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Synthesis and In Vitro Muscarinic Activities of a Series of 1,3-Diazacycloalkyl Carboxaldehyde Oxime Derivatives

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Abstract—A series of 1,3-diazacycloalkyl carboxaldehyde oxime derivatives was synthesized and tested for muscarinic activity 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 measuring 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.

Introduction

The degeneration of forebrain muscarinic cholinergic neurons of patients suffering from Alzheimer's disease has been associated with reduction in cognitive function.[1] In particular, the presynaptic rather than postsynaptic neurons are affected suggesting that a therapeutic strategy based on the cholinergic deficit hypothesis[2] might have potential for the improvement of the cognitive aspects of Alzheimer disease. Treatment of Alzheimer patients with acetylcholine esterase inhibitors such as tacrine,[3] to increase acetylcholine release, indirectly resulted in improvement of only some aspects of cognitive performance, and in association with severe side effects.[3–6] This poor therapeutic outcome after treatment with these acetylcholine esterase inhibitors might be due to stimulation of presynaptic inhibitory autoreceptors following an increase in extracellular acetylcholine, thus limitating the increase in the release of accumulated acetylcholine.

We have concentrated on the stimulation of postsynaptic receptors to correct the cholinergic deficit. Unfortunately, studies involving direct stimulation of cholinergic receptors with non-selective cholinergic agonists have been marred by their side-effect profile (e.g., oxotremorine,[7] RS 86[8]). Careful adjustment of the dose may improve certain cognitive functions without severely compromising side effects,[9,10] indicating the potential of a cholinergic strategy for treatment of Alzheimer patients. Five muscarinic cholinergic receptor subtypes are known and their structure and distribution have been described.[11] These receptors regulate a wide range of peripheral and central functions[12] and consequently it can be expected that treatments with non-selective agonists will in addition to their cognitive activities produce many undesirable effects. Therefore, attention has shifted to subtype selective muscarinic agonists as a means to improve therapeutic and at the same time to reduce cholinergic side effects. Despite some potential problems with this approach,[13] attention has now focused on a pharmacochemical approach aiming for compounds with preferential M_1 or M_1/M_3 agonist properties.[14] Animal studies indeed revealed positive effects of such compounds in tests of learning and memory,[15,16] but more definite conclusions await the outcome for these compounds with such profiles in clinical trials.

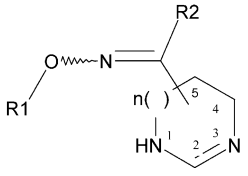

Figure 1.

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The aim of the present study was to design and test a series of compounds with selective effects at specific receptor subtypes in vitro, and then to investigate the central and peripheral effects in vivo including effects on memory. The ultimate objective of these studies is to arrive at compounds with improved efficacy, preferentially action in the central nervous system and associated with a reduced incidence of peripheral side effects (Fig. 1).

We chose arecoline (Fig. 2) as a useful starting point for the design of the title series. arecoline has shown therapeutically beneficial effects, which are, however, of limited use due to its short duration of action.

In our search for a bioisosteric replacement of the ester function of arecoline, we developed several series of potent muscarinic agonists.[17] Moreover, we included the muscarinic agonist isoarecoline as a lead structure in our study. Here, we report the synthesis of the related series of 1,3-diazacycloalkyl carboxaldehyde and ethanone oxime derivatives (Table 1) which was tested for muscarinic activity using [^{3}H]-oxotremorine-M (OXO-M) and [^{3}H]-pirenzepine (PZ) as ligands (Table 2) and was further profiled in our in vitro (Table 3) test battery.[18]

Chemistry

The methods for three different novel approaches of synthesizing of the hitherto unknown 1,4,5,6-tetrahydro-5-pyrimidine carboxaldehyde *O*-alkyloximes, 1,4,5,6-tetrahydro-4-pyrimidine carboxaldehyde *O*-alkyloximes, 4,5,6,7-tetrahydro-1H-1,3-diazepine-4-carboxaldehyde *O*-alkyloximes and 1-(1,4,5,6-tetrahydro-5-pyrimidine)-ethanone *O*-alkyloximes are summarized in Schemes 1–3.

Synthesis of 1,4,5,6-tetrahydro-5-pyrimidine carboxaldehyde *O*-alkyloximes, 8a–c

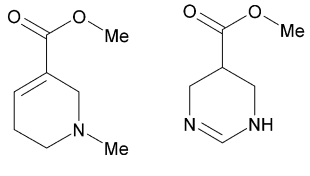
For the synthesis 1,4,5,6-tetrahydro-5-pyrimidine carboxaldehyde, we selected the following strategy starting from the raedily available malononitrile (**1**). Addition of **1** to methylchloroformate gave the potassium salt of methyldicyanoacetate (**2**, Scheme 1).[19] The conditions for the reduction of the dinitrile **2** into the diamino derivative **3** such as the concentration of the reagents and the amount of the used catalyst (10% Pd on carbon) were found to be very important. The method of choice was 2 equivalents 10% Pd/C (w/w)[20] in a diluted (approximately 0.5% w/w) solution of **2** in methanol containing three equivalents of methanolic HCl.[21] A more concentrated reaction mixture or a lower amount of catalyst caused the formation of the undesired enamine **A** as byproduct.[22] The desired methyl 3-amino-2-(aminomethyl)propionate (**3**) was isolated as dihydrochloric acid salt in 58% after recrystallization. The amino groups of **3** were protected using *N*-(benzyloxycarbonyloxy)succinimide as reagent. The corresponding aldehyde **5** was obtained after reduction of the methyl ester moiety of **4** with diisobutylaluminium hydride (DIBAH) as reagent. Treatment with *O*-alkylhydroxylamine derivatives gave the oxime derivatives **6a**–**b**. Deprotection by hydrogenation gave the diamino derivatives **7a**–**b**. Ring closure of **7a**–**b**, using trimethylorthoformate as reagent, gave the desired 1,4,5,6-tetrahydro-5-pyrimidine carboxaldehyde *O*-alkyloximes **8a** and **8b**, respectively, either as pure E-isomers or Z/E mixtures.

After heating the Z/E-mixtures in ethanol, an equilibrium ratio which was in favor of the E-isomer was observed (for details, see Experimental). The mixtures, which were not separable by HPLC, were tested.

This synthetic route turned out to be rather laborious. Consequently, a new, more efficient route was developed. Ring closure of **3** using trimethylorthoformate as reagent gave methyl 1,4,5,6-tetrahydro-5-pyrimidine carboxylate (**9**). Treatment of this methyl ester **9** with diisobutylaluminium hydride, at −78 °C gave 1,4,5,6-tetrahydro-5-pyrimidine carboxaldehyde (**10**). In the final step, the aldehyde was converted in situ at −20 °C into the desired *O*-ethyl oxime derivative **8c**.

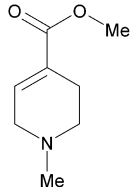
Synthesis of 1,4,5,6-tetrahydro-4-pyrimidine carboxaldehyde oximes and 4,5,6,7-tetrahydro-1H-1,3-diazepine-4-carboxaldehyde oximes, 14a–j

The route for the synthesis of the desired 4-carboxaldehyde derivatives started with the ringclosure reaction of the commercial available α-amino acid derivatives **11a** and **11b** using trimethylorthoformate as reagent (Scheme 2). Esterification with thionylchloride in methanol gave the corresponding methyl esters **12a**–**b**. Subsequently, reduction using diisobutylaluminium hydride, at −78 °C as reagent, as described above, gave 1,4,5,6-tetrahydro-4-pyrimidine carboxaldehyde (**13a**) and the corresponding 4,5,6,7-tetrahydro-1H-1,3-diazepine-4-carboxaldehyde (**13b**). Treatment of these aldehydes in situ with *O*-alkyl hydroxylamine derivatives gave the desired oximes **14a**–**j** as Z/E mixtures (approx. 1:1).

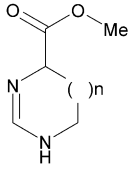


Arecoline

9



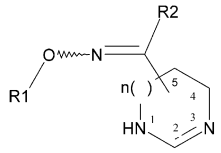
Isoarecoline



12 a, n=1
12 b, n=2

Figure 2.

Table 1. Physical properties 1,3-diazacycloalkyl carboxaldehyde oxime derivatives



Compound	Position	*n*	R_1	R_2	Salt[a]	Molecular formula	Z/E ratio
8a	5	1	Methyl	H	HCl	$C_6H_{11}N_3O{\cdot}HCl$	1:4
8b	5	1	*sec*-Propyl	H	HCl	$C_8H_{15}N_3O{\cdot}HCl$	0:1
8c	5	1	Ethyl	H	HCl	$C_7H_{13}N_3O{\cdot}HCl$	0:1
14a	4	1	H	H	HCl	$C_5H_9N_3O{\cdot}HCl$	0:1
14b	4	1	Methyl	H	HCl	$C_6H_{11}N_3O{\cdot}HCl$	0:1
14c	4	1	Ethyl	H	HCl	$C_7H_{13}N_3O{\cdot}HCl$	2:5
14d	4	1	2-Propynyl	H	HCl	$C_8H_{11}N_3O{\cdot}HCl$	3:7
14e	4	1	*sec*-Propyl	H	HCl	$C_8H_{15}N_3O{\cdot}HCl$	0:1
14f	4	1	(Z)-2-Butenyl	H	HCl	$C_9H_{15}N_3O{\cdot}HCl$	1:10
14g	4	1	*tert*-Butyl	H	mal	$C_9H_{17}N_3O{\cdot}C_4H_4O_4$	1:5
14h	4	1	2-Hexenyl	H	HCl	$C_{11}H_{17}N_3O{\cdot}HCl$	1:5
14i	4	2	H	H	HCl	$C_6H_{11}N_3O{\cdot}HCl$	1:12
14j	4	2	Methyl	H	HCl	$C_7H_{13}N_3O{\cdot}C_4H_4O_4$	1:14
20a	5	1	Methyl	Me	mal	$C_7H_{13}N_3O{\cdot}C_4H_4O_4$	0:1
20b	5	1	Ethyl	Me	mal	$C_8H_{15}N_3O{\cdot}C_4H_4O_4$	0:1
20c	5	1	2-Propynyl	Me	mal	$C_9H_{13}N_3O{\cdot}C_4H_4O_4$	0:1
20d	5	1	(E)-3-Methyl-2-penten-4-ynyl	Me	mal	$C_{12}H_{17}N_3O{\cdot}C_4H_4O_4$	0:1
20e	5	1	H	Me	HCl	$C_6H_{11}N_3O{\cdot}HCl$	0:1

[a]mal, maleic acid.

After heating the Z/E mixtures in ethanol an equilibrium ratio, which was in favor of the E-isomer, was observed (see Experimental).

Synthesis of 1-(1,4,5,6-tetrahydro-5-pyrimidine)ethanone *O*-alkyloximes, 20a–d. For the synthesis of the 1-(1,4,5,6-tetrahydro-5-pyrimidine)ethanone oxime derivatives the following strategy was selected (Scheme 3). Reaction of ethyl acetoacetate **15** with *N*-(hydroxymethyl)-phtalimide **16** in concentrated sulfuric acid gave 2-acetyl-1,3-diphtaloylpropane (**17**).[23] Treatment of the acetyl derivative **17** with the desired *O*-alkylhydroxylamine derivatives gave the corresponding (E)-oximes **18a–e**. Deprotection of both amino functions using hydroxylamine in the presence of sodium methanolate as reagent gave the diamino derivatives **19a–e**. Finally, treatment of **19a–e** with trimethylorthoformate gave the desired 1-(1,4,5,6-tetrahydro-5-pyrimidine)ethanone *O*-alkyloximes **20a–e**.

Pharmacology

Results

The physical properties of the compounds are shown in Table 1. The present series of muscarinic cholinergic ligands was evaluated for potential muscarinic cholinergic agonistic properties by assessing the PZ/OXO-M binding ratio. This ratio of agonist and antagonist binding affinities provides an indication of potential muscarinic cholinergic agonistic properties.[17e] Together with the methyl esters **9**, **12a** and **12b**, which are isosters of either arecoline or isoarecoline, the four *O*-methyloxime derivatives **8a**, **14b**, **14j** and **20a** shared a relatively high affinity in the OXO-M binding with a low affinity in the PZ binding and have OXO-M/PZ binding ratios exceeding 100. Several of these compounds were tested in the three functional in vitro models and displayed muscarinic agonistic activity: **8a**, **12b**, **14b**, **14j**, **20a**. In contrast, only modest affinities were found in the OXO-M binding tests for the OH-oxime derivatives **14a**, **14i**, **20e** with OXO-M/PZ ratios below 30; weak antagonistic properties were found for these compounds

Table 2. Receptor binding affinity and affinity ratios for assays using agonist, oxotremorine-M (OXO-M), and antagonist pirenzepine (PZ) ligands[a]

Compound	^{3}H-OXO-M (K_i in μM)	^{3}H-PZ (K_i in μM)	Ratio
Arecoline	8.1	5.7	250
Isoarecoline	7.3	3.3	10,000
9	7.4	4.1	2000
12a	6.3	3.9	225
12b	7.2	4.4	600
8a	8.1	4.6	3000
8b	6.9	5.7	15
8c	7.9	5.2	500
14a	5.6	4.4	15
14b	8.3	4.5	6000
14c	7.7	5.2	300
14d	7.9	5.3	400
14e	7.4	5.7	50
14f	8.1	5.8	200
14g	7.1	5.8	20
14h	7.3	5.8	30
14i	6.1	5.1	10
14j	7.2	5.1	120
20a	7.5	4.7	600
20b	6.7	5.8	8
20c	6.9	6.1	6
20d	6.3	6.0	2
20e	5.5	5.1	2.5

[a]Data are mean values of 2–4 binding inhibition experiments. For further details, see Experimental.

Table 3. Muscarinic cholinergic activity in guinea pig ileum (M_3), rat left atrium (M_2) and hippocampus (M_1)

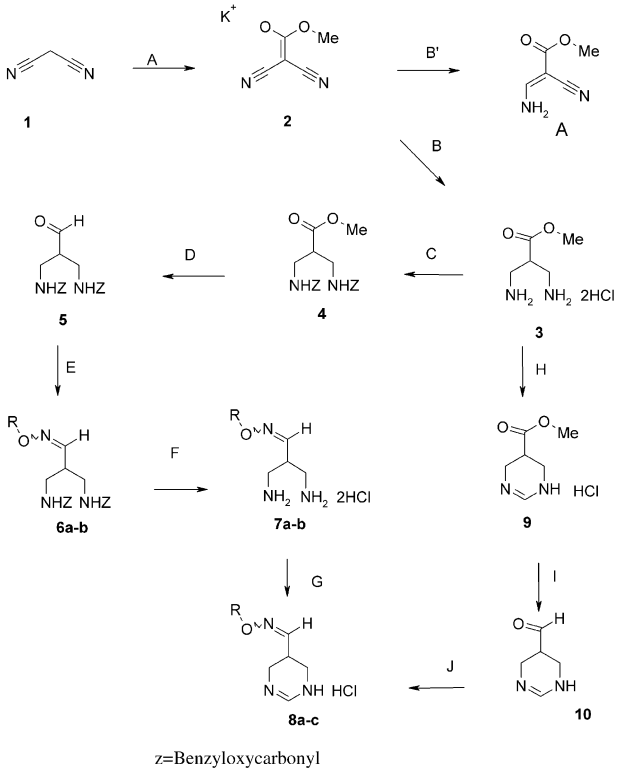
Compound	M_3 (ileum)			M_2 (left atrium)			M_1 (hippocampus)		
	pD_2	α	pA_2	pD_2	α	pA_2	pD_2	α	pA_2
Arecoline	6.5	1.0	—	6.9	1.0	—	5.4	0.72	—
Isoarecoline	5.0	1.3	—	—	—	—	—	—	—
9	5.8	1.1	—	6.1	1.0	—	4.7	0.96	—
12a	nt								
12b	5.2	1.0	—	5.1	1.0	—	4.5	0.49	—
8a	nt			nt			5.0	0.9	—
8b	—		—	4.6	—	—	5.0	<4.0	0
8c	5.6	1.0	—	5.6	1.0	—	4.7	0.77	—
14b	5.6	1.3	—	5.6	1.0	—	4.4	0.92	—
14c	5.6	1.7	—	5.7	1.0	—	4.6	0.75	—
14d	5.9	1.0	—	5.9	1.0	—	5.3	0.9	—
14e	5.1	1.1	—	—	—	5.1	5.3	0.15	—
14f	5.1	0.6	—	—	—	5.3	5.6	0.07	—
14g	—	—	4.6		—	5.5	<3.5	0	—
14h	4.8	0.6	—	—	—	5.1	4.8	0.11	—
14j	5.3	1.2	—	5.7	1.0	—	4.0	0.6	—
20a	5.4	1.3	—	5.4	0.8	—	4.3	0.8	—

nt, not tested; pD_2, agonist values; α, intrinsic activity; pA_2, antagonist values.

(data not shown). Elongation of the *O*-substituted side chain was studied further in series of compounds **14**. The methyl, ethyl and propargyl oxime derivatives (**14b**, **14c**, **14d**) showed OXO-M/PZ binding ratios of 300–6000 in agreement with their full agonist properties for all three muscarinic subtypes. With the compounds where R_1 is s-propyl, (Z)-2-butenyl or 2-hexenyl **14e**, **14f**, **14h** exhibited intermediate OXO-M/PZ binding ratio of approximately 20–50 and displayed differential agonistic/antagonistic properties for the three subtypes. All three compounds showed M_2 antagonistic properties and displayed M_1 agonistic properties with a low intrinsic activity ($\alpha = 0.1$). In contrast, these compounds remained quite active on M_3 receptors with intrinsic activities between 0.6 and 1.1. In these functional models, the compounds **14e**, **14f** and **14h** showed equal affinity or slight preference for the M_1 receptor subtype in contrast with the other analogues **14b**, **14c**, **14d**. For the latter three compounds, the pD_2 values for M_1 were found to be 4- to 15-fold lower for M_1 as compared to M_2 or M_3. In agreement with these observations **14e** and **14f** showed a 15-fold higher affinity in the PZ binding test than **14b**. Only the oxime derivative **14g**, having a bulky *t*-butyl substituent with a low OXO-M/PZ binding ratio of 20, lacked agonistic properties for all three subtypes and instead exhibited antagonistic properties.

Discussion

The present series of 1,3-diazacycloalkyl carboxaldehyde oxime derivatives was compared to the parent compound arecoline in and evaluated for potential muscarinic cholinergic agonistic properties by measuring their affinity in OXOM and PZ binding tests. The PZ/OXO-M binding ratio gives an acccurate prediction of potential muscarinic agonistic properties.[17] This was shown by their characterization in functional models revealing muscarinic subtype related agonistic activities. Indeed OXOM/PZ ratios of 300 or higher are associated with full muscarinic agonistic properties for all three muscarinic cholinergic subtypes, while ratios of 30 or lower predict muscarinic cholinerergic antagonistic properties. Compounds showing intermediate ratios in between 30 and 300 showed partial agonistic properties with agonistic properties being most pronounced for the M_3 subtype. While these compounds shared a slight preference for M_1 receptors in functional models, intrinsic activities in the present M_1 model (measuring



Scheme 1. (A) (1) KOH; (2) methylchloroformate; (B) H_2 Pd/C 10%, MeOH/HCl; (C) Z-succinimide, DMF, triethylamine; (D) DIBAH, CH_2Cl_2, −78 °C; (E) *O*-alkyl hydroxylamine HCl, MeOH; (F) H_2, Pd/C 10%, MeOH/HCl; (G) trimethylorthoformate, MeOH; (H) trimethylorthoformate, MeOH; (I) DIBAH, CH_2Cl_2, −78 °C; (J) *O*-alkyl hydroxylamine HCl, MeOH.

M_1 mediated changes in hippocampal cell firing) are rather low. However, in this series, the slight preference for the M_1 receptor subtype and the M_2-antagonistic properties are lost when the intrinsic activity becomes high as for **8b**, **12b**, **14b** and **14d**.

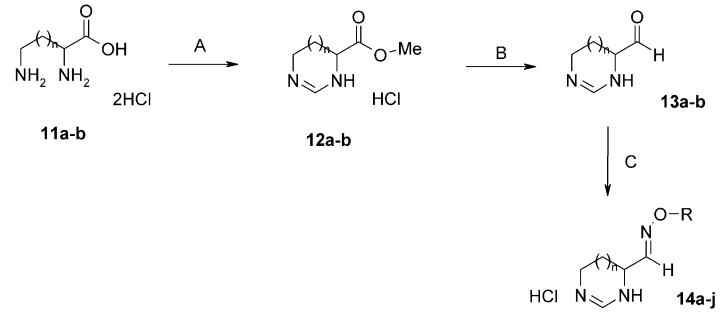
In conclusion, compounds **14e**, **14f** and **14h** show an improved profile compared to arecoline with a combination of M_2 antagonistic and partial muscarinic M_1 agonistic properties. While their affinity for the M_1 receptor was maintained, the affinity for the M_3 receptor as measured in the ileum was 30-fold reduced and the M_2 agonistic properties were eliminated. However, a satisfactory muscarinic profile with better in vivo potential requires a further increase in M_1/M_3 selectivity and intrinsic M_1 activity. The present series offers an interesting opportunity to develop such a profile.

Experimental

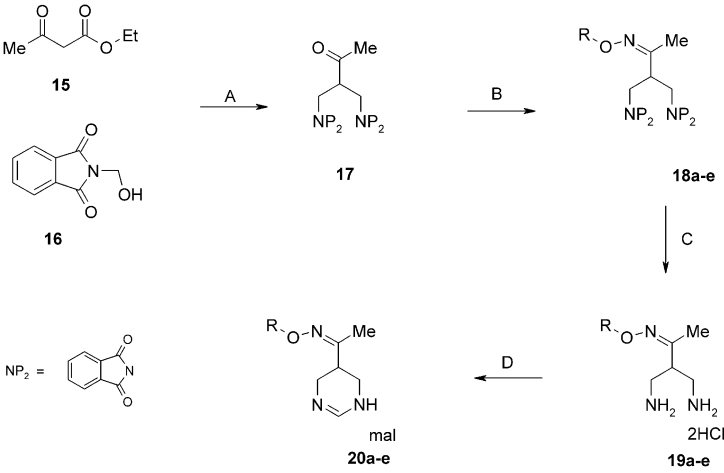
Chemistry

Melting points were taken on a Büchi capillary melting point apparatus and are uncorrected. 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 >7500 (10% valley definition) in the positive ion mode using reference masses 369 and 461 from glycerol. Average exact masses were calculated from at least 10 computer-controlled measurements using the bracketing method. Proton magnetic resonance spectra were measured on a Bruker WP200 or AC200 instrument. 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 silicagel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp and Cl_2/tetramethylbenzidine.

Potassium salt of methyl dicyanoacetate (2). A solution of methylchloroformate **1** (49.5 g, 0.53 mol) and malodinitrile (33 g, 0.5 mol) in 75 mL THF was added in 30 min, with vigorous stirring, to a solution of potassium hydroxide (56.1 g, 1.0 mol) in 50 mL water, keeping the temperature below 40 °C. After 2 h stirring at room temperature, the reaction mixture was cooled to 0 °C. The precipitate was filtered off and washed with ice-cold water and ethanol, to give a white crystalline product **2** (68 g, 0.42 mol, 84%). ^{1}H NMR (D_2O, 200 MHz) δ 3.65 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 176.5 (s, C=O), 125.9 (s, 2 CN), 54.6 (q, CH_3).



Scheme 2. (A) (1) Trimethylorthoformate, MeOH, 18 h reflux; (2) $SOCl_2$, MeOH 3 h reflux; (B) DIBAH, CH_2Cl_2, −78 °C; (C) (1) *O*-alkyl hydroxylamine HCl, MeOH; (2) ethanol reflux.



Scheme 3. (A) Concd H_2SO_4, 24 h rt; (B) *O*-alkyl hydroxylamine HCl; (C) sodium methanolate, hydroxylamine HCl, 3 h rt; (D) (1) trimethylorthoformate, MeOH; (2) NaOH; (3) maleic acid.

Methyl 3-amino-2-(aminomethyl)propionate dihydrochloride (3). A suspension of **2** (12.8 g, 80 mmol) and 26 g 10% Pd/C in 4 L methanol (dry) and 3 equivalents of HCl in methanol was treated with hydrogen. After 24 h, the catalyst was filtered off and the solvent was removed in vacuo. Recrystallization of the crude product from methanol/ethyl acetate gave **3** (9.5 g, 46 mmol, 58%). Mp 178 °C. ^{1}H NMR (D_2O, 200 MHz) δ 3.85 (s, 3H), 3.50–3.20 (m, 5H). ^{13}C NMR (D_2O, 50 MHz) δ 174.5 (s, C=O), 56.4 (q, OCH_3), 43.2 (d, CH), 41.1 (t, 4H, CH_2's).

(Z:E 1:4) 1,4,5,6-Tetrahydro-5-pyrimidinecarboxaldehyde *O*-methyloxime monohydrochloride (8a). A solution of **3** (9.1 g, 44.6 mmol) in dry DMF (250 mL) and triethylamine (12.5 mL, 89.2 mmol) was stirred for 20 min. Then triethylamine (12.5 mL, 89.2 mmol) was added followed by *N*-(benzyloxycarbonyloxy)-succinimide (22.2 g, 89.2 mmol) while keeping the temperature at 20–25 °C. After stirring the reaction mixture at room temperature for 5 h, the precipitate was removed by filtration and the filtrate was evaporated. The residue was partitioned between water and ethyl acetate. The organic layer was washed twice with small portions of water. The dried ($MgSO_4$) organic phase was evaporated in vacuo. The residue was purified by column chromatography on neutral Al_2O_3 using toluene/ethyl acetate (8/2 v/v) as eluent, to give methyl *N*,*N*′-(dibenzyloxycarbonyl)-3-amino-2-(aminomethyl)-propionate **4** (11.0 g, 27.5 mmol, 62%) as an oil.

DIBAH (20.5 mmol, (17.1 mL of a 1.2 M solution in toluene) was added to a cooled (−70 °C) solution of **4** (6.8 g, 17.1 mmol) in dry methylene chloride (50 mL). The reaction mixture was stirred for 3 h and then quenched with methanol (3 mL). The mixture was allowed to warm to room temperature, after which water (20 mL) was added. The mixture was filtered and the organic phase was separated and subsequently dried ($MgSO_4$). The solvents were evaporated in vacuo to give a residue (7.45 g) containing a mixture of *N*,*N*′-(dibenzyloxycarbonyl)-3-amino2-(aminomethyl)propionaldehyde **5** and starting material **4** (30%). Methoxylamine hydrochloride (790 mg, 9.5 mmol) was added to a solution of **5** (3.7 g, crude) in dry methanol (125 mL). After stirring of the reaction mixture for 3.5 h at 65 °C the solvent was evaporated in vacuo. Ethyl acetate was added to the residue. Residual methoxylamine HCl was removed by filtration. The filtrate was evaporated in vacuo to give a residue which was chromatographed on silica (toluene/ethyl acetate 8:2, v/v%) to give *N*,*N*′-(dibenzyloxycarbonyl)-3-amino-2-methylaminopropionaldehyde *O*-methyloxime **6a** (390 mg, 0.98 mmol, 47%) as a 1:4 Z/E isomer. Hydrogen was passed through a solution of **6a** (350 mg, 0.88 mmol) in dry methanol (15 mL) containing 2 equivalents of hydrochloric acid and Pd/C 10%. After 4 h, the reaction mixture was filtered and the filtrate was evaporated in vacuo to give 3-amino-2-methylamino-propionaldehyde *O*-methyloxime dihydrochloride: **7a** in quantitative yield. A solution of **7a** (170 mg, 0.84 mmol) and trimethylorthoformate (10 mL) in dry methanol (25 mL) was refluxed for 24 h. The mixture was evaporated to dryness (120 mg, 0.68 mmol, 80%) as a 1:4 Z/E isomer. ^{1}H NMR (CD_3OD) δ 3.0 (m, 1H), 3.3–3.6 (m, 4H), 3.8 (s, 3H, E-isomer), 3.9 (s, 3H, Z-isomer), 6.65 (d, 1H, Z-isomer), 7.4 (d, 1H (E-isomer)), 8.0 (s, 1H).

The following compounds were prepared in a similar manner as described above.

(E)-1,4,5,6-Tetrahydro-5-pyrimidine carboxaldehyde *O*-(1-methylethyl)oxime monohydrochloride (8b). Yield 27%. Mp 133 °C. ^{1}H NMR (CD_3OD) δ 1.2 (d, 6H), 3.0 (m, 1H), 3.4–3.7 (m, 4H), 4.3 (m, 1H), 7.35 (d, 1H), 8.0 (s, 1H). Exact mass calcd for $[M+H]^+$ 170.1293, found 170.1289.

Methyl-1,4,5,6-tetrahydro-5-pyrimidinecarboxylate monohydrochloride (9). A solution of **3** (8.68 g, 42.3 mmol) and trimethylorthoformate (50 mL) in dry methanol (250 mL) was refluxed for 18 h and evaporated to dryness. Crystallization from methanol/ethyl acetate gave **9** (5.5 g, 31 mmol, 73%) as a yellow solid. Mp 171 °C. ^{1}H NMR (D_2O, 200 MHz) δ 7.95 (s, 1H), 3.80 (s, 3H), 3.70 (d, 4H), 3.40–3.25 (m, 1H). ^{13}C NMR (D_2O, 50 MHz) δ 175.3 (s, C=O) 154.2 (N=C–N), 55.9 (q, CH_3), 41.9 (t, 4H, CH_2's), 36.7 (d, CH)

1,4,5,6-Tetrahydro-5-pyrimidinecarboxaldehyde (10). To a cooled (−78 °C) suspension of **9** (1.5 g, 8.40 mmol) in dry methylene chloride (100 mL) 2.5 equivalents of cold (−78 °C) DIBAH (21 mL, 21 mmol of a 1 M solution of DIBAH in methylene chloride) was added dropwise. The reaction mixture was stirred for 3 h and quenched with cold (−78 °C) methanol (5 mL). Then water (3 mL) was added followed by methanol (20 mL), the reaction mixture was allowed to warm to −20 °C to give the crude aldehyde **10**, which was used directly for the following reaction step.

(E)-1,4,5,6-Tetrahydro-5-pyrimidine carboxaldehyde *O*-ethyloxime monohydrochloride (8c). To aldehyde **10** (8.40 mmol), *O*-ethyl hydroxylamine (819 mg, 8.40 mmol) was added at −20 °C. The reaction mixture was stirred for 20 h at room temperature. Then the aluminum salts were filtered and the filtrate was evaporated.The crude mixture contained a small amount of methylester **9**, which was removed by treatment with sodiumhydroxide in water. Subsequent extraction with methylene chloride and treatment with methanolic hydrochloride afforded **8c** (500 mg, 2.6 mmol, 27%). Mp 139 °C. ^{1}H NMR (CD_3OD) δ 1.25 (t, 3H), 3.0 (m, 1H), 3.4–3.7 (m, 4H), 4.1 (q, 2H), 7.4 (d, 1H), 8.05 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.8, 147.5, 70.5, 41.4, 30.7, 14.8. Exact mass calcd. for $[M+H]^+$ 156.1137, found 156.1108.

Methyl-1,4,5,6-tetrahydro-4-pyrimidinecarboxylate monohydrochloride (12a). A solution of DL-2,4-diaminobutyric acid 2HCl **11a** (49.7 g, 0.26 mol) and trimethylorthoformate (114 mL, 1.04 mol) in dry methanol (500 mL) was refluxed. The crude compound was dissolved in dry methanol (500 mL) and cooled to 0 °C. Thionylchloride (37.1 g, 0.31 mol) was added dropwise, then the solution was refluxed for 3 h and evaporated to dryness. Toluene

was added and the suspension was evaporated to dryness. The product **12a** (46.4 g, 0.26 mol, 100%) was obtained as a white solid. ^{1}H NMR (CD_3OD) δ 2.1–2.4 (m, 2H), 3.3–3.6 (m, 2H), 3.8 (s, 3H), 4.4 (m, 1H), 8.1 (s, 1H).

1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde (13a). To a cooled (−78 °C) suspension of **12a** (2.0 g, 11.2 mmol) in dry methylene chloride (125 mL) 2.5 equivalents of cold (−78 °C) DIBAH (28 mL, 28 mmol of a 1 M solution of DiBA1H in methylene chloride) was added dropwise. The reaction mixture was stirred for 3 h and then quenched with cold (−78 °C) methanol (5 mL). Then water (3 mL) was added followed by methanol (20 mL), the reaction mixture was allowed to warm to −20 °C. to give the crude product **13a**.

(E)-1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-methyloxime monohydrochloride (14b). The cold (−20 °C) solution of **13a** (11.2 mmol) was added to a solution of methoxylamine HCl (940 mg, 11.2 mmol) in methanol (25 mL). The resulting reaction mixture was stirred for 20 h at room temperature. Then the aluminum salts were filtered off and the filtrate was evaporated to dryness to give **14b** (2.0 g, 100%) as a 1/1 Z/E-mixture. A solution of **14b** (2.0 g, 11.2 mmol) in ethanol was heated to reflux for 20 h and evaporated to dryness. Crystallization from methanol/ethyl acetate gave **14b** (1.22 g, 7.42 mmol, 61%) as pure E-isomer. Mp 149 °C. ^{1}H NMR (CD_3OD) δ 1.9–2.3 (m, 2H), 3.45 (m, 2H), 3.85 (s, 3H), 4.35 (m, 1H), 7.45 (d, 1H), 8.1 s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.8, 147.9, 62.5, 37.3, 23.0. Exact mass calcd. for $[M+H]^+$ 142.0980, found 142.0994.

The following compounds were prepared in a similar manner as described above.

(E)-1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde oxime monohydrochloride (14a). Mp 174 °C. Yield 48%. ^{1}H NMR (CD_3OD) δ 1.9–2.3 (m, 2H), 3.35–3.6 (m, 2H), 4.45 (m, 1H), 7.4 (d, 1H), 8.05 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.8, 147.6, 37.4, 23.2. Exact mass calcd. for $[M+H]^+$ 128.0824, found 128.0820.

(Z:E 2:5) 1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-ethyloxime monohydrochloride (14c). Yield 73%. Mp 96 °C. ^{1}H NMR (CD_3OD) δ 1.25 (t, 3H), 2.0–2.3 (m, 2H), 3.45 (m, 2H), 4.1 (q, 2H), 4.4 (m, 1H), 6.85 (d, 1H (Z-isomer)), 7.45 (d, 1H (E-isomer)), 8.1 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.8, 148.4, 147.6, 71.4, 70.9, 37.7, 37.3, 23.1, 22.3, 14.8. Exact mass calcd for $[M+H]^+$ 156.1137, found 156.1113.

(Z:E 3:7) 1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-2-propynyloxime monohydrochloride (14d). Yield 50%. Mp 127 °C. ^{1}H NMR (CD_3OD) δ 2.0–2.3 (m, 2H), 3.0 (m, 1H), 3.4–3.6 (m, 2H), 4.4 (m, 1H), 4.65 (d, 2H (E-isomer)), 4.7 (d, 2H (Z-isomer)), 6.95 (d, 1H (Z-isomer)), 7.55 (d, 1H (E-isomer)), 8.1 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.9, 150.2, 149.6, 76.5, 76.2, 63.2, 62.8, 37.5, 37.1, 22.8, 22.3. Exact mass calcd for $[M+H]^+$ 166.0980, found 166.0984.

(E)-1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-(1-methylethyl)oxime monohydrochloride (14e). Yield 70%. Mp 110 °C. ^{1}H NMR (CD_3OD) δ 1.2 (d, 6H), 1.95–2.3 (m, 2H), 3.4–3.6 (m, 2H), 4.3 (m, 1H), 4.35 (m, 1H), 7.4 (d, 1H), 8.1 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 152.8, 147.1, 77.2, 37.3, 23.1, 21.7. Exact mass calcd for $[M+H]^+$ 170.1293, found 170.1252.

(Z:E 1:10) 1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-(2-(Z)-butenyl)oxime monohydrochloride (14f). Yield 70%. Mp 111 °C. ^{1}H NMR (CD_3OD) δ 1.65 (d, 3H), 2.0–2.3 (m, 2H), 3.4–3.6 (m, 2H), 4.45 (m, 1H), 4.6 (d, 2H (E-isomer)), 4.7 (d, 2H (Z-isomer)), 5.5–5.8 (m, 2H), 6.85 (d, 1H (Z-isomer)), 7.45 (d, 1H (E-isomer)), 8.05 (s, 1H). Exact mass calcd for $[M+H]^+$ 182.1293, found 182.1269.

(Z:E 1:5) 1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-(1,1-dimethylethyl)oxime (Z)-2-butenedioate (14g). Yield 15%. Mp 122 °C. ^{1}H NMR (CD_3OD) δ 1.25 (s, 9H (E-isomer)), 1.3 (s, 9H (Z-isomer)), 2.0–2.3 (m, 2H), 3.5 (m, 2H), 4.4 (m, 1H), 6.25 (s, 2H), 6.8 (d, 1H (Z-isomer)), 7.4 (d, 1H (E-isomer)), 8.05 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 170.8, 152.8, 146.5, 136.8, 80.3, 37.3, 27.7, 23.0. Exact mass calcd for $[M+H]^+$ 184.1450, found 184.1403.

(Z:E 1:5) 1,4,5,6-Tetrahydro-4-pyrimidinecarboxaldehyde *O*-(2-hexynyl)oxime monohydrochloride (14h). Yield 37% oil. ^{1}H NMR (CD_3OD) δ 1.0 (t, 3H), 1.5 (m, 2H), 2.0–2.3 (m, 4H), 3.5 (m, 2H), 4.4 (m, 1H), 4.65 (m, 2H (E-isomer)), 4.7 (m, 2H (Z-isomer)), 6.9 (d, 1H (Z-isomer)), 7.5 (d, 1H (E-isomer)), 8.05 (s, 1H). Exact mass calcd for $[M+H]^+$ 208.1449, found 208.1437.

Methyl-4,5,6,7-tetrahydro-1H-1,3-diazepine-4-carboxylate (12b). A solution of DL-ornithine HCl **11b** (30.4 g, 0.18 mol) and trimethylorthoformate (59 mL, 0.54 mol) in dry methanol (600 mL) was refluxed. After evaporation the crude compound was dissolved in dry methanol (400 mL) and cooled (0 °C). Thionylchloride (53.7 g, 0.45 mol) was added dropwise, then the solution was refluxed for 3 h and evaporated to dryness. Toluene was added and the suspension was evaporated to dryness. The product **2b** (27.5 g, 0.14 mol, 79%) was obtained as a white solid. ^{1}H NMR (CD_3OD) δ 1.9–2.1 (m, 2H), 2.2–2.4 (m, 2H), 3.4–3.7 (m, 2H), 3.75 (s, 3H), 4.65 (m, 1H), 7.8 (s, 1H).

Methyl-4,5,6,7-tetrahydro-1H-1,3-diazepine-4-carboxaldehyde (13b). To a cooled (−78 °C) suspension of **12b** (2.0 g, 10.4 mmol) in dry methylene chloride (125 mL) 2.5 equivalents of cold (−78 °C) DiBA1H (26 mL, 26 mmol of a 1 M solution of DIBAH in methylene chloride) was added dropwise. The reaction mixture was stirred for 3 h and then quenched with cold (−78 °C) methanol (5 mL). Then water (3 mL) was added followed by methanol (20 mL), the reaction mixture was allowed to warm to −20 °C. to give the crude product **13b**.

4,5,6,7-Tetrahydro-1H-1,3-diazepine-4-carboxaldehyde oxime monohydrochloride (14i). The cold (−20 °C) solution of **13b** (10.4 mmol) was added to a solution of

hydroxylamine HCl (723 mg, 10.4 mmol) in methanol (25 mL). The resulting reaction mixture was stirred for 20 h at room temperature. Then the aluminum salts were filtered off and the filtrate was evaporated to dryness to give **14i** (1.7 g, 92%) as a 1/3 Z/E-mixture. A solution of **14i** (1.7 g, 9.6 mmol) in ethanol was heated to reflux for 20 h and evaporated to dryness. Crystallization from ethanol/ethyl acetate gave **14i** (320 mg, 1.8 mmol, 18%) as a 1/12 Z/E mixture.

Mp 173 °C. ^{1}H NMR (CD_3OD) δ 1.8–2.4 (m, 4H), 3.4 (m, 1H), 3.7 (m, 1H), 4.5 (m, 1H), 6.8 (d, 1H (Z-isomer)), 7.4 (d, 1H (E-isomer)), 7.65 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 154.8, 147.7, 57.4, 46.8, 32.0, 25.5, 17.4. Exact mass calcd for $[M+H]^+$ 142.0980, found 142.0992.

The following compounds were prepared in a similar manner as described above.

4,5,6,7-Tetrahydro-1H-1,3-diazepine-4-carboxaldehyde *O*-methyloxime monohydrochloride (14j). Yield 35%. Mp 126 °C. ^{1}H NMR (CD_3OD) δ 1.8–2.3 (m, 4H), 3.4 (m, 1H), 3.7 (m, 1H), 3.8 (s, 3H (E-isomer)), 3.9 (s, 3H (Z-isomer)), 4.5 (m, 1H), 6.85 (d, 1H (Z-isomer)), 7.4 (d, 1H (E-isomer)), 7.65 (s, 1H). Exact mass calcd for $[M+H]^+$ 156.1137, found 156.1109.

2-Acetyl-1,3-diphtaloylpropane (17). Ethylacetoacetate **15** (14.7 g, 113 mmol) was added to cooled (0 °C) concentrated sulfuric acid (240 mL), then *N*-(hydroxymethyl)phtalimide **16** (40 g, 226 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. The dark-red solution was poured on ice-water, the yellow solid was filtered and washed with water. The crude solid was stirred in hot acetone and after cooling collected by filtration to give **17** (32 g, 85 mmol, 75%; yellow solid). Mp 170 °C. ^{1}H NMR ($CDC1_3$) δ 2.35 (s, 3H), 3.65 (m, 1H), 3.8–4.2 (m, 4H), 7.6–7.9 (m, 8H).

3-(Methylphtaloyl)-4-phtaloyl-2-butanone *O*-methyloxime (18a). To a solution of **17** (8.0 g, 21.3 mmol) in methylene chloride (120 mL) methoxylamine HC1 (1.78 g, 21.3 mmol) and methanol (80 mL) were added. The reaction mixture was stirred for 60 h at room temperature and then evaporated to dryness. The crude solid was stirred in hot acetone and after cooling collected by filtration to give **18a** (5.7 g, 14 mmol, 66%) as a yellow solid. ^{1}H NMR ($CDC1_3$) δ 1.9 (s, 3H), 3,3 (m, 1H), 3.5 (s, 3H), 3.7–4.0 (m, 4H), 7.6–7.9 (m, 8H).

The following compounds were prepared in a similar manner as described above.

3-(Methylphtaloyl)-4-phtaloyl-2-butanone *O*-ethyloxime (18b). Yield 65%. ^{1}H NMR ($CDC1_3$) δ 0.9 (t,3H) 1.9 (s, 3H), 3.3 (m, 1H), 3.8 (q, 2H), 3.7–4.0 (m, 4H), 7.6–7.9 (m, 8H).

3-(Methylphtaloyl)-4-phtaloyl-2-butanone 0-2-propynyloxime (18c). Yield 70%. ^{1}H NMR ($CDC1_3$) δ 1.8 (m, 1H), 1.95 (s, 3H), 3.3 (m, 1H), 3.7–4.0 (m, 4H), 4.4 (m, 2H), 7.6–7.9 (m, 8H).

3-(Methylphtaloyl)-4-phtaloyl-2-butanone *O*-(E)-3-methyl-2-penten-4-ynyloxime (18d). Yield 70%. ^{1}H NMR ($CDC1_3$) δ 1.65 (s, 3H), 1.9 (s, 3H), 3.3 (m, 1H), 3.7–4.0 (m, 4H), 4.35 (d, 2H), 5.8 (m, 1H), 7.6–7.9 (m, 8H).

3-(Methylphtaloyl)-4-phtaloyl-2-butanone oxime (18e). Yield 100% (crude).

4-Amino-3-(methylamino)-2-butanone *O*-methyloxime (19a). To a solution of methanol (100 mL) sodium (1.38 g, 60 mmol) and subsequently hydroxylamine HC1 (1.95 g, 28 mmol) were added. After stirring for 15 min, **18a** (5.67 g, 14 mmol) dissolved in dry methylene chloride (150 mL) was added. The dark-red solution was stirred for 3 h at room temperature. A methanolic hydrochloride solution (60 mmol, 32 mL of a 1.9 M solution of hydrochloride in methanol) was added to the reaction mixture. Sodium chloride was filtered from the reaction mixture and the filtrate was evaporated to dryness. Crystallization from methanol/ethyl acetate gave **19a** (2.2 g, 10.1 mmol, 72%) as a yellow solid. ^{1}H NMR (CD_3OD) δ 1.95 (s, 3H), 3.0 (m, 1H), 3.1–3.3 (m, 4H), 3.9 (s, 3H).

The following compounds were prepared in a similar manner as described above.

4-Amino-3-(methylamino)-2-butanone *O*-ethyloxime (19b). Yield 100% (crude). ^{1}H NMR (CD_3OD) δ 1.3 (t, 3H), 1.95 (s, 3H), 3.0 (m, 1H), 3.1–3.3 (m, 4H), 4.2 (q, 2H).

4-Amino-3-(methylamino)-2-butanone *O*-2-propynyloxime (19c). Yield 100% (crude). ^{1}H NMR (CD_3OD) δ 1.95 (s, 3H), 2.9 (m, 1H), 3.0 (m, 1H), 3.1–3.3 (m, 4H), 4.7 (m, 2H).

4-Amino-3-(methylamino)-2-butanone *O*-(E)-3-methyl-2-penten-4-ynyloxime (19d). Yield 100% (crude). ^{1}H NMR (CD_3OD) δ 1.8 (s, 3H) 1.95 (s, 3H), 3.1 (m, 1H), 3.1–3.3 (m, 4H), 4.75 (d, 2H), 6.1 (m, 1H).

4-Amino-3-(methylamino)-2-butanone oxime (19e). Yield 100% (crude).

(E)-1-(1,4,5,6-Tetrahydro-5-pyrimidine)ethanone *O*-methyloxime (20a). A solution of **19a** (2.2 g, 10.1 mmol) and trimethylorthoformate (15 mL) in dry methanol (60 mL) was refluxed for 20 h and concentrated to dryness. The residue was partitioned between an aqueous sodiumhydroxide brine solution (5 mL) and methylene chloride (100 mL) and then the organic layer was concentrated. To a solution of the free base of **20a** (960 mg, 6.2 mmol) in methanol maleic acid (719 mg, 6.2 mmol) was added. Crystallization from methanol/ether gave **20a** (910 mg, 3.3 mmol 33%) as a solid. Mp 138 °C. ^{1}H NMR (CD_3OD) δ 1.9 (s, 3H), 2.9 (m, 1H), 3.4–3.7 (m, 4H), 3.85 (s, 3H), 6.25 (s, 2H), 8.0 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 170.8, 154.5, 152.6, 136.6, 62.1, 41.6, 35.6, 13.4. Exact mass calcd for $[M+H]^+$ 156.1137, found 156.1112.

The following compounds were prepared in a similar manner as described above.

(E)-1-(1,4,5,6-Tetrahydro-5-pyrimidine)ethanone *O*-ethyloxime (Z)-2-butenedioate (20b). Mp 137 °C. ^{1}H NMR (CD_3OD) δ 1.2 (t, 3H), 1.9 (s, 3H), 2.85 (m, 1H), 3.4–3.7 (m, 4H), 4.1 (q, 2H), 6.25 (s, 2H), 8.0 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 170.9, 152.6, 136.8, 70.4, 41.6, 35.7, 14.9, 13.5. Exact mass calcd for $[M+H]^+$ 170.1293, found 170.1294.

(E)-1-(1,4,5,6- Tetrahydro-5-pyrimidine)ethanone *O*-(2-propynyl) oxime (Z)-2-butenedioate (20c). Mp 131 °C. ^{1}H NMR (CD_3OD) δ 1.95 (s, 3H), 2.8 (m, 1H), 2.9 (m, 1H), 3.4–3.7 (m, 4H), 4.6 (d, 2H), 6.25 (s, 2H), 8.0 (s, 1H). Exact mass calcd for $[M+H]^+$ 180.1137, found 180.1104.

(E)-1-(1,4,5,6-Tetrahydro-5-pyrimidine)ethanone *O*-(E)-3-methyl-2-penten-4-ynyloxime (Z)-2-butenedioate (20d). Mp 125 °C. ^{1}H NMR (CD_3OD) δ 1.85 (s, 3H), 1.95 (s, 3H), 2.9 (m, 1H), 3.4–3.8 (m, 4H), 4.6 (d, 2H), 6.0 (m, 1H), 6.25 (s, 2H), 8.0 (s, 1H). ^{13}C NMR (MeOD, 50 MHz) δ 170.8, 155.3, 152.6, 136.7, 134.9, 77.0, 70.6, 41.6, 35.7, 17.8, 13.5. Exact mass calcd for $[M+H]^+$ 220.1450, found 220.1440.

(E)-1-(1,4,5,6-Tetrahydro-5-pyrimidine)ethanone oxime monohydrochloride (20e). Mp 225 °C. ^{1}H NMR (CD_3OD) δ 1.9 (s, 3H), 2.85 (m, 1H), 3.4–3.7 (m, 4H), 8.0 (s, 1H). Exact mass calcd for $[M+H]^+$ 142.0980, found 142.0989.

In Vitro Studies

Agonist and antagonist binding 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.[24] 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. Non-specific binding was defined as the amount of binding of [^{3}H]-OXO-M in the presence of 2 mM atropine sulfate 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.[21] 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. Non-specific binding was defined as the amount of binding of [^{3}H]-PZ in the presence of 1 mM atropine sulfate 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_{50} values by using the Chang–Prusoff equation $K_i = IC_{50}/(1 + C/K_d)$ in which C 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.[25] 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.[22] Carbachol (3E-5 mol/L) causes a 100% decrease (intrinsic activity = 1.0) in the amplitude of the electrically evoked fEPSP, an effect that can be fully antagonized by the M_1 selective antagonist pirenzepine.[26]

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 β_1-adrenoreceptor described previously[27] 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.

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.[23] 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.

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