Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 191–198

BIOORGANIC & MEDICINAL CHEMISTRY

Enzyme-Catalyzed Prodrug Approaches for the Histamine H_3-Receptor Agonist (*R*)-α-Methylhistamine

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Received 30 June 2000; accepted 25 August 2000

Abstract—Five novel prodrug types of the potent and selective histamine H_3-receptor agonist (*R*)-α-methylhistamine (**1**) were prepared and pharmacologically tested in vitro as well as in vivo. In particular, an amide of fatty acid, mono- and dicarbamates, an (acyloxy)alkylcarbamate, and a diphthalidyl derivative were synthesized, all of which require initial prodrug activation through an enzyme-catalyzed reaction in contrast to formerly developed azomethine prodrugs which are cleaved by chemical hydrolysis only. Further drug liberation may ensue spontaneously in a cascade to give **1**. Since they have diverse stabilities the prodrugs were investigated for drug liberation in vitro under neutral, acidic, and basic conditions at different temperatures as well as with liver homogenates. In vivo investigation of prodrugs after oral administration to mice proved that the fatty amide **2**, the N^{α}-methylcarbamate **4a**, and the N^{α}-(1-(acetyloxy)ethylcarbamate) **5** showed moderate to high plasma levels of **1**. Compound **5** displayed even more than 2.5 times the AUC for **1** than that of the reference azomethine prodrug BP2.94 in the periphery and also displayed a detectable drug level in the central nervous system. It was shown that prodrug approaches based on an initial enzyme-catalyzed liberation step are successfully applicable to different pro-moieties for improved bioavailability and prolonged half-live. These approaches may also be used for other aminergic compounds of this class to optimize pharmacokinetic behavior.

Introduction

The classification of histamine receptors by classical pharmacology as well as by molecular biology approaches has well defined three different receptor subtypes named H_1, H_2, and H_3.[1] Whereas in the beginning of 1950 histamine was mainly associated with allergic reactions (H_1) and then, from 1972 onward, expanded to mainly H_2-receptor-mediated reactions on gastric acid secretion, nowadays it is clear that histamine is also a neurotransmitter.[2] Especially the histamine H_3 receptor has been found in the central nervous system (CNS) and in the periphery to be an important regulator of histamine neuron activity[2] as well as a modulator of release of other neurotransmitters, e.g. dopamine,[3] serotonin,[4] noradrenaline,[5] acetylcholine,[6] and various neuropeptides.[7] Many pharmacological investigations on the physiological and pathophysiological functions of H_3 receptors in CNS and peripheral tissues have been made possible by the availability of potent and selective agonists, and different therapeutic applications have been suggested. Due to strong antisecretory and anti-inflammatory properties[8,9] of H_3-receptor agonists on sensory C-fibers and enterochromaffin-like cells, potential clinical targets are ulcers,[10,11] bowl disorders,[8] asthma bronchiale[12] as well as myocardial ischemia.[13] In addition, H_3-receptor agonists display sedative[14] and potential antimigraine actions.[15]

The reference agonist used most frequently is (*R*)-α-methylhistamine (**1**).[1] This hydrophilic compound is a chiral-methylated derivative of the endogenous ligand histamine. Despite its good pharmacodynamic properties, insufficient oral absorption, poor brain penetration, and rapid inactivation in man by histamine *N*-methyltransferase (E.C. 2.1.1.8) limit its clinical use.[8] Most histamine H_3-receptor agonists are 4-monosubstituted imidazole derivatives having in a certain distance a basic moiety which is mainly an aliphatic amino functionality. Specific rigidisation by side-chain branching or ring incorporation increases affinity and selectivity in some cases as described among many others for immepyr,[16] Sch 50971,[17] immepip,[18] and imifuramine (Chart 1).[19] These structure elements are not restricted to agonists only. The partial agonist impentamine also possesses a comparable structure.[20] These structural similarities

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0968-0896/01/$ - see front matter
PII: S0968-0896(00)00237-6

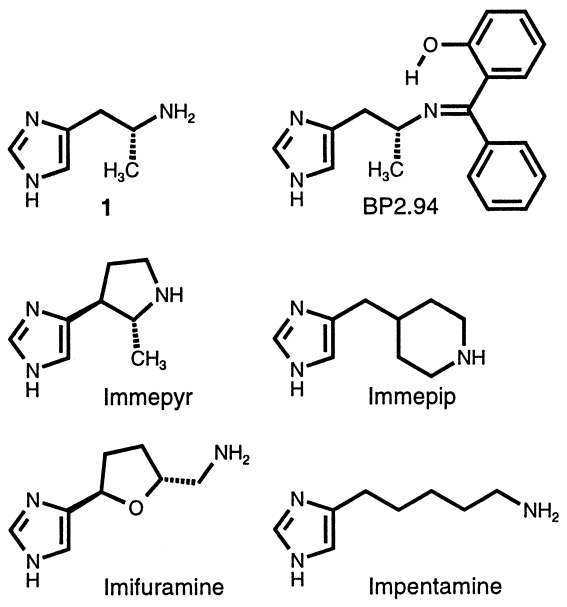

Chart 1.

potentially led to comparable problems with these ligands concerning bioavailability, blood–brain barrier penetration, and metabolism. Azomethine prodrugs of **1** have been described to overcome these pharmacokinetic problems having BP2.94 as parent compound currently in phase II for clinical development.[8,21–24] Furthermore, novel (partial) agonists have been described most recently to display full intrinsic activity in vivo devoid of the basic structural similarity described for former agonists.[25,26] Both these different approaches are promising ways to overcome the problems with the hydrophilic agonists.

To provide a more side-specific alternative to azomethine prodrugs which merely undergo straightforward chemical hydrolysis, different enzyme-dependent prodrug approaches applicable to primary and secondary aliphatic aminergic compounds were developed with **1**. In particular, amide, carbamate, and ester derivatives were prepared having **1** as active agonist element, which was chosen to allow direct comparison with formerly developed azomethine prodrugs and to use the elaborated analytics. In all compounds modification of the amino group of **1** is likely to inhibit first pass inactivation by histamine *N*-methyltransferase as in the case for the azomethine prodrugs.[8]

Prodrug conception is now widely accepted and applied in drug development.[27] Due to the ubiquitous presence of unspecific esterases ester derivatives are most prominent with prodrugs. Monoesters of steroids, antibiotics, neuroleptics etc. are often used as prodrugs with retard effects. Multiple esters like (acyloxy)alkylcarbonates in cefpodoxime-proxetil (Orelox®) are modern developments of prodrugs with improved pharmacokinetics.[28] Despite these developments and the general statement prodrug generation has been successfully performed in most cases on hydroxy functionalities, but only to a much smaller extend on amine moieties. Progabide, an azomethine derivative related to BP2.94, is an example of an aminergic prodrug introduced to therapy, but meanwhile also withdrawn.[29] To make a broad prodrug

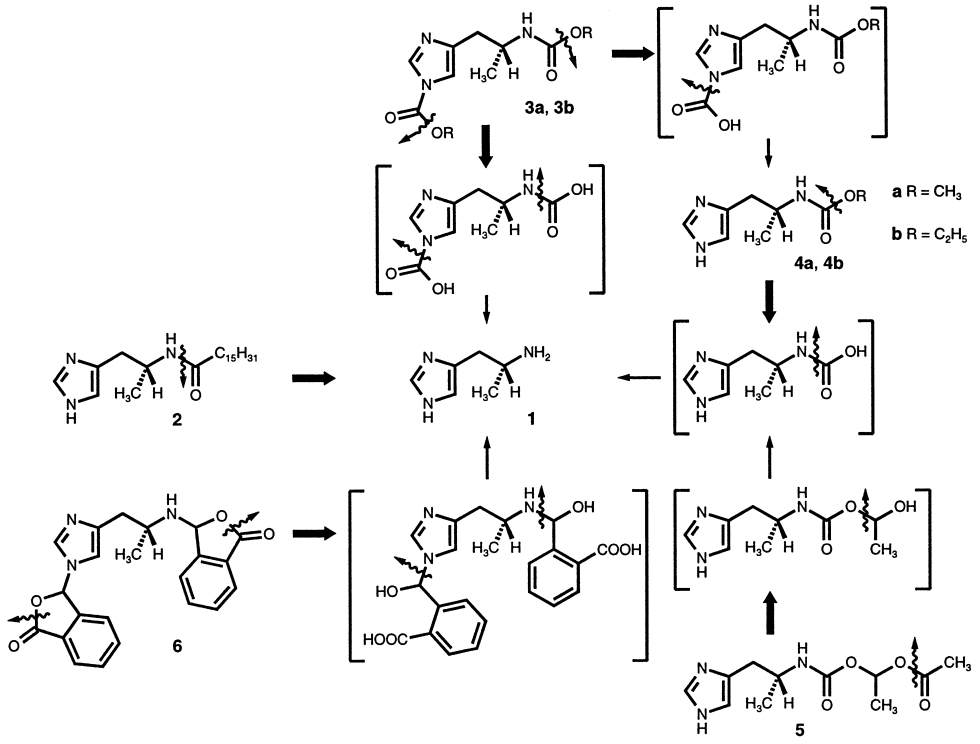

Figure 1. Proposed mechanisms of prodrug activations. Arrows: bold, mainly enzymatic process; regular, spontaneous decomposition; swinging, bond cleavage. Compounds in brackets are unstable and were not characterized.

approach different structures were developed which should include different bioactivation steps mainly depending on amidases and esterases. Derivatization was performed on the aliphatic amino group as well as on the basic imidazole nucleus for some compounds.

Results and Discussion

By conception carboxylic amide **2**, carbamates **3,4**, (acyloxy)alkylcarbamate **5**, and diphthalidyl derivative **6** should firstly undergo enzymatic hydrolysis by unspecific esterases or amidases depending on their pro-moieties. After enzymatic hydrolysis the next step of decomposition for release of **1** should take place spontaneously as shown in Figure 1.

Alkyl carbamates are supposed to be more stable than aryl carbamates.[30] This was the case with the carbamate derivatives in this series (cf. **4a,b** versus **4c**). In related carbamate series with an "inverse" functionality in histamine H_3-receptor antagonists possessing longer alkyl or aromatic substituents, these compounds were found to be stable under in vivo conditions.[31] Therefore, only methyl and ethyl derivatives were prepared since these moieties are supposed to be easily cleaved in vivo by enzymes. Another approach exploiting side-branched alkyl carbamates like *t*-butoxycarbonyl moieties, commonly used as protective groups in peptide chemistry, was unsuccessful with histamine presumably due to early decomposition in the acidic medium of the stomach (results not shown). (Acyloxy)alkylcarbamates are presumably less stable than corresponding carbamates due to an additional obliged-fracture-structure in the acylated hemiacetal.[30] Recently this prodrug conception was successfully applied to guanidine derivatives, too.[32]

Phthalidyl derivatives possess a potential liberation cascade related to the (acyloxy)alkylcarbamates without having a carbamate moiety. Ester hydrolysis liberates the hemiaminal structure thereupon undergoing unprompted hydrolysis to amine and aldehyde (Fig. 1). The imidazole nucleus of the thyreotropine-releasing hormone (TRH) was derived recently by an analogous reaction leading to reduced metabolisation and prolonged halflive.[33]

Chemistry

Five different classes of potential prodrugs have been prepared in this study. Acylation of **1** with equimolar amounts of activated carbonyl compound, i.e. palmitoyl chloride, alkyl chloroformate, or aryl chloroformate, mainly led to N^{α}-monoacylated products due to higher nucleophilicity of the primary amine compared to the imidazole nucleus (Fig. 2). N^{α},N^{τ}-Diacylation took place only to a minor extend, and purification from these byproducts was easily done by salt formation. The comparable reaction with two equivalents led to the desired N^{α},N^{τ}-dicarbamates **3a**, **3b** (Fig. 2). (4-Nitrophenoxy)carbonyl prodrug **4c** decomposed on isolation by standard methods, so that, due to high instability and therefore expected missing prodrug properties, characterization and preparation of the related diacylated product were omitted. Dialkylation of **1** with two equivalents of 3-bromophthalide under similar reaction conditions resulted in low yields of **6** (Fig. 2). Attempts for monoalkylation with one equivalent led to an unseparable mixture of products.

For the preparation of the (acyloxy)alkylcarbamate **5** 4-nitrophenol was reacted with α-chloroethyl chloroformate to the carbonate intermediate, which then underwent nucleophilic substitution in acetic acid by means of Hg(acetate)$_2$ (Fig. 3).[30] Having 4-nitrophenol as an excellent leaving group the aminolysis with **1** can be performed under mild reaction conditions.

Pharmacology

In vitro investigations. All prodrugs were investigated for their ability to release the active drug **1** following incubation under acidic conditions and in presence of liver homogenates, since the latters are presumed to mimic to a certain extent conditions under which drug liberation presumably occurs in vivo. Additional experiments were performed at increased temperature or other

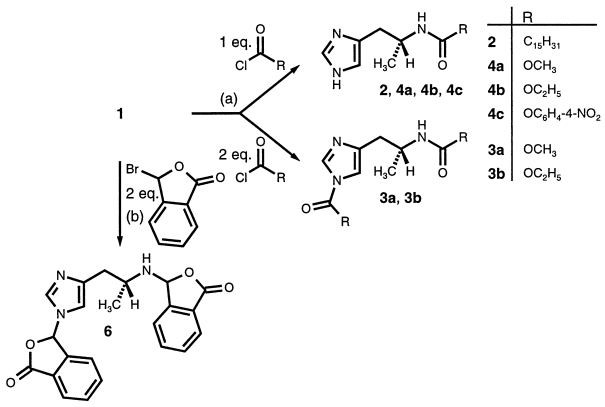

Figure 2. Synthesis of amide (**2**), carbamate (**3**, **4**), and phthalidyl prodrugs (**6**). (a) Et_3N, EtOH or MeOH, EtOAc or MeCN, ambient temp, 2–3 h. (b) Et_3N, MeCN, 65 °C, 7 h.

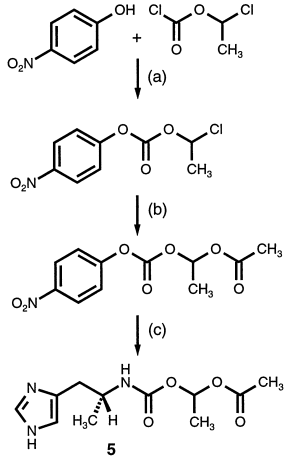

Figure 3. Synthesis of 1-(acetyloxy)ethylcarbamate prodrug **5**. (a) Pyridine, $CHCl_3$, 0 °C→ambient temp, 18 h. (b) Hg(AcO)$_2$, AcOH, ambient temp, 18 h. (c) **1**, HMPT, ambient temp, 18 h.

Table 1. In vitro hydrolysis [%] of prodrugs under various experimental conditions

Conditions	Time (min)	BP 2.94[a]	2	3a	3b	4a	4b	5	6
Neutral medium, 20 °C	0	6.0	1.4			13.6	9.8	4.8	0.5
	15		1.7			12.4	12.1	4.8	0.5
	30	6.0	1.8				10.8	4.8	0.7
	60	6.5	1.7			11.5	9.5	4.8	0.5
	120	15	1.9			13.1	10.0	4.8	0.5
Acidic medium, 20 °C	0	3.5	1.7	0.6	0.7	11.0	11.3	4.6	0.8
	15	4.0	1.6	0.7	0.8	13.1	11.3	4.6	0.5
	30	5.6	1.9	0.8	0.9	11.7	12.8	5.7	0.8
	60	8.7	1.8	0.7	0.7	15.0	11.2	8.2	1.5
	120	14.1	2.0	0.7	0.8	14.9	12.3	8.6	0.8
Acidic medium, 95 °C	15	87	2.5	0.6	0.6	14.2	12.3	85	1.1
	30	94	2.9	0.6	0.6	16.5	11.8	100	0.8
	60	100	4.1	0.9	5.3	17.9	16.0	100	1.8
Alkaline medium, 20 °C	0		1.9					43.8	0.9
	15		1.8					47	1.2
	30		2.3					47	1.1
	60		2.1					47.5	1.0
Liver homogenate, 37 °C	0	3.7	1.6	1.0	1.0	13.1	12.5	23.4	0.6
	15	6.7	2.1	0.6	0.6	10.4	11.5	67.3	0.3
	30	10.4	2.4	0.6	0.8	8.9	6.5	70.8	0.4
	60	19.5	2.0	0.6	0.7	6.2	8.6	73.8	0.6
	120	27.3	2.2	0.6	0.9	11.3	7.0	76.3	0.6

[a]Ref. 8.

incubation media to assess the stability of prodrugs. Data obtained from BP2.94[8] are shown in Tables 1–3 for comparison.

Little liberation of **1** could be found for any compound under incubation in neutral medium and enzyme-free conditions; in presence of the liver extract liberation kinetic was found for compound **5** only (Tables 1 and 2). Slight time-dependent prodrug decomposition could be observed for **2**, **3a**, **3b**, **4b**, and **6** under drastic acidic conditions at 95 °C. Under these conditions, compound **5** was totally hydrolyzed to **1** within 30 min. The in vitro results strongly supported the hypothesis of bioactivation of **5**, but gave disappointing results for the other prodrugs tested concerning the presumed enzyme-catalyzed drug liberation. The reason for these disappointing in vitro results are not clear. Further investigations are necessary to clarify the stability of these compounds, especially in liver homogenate.

Table 2. In vitro hydrolysis [% h^{-1}] of prodrugs under various experimental conditions

Conditions	BP2.94[a]	2	3a	3b	4a	4b	5	6
Neutral medium, 20 °C	4.5	0.3			0	0.1	0	0
Acidic medium, 20 °C	5.3	0.2	0.1	0.1	2.0	0.5	2	0
Acidic medium, 95 °C	100	2.4	0.3	4.6	6.9	4.7	100	1
Alkaline medium, 20 °C		0.2					1.9	0.1
Liver homogenate, 37 °C	11.8	0.3	0	0	0	0	26.5	0

[a]Ref. 8.

In vivo investigations. Whatever the in vitro results, all compounds were investigated in vivo after oral application with regard to prodrug activation measured by released **1** in plasma as well as CNS (Table 3).

As expected the N^{α},N^{τ}-dicarbamates **3a,b**, the N^{α}-monoethylcarbamate **4b**, and the N^{α},N^{τ}-diphthalidyl derivative **6** did not show any drug liberation neither in plasma nor in the CNS. The N^{α}-monomethoxycarbonyl derivative **4a** showed a slight drug level in vivo, but the total detectable amount of 9 ng h mL^{-1} is presumably too small to be considered as pharmacologically relevant.

Surprisingly, the palmityl amide derivative **2** showed good prodrug properties. Although the maximal concentration is much lower than that of the azomethine BP2.94 the total amount (AUC) is comparable to that of BP2.94 due to prolonged drug release (Fig. 4). This result shows that the in vitro models were not able to

Table 3. In vivo data of **1** after p.o. administration of 24 μmol kg^{-1} of prodrug to mice

Tissue		BP2.94[a]	2	3a	3b	4a	4b	5	6
Plasma	c_{max} [ng mL^{-1}]	115±9	73±8	n.d.[b]	n.d.	18±3	n.d.	308±17	n.d.
	t_{max} [min]	30	30	n.d.	n.d.	30	n.d.	30	n.d.
	AUC[c] [ng h mL^{-1}]	186	169	n.d.	n.d.	9	n.d.	482	n.d.
Cerebral cortex	c_{max} [ng mL^{-1}]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.4±2.4	n.d.
	AUC [ng h mL^{-1}]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.9	n.d.

[a]Ref. 8..
[b]n.d., not detectable.
[c]AUC, area under the curve.

predict all in vivo actions. It has also to be considered that **1** was undetectable in the CNS raising hopes that all pharmacological results obtained by this prodrug in future will be mediated by peripheral H_3 receptors only. Furthermore, amide derivatives of fatty acids are a new lead for further prodrug development with aminergic compounds like **1**. One might speculate that these compounds are substrates for amidases after previous biotransformation, e.g. ω-oxidation, what would explain their failure in short time in vitro tests.

The most promising results are obtained from the N^{α}-(1-(acetyloxy)ethylcarbamate) **5**. By oral administration of this prodrug in a dose of 24 μmol kg^{-1} high levels of **1** were readily detectable in plasma and were sustained for more than 6 h (Fig. 4). The AUC is about 2.5 times higher than that obtained with BP2.94 proving a high absorption rate. For a related prodrug, cefpodoxime-proxetil, an enzymatic cleavage by esterases is discussed to take place already during the passage of the gastrointestinal mucosa.[28,34] A comparable mechanism for **5** could explain the high plasma level of **1**. Reasonable drug concentrations are also found in the CNS, and its AUC is even higher than that obtained with compound **4a** in plasma (Table 3). The detectable concentration of **1** in CNS could be related to the extremely high plasma levels. The relation of peripheral to central AUCs supports the hypothesis that prodrugs of the (acyloxy)alkylcarbamate type are acting more strongly at peripheral than at central targets. This type of prodrugs is the most promising approach in this series which does not seem to be restricted to the primary aliphatic amine group of **1**, but can also be applied to other compounds containing primary or secondary amine functionalities.

Conclusions

Five different types of prodrugs for the histamine H_3-receptor agonist (*R*)-α-methylhistamine (**1**) have been described as its N^{α}-position or its N^{α},N^{τ}-position were derivatized simultaneously under mild reaction conditions. In vitro testing under different conditions for the 1-(acetyloxy)ethylcarbamate **5** showed marked lability under enzymatic environment, whereas the amide derivative of palmitic acid **2**, the mono- or dicarbamates (**3**–**4**), and the diphthalidyl derivative **6** were devoid of any hydrolysis kinetic. Despite this stability in vitro the monomethylcarbamate **4a** and the amide **2** showed moderate to promising prodrug properties in vivo after oral application to mice. The palmityl derivative **2** possessed AUC levels comparable to those of the parent azomethine prodrug BP2.94. The most promising compound was **5**, which is supposed to release **1** by a cascade reaction with an initial enzyme-induced hydrolysis. Maximum concentration and the AUC obtained were more than 2.5 times higher than that of the azomethine BP2.94 with an equimolar dose tested. Drug levels could even be detected in CNS. Amides and (acyloxy)alkylcarbamates of the type described are promising prodrugs of **1** and are also interesting leads for further development of aminergic compounds with supposed peripheral activity.

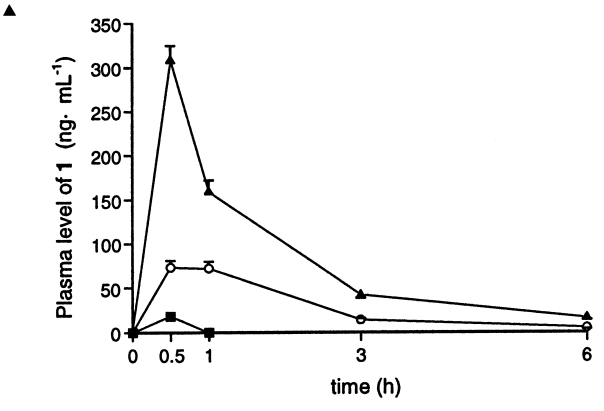

Figure 4. Plasma level of released **1** after p.o. administration of **2** (○), **4a** (■), and **5** (▲) to mice in a dose of 24 μmol kg^{-1}.

Experimental

Chemistry

General procedures. Melting points were determined on an Electrothermal IA 9000 digital or a Büchi 512 apparatus and are uncorrected. For all compounds ^{1}H NMR spectra were recorded on a Bruker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS as reference. ^{1}H NMR data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; q, quintett; m, multiplet; *, exchangeable by D_2O; ** exchangeable by F_3CCOOD; Im, imidazolyl; Mal, maleic acid; Me, methyl; Ph, phenyl), number of protons, and approximate coupling constants in hertz (Hz). Mass spectra were obtained on a Finnigan MAT CH7A (EI–MS) and a Finnigan MAT CH5DF (FAB–MS). Optical rotation was determined on a Perkin–Elmer 241 MC. Elemental analyses (C, H, N) for all compounds were measured on Perkin–Elmer 240 B or Perkin–Elmer 240 C instruments and were within ±0.4% of theoretical values. Column chromatography was carried out using silica gel 63–200 μm (Machery & Nagel). Preparative, centrifugally accelerated, rotatory chromatography was performed using a Chromatotron 7924T (Harrison Research) and glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck). For analytical data on mp. and elemental analyses see Table 4.

Synthesis of prodrugs

Amide of fatty acid

(*R*)-(−)-*N*-(1-(1*H*-Imidazol-4-yl)-2-propyl)hexadecanoic amide (2). A solution of palmitoyl chloride (1.6 mmol, 0.44 g) in 20 mL of MeCN was slowly added to **1** (1.6 mmol, 0.2 g) and Et_3N (1.6 mmol, 0.16 g) in 20 mL of EtOH and 5 mL of MeCN. After stirring for 1 h at ambient temp the reaction mixture was concentrated to 10 mL under reduced pressure. The mixture obtained was diluted with $CHCl_3$, washed (K_2CO_3, H_2O), dried ($MgSO_4$), and evaporated to dryness. Compound **2** was

Table 4. Analytical data of prodrugs **2**–**6**

No.	Formula	MW	Mp [°C]	Calculated			Found		
				C	H	N	C	H	N
2	$C_{22}H_{41}N_3O{\cdot}C_4H_4O_4$	479.7	119	65.1	9.46	8.76	64.9	9.39	8.84
3a	$C_{10}H_{15}N_3O_4$	241.3	79	49.8	6.27	17.4	49.8	6.33	17.5
3b	$C_{12}H_{19}N_3O_4$	269.3	63	53.5	7.11	15.6	53.4	7.31	15.8
4a	$C_8H_{13}N_3O_2{\cdot}C_4H_4O_4$	299.3	104	48.2	5.73	14.0	47.8	5.77	13.7
4b	$C_9H_{15}N_3O_2{\cdot}C_4H_4O_4$	313.3	106	49.8	6.11	13.4	49.8	6.21	13.6
5	$C_{11}H_{17}N_3O_4{\cdot}C_4H_4O_4{\cdot}0.5H_2O$	380.4	86–88	47.4	5.83	11.0	47.7	5.68	10.8
6	$C_{22}H_{19}N_3O_4{\cdot}0.25H_2O$	393.9	163	67.1	4.99	10.7	67.1	4.84	10.7

crystallized as salt of maleic acid in EtOH/Et_2O (yield: 0.5 g, 65%). $[\alpha]_D^{20} = -53.8$ ($c = 1$ g/100 mL in MeOH); ^{1}H NMR ([d_6]DMSO) δ 8.86 (s, 1H, Im-2-H), 7.72 (d**, $J = 8.2$ Hz, 1H, NH), 7.32 (s, 1H, Im-5-H), 6.04 (s, 2H, Mal), 4.06 (m, 1H, CH), 2.72 (d, $J = 6.8$ Hz, 2H, Im-CH_2), 1.99 (t, $J = 7.3$ Hz, 2H, CO-CH_2), 1.41 (m, 2H, CO-CH_2-CH_2), 1.23 (m, 24H, alkyl-H), 1.05 (d, $J =$ 6.6 Hz, 3H, CH-*Me*), 0.85 (t, $J = 7.0$ Hz, 3H, CH_2-*Me*); MS (70 eV), m/z (%) 363 (2) [M^+], 256 (3), 167 (6), 108 (89), 82 (100).

Dicarbamates

(*R*)-(+)-*N*-(1-(1-(Methoxycarbonyl)-1*H*-imidazol-4-yl)-2-propyl)carbamate methylester (3a). A solution of methyl chloroformate (4 mmol, 0.38 g) in 10 mL of ethyl acetate was slowly added to **1** (2 mmol, 0.25 g) and Et_3N (4 mmol, 0.4 g) in 5 mL of MeOH and 10 mL of ethyl acetate. After stirring for 3 h at ambient temp Et_3N·hydrochloride was filtrated and the residue evaporated under reduced pressure. The oily compound **3a** was crystallized as salt of maleic acid in EtOH/Et_2O at −5 °C and was recrystallized in Et_2O (yield: 0.2 g, 41%). $[\alpha]_D^{20} = +2.3$ ($c = 1$ g/100 mL in MeOH); ^{1}H NMR ([d_6]DMSO) δ 8.17 (d, $J = 1.0$ Hz, 1H, Im-2-H), 7.31 (s, 1H, Im-5-H), 7.07 (d**, $J = 8.0$ Hz, 1H, NH), 3.95 (s, 3H, Im-COO-Me), 3.75 (m, 1H, CH), 3.49 (s, 3H, NH-COO-*Me*), 2.62 (d, $J = 6.8$ Hz, 2H, Im-CH_2), 1.02 (d, $J = 6.6$ Hz, 3H, CH-*Me*); MS (70 eV), m/z (%) 241 (1) [M^+], 210 (4) [M^+−MeO], 166 (16), 140 (100), 102 (17), 81 (45).

(*R*)-(+)-*N*-(1-(1-(Ethoxycarbonyl)-1*H*-imidazol-4-yl)-2-propyl)carbamate ethylester (3b). The synthesis was made according to **3a** with ethyl chloroformate and EtOH instead of MeOH. Crystallization was performed in Et_2O (yield: 0.24 g, 44%). $[\alpha]_D^{20} = +1.8$ ($c = 1$ g/100 mL in MeOH); ^{1}H NMR ([d_6]DMSO) δ 8.16 (d, $J = 1.0$ Hz, 1H, Im-2-H), 7.29 (s, 1H, Im-5-H), 7.02 (d**, $J = 8.1$ Hz, NH), 4.39 (q, $J = 7.1$ Hz, 2H, Im-COO-CH_2), 3.94 (q, $J = 7.1$ Hz, 2H, NH-COO-CH_2), 3.77 (m, 1H, CH), 2.62 (d, $J = 6.4$ Hz, 2H, Im-CH_2), 1.33 (t, $J =$ 7.1 Hz, 3H, Im-COO-CH_2-*Me*), 1.13 (t, $J = 7.1$ Hz, 3H, NH-COO-CH_2-*Me*), 1.02 (d, $J = 6.5$ Hz, 3H, CH-*Me*); MS (70 eV), m/z (%) 269 (2) [M^+], 224 (5), 180 (17), 154 (100), 116 (11), 81 (77).

Monocarbamates

(*R*)-(+)-*N*-(1-(1*H*-Imidazol-4-yl)-2-propyl)carbamate methylester (4a). The synthesis was performed according to **3a** with methyl chloroformate (2 mmol, 0.19 g). Compound **4a** was crystallized as salt of maleic acid in EtOH/Et_2O (yield: 0.4 g, 66%). $[\alpha]_D^{20} = +9.2$ ($c = 1$ g/100 mL in MeOH); ^{1}H NMR ([d_6]DMSO) δ 8.90 (s, 1H, Im-2-H), 7.36 (s, 1H, Im-5-H), 7.16 (d**, $J = 8.1$ Hz, 1H, NH-CO), 6.06 (s, 2H, Mal), 3.81 (m, 1H, CH), 3.49 (s, 3H, O-Me), 2.73 (d, $J = 6.7$ Hz, 2H, Im-CH_2), 1.07 (d, $J = 6.6$ Hz, 3H, CH-*Me*); MS (70 eV), m/z (%) 183 (1) [M^+], 140 (12), 101 (16), 86 (100), 82 (16).

(*R*)-(+)-*N*-(1-(1*H*-Imidazol-4-yl)-2-propyl)carbamate ethylester (4b). The synthesis was performed according to **3b** with ethyl chloroformate (2 mmol, 0.19 g). Compound **4b** was crystallized as salt of maleic acid in EtOH/Et_2O (yield: 0.4 g, 63%). $[\alpha]_D^{20} = +7.4$ ($c = 1$ g/100 mL in MeOH); ^{1}H NMR ([d_6]DMSO) δ 8.91 (s, 1H, Im-2-H), 7.35 (s, 1H, Im-5-H), 7.12 (d**, $J = 8.2$ Hz, NH-CO), 6.05 (s, 2H, Mal), 3.91 (q, $J = 7.0$ Hz, 2H, O-CH_2), 3.81 (m, 1H, CH), 2.72 (d, $J = 6.7$ Hz, 2H, Im-CH_2), 1.11 (t, $J = 7.0$ Hz, 3H, O-CH_2-*Me*), 1.06 (d, $J =$ 6.6 Hz, 3H, CH-*Me*); MS (70 eV), m/z (%) 197 (1) [M^+], 154 (84), 109 (13), 86 (32), 81 (100).

(Acetyloxy)alkylcarbamate

(1-Chloroethyl)-(4-nitrophenyl)carbonate.[30] α-Chloroethyl chloroformate (35 mmol, 5 g) was added slowly at 0 °C to 4-nitrophenol (35 mmol, 4.87 g) and pyridine (35 mmol, 2,77 g) in 100 mL of $CHCl_3$. After 30 min at 0 °C the mixture was stirred at ambient temp for 18 h. Washing of the mixture (H_2O, 0.5% NaOH, H_2O), drying (Na_2SO_4), and evaporation to dryness resulted in an oil, which slowly crystallized at −5 °C. Recrystallization in ethyl acetate/petrol ether gave 81% yield (7 g), which can be used directly for further reactions. Mp. 70–71 °C; ^{1}H NMR ([d_6]DMSO) δ 8.35 (dd, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 2H, Ph-3-H, Ph-5-H), 7.62 (dd, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 2H, Ph-2-H, Ph-6-H), 6.61 (q, $J = 5.8$ Hz, 1H, CH), 1.86 (d, $J = 5.7$ Hz, 3H, Me); MS (70 eV), m/z (%) 245 (1) [M^+], 210 (8) [M^+−Cl], 166 (14), 139 (99), 122 (19), 109 (28), 93 (13), 81 (11), 76 (11); ($C_9H_8ClNO_5$).

(1-(Acetoxy)ethyl)-(4-nitrophenyl)carbonate.[30] The product obtained above (28 mmol, 6.87 g) in 50 mL of acetic acid was mixed with $Hg(H_3CCOO)_2$ (34 mmol, 10.83 g) and stirred for 18 h at ambient temp. After evaporation to dryness the residue was dissolved in Et_2O, filtrated, washed (H_2O, $NaHCO_3$, H_2O), dried (Na_2SO_4), and once more evaporated to dryness. The oil obtained was purified by column chromatography (eluent:

CH_2Cl_2:petrol ether; 1:1) (yield: 5.5 g, 73%); ^1H NMR ($CDCl_3$) δ 8.29 (d, $J=9.1$ Hz, 2H, Ph-3-H, Ph-5-H), 7.41 (d, $J=9.1$ Hz, 2H, Ph-2-H, Ph-6-H), 6.84 (q, $J=5.5$ Hz, 1H, CH), 2.14 (s, 3H, CO-Me), 1.62 (d, $J=5.4$ Hz, 3H, CH-*Me*); MS (70 eV), *m*/*z* (%) 268 (0.3) [M^+-H], 210 (2) [M^+-AcO], 181 (1), 166 (4), 139 (2), 122 (5), 87 (33), 43 (100); ($C_{11}H_{11}NO_7$).

(2*R*)-(+)-*N*-(1-(1*H*-Imidazol-4-yl)-2-propyl)carbamate 1-(acetyloxy)ethylester (5). The product obtained above (1.6 mmol, 0.43 g) and **1** (1.6 mmol, 0.2 g) were dissolved in 30 mL of HMPT and stirred for 18 h at ambient temp. After addition of 70 mL of H_2O the mixture was extracted with ethyl acetate. After evaporation to dryness of the combined organic extracts the resulting oil was purified by preparative rotatory chromatography (gradient eluent: CH_2Cl_2:MeOH, 95–85:5–15). Compound **5** was crystallized as salt of maleic acid in EtOH/Et_2O (yield: 0.2 g, 32%). $[\alpha]_D^{20}=+28.0$ ($c=1$ g/100 mL in MeOH); ^1H NMR ([d_6]DMSO) δ 8.97 (s, 1H, Im-2-H), 7.51 (d**, $J=8.3$ Hz, 1H, NH-CO), 7.34 (s, 1H, Im-5-H), 6.59 (q, $J=5.4$ Hz, O-CH), 6.12 (s, 2H, Mal), 3.83 (m, 1H, CH_2-C*H*-Me), 2.74 (d, $J=6.7$ Hz, CH_2), 1.99 (s, 3H, CO-Me), 1.36 (d, $J=5.4$ Hz, O-CH-*Me*), 1.08 (d, $J=6.6$ Hz, CH_2-CH-*Me*); FAB^+-MS (Xe, DMSO/Glycerin), *m*/*z* (%) 511 (1) [$M_2^+ + H$], 256 (66) [$M^+ + H$], 152 (100), 126 (15), 109 (31), 82 (24).

N^{α},N^{τ}-Diphthalidyl derivate

(±)-(2*R*)-3-(*N*-(1-(1-(3-Phthalidyl)-1*H*-imidazol-4-yl)-2-propyl)amino)phthalide (6). 3-Bromophthalide (3.5 mmol, 0.21 g) was added to a solution of **1** (1.7 mmol, 0.21 g) and Et_3N (3.5 mmol, 0.35 g) in 40 mL of MeCN. After stirring for 7 h at 65 °C the mixture was evaporated to dryness. The oily residue was purified by preparative rotatory chromatography (eluent: CH_2Cl_2:MeOH, 95:5). The product **6** was crystallized in Et_2O (yield: 0.135 g, 20%). ^1H NMR ([d_6]DMSO) δ 7.98–7.38 (m, 10H, 8 Ph-H, Im-2-H, Im-5-H), 6.70 (s, 1H, Im-CH), 6.50 (m*, 1H, NH), 5.98 (m, 1H, NH-C*H*), 4.37 (m, 1H, CH_2-C*H*), 2.91 (m, 2H, CH_2), 1.27 (d, $J=6.7$ Hz, 3H, Me); MS (70 eV), *m*/*z* (%) 389 (2) [M^+], 371 (5) [M^+-H_2O], 239 (45), 214 (25), 176 (14), 159 (88), 149 (18), 133 (100), 121 (16), 108 (60), 81 (29).

Pharmacology

Determination of prodrug hydrolysis rates in vitro. A 10 mM prodrug solution prepared extemporaneously in DMSO was diluted to a final concentration of 4 μM in 0.4 $HClO_4$ (acidic conditions), in 0.1 N NaOH (alkaline condition), in 0.05 M K_2HPO_4/KH_2PO_4 buffer, pH 7.4 (neutral conditions), or in liver homogenate obtained from rat tissues and incubated at different temperatures. At various time intervals, an aliquot was taken, diluted, and brought up to a final concentration of 0.4 N $HClO_4$. Immediately after the incubation, **1** was measured by a sensitive and specific radioimmuno assay (RIA) developed according to a principle already described.[35] Briefly, samples or standards were derivatized with *p*-benzoquinone during a 30 min incubation at room temp. The derivatized samples were then incubated with an antiserum raised in rabbits for 1 h at 37 °C and left at 4 °C overnight after transfer in swine anti-rabbit IgG-coated 96-well plates and addition of an [^{125}I]iodinated tracer. The radioactivity bound to the wells was then counted in a γ-spectrometer with an efficiency of 82%. The percentage ratio of level of **1** measured in the sample and that corresponding to complete hydrolysis of prodrug was calculated at various times. A blank value corresponding to a level of **1** at zero time incubation (and representing hydrolysis occurring during the RIA) was determined and subtracted. The hydrolysis rate of prodrugs was then expressed as percent compound hydrolyzed per h.

Determination of 1 and prodrug levels in plasma and cerebral cortex of mice treated with the varius prodrugs.[8] Male Swiss mice (20–25 g; Iffa-Credo, France) that were given food and water ad libitum received an oral dose of 24 μmol kg^{-1} of prodrug in 1% methyl cellulose and were sacrificed by decapitation 0.5, 1, 3, or 6 h later. Controls received the vehicle only. For determination of **1** and prodrug levels in plasma and in cerebral cortex, blood was collected after decapitation and centrifuged (15,000 *g* for 1 min), and the cerebral cortex was dissected out rapidly and homogenized in 10 vol (w/v) of ice-cold 0.4 N $HClO_4$. Plasma (brought to a final concentration of 0.4 N $HClO_4$) and cerebral extracts were then centrifuged, and the clear supernatant was used for the RIA immediately or stored at −20 °C. Compound **1** was derivatized and radioimmunoassayed. The plasma of nontreated mice was also assayed in order to estimate the interference of plasma in the RIA for **1**. The determinations of **1** for treated mice were then corrected accordingly. Each determination was perfomed at least in three different animals.

Acknowledgements

We greatly acknowledge the gift of (*R*)-α-methylhistamine kindly provided by Dr. J.-M. Lecomte (Bioprojet Pharma, Paris, France). This work was supported by the Biomedical & Health Research Programme (BIOMED) of the European Union and the Fonds der Chemischen Industrie, Verband der Chemischen Industrie, Frankfurt/Main, Germany.

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