, 2H, CH_2), 3.90 (s, 3H, O-CH_3). Exact mass calcd for $C_5H_9N_3O$ $[M+H]^+$ 128.0779, found: 128.0824. Anal. calcd ($C_5H_9N_3O$·HCl): 36.71% C, 6.16% H, 25.68% N. Found: 36.44% C,6.19% H, 25.21% N.

According to the general procedure 1 the following derivatives were prepared

1,6-Dihydro-5-(4*H*)-pyrimidinone oxime hydrochloride (4). Starting from diamino-acetone dihydrochloride monohydrate and hydroxylamine·HCl the diamino

derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 3.95 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethyl-orthoformate gave **4** in 54% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2). Exact mass calcd for $C_4H_7N_3O$ [M+H] 114.0634, found: 114.0667. Anal. calcd ($C_4H_7N_3O$·HCl): 32.11% C, 5.39% H, 28.09% N. Found: 31.85% C, 5.19% 14, 27.41% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-ethyloxime hydrochloride (6). Starting from diamino acetone dihydrochloride monohydrate and *O*-ethylhydroxylamine·HCl the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.15–4.05 (q, 2H, OCH_2), 3.85 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 1.25 (t, 3H, CH_3). Cyclisation with trimethylorthoformate gave 6 in 48 % overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.30 (s, 21-1, CH_2),4.10 (s, 2H, CH_2), 4.25–4.10 (q, 2H, OCH_2), 1.25 (t, 3H, CH_3). Exact mass calcd for $C_6H_{11}N_3O$ $[M+H]^+$ 142.0938, found: 142.0980. Anal. calcd ($C_6H_{11}N_3O$·HCl): 40.57% C, 6.81% H, 23.66% N. Found: 40.59% C, 6.73% H, 23.54% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-propyloxime hydrochloride (7). Starting from diamino acetone dihydrochloride monohydrate and *O*-propylhydroxylamine. HCl[19] the diamino derivative was prepared. ^{1}H NMR:(MeOD, 200 MHz) δ 4.00 (t, 2H, OCH_2), 3.95 (s, 21-1, CH_2), 3.85 (s, 2H, CH_2), 1.75–1.60 (m, 2H, CH,), 1.00 (t, 3H, CH_3). Cyclisation with trimethylorthoformate gave **7** in 45% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 4.00 (t, 2H, OCH_2), 1.75–1.60 (m, 2H, -CH_2), 1.00 (t, 3H, CH_3). Exact mass calcd for $C_7H_{11}N_3O$ $[M+H]_+$ 156.1112, found: 156.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-butyloxime hydrochloride (8). Starting from diamino acetone dihydrochloride monohydrate and *O*-butylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.05 (t, 2H, OCH_2), 4.20–4.10 (m, 2H, OCH_2), 3.85 (s, 2H, CH_2), 1.70–1.55 (m, 2H, CH_2), 1.50.1.10 (m, 2H, CH_2), 0.90 (t, 3 H, CH_3). Cyclisation with trimethylorthoformate gave **8** in 49% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.35 (s, 2H, CH_2), 4.20–4.10 (m, 2H, OCH_2), 4.15 (s, 2H, CH_2), 1.70–1.55 (m, 2H, -CH_2), 1.50–1.10 (m, 2H, CH_2), 0.90 (t, 3H, CH_3). Exact mass calcd for $C_8H_{15}N_3O$ $[M+H]^+$ 170.1288, found: 170.1293.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(1-methylethyl)oxime hydrochloride (9). Starting from diamino acetone dihydrochloride monohydrate and *O*-1-methyl-ethylhydroxylamine·HCl[21] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.60–4.3 0 (m, 1H, CH), 3.75 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 1.25 (d, 6H, CH_3′s). Cyclisation with trimethylorthoformate gave **9** in 47% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13(s, 1H, N=CH), 4.50–4.20 (m, 1H, CH), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 1.25 (d, 6H, CH_3′s). Exact mass calcd for $C_7H_{11}N_3O$ $[M+H]^+$ 156.1113, found: 156.1137. Anal. calcd ($C_7H_{13}N_3O$·HCl): 43.87% C, 7.36% H, 21.92% N. Found: 43.27% C, 7.01 % H, 21.39% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-methyl-2-propyl)-oxime hydrochloride (10). Starting from diamino acetone dihydrochloride monohydrate and 2-methyl-2-propylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 3.95 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.35 (s, 9H, CH_3′s). Cyclisation with trimethylorthoformate gave **10** in 49% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.85 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.35 (d, 9H, CH_3′s). Exact mass calcd for $C_8H_{15}N_3O$ $[M+H]^+$ 170.1276, 170.1293. Anal. calcd ($C_8H_{15}N_3O$·HCl): 46.71% C, 7.84% H, 20.42% N. Found: 46.87% C, 7.63% H, 20.73% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(1-methyl-1-propyl)-oxime hydrochloride (11). Starting from diamino acetone dihydrochloride monohydrate and 1-methyl-1-propylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.40–4.10 (m, 1H, CH), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.80–1.45 (m, 2H, CH_2), 1.20 (d, 3H, CH_2), 0.95 (t, 3H, CH_3). Cyclisation with trimethylorthoformate gave **11** in 40% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.40–4.10 (m, 1H, CH), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.80–1.45 (m, 2H, CH_2), 1.20 (d, 3H, CH_3), 0.95 (t, 3H, CH_3). Exact mass calcd for $C_8H_{15}N_3O$ $[M+H]+$ 170.1277, found: 170.1293.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(methylcyclopropyl)-oxime hydrochloride (12). Starting from diamino acetone dihydrochloride monohydrate and *O*-methylcyclopropyihydroxylamine·HCl[19] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 3.70 (d, 2H, OCH_2), 3.75 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 1.30–1.00 (m, 1H, CH_2), 0.80–0.60 (m, 2H, CH_2), 0.50–0.30 (m, 2H, CH_2). Cyclisation with trimethylorthoformate gave **12** in 57% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.10 (s, 1H, N=CH), 4.40 (d, 2H, CH_2), 4.20 (s, 2H, CH_2), 4.00–3.90 (d, 2H, OCH_2), 1.30–1.00 (m, 1H, CH_2), 0.80–0.60 (m, 2H, CH_2), 0.50–0.30 (m, 2H, CH_2). Exact mass calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1127, found: 168.1137. Anal. calcd ($C_8H_{13}N_3O$·HCl): 47.18% C, 6.93% H, 20.63% N. Found:47.35% C, 6.59% H, 20.81% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-propenyl)oxime hydrochloride (13). Starting from diamino acetone dihydrochloride monohydrate and 2-propenylhydroxyl-

amine·HCl the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.20–5.80 (m, 1H, =CH), 5.40–5.20 (m, 2H, $=CH_2$), 4.65 (d, 2H, OCH_2), 4.00 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **13** in 57% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1 H, N=CH), 6.10–5.80 (m, 1H, =CH), 5.40–5.25 (m, 2H, $=CH_2$-), 4.65 (d, 2H, OCH_2), 4.40 (s, 2H, CH_2), 4.15 (s, 2H, CH_2). Exact mass calcd for $C_7H_{11}N_3O$ $[M+H]^+$ 154.0969, found: 154.0980.

(*E*)-1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-butenyl)oxime hydrochloride (14). Starting from diamino acetone dihydrochloride monohydrate and (*E*)-2-butenylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 5.90–5.50 (m, 2H, CH=CH), 4.50 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85(s, 2H, CH_2), 1.70 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **14** in 16% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 5.90–5.50 (m, 2H, CH=CH), 4.50 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.70 (d, 3H, CH_3). Exact mass calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1128, found: 168.1137.

(*Z*)-1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-butenyl)oxime hydrochloride (15). Starting from diamino acetone dihydrochloride monohydrate and (*Z*)-2-butenythydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 5.70–5.30 (m, 2H,CH=CH), 4.60 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85(s, 2H, CH_2), 1.50 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **15** in 32% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 5.70–5.30 (m, 2H, CH=CH), 4.50 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.50 (d, 3H, CH_3). Exact mass calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1116, found: 168.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(1-methyl-2-propenyl)-oxime hydrochloride (16). Starting from diamino acetone dihydrochloride monohydrate and 1-methyl-2-propenylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.00–5.80 (m, 1H, CH=), 5.30–5.10 (m, 2H, $=CH_2$), 4.80–4.60 (m, 1H, OCH), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.35 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **16** in 8% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 6.00–5.80 (m, 1H, HC=), 5.30–5.10 (m, 2H, $=CH_2$), 4.80–4.60 (m, 1H, OCH), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.35 (d, 3H, CH_3). Exact mass calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1107, found: 168.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-methyl-2-propenyl)-oxime hydrochloride (17). Starting from diamino acetone dihydrochloride monohydrate and 2-methyl-2-propenylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 5.00 (m, 2H, $=CH_2$), 4.60–4.50 (s, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.75 (s, 3H, CH_3). Cyclisation with trimethylorthoformate gave **17** in 9% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 5.00 (m, 2H, $=CH_2$), 4.60–4.50 (s, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.75 (s, 3H, CH_3). Exact mass calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1108, found: 168.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(3-methyl-2-butenyl)-oxime hydrochloride (18). Starting from diamino acetone dihydrochloride monohydrate and 3-methyl-2-butenylhydroxylamine·HCl[20] in *tert*-butanol the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 5.50 (m, 1H, =CH), 4.80 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.75 (d, 6H, $=(CH_3)_2$). Cyclisation with trimethylorthoformate gave **18** in 44% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.05 (s, 1H, N=CH), 5.40 (m, 1H, =CH), 4.60 (d, 2H, OCH_2), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 1.75 (d, 6H, $=(CH_3)_2$). Exact mass calcd for $C_9H_{15}N_3O$ $[M+H]^+$ 182.1255, found: 182.1293.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2,4-hexendienyl)-oxime hydrochloride (19). Starting from diamino acetone dihydrochloride monohydrate and 2,4-hexendienylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.30–6.20 (m, 1H, =CH), 6.15–6.00 (t, 1H, =CH), 5.90–5.65 (m, 2H, =CH), 4.75 (d, 2H, OCH_2), 4.00 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 1.75 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **19** in 37% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.10 (s, 1H, N=CH), 6.30–6.00 (m, 2H, HC=CH), 5.85–5.60 (m, 2H, HC=CH), 4.65 (d, 2H, OCH_2), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 1.75 (d, 3H, CH_3). Exact mass calcd for $C_{10}H_{15}N_3O$ $[M+H]^+$ 194.1263, found: 194.1293.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-propynyl)oxime hydrochloride (20). Starting from diamino acetone dihydrochloride monohydrate and 2-propynylhydroxylamine·HCl the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.80 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 3.40 (t, 1H, (CH). Cyclisation with trimethylorthoformate gave **20** in 36% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.75 (d, 2H, OCH_2) 4.3 5 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 2.95(t, 3H, ≡CH). Exact mass calcd for $C_7H_9N_3O$ $[M+H]^+$ 152.0800, found: 152.0824. Anal. caled ($C_7H_9N_3O$·HCl): 44.81% C, 5.37% H, 22.40% N. Found: 45.05% C, 5.22% H, 22.23% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-butynyl)oxime hydrochloride (21). Starting from diamino acetone dihydrochloride monohydrate and 2-butynylhydroxylamine·HCl[20] the diamino derivative was prepared.

^{1}H NMR: (MeOD, 200 MHz) δ 4.65 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.80 (s, 3H, CH_3). Cyclisation with trimethylorthoformate gave **21** in 35% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.65 (d, 2H, OCH_2) 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.80 (s, 3H, CH_3). Exact mass calcd for $C_8H_{11}N_3O$ $[M+H]^+$ 166.0966, found: 166.0980.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-pentynyl)oxime hydrochloride (22). Starting from diamino acetone dihydrochloride monohydrate and 2-pentynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.70 (t, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 2.30–2.10 (m, 2H, ≡C-CH_2), 1.10 (t, 3H, CH_3). Cyclisation with trimethylorthoformate gave **22** in 40% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.70 (t, 2H, OCH_2) 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH,), 2.30–2.10 (m, 2H, ≡C-CH_2), 1.10 (t, 3H, CH_3). Exact mass calcd for $C_9H_{13}N_3O$ $[M+H]^+$ 180.1120, found: 180.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-hexynyl)oxime hydrochloride (23). Starting from diamino acetone dihydrochloride monohydrate and 2-hexynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 4.55 (m, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 2.20 (m, 2H, ≡CH), 1.50 (m, 2H, -CH_2-), 1.00 (t, 3H, -CH_3). Cyclisation with trimethyforthoformate gave **23** in 52% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.55 (m, 2H, OCH_2) 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 2.20 (m, 2H, ≡CH), 1.50 (m, 2H, -CH_2-), 1.00 (t, 3H, CH_3). Exact mass calcd for $C_{10}H_{15}N_3O$ $[M+H]^+$ 194.1303, found: 194.1293.

(*E*)-(1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-penten-4-ynyl)-oxime hydrochloride (24). Starting from diamino acetone dihydrochloride monohydrate and (*E*)-2-penten-4-ynylhydroxylamine·HCl[19] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.40 (m, 1H, =CH), 5.90–5.80 (m, 1H, =CH), 4.70 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 3.20 (d, 1H, (CH). Cyclisation with trimethylorthoformate gave **24** in 58% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, CH=N), 6.40 (m, 1H, =CH), 5.80–5.70 (m, 1H, =CH), 4.65 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 3.30 (d, 1H, (CH). Exact mass calcd for $C_9H_{11}N_3O$ $[M+H]^+$ 178.0991, found: 178.0980.

(*E*)-(1,6-Dihydro-5-(4*H*)-pyrimidinone-*O*-(3-methyl-2-penten-4-ynyl)oxime hydrochloride (25). Starting from diamino acetone dihydrochloride monohydrate and (*E*)-3-methyl-2-penten-4-ynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.00 (t, 1H, =CH), 4.75 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.85 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **25** in 32% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, CH=N), 6.00 (t, 1H, =CH), 4.65 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.85 (d, 3H, CH_3). Exact mass calcd for $C_{10}H_{13}N_3O$ $[M+H]^+$ 192.1120, found: 192.1137.

(*Z*)-1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(3-methyl-2-penten-4-ynyl)oxime hydrochloride (26). Starting from diamino acetone dihydrochloride monohydrate and (*Z*)-3-methyl-2-penten-4-ynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.00 (t, 1H, =CH), 4.85 (d, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 1.85 (d, 3H, CH_3). Cyclisation with trimethylorthoformate gave **26** in 42% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, CH=N), 6.00 (t, 1H, =CH), 4.85 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 1.85 (d, 3H, CH_3). Exact mass calcd for $C_{10}H_{13}N_3O$ $[M+H]^+$ 192.1132, found: 192.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(l-methyl-2-propynyl)-oxime hydrochloride (27). Starting from diamino acetone dihydrochloride monohydrate and 1-methyl-2-propynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 5.00 (m, 1H, OCH), 4.05 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 2.90 (m, 1H, ≡CH). Cyclisation with trimethylorthoformate gave **27** in 10% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, CH=N), 5.00 (m, 1H, OCH), 4.35 (s, 2H, CH_2),4.15 (s, 2H, CH_2), 2.90 (m, 1H, ≡CH). Exact mass calcd for $C_8H_{11}N_3O$ $[M+H]^+$ 166.0947, found: 166.0980.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(phenylmethyl)oxime hydrochloride (28). Starting from diamino acetone dihydrochloride monohydrate and *O*-phenylmethylhydroxylamine·HCl the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.50–7.00 (m, 5H, Ar.), 5.20 (s, 2H, OCH_2), 3.95 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **28** in 60% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 7.50–7.20 (m, 5 H, Ar.), 5.20 (s, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.10 (s, 2H, CH_2). Exact mass calcd for $C_{11}H_{13}N_3O$ $[M+H]^+$ 204.1137, found: 204.1142. Anal. calcd ($C^{11}H^{13}N_3O$·HCl): 55.12% C, 5.8 9% H, 17.5 3 % N. Found: 54.84% C, 5.85% H, 17.81% N.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-[(2-chlorophenyl)-methylloxime hydrochloride (29). Starting from diamino acetone dihydrochloride monohydrate and *O*-(2-chlorophenyl)methylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.50–7.00 (m, 4H, Ar.), 5.20 (s, 2H, OCH_2), 3.95 (s, 2H,

CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **29** in 26% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N = CH) 7.50–7.00 (m, 4H, Ar), 5.20 (s, 2H, OCH_2), 4.35 (s, 2H, CH_2), 4.10 (s, 2H, CH_2). Exact mass calcd for $C_{11}H_{12}ClN_3O$ $[M+H]^+$ 238.0716, found: 238.0747.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-[(2-fluorophenyl) methylloxime hydrochloride (30). Starting from diamino acetone dihydrochloride monohydrate and *O*-(2-fluorophenyl)methylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.50–7.00 (m, 4H, Ar), 5.20 (s, 2H, OCH_2), 3.95 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **30** in 50% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N = CH) 7.50–7.00 (m, 4H, Ar.), 5.20 (s, 2H, OCH_2), 4.3 5 (s, 2H, CH_2), 4.10 (s, 2H, CH_2). Exact mass calcd for $C_{11}H_{12}FN_3O$ $[M+H]^+$ 222.1042, found: 222.103 7.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-[(2-trifluoromethylphenyl)methylloxime hydrochloride (31). Starting from diamino acetone dihydrochloride monohydrate and *O*-(2-trifluoromethylphenyl)methylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.60–7.20 (m, 4H, ArH's), 5.20 (s, 2H, OCH_2), 3.95 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **31** in 7% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 7.95 (s, 1H, N = CH) 7.607.20 (m, 4H, Ar.), 5.20 (s, 2H, OCH_2), 4.20 (s, 2H, CH_2), 3.95 (s, 2H, CH_2). Exact mass calcd for $C_{12}H_{12}F_3N_3O$ $[M+H]^+$ 272.1010, found: 272.1002.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(3-phenyl-2-propynyl)-oxime hydrochloride (32). Starting from diamino acetone dihydrochloride monohydrate and 3-phcnyl-2-propynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.50–7.30 (m, 5H, Ar H's), 5.10 (s, 2H, OCH_2), 4.05 (s, 2H, CH_2), 3.90 (s, 2H, CH_2). Cyclisation with trimethylorthoformate gave **32** in 55% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.05 (s, 1H, N = CH) 7.50–7.30 (in, 5H, Ar H's), 5.00 (s, 2H, OCH_2), 4.40 (s, 2H, CH_2), 4.15 (s, 2H, CH_2). Exact mass calcd for $C_{13}H_{13}N_3O[M+H]^+$ 228.1089, found: 228.1137.

1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-[3-(3-methoxyphenyl)-2-propynylloxime hydrochloride (33). Starting from diamino acetone dihydrochloride monohydrate and 3-(3-methoxyphenyl)-2-propynylhydroxylamine·HCl[20] the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 7.30–6.90 (m, 4H, Ar H's), 5.10 (s, 2H, OCH_2), 4.00 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3). Cyclisation with trimethylorthoformate gave **33** in 44% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.15 (s, 1H, N = CH) 7.30–6.90 (m, 4H, Ar H's), 5.00 (s, 2H, OCH_2), 4.40 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3). Exact mass calcd for $C_{14}H_{15}N_3O_2$ $[M+H]^+$ 258.1242, found: 258.1239.

1,6-Dihydro-5-(4*H*)-pyrimidinone derivatives from dichloroacetone (*E*)-1,3-Dichloroacetone *O*-(2-penten-4-ynyl)-oxime (2a). (*E*)-(2-Penten-4-ynyl)hydroxylamine (**B**) (6.7 g, 50 mmol) and 1,3-dichloroacetone (6.1 g, 48 mmol) were dissolved in 250 mL dry methanol and heated to 40 °C. After 2 h the solvent was evaporated and the residue dissolved in 100 mL water. The solution was extracted three times with 75 mL ethylacetate. The combined organic layers were dried ($MgSO_4$) and evaporated and gave **2a** quantitatively as an oil. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 6.30 (dt, 1H, = CH), 5.70 (dd, 1H, = CH), 4.70 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2Cl), 4.25 (s, 2H, CH_2Cl), 2.95 (s, 1H, (CH).

(*E*)-1,3-Diphthaloylacetone *O*-(2-penten-4-ynyl)oxime (3a). Potassiumphthalimide (20.4 g, 110 mmol) was added to a solution of **2a** (50 mmol) in 75 mL dry DMF. The mixture was heated to 100 °C for 3 h. After cooling, the solution was diluted with 150 mL water and extracted three times with 150 mL dichloromethane. The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The residue was subjected to column chromatography (Silicalgel 60, toluene:ethanol, 9:1) to give the crude product. Recrystallisation from heptane/ethylacetate gave **3a** in 43% yield. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 7.90–7.65 (m, 8H, AR H's), 6.25 (dt, 1H, = CH), 5.55 (d, 1H, = CH), 4.60 (s, 2H, CH_2N), 4.55 (d, 2H, OCH_2), 4.45 (s, 2H, CH_2N), 2.85 (s, 1H, (CH).

(*E*)-1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(2-penten-4-ynyl)-oxime (*Z*)-2-butenedioate (24). Sodium (1.7 g, 74 mmol) was dissolved in 150 mL dry methanol and hydroxylamine·HCl (2.66, 37 mmol) was added. After 1 h stirring at room temperature, a solution of **3a** in 100 mL dry dichloromethane was added slowly. A dark red suspension appeared. After 2 h, maleic acid (8.6 g, 74 mmol) was added. The solvent of the now white suspension was evaporated. Recrystallisation of the crude residue in (ethylacetate:ethanol, 9:1) gave the diamino oxime derivative as a crude product. Cyclisation with trimethylorthoformate gave **24** as a maleic acid salt. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 8.15 (s, 1H, CH = N), 6.25 (m, 1H, = CH), 6.25 (s, 2H, mal), 5.75 (d, 1H, = CH), 4.70 (d, 2H, OCH_2), 4.35 (s, 2H, CH_2N), 4.10 (s, 2H, CH_2N), 3.30 (s, 1H, CH).

(*E*)-1,3-Dichloroacetone *O*-(3-phenyl-2-propenyl)oxime (2b). Starting from 1,3-dichloro-acetone and (*E*)-3-phenyl-2-propenylhydroxylamine·HCl[1] the dichloro derivative **2b** was prepared according the procedure described for **2a** in 100% crude yield. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 7.50–7.20 (m, 5H, Ar H's), 6.70 (d, 1H,

=CH), 6.40 (dt, 1H, =CH), 4.70 (d, 2H, OCH_2), 4.45 (s, 2H, CH_2Cl), 4.35 (s, 2H, CH_2Cl).

(*E*)-1,3-Diphthaloylacetone *O*-(3-phenyl-2-propenyl)oxime (3b). Starting from potassium phthalimide and **2b** the diphthaloyl derivative **3b** was prepared according to the procedure described for **3a** in 58% yield. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 7.85–7.60 (m, 8H, Ar H's), 7.50–7.20 (m, 5H, Ar H's), 6.45 (d, 1H, =CH), 6.20 (dt, 1H, =CH), 4.70 (d, 2H, OCH_2), 4.60 (s, 2H, CH_2N), 4.50 (s, 2H, CH_2N).

(*E*)-1,6-Dihydro-5-(4*H*)-pyrimidinone *O*-(3-phenyl-2-propenyl)oxime (*Z*)-2-butenedioate (34). According to the procedure described for **24** the dihydropyrimidine derivative **34** was prepared in 39% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.15 (s, 1H, CH=N), 7.50–7.70 (m, 5H, Ar H's), 6.65 (d, 2H, =CH), 6.40 (dt, 2H, =CH2), 6.25 (s, 2H, mal), 4.80 (d, 2H, OCH_2), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2). Exact mass calcd for $C_{13}H_{15}N_3O$ $[M+H]^+$ 230.1277, found: 230.1293.

(*E*)-1,6-Dihydro-2-methyl-5-(4*H*)-pyrimidinone *O*-(3-phenyl-2-propenyl)oxime hydrochloride (35). Starting from diamino acetone dihydrochloride monohydrate and 2-propenylhydroxylamine·HCl the diamino derivative was prepared. ^{1}H NMR: (MeOD, 200 MHz) δ 6.20–5.80 (m, 1H, =CH), 5.40–5.20 (m, 2H, $=CH_2$), 4.65 (d, 2H, $-OCH_2$), 4.00 (s, 2H, CH_2), 3.85 (s, 2H, CH_2). Treatment with trimethylorthoacetate gave **35** in 32% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 6.10–5.85 (m, 1H, =CH), 5.30–5.10 (m, 2H, $=CH_2$), 4.60 (d, 2H, OCH_2), 4.30 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 2.25 (s, 3H, CH_3). Exact mass calcd for C,H,N,O $[M+H]^+$ 168.1129, found: 168.1137.

(*E*)-1,6-Dihydro-4-methyl-5-(4*H*)-pyrimidinone *O*-(3-phenyl-2-propenyl)oxime hydrochloride (37). Starting from 1,3-diamino-1-methylacetone and *O*-ethylhydroxylamine the diamino derivative **36** was prepared. Cyclisation with trimethyl orthoformate gave **37** in 45% overall yield. ^{1}H NMR: (MeOD, 200 MHz) δ 7.95 (s, 1H, CH=N), 4.15 (s, 2H, $-CH_2-$), 4.00 (q, 2H, OCH_2), 1.30 (d, 3H, CH_3), 1.05 (t, 3H, CH_3). Exact mass calcd for $C_7H_{13}N_3O$ $[M+H]^+$ 156.1110, found: 156.1137.

General procedure II: Tetrahydro-*N*-hydroxy-5-pyrimidinamines

3,4,5,6-Tetrahydro-*N*-hydroxy-5-pyrimidinamine dihydrochloride (38). A solution of 1,6-dihydro-5-(4*H*)pyrimidinone oxime hydrochloride **4** (0.980 g, 6.6 mmol) in dry methanol was added dropwise to a stirred solution of HC1/methanol (6.5 mL of a 5.2 N solution) and trimethylamine borohydride (0.525 g, 7.2 mmol) at room temperature under a nitrogen atmosphere. After 24 h the reaction product crystallized spontaneously. Crystallisation from methanol/ethylacetate gave **38** in 18% yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.25 (m, 1H, CH), 3.85 (d, 4H, CH_2). Exact mass calcd for $C_4H_9N_3O$ $[M+H]^+$ 116.0820, found: 116.0824. Anal. calcd $C_4H_9N_3O{\cdot}2HC1$): 25.54% C, 5.89% H, 22.34% N. Found: 25.95% C, 5.64% H, 22.45% N.

According to the general procedure II the following derivatives were prepared

1,4,5,6-Tetrahydro-*N*-methoxy-5-pyrimidinamine dihydrochloride (39). Starting from **5** the tetrahydropyrimidinamine **39** was prepared in 38% yield. ^{1}H NMR: (MeOD, 200 MHz) δ 8.13 (s, 1H, N=CH), 4.10 (m, 1H, CH), 3.90 (s, 3H, OCH_3), 3.80–3.65 (m, 4H, CH_2). Exact mass calcd for $C_5H_{11}N_3O$ $[M+H]^+$ 130.0991, found: 130.0980. Anal. calcd ($C_5H_{11}N_3O{\cdot}2HC1$): 29.72% C, 6.48% H, 20.79% N. Found: 28.95% C, 6.57% H, 19.86% N.

1,4,5,6-Tetrahydro-*N*-ethoxy-5-pyrimidinamine dihydrochloride (40). Starting from **6** the tetrahydropyrimidinamine **40** was prepared in 76% yield. ^{1}H NMR: (D_2O, 200 MHz) δ 8.05 (s, 1H, N=CH), 4.20–4.00 (m, 1H, CH), 3.80–3.65 (d, 4H, CH_2), 1.25 (t, 3H, CH_3). Exact mass calcd for $C_6H_{13}N_3O$ $[M+H]^+$ 144.1949, found: 144.1961. Anal. calcd ($C_6H_{13}N_3O{\cdot}2HC1$): 33.34% C, 6.99% H, 19.44% N. Found: 32.40% C, 6.85% H, 18.68% N.

In Vitro Studies

Agonist and antagonist binding studies

Binding of [*N*-methyl-^{3}H]-oxotremorine-M acetate (^{3}H-Oxo-M) in homogenates of frontal cortex. The rapid filtration method of Freedman et al.[32] 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 mM 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.[32] 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 mM atropine sulphate and represented about 20% of total binding.

Evaluation of the 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_0 values by using the Cheng–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 muscarinic subtype mediated responses in isolated organs

M_1-mediated activity in the hippocampal slice. Muscarinic cholinergic agonists effectively suppress the electrically evoked field excitatory postsynaptic potential (FEPSP) in the rat hippocampal slice.[33] This effect is due to a decrease in release of excitatory amino acids from the Schaffer collateral/commissural fibers, probably mediated by a presynaptic M_1 muscarinic receptor.[33] Carbachol (VI) (3E-5 mol/L^{-1}) causes a 100% decrease (intrinsic activity = 1.0) in the amplitude of the electrically evoked FEPSP an effect can be fully antagonized by the M_1 selective antagonist pirenzepine.

Preparation of hippocampal slices. Rats (200–350 g; strain: Hsd/Cpb:WU, SPF bred by Harlan CPB, Zeist, The Netherlands) are killed by decapitation using a small guillotine. The brain is taken from the skull and rinsed in ice-cold (1–2 °C) artificial CSF gassed with carbogen (95% 0_2 + 5% CO_2). The hippocampus is taken from either hemisphere and transverse slices (thickness 500 μm) are chopped using a McIlwain tissue chopper. Slices from the middle one third of the hippocampus are separated using two fine nylon paint brushes and subsequently transferred to a slice holding chamber containing ACSF at room temperature (approx. 22 °C).

Electrical stimulation and recording. After a recovery period of at least 1 h a single slice is placed in the temperature controlled recording chamber (capacity approx. 0.8 mL, T = 34–35 °C) and sandwiched between two nylon meshes by adjusting the top mesh in height with a micromanipulator. The slice is continuously gravity fed superfused with aCSF (gassed with carbogen) resulting in 3–4 turnovers per min. The effluent is removed by vacuum. A bipolar concentric stimulation electrode is placed in the stratum radiatum of CA1 near the border with CA2/CA3.

Glass recording electrodes are pulled from 1.0 or 1.5 mm boro-silicate capillaries with filament, using a conventional horizontal puller. These electrodes are filled with NaCl solution in water (4 mol/L) resulting in an electrode resistance of approx. 5 MOhm. The recording electrode is placed approx. 200 μm below the top surface of the slice and at a distance of approx 600 μm from the stimulation electrode in the stratum radiatum of CA1. Synaptic responses are elicited by 100 μs, constant current, monophasic square wave pulses at a frequency of 0.033 Hz. The slice is stimulated at submaximal intensity until a stable response (1–3 mV fEPSP amplitude) is obtained. The stimulus intensity is then adjusted so that the evoked fEPSP apparently does not trigger postsynaptic cells to fire an action potential, i.e. no 'Population Spike' is observed. Signals are amplified (gain 1000), filtered (DC-5 kHz), monitored on an oscilloscope and taped for off-line analysis using a digital tape recorder (DTR 1800).

Procedure. At least two control cycles consisting of four stimulation pulses (1/15 Hz) are run before drug administration. If the control cycles are comparable, the test or reference compound is applied to the slice by switching between the non drug and drug containing solutions using a miniature Hamilton valve. The slice is exposed to a drug concentration for a maximum of 10 min. At 2, 5, and 8 min of application a test cycle consisting of four stimulation pulses (1/15 Hz) is run. Each test or reference compound is initially tested at successively-administered concentrations of 1E-6, 1E-5, and IE-4 mol/L in one slice to obtain a cumulative concentration–response curve. Dependent on the activity found the concentration steps are adapted for a follow up experiment in another slice. If the fEPSP is maximal suppressed or the concentration reaches 3E-4 mol/L the slice is replaced. Each test compound is tested in at least two slices and a washout response is generally obtained.

Evaluation of responses. The effects of the test compounds are evaluated on the basis of amplitude measurements of the fEPSP before (control) and during their application. The fEPSP amplitude during application is expressed as a percentage of the fEPSP amplitude before application. A line is fitted through the data points of the log drug concentration–effect relationship and the pD_2 value (negative logarithm of agonist concentration causing 50% inhibition) and α (efficacy) calculated.

M_2-mediated effects. 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 (1-adrenoreceptor described previously[34] but with the following adaptation. The inhibition by cholinergic agonists of the electrical stimulation evoked switch of the atrium was measured using carbachol (VI) 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 AFDX 1 16. 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.

M_3-mediated effects. Interactions with M_3-muscarinic cholinergic receptors were measured in the isolated guinea pig ileum. A fully automated method was used as described previously.[34] Contractions induced with acetylcholine as an agonist (pD_2-values between 6 and 7) 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.[35] Male Wistar rats (250–300 g, Hsd/Cpb:WU, Harlan, Zeist, Netherlands) were anaesthetized 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 μL 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 mice (30 g, CD-1, Charles Rivers, Germany) were anaesthetized 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 equated 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[36] and the two island swim task in the Morris swim maze as used by Hagan et al.[37] For both tasks separate groups of male rats (350 g, Hsd/Cpb: Long Evans, Harlan, Zeist, Netherlands) were used. Training in the delayed matching to position task has been described in detail elsewhere.[37] Rats were trained on the spatial matching task in standard operant chambers (Coulbourn Instruments, PA, USA) connected to a MEDLab interface (Med Associates, VT, USA) and controlled by an IBM PS2 PC. Training in the swim maze followed the procedure described in detail;[37] the same 2.1 m diameter water maze, cues and islands used by Hagan et al. were used in this study. After stabilization training, the rats were implanted with guide cannula into the lateral ventricle as described previously.[37,38] For the delayed matching experiment, rats were infused with 5 μL of merlys, or 5 μL of merlys containing 1 μg of the cholinergic depleting agent hemicholinium-3, 1 h before testing in the operant chambers. Rats in the swim maze received the same vehicle and infusion volume, but a concentration of 5 μg of 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 sc in a saline vehicle. For both the delayed matching and swim maze experiments five drug treatments were given to groups of 8–10 rats in a latin square design: typically: placebo icv and sc; hemicholinium-3 icv and placebo sc; and hemicholinium-3 icv and three separate doses of the test compound. Several parameters were recorded in both procedures. Data for correct responses, trials completed and response time latencies for both swim maze and delayed matching data were analyzed using ANOVA and, where significance was attained, by appropriate post hoc testing.

Effects on sleep electroencephalograph (EEG) activity

Different stages of sleep–waking behaviour (active waking, quiet waking, quiet sleep, deep sleep, pre-REM sleep and REM sleep) can be identified on the basis of EEG recordings from the cerebral cortex, a measure of muscle tone and a movement index. Most psychotropic compounds alter sleep in a specific way and can be classified either according to drug class (anxiolytic) or specific drug effect (benzodiazepine); see ref. 38 for discussion.

Full details of the procedure have been described in detail elsewhere.[39] Briefly, epidural screw electrodes were implanted over the parietal-occipital cortex of male rats (Hsd/Cpb:Wu, Harlan, Zeist, Netherlands; 250–300 g) for recording of EEG against a frontal reference electrode. Stainless steel electrodes were inserted into the dorsal neck muscles for recording of electromyogram. Twenty-nine hour EEG and EMG recordings were made in sound attenuated Faraday cages. Movements were detected as capacitative arterartefacts generated in an open-ended wire of the cable connecting the rats to the swivel commutator. The swivel was connected to amplification and A/D conversion units

attached to a dedicated PDP-11/83 minicomputer system for on-line spectral EEG and data compression.

Off-line sleep staging was done for 2s epochs based on five spectral EEG band values,[39] the integrated EMG and movement level. A first sleep stage assignment per epoch is done by application of a discriminant function to these epoch values. A moving average EEG smoothing procedure and a set of syntactic classification rules are then used to give the final sleep stage assignments to each specific EEG epoch. Six sleep–wake states are distinguished including two waking states: active waking, characterized by movement, theta activity and high EMG, and quiet waking, without movement. Four sleep stages are discriminated: quiet sleep, characterized by EEG spindles; deep slow wave sleep with prominent delta activity; preREM sleep with spindles against a background of theta activity and low EMG; REM sleep with theta activity and low EMG.

Drugs are administered at the beginning of the light cycle of the rats. Drug effects are assessed on a number of parameters including percentage time spent in each of the sleep stages per 30 min period, allowing a profile of changes over sleep stages and over time.

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