Arpromidine-Related Acylguanidines: Synthesis and Structure-Activity Relationships of a New Class of Guanidine-Type Histamine H_2 Receptor Agonists with Reduced Basicity

Dissertation

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To my parents.....

Take up one idea. Make that one idea your life - think of it, dream of it, live on idea. Let the brain, muscles, nerves, every part of your body, be full of that idea, and just leave every other idea alone. This is the way to success.

.....Swami Vivekananda

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I am forever grateful to my parents and brother who gently offer a counsel and unconditional support at each turn of the road.

Table of Contents

List of abbreviations

Abs.	absolute
Ac	acetyl
Bn	benzyl
Boc	*tert*-Butoxycarbonyl
Bu	butyl
BuLi	butyl lithium
Cbz	benzyloxycarbonyl
CD	bircular dichroism
CDI	*N*,*N*'-carbonyldiimidazole
CNS	central nervous system
DCM	dichloromethane (CH_2Cl_2)
DCC	dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	p-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
EC_{50}	The molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist.
ECL	extracellular loop
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
ee	enantiomeric excess
EI	electron impact (MS)
GDP	guanosinediphosphate
GTP	guanosinetriphosphate
GPCR	G-protein coupled receptor
h	hour(s)
h, gp	in context with a receptor name: human, guinea pig

List of abbreviations

H_1R, H_2R, H_3R, H_4R	histamine receptor subtypes
HOBt	1-hydroxybenzotriazole Hydrate
HPLC	high performance (pressure) liquid chromatography
HRMS	high resolution mass spectroscopy
i.a.	intrinsic activity
IC_{50}	antagonist concentration which suppresses 50% of an agonist induced effect
ICL	intracellular loop
IR	infrared(spectroscopy)
Ki	dissociation constant (competition binding assay)
L	ligand
Me	methyl
Mes	mesyl
min	minute(s)
mL	milliliter
MS	mass spectrometry
NMR	nuclear magnetic resonance
NPY	neuropeptide Y
pEC_{50}	negative logarithm of EC_{50}
Ph	phenyl
pH	negative logarithm of the hydrogen ion concentration
Ph	phenyl
ppm	parts per million
quant.	quantitative
quart.	quarternary (carbon)
rt	room temperature
SEM	standard error of the mean
Sf9	insect cell line
t (tert)	tertiary

List of abbreviations

Triflat (Tf)	trifluoromethansulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TM	transmembrane domain
TMS	tetramethyl silane
UV	ultra violet

Chapter 1

Introduction

To most of the modern pharmacologists the receptor is like a beutiful but remote lady. He has written her many a letter and quite often she has answered the letters. From these answers the pharmacologists has built himself an image of this fair lady. He cannot, however, truly claim ever to have seen her, although one day he may do so.

D. K. de Jongh (1964)

1.1 Histamine and its receptors

Histamine[1] (**1, Fig. 1.1**), 2-(1*H*-imidazol-4-yl)ethylamine, is a biogenic amine formed by enzymatic decarboxylation of the amino acid L-histidine. It is found in many plants and present in nearly all animal tissues where it is detected in many cell types, e.g., mast cells, basophils, platelets, endothelial and neuronal cells.[2] Histamine plays a role as a chemical messenger, i.e., it transfers signals from one cell to another. As such, it is able to induce numerous (patho)physiological effects, like smooth muscle contraction, stimulation of hormone release, modulation of immune responses, gastric acid secretion, induction of sleep and modulation of cognitive processes. Histamine release from mast cells and basophils is notorious for the pathophysiological effects of the biogenic amine in allergic diseases and inflammation, for instance, the well known symptoms of irritation in skin and airways, bronchospams and vasodilation.[3,4] In 1937, Bovet and Staub discovered compounds that antagonised several of these effects of histamine.[5] Subsequently, many other chemicals that have similar actions have been identified. Some of these compounds (e.g., mepyramine, diphenhydramine) were introduced to clinical use in allergic conditions like hay fever. As these classical "antihistamines" were not able to block certain effects of histamine such as the stimulation of gastric acid secretion, in the sixties, Ash and Schild suggested that histamine acts via two distinct receptor subtypes.[6] This prediction was confirmed when Black and co-workers developed burimamide, the first compound described to antagonize the histamine-stimulated gastric acid secretion or the histamine-induced relaxation of the electrically stimulated rat uterus.[7] This led to the classification of two subtypes of histamine receptors termed H_1, blocked by classical antihistamines, and H_2

receptors, blocked by burimamide. As the structural requirement for compounds having H_1 or H_2 receptor affinity are different, selective H_2 receptor ligands could be developed and H_2 antagonists became blockbuster drugs for the treatment of gastric and duodenal ulcers (e.g., cimetidine, ranitidine).

Histamine is widely distributed within the mammalian central nervous system (CNS). Mapping of the histaminergic pathways in the rat and guinea-pig revealed that the arrangement of the histaminergic fibres were similar to, e.g., the noradenaline and 5-hydroxytryptamine pathways.[8,9] The role of histamine as a neurotransmitter became apparent in 1983 when Jean-Charles Schwartz and co-workers discovered that histamine inhibits its own release from depolarized slices of rat cortex.[10] Such feedback mechanisms mediated through presynaptic receptors are crucial to neurotransmission. These effects were found not to be mediated by H_1 or H_2 receptors since neither H_1 nor H_2 activity of the histaminergic ligands correlated with the inhibitory effect. Hence the results indicated a third histamine receptor subtype. In 1987, the existence of this H_3-receptor was validated by the development of the potent and selective H_3 antagonist thioperamide.[11] Subsequent pharmacological characterization of the receptor using selective histamine H_3 receptor ligands, confirmed the H_3 receptor as being located presynaptically on the histaminergic (autoreceptors) and other nerve endings (heteroreceptors). Histamine H_3 receptors are predominantly involved in the function of central nervous system (CNS). Various biological responses (e.g., arousal, circadian rhythm) are supposed to be associated with histamine acting in the CNS. Besides H_3-receptor, H_1 and H_2-receptors are also widely expressed in the mammalian brain.

H_1, H_2, and H_3-receptors were initially characterized by pharmacological means and the cloning of their respective genes[12] has been performed later. By using cloning approaches, most recently, a new histamine receptor subtype was discovered.[13] Several groups independently identified a DNA sequence with homology to the H_3 histamine (37-43%), that was cloned and later termed the histamine H_4-receptor. The functions of this receptor are not yet known in detail, but its detection in the bone marrow, in spleen and leukocytes (particularly eosinophils and neurophils) may allude to a possible involvement in

immunological processes and the regulation of hematopoiesis[14]. A summary of the most important characteristics of histamine receptors is presented in **Table 1.1**.

Table 1.1: Histamine receptors and some biological effects
H_1 - contraction of smooth muscles (e. g., gut, bronchi) - increase in the permeability of the capillaries - vasoconstriction (via receptors at vascular smooth muscles) - vasodilatation (via EDRF release from endothelial cells)
H_2 - stimulation of gastric acid secretion - Positiv inotropic and chronotropic effect on the heart - vasodilatation
H_3 - inhibition of neuronal synthesis and release of histamine (presynaptic autoreceptor) - modulation of various neurotransmitters (e. g. acetylcholine, serotonin, noradrenaline, dopamine) *via* presynaptic heteroreceptors
H_4 - biological function? (H_4 receptors were found, e. g., in leukocytes, spleen, thymus, colon)

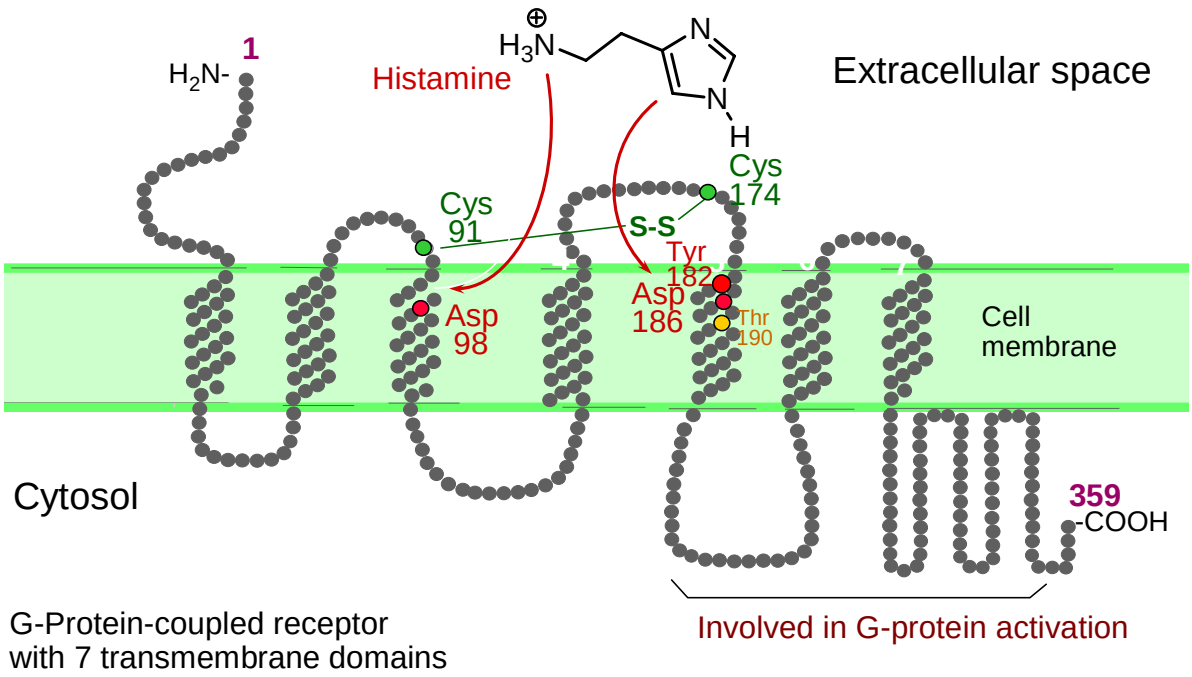


Fig. 1.1. Schematic representation of the histamine H_2 receptor. Interactions of amino acids Asp 98, Tyr 182 and Asp 186 in transmembrane domains TM3 and TM5, respectively, are considered to be involved in receptor activation.

All histamine receptors belong to the G-protein coupled receptor (GPCR) superfamily that possesses seven transmembrane domains, three extracellular and three intracellular loops. The transmembrane domains contain stretches of 20-25 hydrophobic amino acids that are predicted to form α-helices that span the cell membrane. The third (TM-III) and fifth (TM-V) transmembrane domains of heptahelical receptors appear to be especially important for ligand-binding as schematically shown for the histamine H_2 receptor in **Fig. 1.1**. H_1 receptors are coupling to $G_{q/11}$ (IP_3 ↑, Ca^{2+}↑), H_2 to G_s (cAMP ↑), H_3 and H_4 receptors to $G_{i/o}$ proteins (cAMP ↓).[15]

1.2 Histamine H_2 receptors and their ligands

As mentioned, the histamine H_2 receptors was pharmacologically characterized by Black et al. in 1972.[7] With the high affinity (K_D=0.3 nM) radioligand [^{125}I]iodoaminopotentidine[16-19] the H_2 receptor was found to be widely distributed in the brain, with highest densities in the basal ganglia, hippocampus, amygdala, and cerebral cortex.[19] Lowest densities were detected in cerebellum and hypothalamus.[19] In the periphery the H_2 receptor was found, for example, in gastric cells, cardiac tissue, airway, uterine, and vascular smooth muscle.[15] H_2 receptors have a potent stimulatory effect on gastric acid secretion, and the inhibition of this secretory process by H_2 receptor antagonists has provided evidence for an important physiological role of histamine in the regulation of gastric acid secretion.[7] The cloned rat, human, guinea pig, mouse and canine H_2 receptor include 358 or 359 amino acids.

1.2.1 Histamine H_2 receptor antagonists

After the characterization of the histamine H_2-receptor by Black et al[7], there was a great effort to search for H_2 receptor selective antagonists for the treatment of peptic ulcer. The histamine derivative 5-methylhistamine, the first compound described to exhibit some selectivity for the histamine H_2 receptor[7,20,21]. The extension of the side chain and replacement of the basic amino group with polar, planar groups (e.g. thiourea or cyanoguanidine) that are uncharged at physiological pH led to discovery of burimamide and the more active H_2 antagonsts which are therapeutically used as antiulcer drugs. After the discovery of the histamine H_3 receptor the weak H_2 antagonist burimamide was found to be a more potent antagonist at the H_3 receptor.[10] Cimetidine and metiamide were developed

directly from burimamide.[22-24] Cimetidine was the first H_2 blocker launched onto the market for the treatment of gastric and duodenal ulcer. Since then a large number of H_2-receptor antagonist such as ranitidine, tiotidine, famotidine, nizatidine, roxatidine acetate and mifentidine have been developed as antiulcer drugs.[15] Most H_2-receptor antagonists are polar compounds and penetrate poorly into the central nervous system (CNS). On one hand this property is useful to obtain peripherally acting drugs (e.g. gastric secretion inhibitors), on the other hand the lack of penetration into the brain is limiting the use of H_2 antagonists for in vivo pharmacological investigations of the role of H_2 receptors in the CNS.

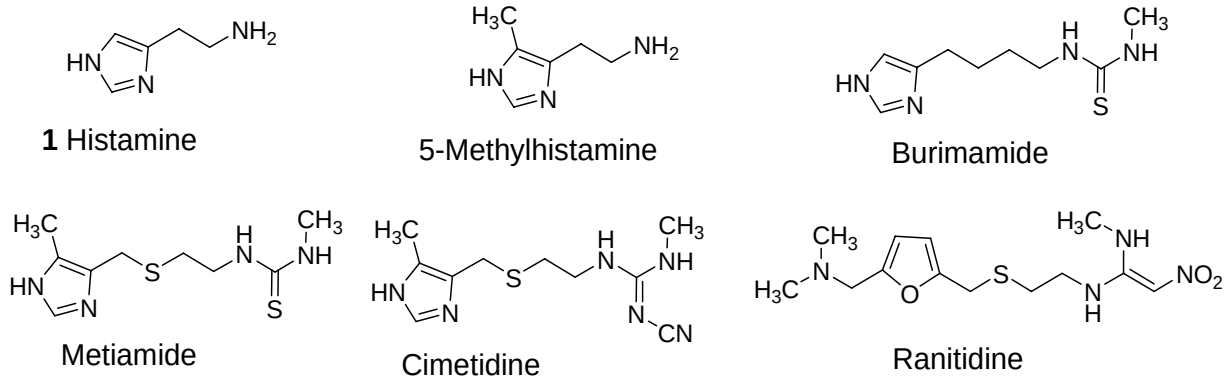


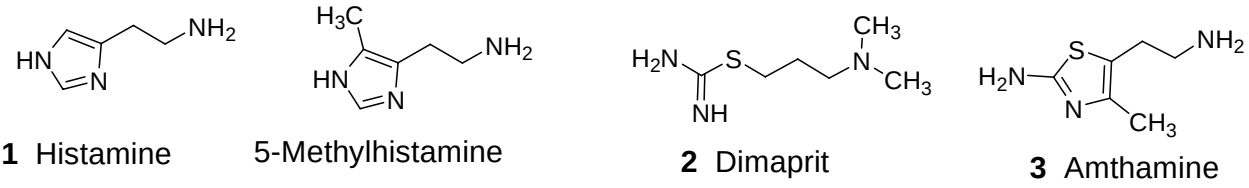
Fig. 1.2: Structures of histamine, the selective H_2 receptor agonist 5-methylhistamine and the H_2 receptor antagonists burimamide, metiamide, cimetidine and ranitidine.

1.2.2 Histamine H_2 receptor agonists

From the therapeutic point of view the interest in drugs acting on histamine receptors is focused on antagonists. Nevertheless, there has been increasing evidence that histamine receptor stimulation might be an interesting aspect for the development of future drugs as well.

5-Methylhistamine was the first histamine H_2 receptor agonist described in literature.[7] Another H_2 receptor selective ligand devoid of an imidazole ring is dimaprit (**2**)[25] which is about as potent as histamine. Cyclization of the isothiourea partial structure of dimaprit led to the development of amthamine (**3**).[26] Most interesting thing was this kind of ligand is devoid of H_1 and H_3-receptor activity. Further replacement of the sulphur atom by a selenium atom (amselamine) was found to result in similar activity. All these small

molecules are similar to histamine concerning both structural criteria and H_2 receptor agonistic potency, but are selective for the H_2-receptor.



1 Histamine 5-Methylhistamine **2** Dimaprit **3** Amthamine

Fig. 1.3: Examples of amines with histamine H_2 receptor agonistic activity

In addition to the synthesis and pharmacological characterization of histamine analogues and related small molecules, theoretical investigations were performed to identify the putative active species of the H_2-receptor agonists (i.e., protonation sites(s) at physiological pH, the conformeric and tautomeric form) and its possible interaction with the receptor protein.[27,28] The histamine binding site of the H_2 receptor was identified by in vitro mutagenesis studies and molecular modeling approaches based on the 3D structure of bacteriorhodopsin and bovine rhodopsin, respectively. Investigations of H_2 receptor mutants supported an ionic interaction of the protonated amino group with Asp98 (TM3).[29] The second and the third site of the widely accepted three-point model for biogenic amine/GPCR interaction could principally be formed by the couples Asp186/Thr190 or Tyr182/Asp186 in TM5.[30-32] (**Fig. 1.4**) Based on a pure alpha helical TM5, the proposed two hydrogen bonds of the imidazole ring with the H_2-receptor are only possible with Tyr182 and Asp186. This assumption is also in agreement with pH-dependant model of H_2-receptor activation that suggests tautomerisation of the imidazole into the N^{π}-H form caused by neutralization of histamine upon binding and accompanied by proton transfers from Tyr182 to N^{π} and N^{τ} to Asp186, respectively.[33]

The first highly potent H_2 receptor agonist impromidine (**4, Fig. 1.5**) resulted from histamine, by extending the chain length by one additional methylene group, replacing the amine functionality by the strongly basic guanidine group and combining the structure with the 2-[(5-methyl-1*H*-imidazole-4-yl)methylthio]ethyl moiety known from the H_2 receptor antagonist cimetidine.[34]

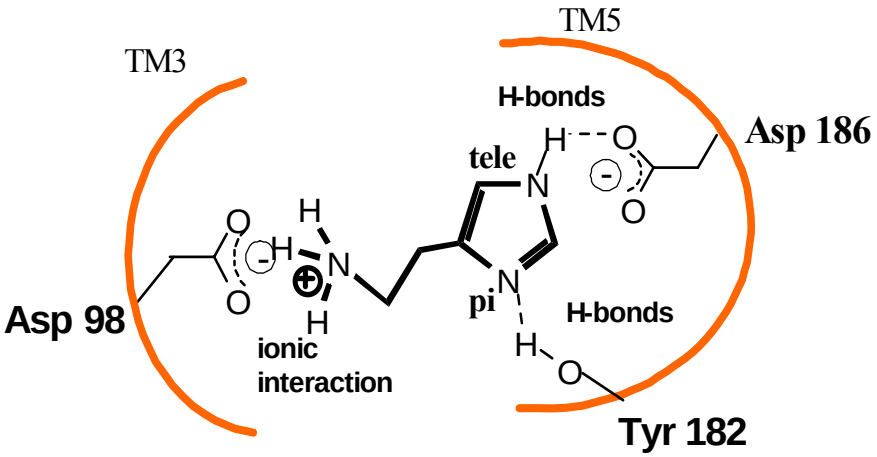


Fig. 1.4: Interaction model for the binding of histamine to the human histamine H_2 receptor This prototypical guanidine-type H_2 receptor agonist is nearly 50 times more potent than histamine on the isolated spontaneously beating guinea-pig right atrium (positive chronotropic effect), a standard model used for the pharmacological characterization of H_2 receptor ligands. Impromidine is a full agonist on the atrium but, depending on species and the tissue studied, its intrinsic activity may be lower.[15,35-40]

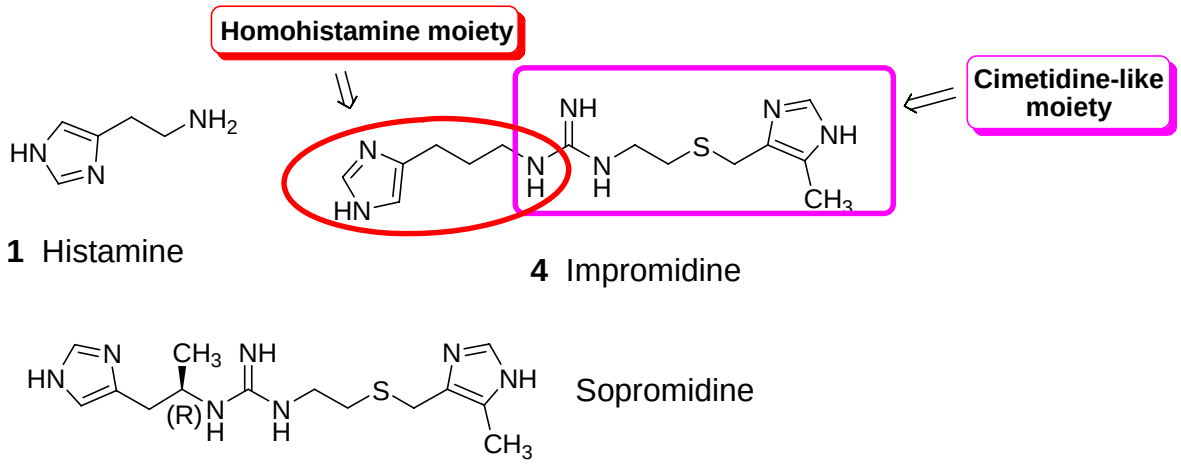


Fig. 1.5: Examples of H_2-receptor agonists: Impromidine.

Numerous impromidine analogues have been synthesized and analysed for agonistic activity at H_2-receptor[41,42] Highest potency is observed for compounds with a three- instead of a two- carbon chain as in histamine connecting the imidazole ring and the basic

guanidine group, although the corresponding partial structures of impromidine and histamine are considered as functionally equivalent groups that are important for the receptor activation. The lower homologue of impromidine is considerably less potent than impromidine. However, an unique stereochemical differentiation was found for the (*R*)-configured methyl branched imidazolylethylguanidine, sopromidine, which proved to be an H_2 receptor agonist achieving nearly 7 times the potency of histamine in gunea-pig atrium, whereas the (*S*)-enantiomer has weak H_2 receptor antagonistic properties.[43] Baumann and co-workers demonstrated that H_2 receptor agonists may be useful as positive inotropic drugs for the treatment of catecholamine insensitive congestive heart failure, by intravenous administration of impromidine to severely ill patients.[44] Unfortunately, the compound is also a powerful stimulant of gastric acid secretion, it is inactive after oral administration, and it produces pronounced disrhythmias, even in very low concentrations. Thus, for general therapeutic application H_2 agonists with a more beneficial profile of action, e.g., less pronounced induction of rhythm disturbances, lower positive chronotropic effect, and more favorable heart/stomach activity ratio than that of impromidine as well as activity after oral dosage, are required.

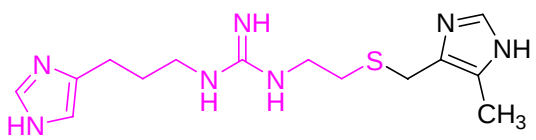
Therefore, at the beginning of an extensive research programme impromidine (**4**) was considered the chemical lead. The imidazolylpropylguanidine moiety appears to be essential for the agonistic activity.[45] Several attempts to replace the homohistamine part by other groups resulted in a decrease in activity. However, variations of the other partial structures were tolerated in a wide range. The molecule was mainly modified in two ways:

(I) The “cimetidine-like” part, which is considered to confer the high receptor affinity on impromidine, was replaced by alternative groups which are known from antagonists to contribute H_2 receptor affinity, such as imidazoles, thiazoles, piperidinomethylphenoxy groups, etc.[42,46,47]

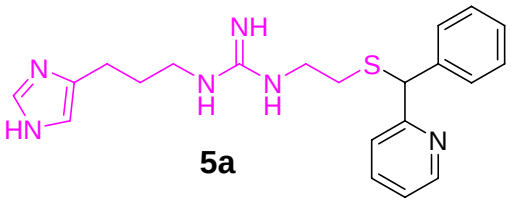
(II) The “cimetidine-like” part was replaced by lipophilic H_2 nonspecific structures.

Although potent H_2 receptor selective agonists were obtained in both ways, the most interesting compounds were found in the latter group. Replacement of 5-methyl-4-imidazolylmethylthio moiety in impromidine with aryl, and arylmethyl ethers and thioethers resulted H_2 agonists with up to 5 times higher potency than that of histamine in the guinea-pig atrium. Additionally, these compounds proved to be weak H_1 receptor

antagonists in the guinea-pig ileum. The H_2 agonistic potency could be retained and the H_1 receptor antagonistic activity further increased by replacement of the monoaryl with diaryl- or aryl(heteroaryl)methylthio groups. The guanidine **5a** was found to be about 4 times more potent than histamine on H_2 receptors and, additionally, to be a moderately active H_1 antagonist, achieving about 10 % of the potency of diphenhydramine or pheniramine.[48]



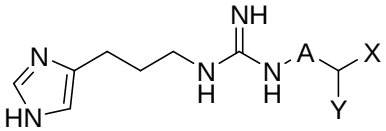
4 Impromidine



5a

Fig. 1.6: Structure of impromidine (**4**) and compound **5a**: replacement of the cimetidine-like moiety in **4** with a pheniramine-like phenyl(pyridyl)methyl group.

Table 1.2: Structures and histamine H_2 receptor agonistic activities of the arpromidine-like imidazolylpropylguanidines **5a-i**[49]

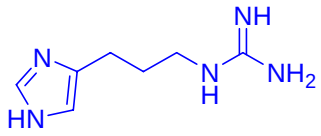


5a-k

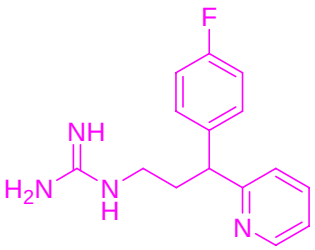
No.	A	X	Y	$_pEC_{50}$	Rel. potency
Histamine (**1**)				6.00	1.0
Impromidine (**4**)				7.70	50.1
5a	CH_2CH_2S	2-pyridyl	Ph	6.60	4.0
5b	$(CH_2)_3$	2-pyridyl	Ph	6.61	4.1
5c	$(CH_2)_2$	2-pyridyl	Ph	7.39	25.5
Arpromidine (**5d**)	$(CH_2)_2$	2-pyridyl	4-FC_6H_4	8.01	102.3
5e	$(CH_2)_2$	2-pyridyl	3,4-$F_2C_6H_3$	8.12	131.8
5f	$(CH_2)_2$	2-pyridyl	3,5-$F_2C_6H_3$	8.05	112.2
5g	$(CH_2)_2$	2-pyridyl	3,4-$Cl_2C_6H_3$	8.19	154.9
5h	$(CH_2)_2$	2-pyridyl	3,5-$Cl_2C_6H_3$	7.37	23.4
5i	$(CH_2)_2$	3-pyridyl	4-FC_6H_4	8.01	123.0

Bioisosteric replacement of 'S' by 'CH_2' and optimization of the chain led to **5b**, the parent molecule of H_2 agonists with pheniramine-like moieties. This compound is about 25 times more potent than histamine and is an H_1 receptor antagonist with about 16 % of the activity of pheniramine, additionally.[48,49]

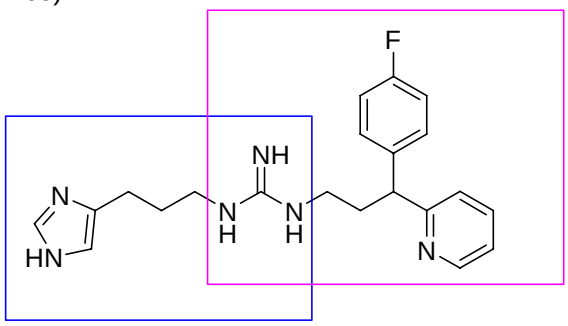
In a series of ring substituted analogues the p-fluoro compound (**5d**, arpromidine) was found to be the optimum at both histamine receptors.[49] Arpromidine (**5d**) is composed of the weakly active partial H_2 receptor agonist imidazolylpropylguanidine (**X, Fig. 1.7**) (SK&F 91486)[50] and the pheniramine-like guanidine (**Y**), which is a weak H_1 antagonist.[49] The hybrid molecule possesses about 100 times histamine's potency on the guinea-pig atrium and H_1-antagonistic activity comparable to pheniramine on the guinea-pig ileum. Thus the pheniramine part provides additional binding to the H_2 receptor and the homohistamine group increases the H_1 receptor affinity of the fragment **X**. Eventually, Arpromidine became a promising new chemical lead for the development of 'cardiohistaminergics'.[51]



X Weak partial H_2-agaonist
(0.04 x histamine, i.a. = 0.3;
pD_2 = 4.65)



Y Weak H_1-antagonist
(pA_2 ca. 6.6; noncompetitive)



5d H_1-antagonist:pA_2 = 7.65
Potent H_2-agonist, 100 x histamine

Fig. 1.7: Symbiotic approach for the design of arpromidine[49]

Table 1.2 shows the results for arpromidine analogues substituted at the phenyl nucleus. Ortho halogenation does not affect the agonistic activity. By contrast, *meta* or *para*

substitution with Cl or F results in up to a 4-fold increase in potency, arpromidine being most potent in the monosubstituted series. Further increase in agonistic activity is achieved with the 3,4-difluoro, 3,5-difluoro and 3,4-dichloro analogues (**5e-h**), which are 100-160 times as potent as histamine. An exception is the 3,5-dichloro compound **5h**, which is considerably less active than its isomer **5g**. The 3-pyridyl isomer (**5i**) of arpromidine is about equipotent on the atrium, but 10 times less active as H_1 antagonist on the ileum. These arpromidine analogues, so-called 'cardiohistaminergics', were developed as positive inotropic vasodilators for the treatment of severe congestive heart failure.[47,51-55] The 3,4- and 3,5-difluorinated compounds **5e** and **5f** proved to be superior to impromidine in potency, hemodynamic profile and side effects when tested in the guinea-pig under physiological conditions and in a pathophysological model of severe congestive heart failure (vasopression-induced acute heart failure).[55,56] Moreover, arpromidine-like compounds having both H_2 receptor agonistic and H_1 receptor antagonistic properties significantly increased the survival time in rat endotoxic shock.[57] Independent from H_2 agonism, such compounds were described as first competitive non-peptidic neuropeptide Y (NPY) Y_1 receptor antagonists.[58-60] These compounds exhibited only low moderate Y_1 receptor affinity (pK_i up to 6.5), however, they proved to be useful as chemical leads for the development of more potent non-peptidic NPY receptor antagonists.[61]

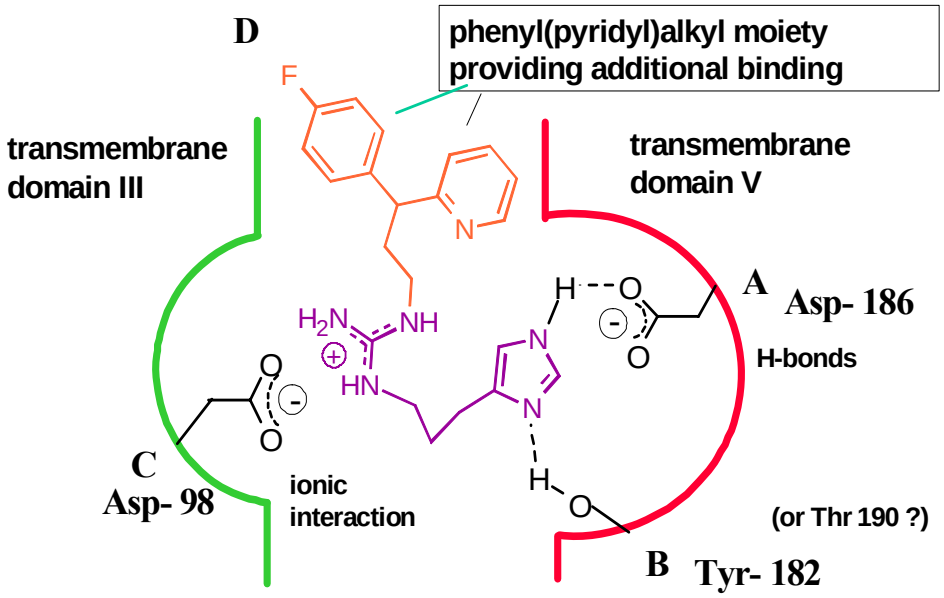


Fig. 1.8: Possible interactions of arpromidine with the guinea-pig H_2 receptor[49]

The interaction of guanidine-type agonists with the histamine H_2 receptor may be interpreted by analogy with the model proposed for histamine by Weistein et al.[27,62]

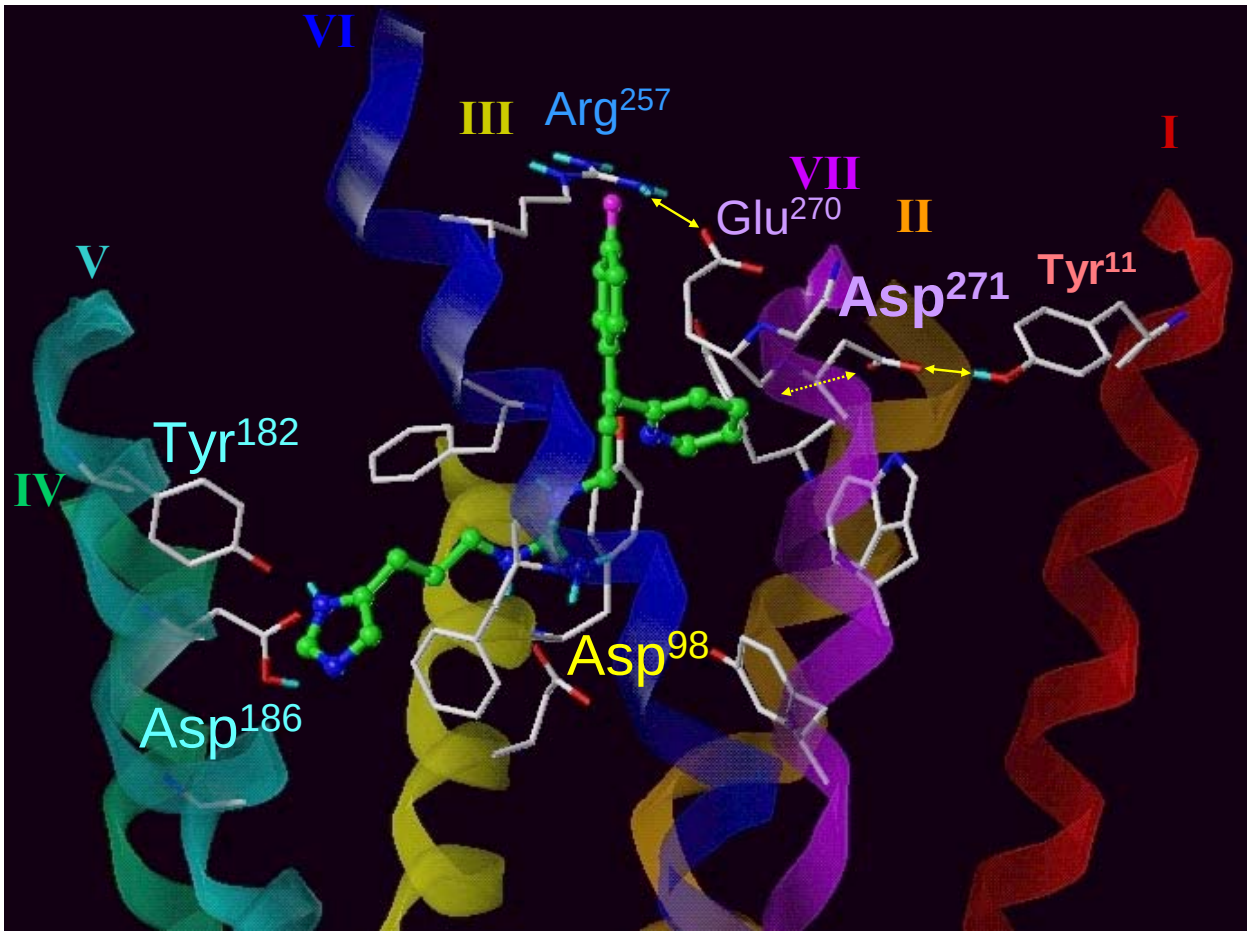


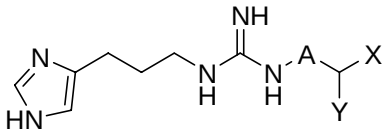
Fig. 1.9: Molecular modeling representation of the binding of arpromidine (C-atoms green) at the guinea-pig histamine H_2 receptor. Side chains and α-C atoms are drawn only for the putative binding site and residues of the upper parts of TM1 and TM7, wich are different from the human H_2-receptor.[42]

The imidazole ring may be involved in a proton transfer mechanism by interacting with hydrogen bonding regions (A, B) (**Fig. 1.8**). A shift in the tautomeric system could be induced by interaction of the guanidinium cation with a negatively charged group (e. g., a carboxylate group) (C). Since imidazolylpropylguanidine (**X**) is only a weakly active partial H_2 agonists, the phenyl(pyridyl)propyl substituent of arpromidine (or a corresponding class

of potent compounds) appears to increase the receptor binding to a site D. On the basis of molecular modeling investigations (**Fig. 1.8**) some amino acids of transmembrane domains **6** and **7** were suggested to be involved in this interaction.[63]

The diphenylpropylguanidine (**6a**) analogues of arpromidine are known to produce a similar dual mode of action but at a lower level of potency. Two aromatic rings are not required. For example, one or both of the aryl rings may be replaced by a cyclohexyl (**6f-h**) and a methyl group (**6e**) resulting in H_2 agonists which are more potent than histamine.[64]

Table 1.3: Structures and histamine H_2 receptor agonistic activities of diphenylalkyl-, phenylalkyl- and cyclohexylalkyl-substituted imidazolylpropylguanidines[64]



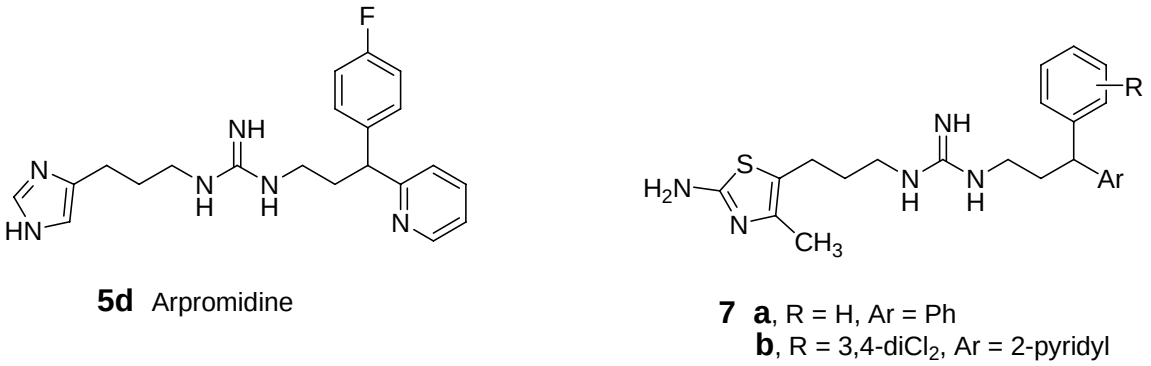
6a-h

No.	A	X	Y	pEC_{50}	rel. potency
Histamine (**1**)				6.00	1.0
Impromidine (**4**)				7.70	50.1
Arpromidine (**3d**)	$(CH_2)_2$	2-pyridyl	4-FC_6H_4	8.01	102.3
6a	$(CH_2)_2$	Ph	Ph	7.15	14.1
6b	CH_2	Ph	Ph	6.20	1.6
6c	$(CH_2)_2$	Ph	4-FC_6H_4	7.75	56.2
6d	$(CH_2)_2$	4-FC_6H_4	4-FC_6H_4	7.75	56.2
6e	$(CH_2)_2$	Ph	Me	7.50	31.6
6f	$(CH_2)_2$	c-Hex	H	6.80	6.3
6g	$(CH_2)_3$	c-Hex	H	6.40	2.5
6h	$(CH_2)_2$	c-Hex	Me	7.50	31.6

Very recently, the stereoisomers of the 3,4-difluorinated and the 3,4-dichlorinated arpromidine analogues (**5e**, **5g**) were isolated and tested on the gunea pig right atrium. The (*S*)-enantiomers were found to be the eutomers with eudismic ratios of up to 40.[65,66] These

compounds are up to 400 times more potent than histamine on the guinea-pig right atrium and are the most potent H_2-receptor agonists known so far.

However, in addition to their agonistic activity at the H_2 receptor and weak to moderate antagonistic activity at H_1 receptor, the impromidine and arpromidine-like compounds have also remarkable H_3 antagonistic properties (in a low nanomolar range) as examplified for some representative derivatives. Eriks et al. have found that the imidazole ring of the arpromidine-like molecule is not essential for H_2 agonism, but can be replaced with an amthamine-like 2-amino-4-methylthiazol-2-yl substructure (**7a,b**; **Fig. 1.10**).[26] The resulting compounds were reported to be superior to the imidazole analogues concerning H_2 receptor selectivity, in particular vs. the H_3 receptor.[26]



5d Arpromidine

7 **a**, R = H, Ar = Ph
b, R = 3,4-diCl_2, Ar = 2-pyridyl

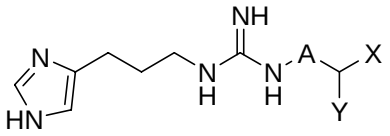
Fig. 1.10: Arpromidine and analogous N-[3-(2-amino-4-methylthiazol-2-yl)propyl]-guanidines (**7a,b**)

1.2.3 Species selectivity of histamine H_2 receptor agonists

The guinea-pig right atrium was successfully used as a pharmacological *in vitro* model for the characterization of H_2 receptor ligands including the antagonists developed as antiulcer drugs. Nevertheless, discrepancies in potencies and efficacies of H_2 receptor agonists as well as different H_2 receptor blocking activities e. g. in models of gastric acid secretion, were found on different tissues of various species and appeared to be dependent on the lipophilicity of the compounds.[35,51,67,68] Previous investigations of guanidine-type agonists on the human H_2 receptor (hH_2R), using neutrophils as a model, and on the guinea pig H_2 receptor (gpH_2R), using the isolated right atrium, showed that, in contrast to H_2 antagonists and amine type H_2 agonists, there were considerable differences in the potencies of

guanidines between both species. Specifically on the hH_2R the arpromidine-type compounds were less potent than expected and generally only partial agonists.[35,49,69] However, interpretation of the result was difficult because human neutrophils and guinea pig atrium represent very different analysis systems concerning receptor expression levels and diffusion of the compounds.[35]

Table 1.4. **Agonist efficacies and potencies at hH_2R-Gsαs, gpH_2R-Gsαs fusion proteins and at the hH_2R-A271D-Gsαs mutant expressed in Sf9 cells (results from GTPase assay on cell membrane preparations)[63]**



5b-d

No.[a]				hH_2R-Gsαs		gpH_2R-Gsαs		hH_2R-A271D-Gsαs	
				Efficacy	**pD_2**	**Efficacy**	**pD_2**	**Efficacy**	**pD_2**
	Amines[b]								
1	histamine			1.00	5.90	1.00	5.92	1.00	6.46
2	dimaprit			0.85	5.71	0.93	5.40	-	-
3	amthamine			0.90	6.35	1.40	6.36	-	-
	Imidazolylpropylguanidines[c]								
	A	**X**	**Y**						
4	impromidine			0.84	6.70	1.00	7.41	0.85	7.57
5b	$(CH_2)_3$	2-pyridyl	Ph	0.56	6.55	0.93	6.72	0.76	6.55
5c	$(CH_2)_2$	2-pyridyl	Ph	0.86	6.38	1.02	7.14	0.95	7.10
5d	$(CH_2)_2$	2-pyridyl	4-FC_6F_4	0.79	6.72	1.02	7.12	0.91	7.22

[a]Numbers of amine as in **Fig. 1.3,** numbers of imidazolylpropylguanidines as in **Table 1.2,** [b]For structure of amines, see **Fig. 1.3**; [c]For structure of imidazolylpropylguanidines see **Fig. 1.6**.

Very recently, the groups of Seifert and Buschauer[63] investigated in detail the species differences of H_2 receptor agonists of different structural classes by using the GPCR-Gα fusion protein technique[70,71]. The coupling of hH_2R and gpH_2R to GsαS could be compared under identical experimental conditions, so that an unequivocal dissection of the pharmacological difference between hH_2R and gpH_2R with respect to agonistic activity of amine and guanidine was possible. This is demonstrated by the efficacies and potencies of representative H_2 receptor agonists of each structural class in the steady state GTPase assay (**Table 1.4**).

The efficacies of histamine, dimaprit and amthamine were similar at hH_2R-GsαS and gpH_2R-GsαS, whereas the guanidines were significantly less efficacious at hH_2R-GsαS than at gpH_2R-GsαS. Elongation of the alkyl chain between the guanidino group and the phenyl ring (**5c** *vs*. **5b**) and introduction of Br or of multiple Cl atoms into the phenyl ring strongly decreased agonist efficacy at hH_2R-GsαS but not at gpH_2R-GsαS. These results indicate that the hH_2R-GsαS and gpH_2R-GsαS conformations stabilized by one of the small amines similarly promote GDP/GTP exchange. In contrast, the guanidines stabilize a hH_2R-GsαS conformation considerably less efficient for GDP/GTP exchange than the corresponding gpH_2R-GsαS conformation.[63]

The potencies of amines differed by not more than 0.21 log units between hH_2R-GsαS and gpH_2R-GsαS (**Table 1.4**). All guanidines except **5b** were significantly less potent at hH_2R-GsαS than at gpH_2R-GsαS. The pD_2 differences between hH_2R-GsαS and gpH_2R-GsαS are rather similar (ca. 0.4 – 0.8), indicating a nearly constant contribution of the guanidino-alkylaryl moiety to the different ligand interactions with hH_2R and gpH_2R. Agonist potency was decreased by almost three-fold at gpH_2R-GsαS by elongation of the alkyl chain between the guanidino group and the phenyl ring (**5c** *vs.* **5b**), but slightly increased at hH_2R-GsαS. Taken together, guanidines stabilize an active conformation in gpH_2R not only more efficiently but also with higher affinity than in hH_2R, and the structure-activity relationships for guanidines at hH_2R and gpH_2R are slightly different.[63]

Considerations from a sequence alignment of hH_2R and gpH_2R and from gpH_2R models suggested that an exchange of Ala271 (hH_2R) against Asp271 (gpH_2R) in TM7 should be the main difference of the agonist binding site between both receptor species. Results on a hH_2R-A271D-GsαS fusion protein mutant (**Fig. 1.11**)[63] confirmed this hypothesis (**Table 1.4**).

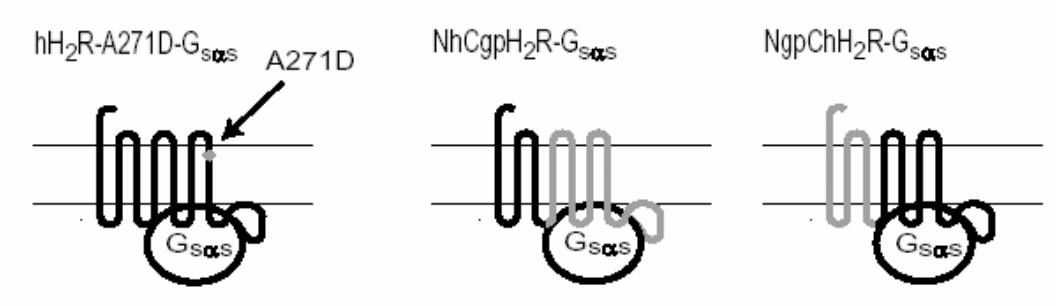


Fig. 1.11. Schematic representation of hH_2R-A271D-$G_{s\alpha S}$ and chimeric H_2 receptor fusion proteins[63]

The pD_2 values of guanidines at hH_2RA271D-GsαS and gpH_2R-GsαS are nearly identical. Thus, the Ala-271→Asp-271 mutation increased the potency of hH_2R for guanidines to the level of gpH_2R. These findings indicate that ion-dipole or H-bond interactions with Asp271 may play a role. Such interactions cannot occur with Ala271 in hH_2R, explaining why the guanidines exhibit substantially lower potencies at hH_2R than at gpH_2R. Regarding the properties of some specific agonists, it becomes obvious that elongation of the alkyl chain between the guanidino group and the phenyl ring (**5c** *vs.* **5b**) decreased agonist potency at hH_2R-A271D-GsαS by 0.55 log units (**Table 1.4**). This decrease in potency is similar to that observed at gpH_2R-GsαS.

Conversely, at hH_2R-GsαS the longer alkyl chain slightly increased agonist potency. These data suggest that the amino acid at position 271 of H_2Rs affects the size and flexibility of the guanidine binding pocket. With Ala271, the binding pocket is wider, more flexible and accommodates the longer (**5b**) as well as the shorter guanidine (**5c**). In contrast, with Asp271, the fit of the longer guanidine must probably be enforced by conformational strain.

Among all guanidines studied, the amino acid substitution at position 271 had the greatest and most consistent impact on the potency of impromidine (**4**). With Asp271 the potencies (pD_2 values) of impromidine (**4**) were consistently about 0.7 to 0.9 log units higher than with Ala271. For other guanidines, the impact of the amino acid substitution at position 271 was less consistent. These data indicate that the binding of impromidine to H_2Rs is considerably more dependent on interaction with Asp271 than the binding of other guanidines to H_2R.

So, guanidines stabilize an active conformation in gpH_2R more efficiently and potently than in hH_2R. Studies on a hH_2R-A271D-GsαS fusion protein mutant and also on chimeric hH_2R/gpH_2R receptors confirmed that Asp271 accounts for the high potency of the guanidines. However, their high efficacy observed in gpH_2R was not restored by the mutants. The data show that hH_2R and gpH_2R selectively interact with a single class of synthetic agonists, that high agonist potency is mainly due to interaction with a single amino acid and agonist potency and efficacy are regulated independently of each other. The inverse order of potency of compounds **5c** and **5b** at hH_2R and gpH_2R, respectively, indicates that it is possible to develop guanidines with high, selective potency and efficacy at hH_2R. Such compounds could be useful for the treatment of cardiac failure, acute myelogenous leukemia and inflammatory diseases.

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Chapter 2

Objectives

The strongly basic guanidino group is essential for the histamine H_2 receptor (H_2R) agonistic activity of arpromidine and analogues,[1,2] but it is also responsible for very low oral bioavailability and the lack of penetration across the blood-brain barrier.[3] Impromidine, arpromidine and related guanidines are nearly quantitatively protonated at physiological pH and are virtually inactive after oral administration. It has been shown in previous work that prodrugs of the arpromidines can be prepared by changing the physicochemical properties through introducing electron-withdrawing substituents such as an ethyl ester group at the guanidine.[4] These compounds were inactive as H_2R agonists *in vitro* but proved to be active after introduodenal application in guinea pigs. The ester group is cleaved *in vivo* and the active molecule, free guanidine, is formed by spontaneous decarboxylation of the intermediate guanidine-N-carboxylic acid. This approach was successful to obtain peripherally active H_2R agonists, which may be useful, for example, for the treatment of congestive heart failure. However, centrally active guanidine-type histamine H_2R agonists are not accessible in the same way. Centrally active H_2R agonists could be valuable pharmacological tools to investigate the role of H_2Rs in the brain. Such compounds are not available so far.

The objective of this work was to design, synthesize and characterize histamine H_2 receptor agonists, structurally related to arpromidine, but with lower basicity in order to achieve more favourable pharmacokinetic properties, in particular, oral bioavailability and penetration across the blood-brain barrier. Previous preliminary investigations from our group gave a first hint to a possibly successful general strategy for the structural modification of arpromidine-type H_2R agonists: a few synthesized diphenylalkanoyl-guanidines were found to have about the same H_2R agonistic potency as the corresponding diphenylalkylguanidines regardless of considerably reduced pKa values of the guanidino group (pKa $\approx$ 8 compared to $\approx$ 13 for guanidine). Obviously, the acylguanidine may be

considered a bioisostere of the alkylguanidine moiety in arpromidine-like compounds. Based on this working hypothesis the preparation of a large series of new H_2R agonists including the analogues of the most potent arpromidine analogues and the investigation of the structure activity relationships were in the focus of the present work.

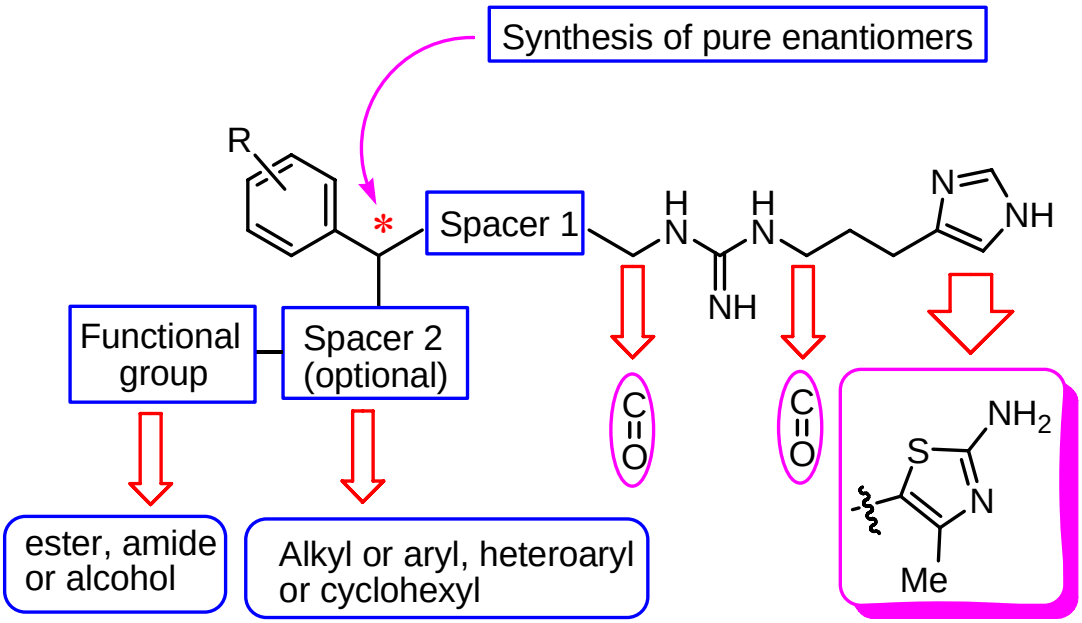

Fig. 2.1: General structure of potential histamine H_2 receptor agonists to be synthesized

- The first part of this project was to develop a general synthetic route for the synthesis of N^G-acylated imidazolylpropylguanidines, i. e. a methylene had to be replaced with a carbonyl group in the connecting chain between the diaryl and guanidine group.
- Then the substitution patterns had to be optimised according to the structure activity relationships gained previously from a large series of diphenylpropylguanidines, in particular, fluorine in p-position should be introduced because fluorinated compounds are known to be at least half an order of magnitude more active H_2R agonists.
- As an alternative the carbonyl group should be introduced in the imidazolylpropyl portion of the molecule to verify the role of the other guanidine nitrogen atoms in the interaction with Asp 98 in transmembrane domain 3 of the H_2R protein.
- Furthermore, one of the phenyl rings should be omitted or replaced by heterocycle, alkyl or cyclohexyl groups or by partial structures having functional groups capable of

interacting with the H_2 receptor protein in the region identified as important for the binding of arpromidine and impromidine.

- The 3-(imidazol-4-yl)propyl moiety of N^G-acylated imidazolylpropylguanidines should be replaced by a 3-(2-amino-4-methylthiazol-5-yl)propyl side chain to reduce the H_3 receptor activity.
- With respect to the development of models for the ligand receptor interactions enantiomerically pure agonists of defined 3D structure are valuable tools to explain the species dependent potency and to optimise the structure of the substances. So, the enantiomers of several chiral new H_2R agonists should be selectively prepared or separated.
- The synthesized compounds should be pharmacologically studied for histamine H_2R agonism and selectivity for H_2R *versus* the other histamine receptors. Moreover, the compounds should be investigated at H_2R agonists of different species (human and guinea pig H_2R) to study the species dependent structure-activity relationships and to refine the H_2R model[3] based on bovine rhodopsin.[a]
- Moreover, selected compounds should be investigated for absorption from the gastrointestinal tract and for penetration into the brain.[b]

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[a] Cooperation with Prof. Dr. S. Elz and Prof. Dr. R. Seifert (pharmacological investigations) and Prof. Dr. S. Dove (Molecular Modelling).

[b] These investigations (HPLC-MS studies) were subject of a diploma thesis by Max Keller.

Chapter 3
Synthesis and Pharmacological Activity of N^G-Acylated Imidazolylpropylguanidines

The only thing that I know is that I don't know anything **Plato**

3.1. Introduction

Histamine (**1**) is a neurotransmitter, an autacoid and involved in numerous biological processes in the CNS and in peripheral tissues.[1-5] The biological effects of histamine are mediated by four receptor subtypes, H_1, H_2, H_3 and H_4 receptors, which all belong to the G-protein coupled receptor (GPCR) superfamily.[1] The histamine H_2 receptor (H_2R) couples to the G-protein G_s to mediate adenylyl cyclase activation[1] The discovery and pharmacological characterisation of the H_2R by Black and collaborators[6] in 1972, the development and clinical use of drugs for the treatment of gastric and duodenal ulcer have provided evidence for an important physiological role of histamine (**1**, **Fig. 3.1**) in the regulation of gastric acid secretion. Meanwhile, H_2Rs were detected in numerous other peripheral tissues and cells, for example, in leukocytes, the heart, airways, uterus and vascular smooth muscle, and in the brain[1,7-10] Subsequently, the H_2R was cloned from several species including rat, guinea pig, mouse, dog and humans.[11]

Numerous histamine H_2 receptor agonists[12-15] are known which may be roughly classified into two classes: the amine- and the guanidine-type H_2R agonists corresponding to histamine-like (**Fig. 3.1**, **1-3**) and impromidine-like compounds (**Fig 3.1**, **4 and 5**). Impromidine[16], the prototypical guanidine-type H_2R agonist, is nearly 50-fold more potent than histamine on the isolated spontaneously beating guinea pig right atrium, a standard model for the pharmacological investigation of H_2R ligands. By using Impromidine, Baumann and his co-workers demonstrated that H_2R stimulation may be an effective treatment for patients suffering from severe catecholamine-insensitive congestive heart failure.[17]

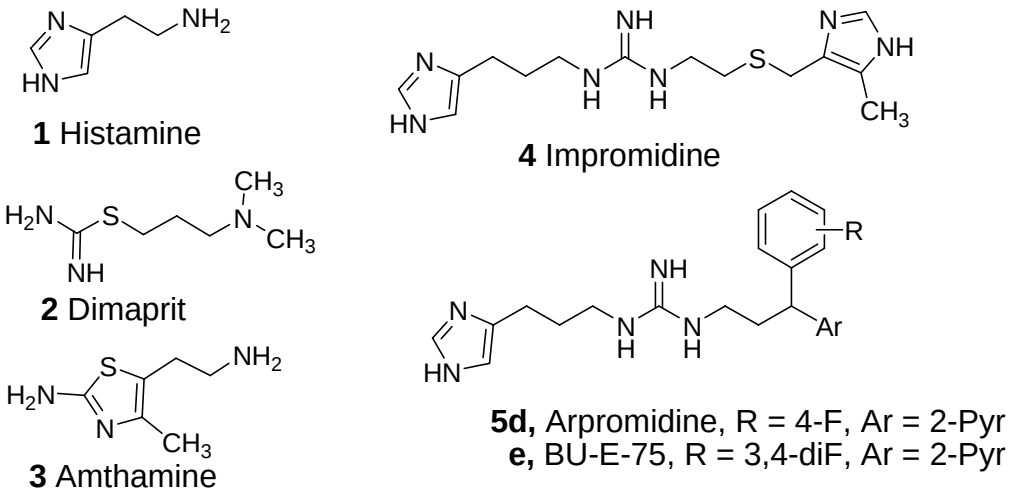


Fig. 3.1: Structure of some histamine H_2 receptor agonists

This proof of concept encouraged to search for new H_2R agonists with better pharmacological profile than that of impromidine. Buschauer and his co-workers developed Arpromidine[14] (**5d**) and a large series of related imidazolylpropylguanidines [**5e**] ('cardiohistaminergics'), as positive inotropic vasodialators.[14,18-20] The most active analogues achieve about 400 times the activity of histamine on the isolated spontaneously beating guinea pig right atrium. These arpromidine-like H_2R agonists, in particular 3,4- and 3,5 difluorinated analogues proved to be superior to impromidine in potency, hemodynamic profile and side effects when tested in the guinea pig under physiological conditions and in a pathological model of severe congestive heart failure[20]. Moreover, it has been speculated that H_2 agonists might be useful as anticancer agents in leukemia to induce cell differentiation[21] and as anti-inflammmatory drugs.[22,23]

The arpromidines proved to be less potent or to act as partial agonists at human (hH_2R) compared to guinea pig H_2 receptors (gpH_2R)[24]. It has been suggested from in vitro mutagenesis combined with molecular modelling studies that the presence of Ala-271 in the hH_2R in place of Asp-271 in the gpH_2R accounts for this species difference. The strongly basic guanidino group (pK_a value about 13), which is supposed to interact with Asp-98 in transmembrane domain 3, is essential for the agonistic activity of **5** and analogues but it is also the main reason for very low oral bioavailability, non-H_2R-mediated effects and lack of penetration across the blood-brain barrier. Impromidine, arpromidine and related guanidines are nearly quantitatively protonated at physiological pH and are virtually inactive following

oral administration. So our aim was to change the physciochemical properties by introducing electron withdrawing substituents, for instance a carbonyl group, at the guanidine group to increase oral bioavailability, reduce the non-H_2R-mediated effects and to have better penetration across the blood-brain barrier.

Here we report the successful synthetic route for the preparation and the pharmacology of N^G-acylated imidazolylpropylguanidines, which turned out to be a novel class of H_2 receptor agonists.

3.2. Chemistry

3.2.1. Retrosynthetic analysis of N^G-acylated imidazolylalkylguanidines

According to the concept of retrosynthesis the target molecules (**R**) could be assembled as schematically shown in **Fig. 3.2**. The acyl part (**R^{I}**) was thought to be attached to an iminium precursor (**R^{II}**) to give a *N*-acyl iminium precursor (**R^{III}**), which could be converted to the target R by nucleophilic addition elimination with imidazolylalkylamine (**R^{IV}**).

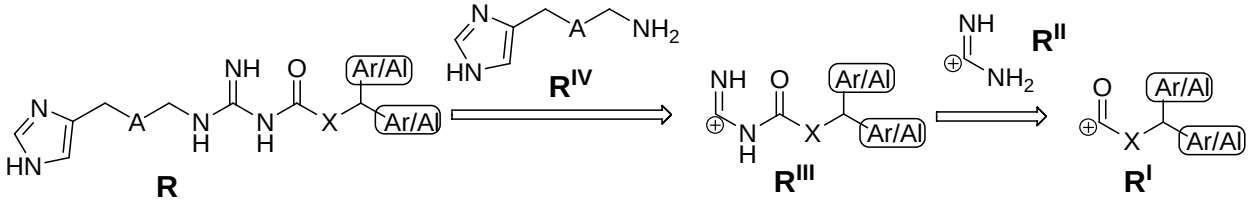
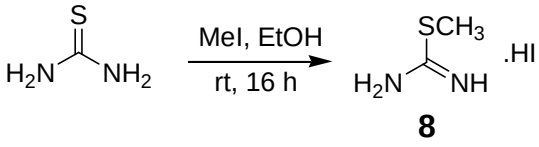


Fig. 3.2: Retrosynthetic analysis of ***N*^{G}**-acylated guanidines: Approach I

3.2.2. Synthesis of S-methyl thiourea

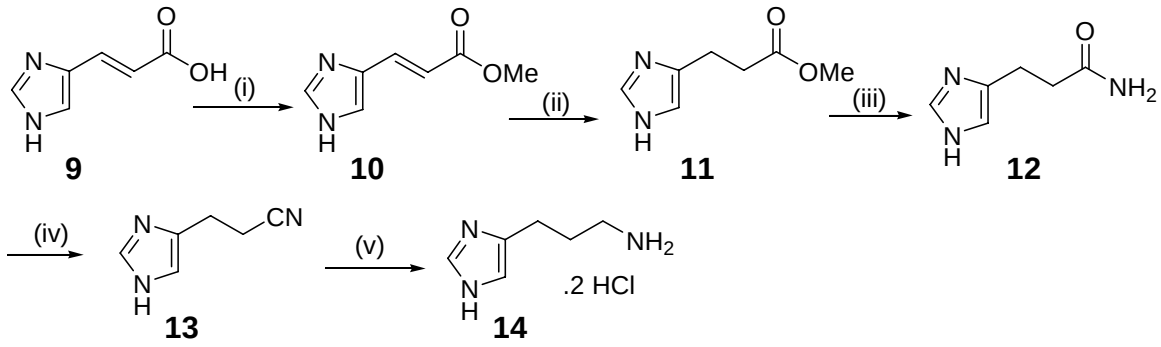
S-Methyl thiourea (S-methyl isothiourea, **8**), an iminium cation precursor was best prepared from thiourea and methyl iodide according to the known procedure (**Scheme 3.1**).[25]



Scheme 3.1: Synthesis of S-methyl thiourea

3.2.3. Synthesis of homohistamine

Starting from the commercially available urocanic acid (**9**), the intermediate ester **11** can be obtained via esterification in absolute methanol at a constant flow of dry HCl, followed by hydrogenation of the double bond over Pd/C catalyst [**Scheme 3.2**].[26]



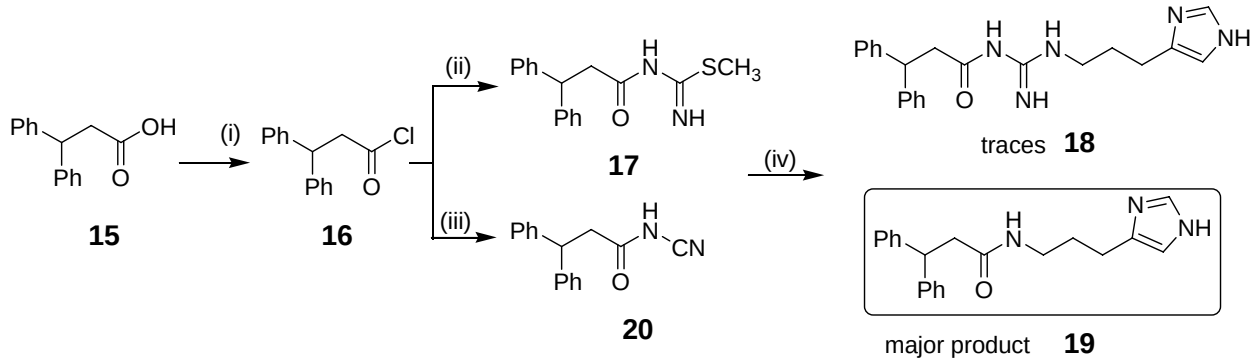
Reagents: (i) MeOH, dry HCl, 2 h reflux; (ii) H_2, Pd/C (10 %), MeOH; (iii) NH_3, reflux, 70 °C; (iv) $SOCl_2$, DMF, 60 °C; (v) H_2, Ra-Ni, 25 bar, NH_3, reflux.

Scheme 3.2: Synthesis of homohistamine from urocanic acid

The ammonolysis of the ester **11** in aqueous ammonia produced amide **12** which on treatment with thionyl chloride gave nitrile **13**. Subsequent reduction of nitrile with hydrogen over Raney-Ni in liquid ammonia provided homohistamine (**14**).[25]

3.2.4. Synthesis of N^G-acylated imidazolylpropylguanidines via the S-methyl thiourea and cyanamide route

3,3-Diphenylpropanoic acid (**15**) was activated with excess oxalylchloride to yield the acyl chloride intermediate **16**. Then without purification the S-methyl thiourea **8** was added to obtain the acylated intermediate **17**.



Reagents: (i) excess $(COCl)_2$, rt, 2 h; (ii) **8**, Et_3N, DCM, 0 °C, rt, 24 h; (iii)H_2N-CN, NaOH, acetone, 0°C, 4h; (iv) **8**, KO^tBu, HO^tBu

Scheme 3.3: Towards synthesis of N^G-acylated imidazolylpropylguanidine

Unfortunately, when homohistamine (**14**) was treated with **17**, in spite of producing the desired acylguanidine **18**, the amide **19** was formed as major product as a result of the nucleophilic attack to the carbonyl instead of the isothiourea group.

As an alternative, cyanamide was used as an iminium cation equivalent. By analogy with the aforementioned procedure the acid **15** was activated with excess oxyl chloride followed by addition of cyanamide to obtain the acyl cyanamide **20**. Unfortunately, treatment with homohistamine (**14**) as in case of the acylated thiourea gave **19** as the major product and only traces of the desired product (**18)**.

Therefore, we changed our strategy and developed an alternative synthesis route, which was retrosynthetically analysed as: first introduction of guanidine group to the imidazolylalkyl chain then acylation at the guanidine group, then acylation of the guanidine group.

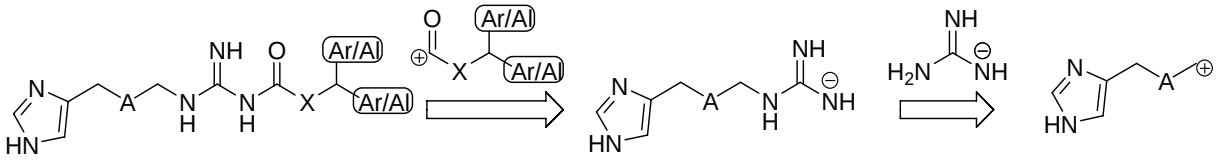
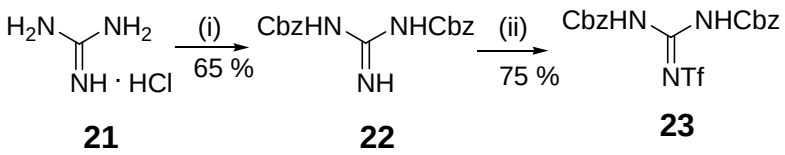


Fig. 3.3: Retrosynthetic analysis of N^G-acylated guanidines: Approach II

3.2.5. Protection of guanidine

Guanidine is a strong base and only available as salt. It is generally not soluble in organic solvents, so it was protected with benzyl formate to yield the di-Cbz protected guanidine (**22**), which is soluble in organic solvents and can be purified either by column chromatography or recrystallisation (**Scheme 3.4**).[27] This is a versatile nucleophilic guanidinylation[28] reagent towards alcohols. Further, a triflate group was introduced to the di-Cbz protected guanidine **22** to provide compound **23**, which is an electrophilic guanidinylation reagent towards amines.



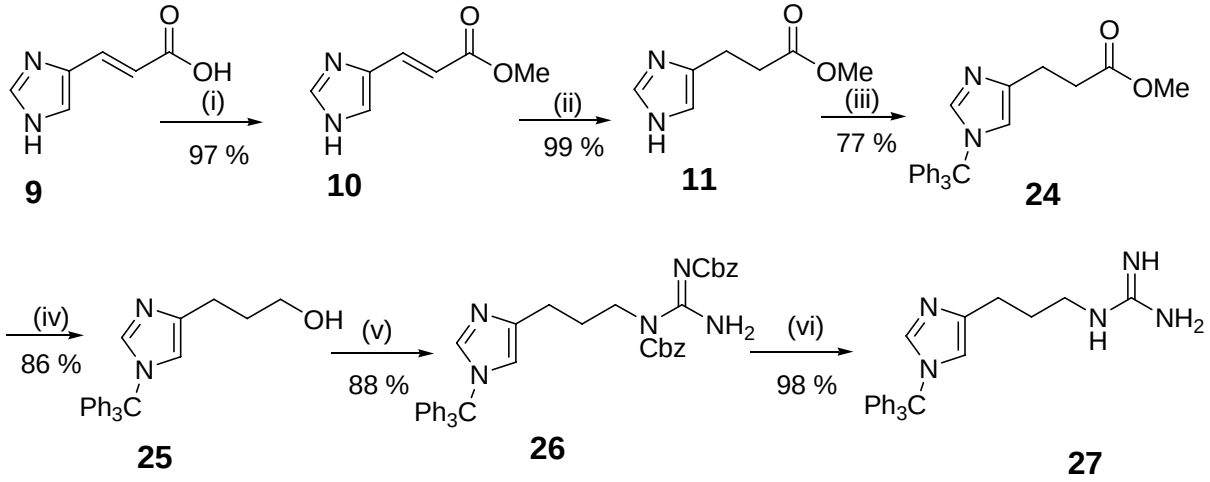
21 **22** **23**

Reagents: (i) CbzCl, NaOH, DCM / H_2O (4:2), 0 °C, 20 h; (ii) Tf_2O, NaH, THF, -45 to 0 °C, 16 h

Scheme 3.4: Protection of the guanidine group

3.2.6. Synthesis of the imidazolylpropylguanidine building block

Towards the synthesis of imidazolylpropyl guanidine, the intermediate imidazolylpropanol (**25**) was synthesized from urocanic acid (**9**) via esterification, hydrogenation of the double bond, trityl protection of the imidazole N-H to obtain a more lipophilic and easily separable intermediate, and finally reduction of the ester functionality is done by using lithium alanate.[26] Thereafter, the protected guanidine (**22**) was coupled to the imidazolylpropyl chain of the primary alcohol **25** under Mitsunobu conditions[27] with excellent yield, using triphenyl-phosphine and DIAD. Subsequent quantitative cleavage of the Cbz groups by hydrogenolysis produced trityl protected imidazolylpropylguanidine (**27**) [**Scheme 3.5**].



9 **10** **11** **24**

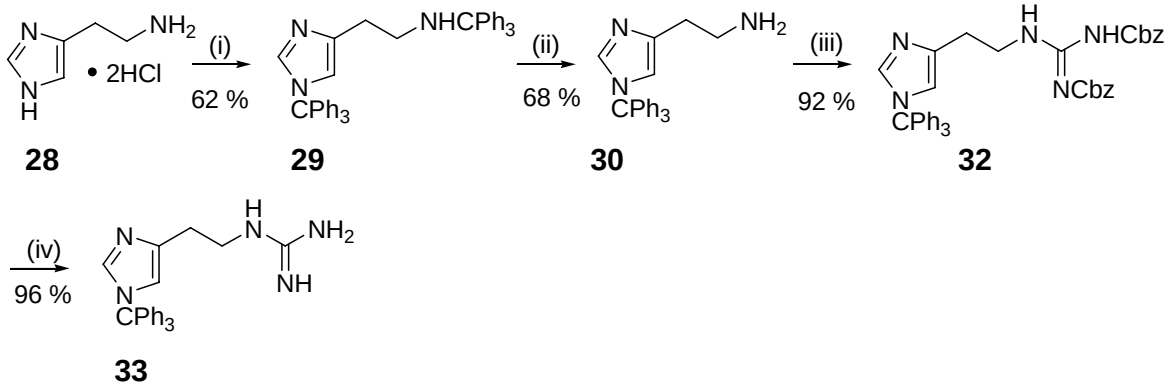
25 **26** **27**

Reagents: (i) MeOH, dry HCl, 2 h reflux; (ii) H_2, Pd/C (10 %), MeOH, 20 h; (iii) TrCl, MeCN, 12 h; (iv) LAH, THF, 2 h; (v) **22**, Ph_3P, DIAD, THF, overnight; (vi) H_2, 5 bar, Pd/C (10 %), THF, 9 h.

Scheme 3.5: Synthesis of the imidazolylpropylguanidine building block

3.2.7. Synthesis of the imidazolylethylguanidine building block

For the synthesis of the trityl protected imidazolylethylguanidine building block (**33**), we started from histamine (**28**) (**Scheme 3.6**).



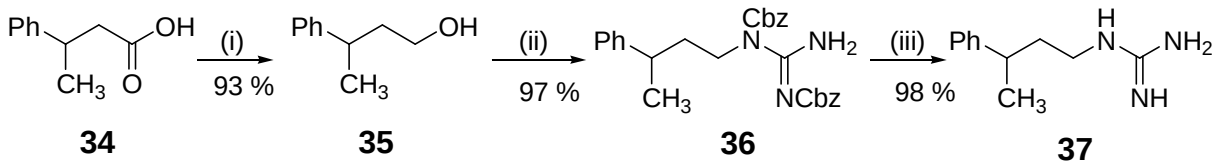
Reagents: (i) TrCl, Et_3N; (ii) 5 % TFA in DCM, 30 min, rt; (iii) 5 , Et_3N, 3 h; (iv) H_2, Pd/C (10 %), THF, overnight.

Scheme 3.6: Synthesis of the imidazolylethylguanidine building block

The trityl protection of both imidazole N-H and primary NH_2 of the chain was carried out according to a known procedure[29]. Then the side chain amino group was detritylated at low concentration of TFA for a short period to give **30**. The difference in acidity of imidazole N-H and primary NH_2 made it possible to selectively deprotect the trityl group. Subsequently, the introduction of the guanidine group was carried out by following the Goodmann's procedure[27] for guanidinylation of amines, using di-Cbz- and Tf-protected guanidine (**23**). Then, the Cbz groups of **32** were quantitatively removed by hydrogenation over Pd/C catalyst to obtain compound **33**.

3.2.8. Synthesis of the arylalkylguanidine building block

The preparation of the arylalkylguanidine building block **37** (**Scheme 3.7**) was performed by analogy with the aforementioned procedure for the synthesis of compound **32**.



34 **35** **36** **37**

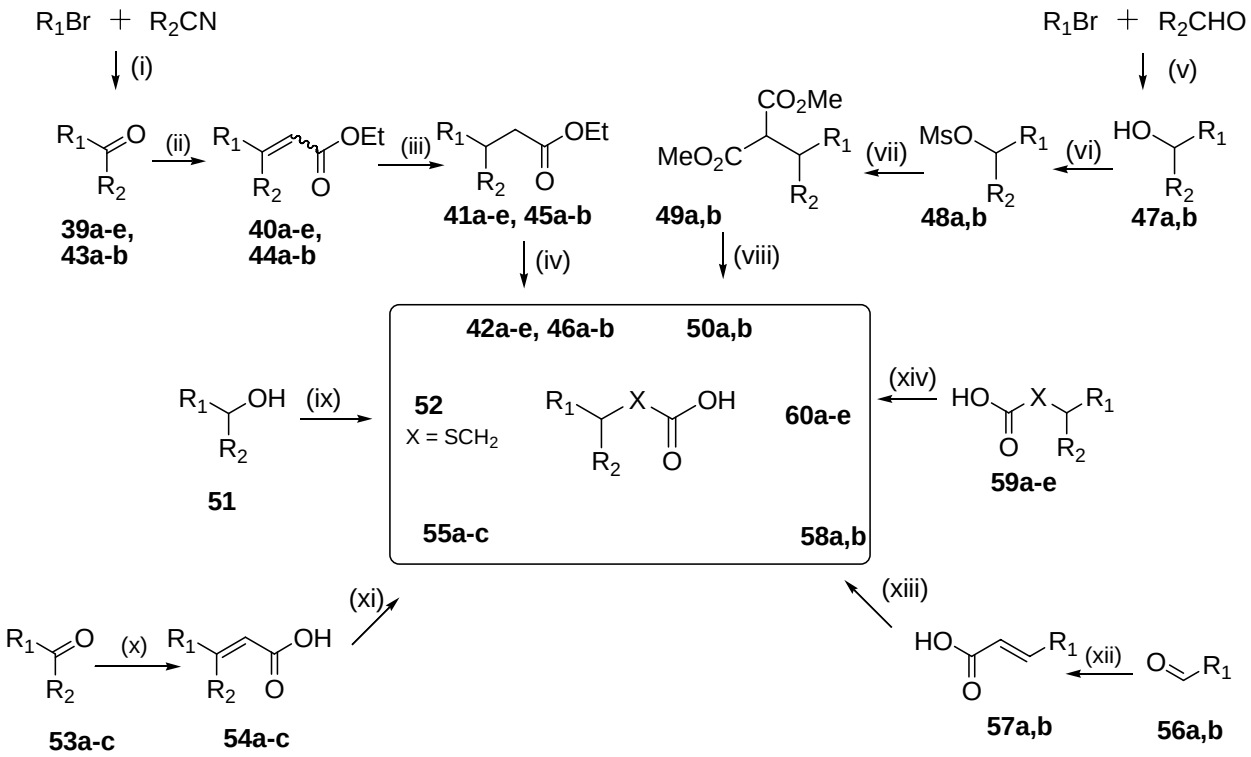
Reagents: (i) LAH, THF, 2 h; (ii) **22**, Ph_3P, DIAD, THF, 12 h; (iii) H_2 5 bar, Pd/C (10 %), THF, 10 h.

Scheme 3.7: Synthesis of the arylalkylguanidine building block

3.2.9. Synthesis of the alkanoic acids

A large series of alkanoic acids were synthesized by applying standard synthetic methods as summarized in **Scheme 3.8**. The ketones **39a-d** were prepared from nitriles via addition of lithium organyls, which were obtained from the corresponding heteroaryl bromides, followed by acid hydrolysis[30] (**Scheme 3.8**). The resulting ketones **39a-d** as well as the commercially available ketones **43a,b** were treated with triethyl phosphonoacetate to give the corresponding 3,3-disubstituted compounds **40a-d**[31] and **44a,b**[32]**.** The hydrogenation of the alkenoates to the corresponding alkanoic acid ethyl esters **41a-d** and **45a,b**, followed by hydrolysis produced the 3,3-disubstituted propanoic acids **42a-d** and **46a,b**.

1-(Pyridin-2-yl)ethanol (**47a**) was prepared from methyl magnesium bromide and pyridine-2-carbaldehyde by using a known procedure.[33] 1-(Thiazol-2-yl)ethanol (**47b**) was prepared by Grignard reaction from 2-bromothiazole and acetaldehyde.[34]



No.	R_1	R_2
39-42 a	Ph	2-pyridyl
b	4-F-C_6H_4	2-pyridyl
c	Ph	2-thiazolyl
d	3,4-diF-C_6H_3	2-thiazolyl
e	3,4-diF-C_6H_3	1-PhCH_2-2-imidazolyl
43-46 a	CH_3	2-pyridyl
b	CH_3	2-thienyl
47-50 a	CH_3	2-pyridyl
b	CH_3	2-thiazolyl
51-52	H	5-Me-4-imida zolyl

53-55 a	4-F-C_6H_4	4-F-C_6H_4	
b	Ph	4-F-C_6H_4	
c	Ph	3,4-diF-C_6H_3	
56-58a[25]	4-Cl-C_6H_4	4-Cl-C_6H_4	
b	4-Br-C_6H_4	4-Br-C_6H_4	
			X
59[b]**a**	Ph	CH_3	CH_2
b	Ph	H	-
c	Ph	CH_3	-
60[b]**a**	c-hexyl	CH_3	CH_2
b	c-hexyl	H	-
c	c-hexyl	CH_3	-
61[b]	1-trityl-2-imidazolyl	H	$(CH_2)_2$

[b] prepared by Anja Kraus[37]

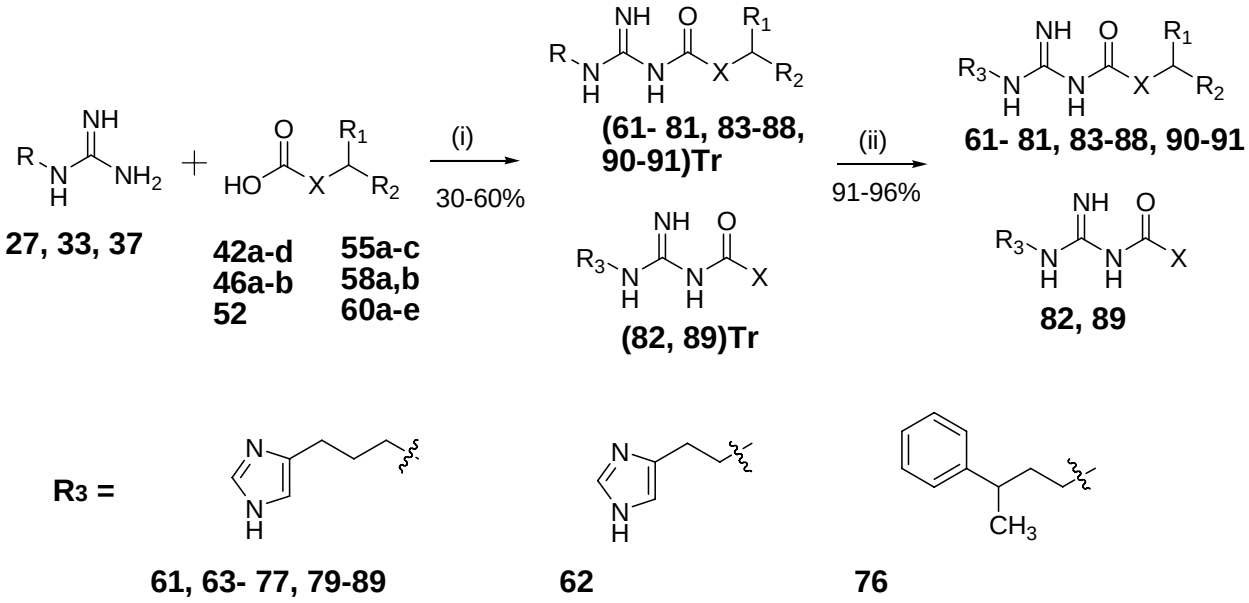
Reagents: (i) n-BuLi, THF/Et_2O, 0 °C, H_30^+; (ii) triethyl phosphonoacetate, NaH, THF, reflux; (iii) H_2, Pd/C (10 %), MeOH, rt; (iv) LiOH, DMEG or THF/H_2O, 5-12 h;(v) A: Mg, THF, rt; B: BuLi, THF, -78 °C; (vi) MsCl, DMAP, DCM; (vii) DMM, NaH, THF, rt, 12 h; (viii) a. NaOH, reflux; b. HCl, reflux, 12 h; (ix) $HSCH_2COOH$, Na_2CO_3, AcOH, reflux, 24 h; (X) triethyl phosphonoacetate, KO^{t}Bu, ^{t}BuOH, reflux, 12-16 h; (xi) H_2, Pd/C (10 %), MeOH, rt; (xi) triethyl phosphonoacetate, KO^{t}Bu, ^{t}BuOH, reflux, 12-16 h; (xii) ArX, $AlCl_3$; (xiii) Rh/Al_2O_3, AcOH, H_2 3 bar, 36-40 h.

Scheme 3.8: General procedure for the synthesis of acids

The alcohols were then converted to the mesitylate[35] **48a**,**b**, followed by nucleophilic displacement with dimethyl malonate[36], basic hydrolysis, and decarboxylation to produce the corresponding 3-substituted propanoic acids **50a,b**. Acid **52** was prepared according to the known procedure from **51**. The 3,3-diarylpropanoic acids **55a-c** were prepared by a very straightforward method via Wittig-Horner Reaction of corresponding ketones **53a-c** treated with triethyl phosphonoacetate, followed by *in situ* hydrolysis[31] to acids **54a-c** and final hydrogenation over Pd/C catalyst. The acids **58a,b** and **60a-e** were synthesized by Carsten Götte[25] and Anja Kraus[37] respectively.

3.2.10. Synthesis of the acylguanidines

The most critical step was the coupling of the guanidine building blocks **27, 33,** and **37** with acids. *N,N'*–carbonyldiimidazole (CDI), an acylation activating agent, was used for its high reactivity towards nucleophilic residues[38,39] The activated acids were reacted with free guanidines which led to acyl guanidines **(61-91)Tr,** where **Tr** indicates the trityl protection. The deprotection of the trityl group with trifluoroacetic acid (TFA)[40] yielded the acyl guanidines **61-91**, as a TFA salt.



Reagents: (i) CDI, THF, rt, 2-6 h, 30-60 %; (ii) 20 % TFA in DCM, rt, 6-16 h, 90-95 %.

Scheme 3.9: General procedure for coupling of acids with guanidine building blocks

Table 3.1: Synthesized compounds

No.	R_1	R_2	X	Yield (%)[a]	Formula[b]	HRMS: *m/z* found[f] (calcd.)	t_r [c] (0.05%TFA: CH_3CN)
61	5-Me-4-imidazolyl	H	CH_2S	83	$C_{14}H_{21}N_7OS$ (3 TFA)	335.1607 (335.1528)	8.75
62	Ph	Ph	CH_2	92	$C_{21}H_{23}N_5O$ (2 TFA)	361.1901 (361.1903)	14.66
63	Ph	Ph	CH_2	92	$C_{22}H_{25}N_5O$ (2 TFA)	376.2137 (376.2138)	16.51
64	Ph	Ph	-	92	$C_{21}H_{23}N_5O$ (2 TFA)	361.1903 (361.1903)	14.72
65	4-F-C_6H_4	4-F-C_6H_4	CH_2	85	$C_{22}H_{23}F_2N_5O$ (2 TFA	411.1866 (411.1871)	19.50
66	Ph	4-F-C_6H_4	CH_2	91	$C_{22}H_{24}FN_5O$ (2 TFA)	393.1964 (393.1965)	18.18
67	Ph	3,4-diF-C_6H_3	CH_2	91	$C_{22}H_{23}F_2N_5O$ (2 TFA)	411.1865 (411.1865)	18.59
68 [d]	4-Cl-C_6H_4	4-Cl-C_6H_4	CH_2	88	$C_{22}H_{23}Cl_2N_5O$ (2 TFA)	444.1357 (444.1351)	8.36
69 [d]	4-Br-C_6H_4	4-Br-C_6H_4	CH_2	83	$C_{22}H_{23}Br_2N_5O$ (2 TFA)	532.0347 (532.0347)	9.42
70	2-pyridyl	Ph	CH_2	85	$C_{21}H_{24}N_6O$ (3 TFA)	376.2002 (376.2012)	14.27
71	2-pyridyl	4-F-C_6H_4	CH_2	79	$C_{21}H_{23}FN_6O$ (3 TFA)	394.1917 (394.1917)	17.51
72	2-thiazolyl	Ph	CH_2	85	$C_{19}H_{22}N_6OS$ (2 TFA)	382.1576 (382.1576)	10.91
73	2-thiazolyl	3,4-diF-C_6H_3	CH_2	86	$C_{19}H_{20}F_2N_6OS$ (2 TFA)	418.1387 (418.1387)	12.62
74	1-PhCH_2-2-imidazolyl	3,4-diF-C_6H_3	CH_2	87	$C_{26}H_{27}F_2N_7O$ (2 TFA)	491.2234 (491.2245)	13.78
75	2-imidazolyl	3,4-diF-C_6H_3	CH_2	88	$C_{19}H_{21}F_2N_7O$ (3 TFA)	402.1842 (402.1776)	9.12
76 [e]	4-imidazolyl	H	CH_2	82	$C_{17}H_{23}N_5O$ (3 TFA)	-	-
77 [e]	CH_3	Ph	-	83	$C_{16}H_{21}N_5O$ (2 TFA)	299.1745 (299.1746)	11.59
78 [e]	CH_3	Ph	CH_2	92	$C_{17}H_{23}N_5O$ (2 TFA)	313.1902 (313.1902)	9.54
79	CH_3	2-thienyl	CH_2	92	$C_{15}H_{21}N_5OS$ (2 TFA)	319.1469 (319.1467)	9.03
80	CH_3	2-pyridyl	CH_2	89	$C_{16}H_{22}N_6O$ (3 TFA)	314.1863 (314.1855)	9.29
81	CH_3	2-thiazolyl	CH_2	84	$C_{14}H_{20}N_6OS$ (2 TFA)	321.1508 (321.1419)	11.95
82 [e]	-	-	Ph	89	$C_{14}H_{17}N_5O$ (2 TFA)	271.1433	16.23

						(271.1433)	
83 [e]	H	Ph	-	77	$C_{15}H_{19}N_5O$ (2 TFA)	285.1590 (285.1590)	15.10
84 [e]	H	Ph	CH_2	78	$C_{16}H_{21}N_5O$ (2 TFA)	-	14.75
85	H	Ph	$(CH_2)_2$	72	$C_{17}H_{23}N_5O$ (2 TFA)	313.1903 (313.1903)	-
86 [e]	H	Ph	$(CH_2)_3$	74	$C_{18}H_{25}N_5O$ (2 TFA)	-	13.98
87 [e]	CH_3	c-hexyl		86	$C_{16}H_{27}N_5O$ (2 TFA)	305.2212 (305.2216)	12.18
88 [e]	CH_3	c-hexyl	CH_2	91	$C_{17}H_{29}N_5O$ (2 TFA)	319.2372 (319.2372)	17.12
89 [e]	-	-	c-hexyl	79	$C_{14}H_{23}N_5O$ (2 TFA)	277.1900 (277.1900)	13.14
90 [e]	H	c-hexyl		75	$C_{15}H_{25}N_5O$ (2 TFA)	-	17.15
91 [e]	H	c-hexyl	CH_2	81	$C_{16}H_{27}N_5O$ (2 TFA)	305.2213 (305.2213)	14.60

[a] After preparative HPLC; [b] isolated as trifluoroacetic acid salt; [c] retention time in minutes; the solvent system (0.05 % TFA / acetonitrile; gradient) is presented in experimental section and the experimental setup is in the experimental section; representative chromatogram in **Fig. 5** , [d] Ph.D. thesis of C. Götte[25]; [e] Diploma thesis of Anja Kraus; [37] [f] Techniques for HRMS is described in experimental section of respective compounds;

The intermediate **64-(Tr)** was isolated and recrystalized for x-ray analysis. The crystal structure (view **A** in **Fig. 3.4**) shows that a hydrogen bond is possible between oxygen of amide C=O and the hydrogen of guanidine nitrogen, which is attached to alkyl group. In view **B** the co-planar orientation of guanidine and C=O group is obvious.

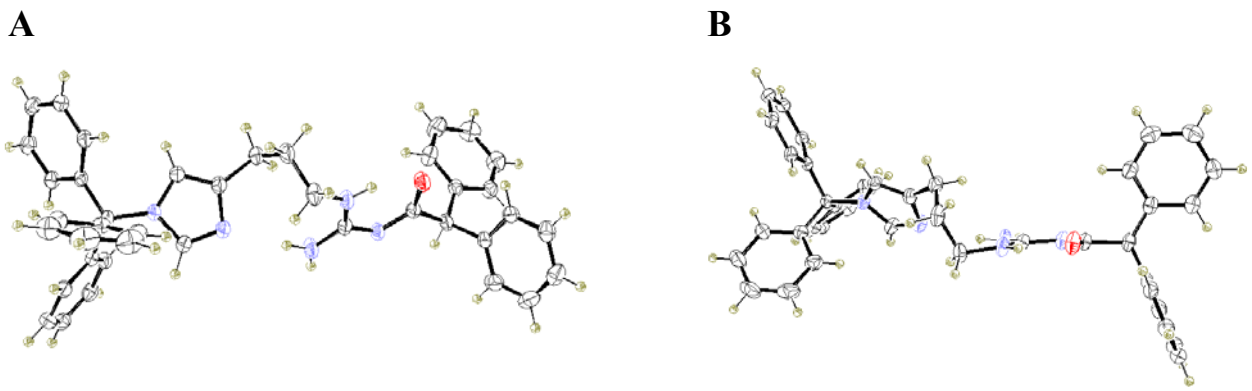


Fig. 3.4: Crystal structure (ORTEP diagram) of trityl protected acylguanidine **64-(*Tr*)**.

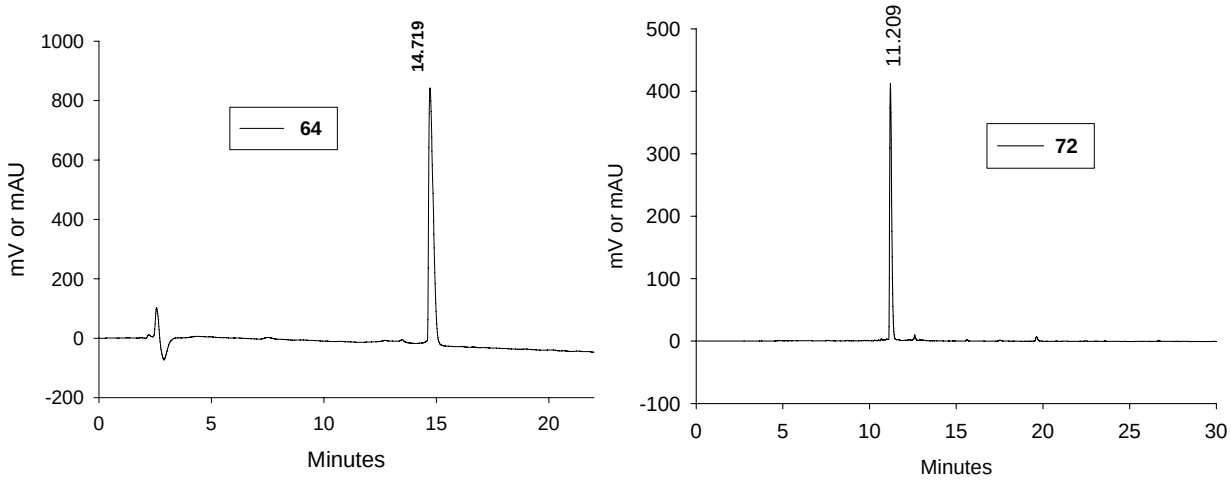


Fig. 3.5: Representative analytical HPLC chromatograms of **64** (Rt: 14.72 min; UV detection at 210 nm) and **72** (Rt: 11.21 min; UV detection at 254 nm). Detailed description is given in experimental section.

3.3. Pharmacological results and discussion

The synthesized compounds were tested for histamine H_2 receptor (H_2R) agonistic activity[a] on the isolated spontaneously beating guinea pig right atrium[6] (positive chronotropic response). Most of the acylguanidines were also investigated for H_1 antagonism on the isolated guinea pig ileum and on U-373 MG human cells. The results are summarized in **Table 3.2**. Moreover, the agonistic activity was studied in the GTPase assay using guinea pig and human H_2R expressing Sf9 insect cells[b] (see **Table 3.3**). Acylguanidines **73** and **78** were investigated in mice for absorption after peroral administration and for penetration into the brain.[41] Additionally, selected compounds were studied for histamine H_3 receptor antagonism on the isolated electrically stimulated guinea pig ileum (results reported in **Chapter 4**) and for activity on histamine H_4 receptors using H_4R expressing Sf9 cells (GTPase assay). In the latter experiments highly potent H_4R agonists were identified (R. Seifert, personal communcation), however, data was not included in this thesis, as these studies are subject of an ongoing project.

[a] Co-operation with Prof. Dr. S. Elz, Dept. Pharm./Medicinal Chemistry I, University of Regensburg
[b] Co-operation with Prof. Dr. R. Seifert, Dept. Pharmacology & Toxicology, University of Regensburg

Table 3.2: Histamine H_2 receptor agonism on guinea pig right atrium, H_1 receptor antagonism on isolated guinea pig ileum and U-373 MG human cells (Ca^{2+}-assay)

	Histamine H_2 receptor agonism				Histamine H_1 receptor antagonism		
	isolated guinea pig right atrium				guinea pig ileum		U-373 MG cells (Ca^{2+}-assay)
No.	pEC_{50}[a] ± SEM[J]	rel. pot.[b]	E_{max} (%)[c]	N[d]	$_pA_2$[a,c] ± SEM [or pD'$_2$[a,c] ± SEM][e]	N[d]	IC_{50} [µM][1]
1, HIS	6.00 ± 0.10	100	100 ± 2	>50			
4	7.70 ± 0.10	5000	100	4	5.47 ± 0.01	-	-
5d	8.01 ± 0.10	10200	100	5	7.65 ± 0.01	7	-
61	7.38 ± 0.09	2370	101 ± 2	4	5.37 ± 0.05	12	56
62	6.06 ± 017	114	64 ± 3	3	5.01 ± 0.10 [4.55 ± 0.06]	11 16	57
63	7.22 ± 0.09	1650	85 ± 3	5	5.93 ± 0.05 [5.00 ± 0.09]	10 18	-
64	5.85 ± 0.18	70	69 ± 5	3	-	-	26
65	6.37 ± 0.07	234	76 ± 2	4	[5.76 ± 0.15]	4	9
66	6.93 ± 0.09	857	95 ± 3	4	[5.19 ± 0.03]	4	
67	6.95 ± 0.05	887	81 ± 3	4	[5.87 ± 0.14]	4	8
68	6.51 ± 0.10	320	60 ± x	3	-		-
69	6.11 ± 0.10	130	60 ± x	3	-		-
70	7.16 ± 0.03	1455	97 ± 1	4	4.95 ± 0.04	10	32
71	7.34 ± 0.12	2170	100 ± 1	3	5.43 ± 0.03	12	34
72	7.29 ± 0.03	1950	100 ± 1	3	5.33 ± 0.05	18	45
73	7.17 ± 0.06	1480	90 ± 4	3	5.33 ± 0.07	16	30
74	6.26 ± 0.08	186	54 ± 5	3	-		19
75	6.88 ± 0.16	764	100 ± 4	3	-		41
76	5.10 ± 0.19[K]	-	7 ± 1[I]	3	-	-	-
77	5.06 ± 0.20	12	88 ± 5	3	<5.0	2	58.1
78	7.45 ± 0.07	2710	99 ± 2	4	6.31 ± 0.06	2	19.5
79	7.29 ± 0.10	1950	99 ± 2	3	-		14
80	7.29 ± 0.06	1940	98 ± 2	3	-		49
81	7.28 ± 0.11	1920	91 ± 2	4	-		73
82	5.53 ± 0.12	34	89 ± 4	3	<5.0	2	104
83	6.35 ± 0.01	222	97 ± 2	3	5.98 ± 0.02	2	18.7
84	6.40 ± 0.10	253	97 ± 5	3	5.42 ± 0.14	5	60.8
85	6.88 ± 0.10	759	94 ± 2	4	5.50 ± 0.20	2	46.9
86	5.96 ± 0.03	92	86 ± 5	4	6.02 ± 0.03	4	-
87	6.49 ± 0.11	311	86 ± 3	3	5.39 ± 0.01	4	57.7
88	6.81 ± 0.07	641	101 ± 3	3	5.43 ± 0.09	5	0.95
89	6.93 ± 0.07	851	102 ± 1	3	5.03 ± 0.07	2	39.2
90	6.62 ± 0.10	417	95 ± 1	3	5.36 ± 0.07	4	28.2
91	6.84 ± 0.11	687	98 ± 3	3	4.97 ± 0.26	2	10.2

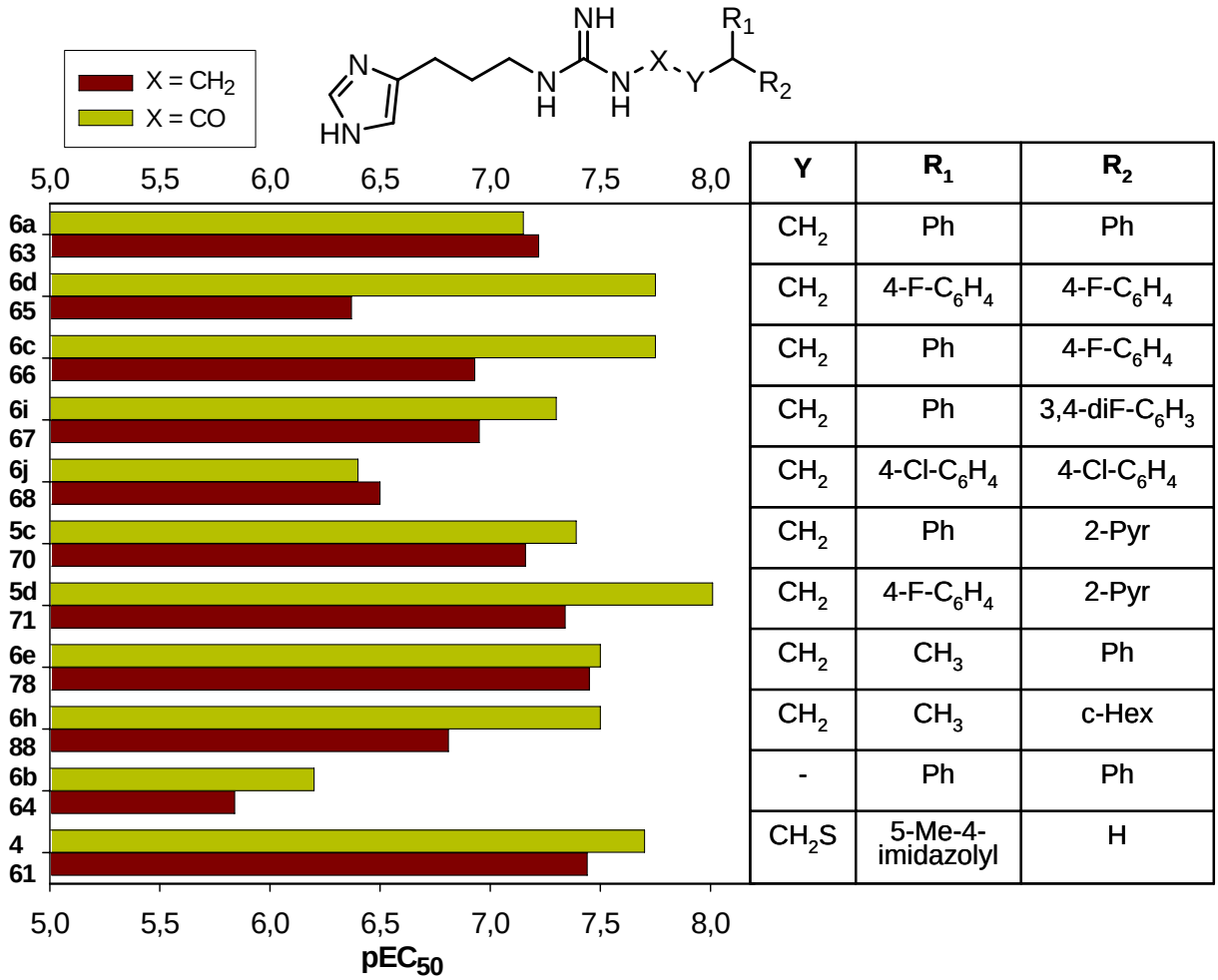
[a] Explanation of abbreviations see appendix; [b] Potency relative to histamine = 100 %, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; [d] Number of experiments; [e] pD'_2 values given in brackets for compounds producing a significant, concentration-dependent reduction of histamine's maximal response; [f] non-surmountable; [g] competitive/surmountable ; [h] competitive/non-surmountable; [i] IC_{50} values for the inhibition of the histamine (30 µM) induced increase in intracellular calcium; mean of at least 3 independent experiments; SEM < 10 %; [j] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by the equation: $pEC_{50} = 6.00 + \Delta pEC_{50}$. The *SEM* given for pEC_{50} is the *SEM* calculated for ΔpEC_{50}; [K] Antagonist (pA_2); [l] Emax at 100 µM; E_{max} of histamine in the presence of 100 µM **76** was 45 ± 5%.

Most of the synthesized acylguanidines (**61-91**, **Table 3.2**) proved to be full or nearly full histamine H_2R agonists on the spontaneously beating guinea pig right atrium, supporting the working hypothesis that the strongly basic guanidine group may be replaced by a considerably less basic acylguanidine without loss of H_2R agonistic activity. In contrast, on the guinea pig ileum as well as on human U373 cells all tested compounds proved to be devoid of histamine H_1 receptor agonistic activity. In both test systems weak H_1R antagonism was found, for instance, on the guinea pig ileum the compounds were by 1 - 2.5 orders of magnitude less active than arpromidine.

Histamine H_2R agonism on the isolated guinea pig right atrium:

The suitability of a carbonyl group as a bioisosteric substitute for a methylene group is strongly depending on the substitution pattern of the H_2R agonist molecule, i.e. the substituents R_1, R_2 and the chain length, as shown in **Fig. 3.6** for a selection of pairs of compounds. As arpromidine and related phenyl(heteroaryl)alkyl-substituted imidazolyl-propylguanidines are the most potent H_2R agonists known so far, we expected highest activity to reside in the corresponding acylated analogues, too. "Oxo-arpromidine" (**71**) was found to be about 5 times less potent than arpromidine or half as potent as impromidine, respectively. This tendency to lower pEC_{50} values compared to the alkylguanidines was confirmed by investigation of related compounds such as **70**, **72**, **73** and **75** and was most obvious for compounds having a mono- or two-fold fluorinated phenyl ring such as **71**, **73** and the fluorinated diphenylpropanoylguanidines **65-67**. Taking into consideration that improved pharmacokinetic properties of the aforementioned substances can compensate for that, in principle, a moderate decrease in potency can be accepted. However, a decrease in activity is not inevitable: the compounds **63**, **68**, and in particular the 3-(hetero)arylbutanoyl-substituted guanidines **78-81** were found to be as potent as the corresponding 3-

(hetero)arylbutylguanidines, i. e., when one of the aryl group is replaced with a methyl group, the potencies become similar comparing the alkyl and the acylguanidine series. As in the arpromidine series a three-membered carbon chain between guanidine and aromatic ring is optimum. Among these H_2R agonists **78** is about 28 times, and the thienyl, pyridyl and thiazolyl analogues (**79-81**) are about 20 times more potent than histamine on the isolated guinea pig right atrium. The corresponding cyclohexyl analogue **88** is only 6 times more potent than the reference compound. Interestingly, substance **61**, the oxo-derivative of impromidine, was about half as potent as the parent compound. This result further supports the idea that the acylguanidines and the alkylguanidines bind to the H_2R in a similar way. It is conceivable that the acylated guanidine forms a charge-assisted hydrogen bond with Asp^{98} in transmembrane domain 3 of the H_2R.



Y	R_1	R_2
CH_2	Ph	Ph
CH_2	4-F-C_6H_4	4-F-C_6H_4
CH_2	Ph	4-F-C_6H_4
CH_2	Ph	3,4-diF-C_6H_3
CH_2	4-Cl-C_6H_4	4-Cl-C_6H_4
CH_2	Ph	2-Pyr
CH_2	4-F-C_6H_4	2-Pyr
CH_2	CH_3	Ph
CH_2	CH_3	c-Hex
-	Ph	Ph
CH_2S	5-Me-4-imidazolyl	H

Fig 3.6: H_2R agonistic potency of alkyl (X = CH_2) and acyl guanidines (X = CO) on the isolated spontaneously beating guinea pig right atrium (pEC_{50} values).

A concentration-response curve is exemplarily shown for histamine and compound **78** in **Fig. 3.7**. The curve shows that the compound **78** is 28 times more potent than the reference compound, histamine. The positive chronotropic response to compound **78** is inhibited by the H_2R cimetidine resulting in a rightward shift of the concentration-response curve (see **Fig. 3.7**). The pA_2 determined for cimetidine (6.31) *versus* **78** as the agonist is not significantly different from the values obtained *versus* other agonists like histamine, impromidine or arpromidine. As **78** is a potent chiral H_2R agonist the compound was one of the acylguanidines selected for the synthesis of the enantiomers (see **Chapter 5**).

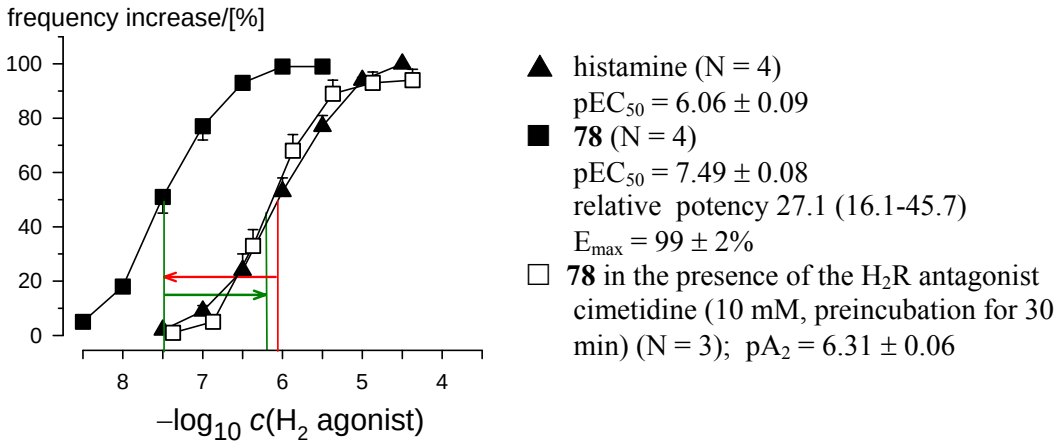


Fig 3.7: Concentration-response curves of histamine and compound **78** on the guinea pig right atrium.

Species selectivity - agonism on guinea pig and human H_2R-$G_{s\alpha}$ fusion proteins

The isolated guinea pig right atrium was successfully used as a predictive standard model for the pharmacological characterisation of H_2R antagonists developed as antiulcer agents. Recently, a discrepancy between activity on guinea pig and human H_2R was demonstrated for arpromidine and related H_2R agonists, whereas the differences between agonistic potencies of small amine-like agonists at both species was negligible. Moreover, a binding mode for arpromidine-like H_2R agonists was suggested on the basis of site-directed mutagenesis and molecular modelling. In order to study the species selectivity and to investigate the structure-activity relationships in comparison to the arpromidines, the synthesized compounds were also tested for potency and efficacy on hH_2R-$G_{s\alpha s}$ and gpH_2R-$G_{s\alpha s}$ fusion proteins expressed

in Sf9 cells (investigations on cell membrane preparations according to the procedure reported by Kelley *et al.*[24]). The results are summarized in **Table 3.3**. Moreover, graphical presentations of efficacies and potencies at H_2R of both species, concentration-response curves of a number of acylguanidines as well as radioligand displacement curves for selected compounds are shown in **Figures 3.8** and **3.9**.

Table 3.3: Activity on the human and guinea pig receptor (GTPase assay* on human and guinea pig H_2R-$G_{s\alpha}$ fusion proteins)

	hH_2R-$G_{s\alpha S}$				**gpH_2R-$G_{s\alpha S}$**				$\frac{EC_{50}\ hH_2R\text{-}G_{s\alpha S}}{EC_{50}\ gpH_2R\text{-}G_{s\alpha S}}$
No	Efficacy	EC_{50}, nM	pEC_{50}	Rel. Pot.	Efficacy	EC_{50}, nM	pEC_{50}	Rel. Pot.	
HIS[a]	1,00	1260 ± 250	5.90	100	1,00	1200 ± 240	5.92	100	1.05
4[a]	0.84 ± 0.04	200 ± 20	6.70	641	1.00 ± 0.12	40 ± 10	7.41	3060	5.00
5d[a]	0.79 ± 0.07	0.19 ± 0.04	6.72	659	1.02 ± 0.04	70 ± 10	7.15	1600	2.71
61	0.79 ± 0.04	267 ± 38	6.57	480	0.93 ± 0.01	60 ± 1	7.22	2000	4.44
62	0.17 ± 0.01	186 ± 9	6.73	680	0.54 ± 0.02	175 ± 27	6.76	700	1.06
63	0.69 ± 0.09	78 ± 42	7.11	1600	0.93 ± 0.32	6 ± 1	8.22	19000	12.1
64	0.08 ± 0.04	-	-	740	0.60 ± 0.11	400 ± 86	6.40	300	0.42
65	0.61 ± 0.02	48 ± 16	7.32	2600	0.81 ± 0.13	25 ± 17	7.60	4800	1.88
66	0.67 ± 0.07	113 ± 34	6.95	1100	1.00 ± 0.11	20 ± 15	7.70	5900	5.56
67	0.72 ± 0.09	61 ± 15	7.22	2100	1.02 ± 0.11	7 ± 1	8.15	16000	8.23
70	0.86 ± 0.01	547 ± 33	6.26	230	1.03 ± 0.07	60 ± 14	7.22	2000	9.17
71	0.73 ± 0.03	415 ± 90	6.38	320	0.93 ± 0.04	45 ± 4	7.35	2700	9.21
72	0.93 ± 0.08	550 ± 80	6.26	260	1.08 ± 0.27	20 ± 10	7.70	6000	23.1
73	0.76 ± 0.01	298 ± 4	6.53	420	1.01 ± 0.09	75 ± 5	7.12	1600	3.96
74	0.38 ± 0.01	782 ± 52	6.11	160	0.28 ± 0.01	359 ± 38	6.44	340	2.18
75	0.88 ± 0.10	654 ± 92	6.18	200	0.90 ± 0.01	261 ± 10	6.58	460	2.51
77	0.81 ± 0.03	75 ± 26	7.12	1680	0.89 ± 0.03	29 ± 7	7.54	3680	2.59
78	0.87 ± 0.01	67 ± 2	7.17	1880	1.03 ± 0.06	12 ± 1	7.92	10300	5.58
79	0.97 ± 0.01	109 ± 31	6.96	1156	1.05 ± 0.13	21 ± 19	7.67	5714	4.97
80	1.11 ± 0.06	520 ± 140	6.28	246	1.22 ± 0.08	130 ± 11	6.89	923	3.75
81	0.92 ± 0.01	188 ± 99	6.72	670	0.94 ± 0.04	33 ± 3	7.48	3636	5.43
82	0.50 ± 0.01	1390 ± 37	5.86	91	0.84 ± 0.06	636 ± 99	6.20	158	2.18
83	0.76 ± 0.01	147 ± 8	6.83	857	0.90 ± 0.01	76 ± 13	7.12	1420	1.93
84	0.84 ± 0.03	100 ± 16	7.00	1260	1.05 ± 0.11	23 ± 1	7.64	5480	4.35
85	0.61 ± 0.01	67 ± 9	7.17	1880	0.84 ± 0.01	21 ± 2	7.68	4800	3.19
86	0.60 ± 0.01	72 ± 23	7.14	1750	0.80 ± 0.06	56 ± 5	7.25	1710	1.29
87	0.74 ± 0.06	99 ± 8	7.00	1270	0.90 ± 0.08	37 ± 8	7.43	2920	2.68
88	0.87 ± 0.05	23 ± 3	7.64	5480	1.11 ± 0.16	9 ± 1	8.04	14800	2.56
89	0.76 ± 0.05	38 ± 4	7.42	3320	0.99 ± 0.08	21 ± 1	7.68	5660	1.81
90	0.87 ± 0.04	62 ± 6	7.21	2030	1.01 ± 0.13	23 ± 1	7.64	5270	2.70
91	0.76 ± 0.04	46 ± 2	7.34	2740	1.02 ± 0.11	22 ± 1	7.66	5560	2.09

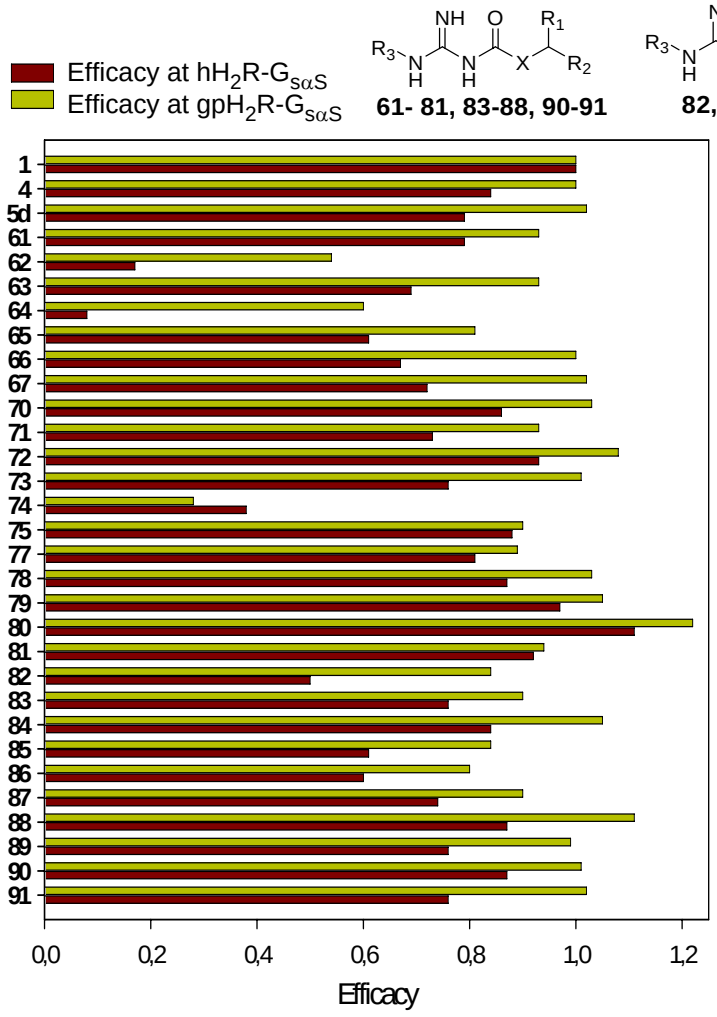
[a] Data from ref.[24]

***** Potencies and efficacies of ligands at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were determined in the GTPase assay. GTP hydrolysis was determined as described in literature. Reaction mixtures contained membranes of Sf9 cells expressing fusion proteins and agonists at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Curves were analyzed by nonlinear regression. Typical basal GTPase activities ranged between

~1 and 2 pmol/mg/min, and typical GTPase activities stimulated by HIS (100 μM) ranged between ~4 and 8 pmol/mg/min. To calculate agonist efficacies, the maximum stimulatory effect of HIS was set at 1.00, and the stimulatory effects of other agonists were referred to this value. Data shown are the means ± SD of four to six experiments performed in duplicate. The relative potency (Rel. Pot.) of HIS was set at 100, and the potencies of other agonists were referred to this value to facilitate comparison of agonist potencies. Efficacies and potencies, respectively, of ligands at hH_2R-$G_{s\alpha S}$ were compared with the corresponding parameters at gpH_2R-$G_{s\alpha S}$ using the t test.

Efficacy at hH_2R and gpH_2R. Similar to the results published for arpromidine-like guanidines the efficacies at the human H_2R-$G_{s\alpha S}$ were significantly lower than at guinea pig H_2R-$G_{s\alpha S}$ (**Fig. 3.8**). As demonstrated by Kelley *et al*.[24] the efficacy of agonists strongly depends on the presence of Tyr or Cys in position 17 of the guinea pig and the human H_2R, respectively. The difference is more pronounced for the diaryl (e. g., **63-73**) and the cyclohexyl analogues (**87-91**) and smaller for the monoaryl analogues, especially the methyl branched compounds (**77-81**). Compounds **62** and **64**, the shorter homologues of compound **63** with either an imidazolylethylguanidine or a diphenylacetyl moiety, have lower efficacies than **63** and very low intrinsic acitivity at the hH_2R. As the number of halogen substituents in the ring increases the difference in efficacy increases (**63**, **65-67, 70** vs **71, 72** vs **73**). In case of benzyl protected imidazole analogue **74**, the efficacy is much lower in comparison to its deprotected **75**, which has the same efficacy at both hH_2R and gpH_2R.

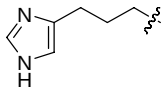
Potency at hH_2R and gpH_2R. Generally, acylguanidines are more potent at gpH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$. This result is in accordance with the structure-activity relationship of arpromidine, impromidine and related imidazolylpropylguanidine-type H_2R agonists (Kelley *et al*.[24]), suggesting a similar binding mode of the affinity-conferring group regardless of the presence of a CH_2 or a CO group adjacent to the guanidine nitrogen. The differences are depending on the substitution pattern (e.g., diaryl, aryl/heteroaryl, mono(hetero)aryl, or cyclohexyl series; see **Fig. 3.8,** diagram taken from data in **Table 3.3**). For instance, the species selectivities of "oxo-arpromidine" (**71**), diarylalkanoylguanidines (**63-67**) and "oxo-impromidine" (**61**) are comparable with those of the corresponding alkylguanidines.



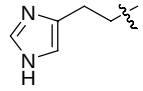
61- 81, 83-88, 90-91

82, 89

R3 =



61, 63- 91

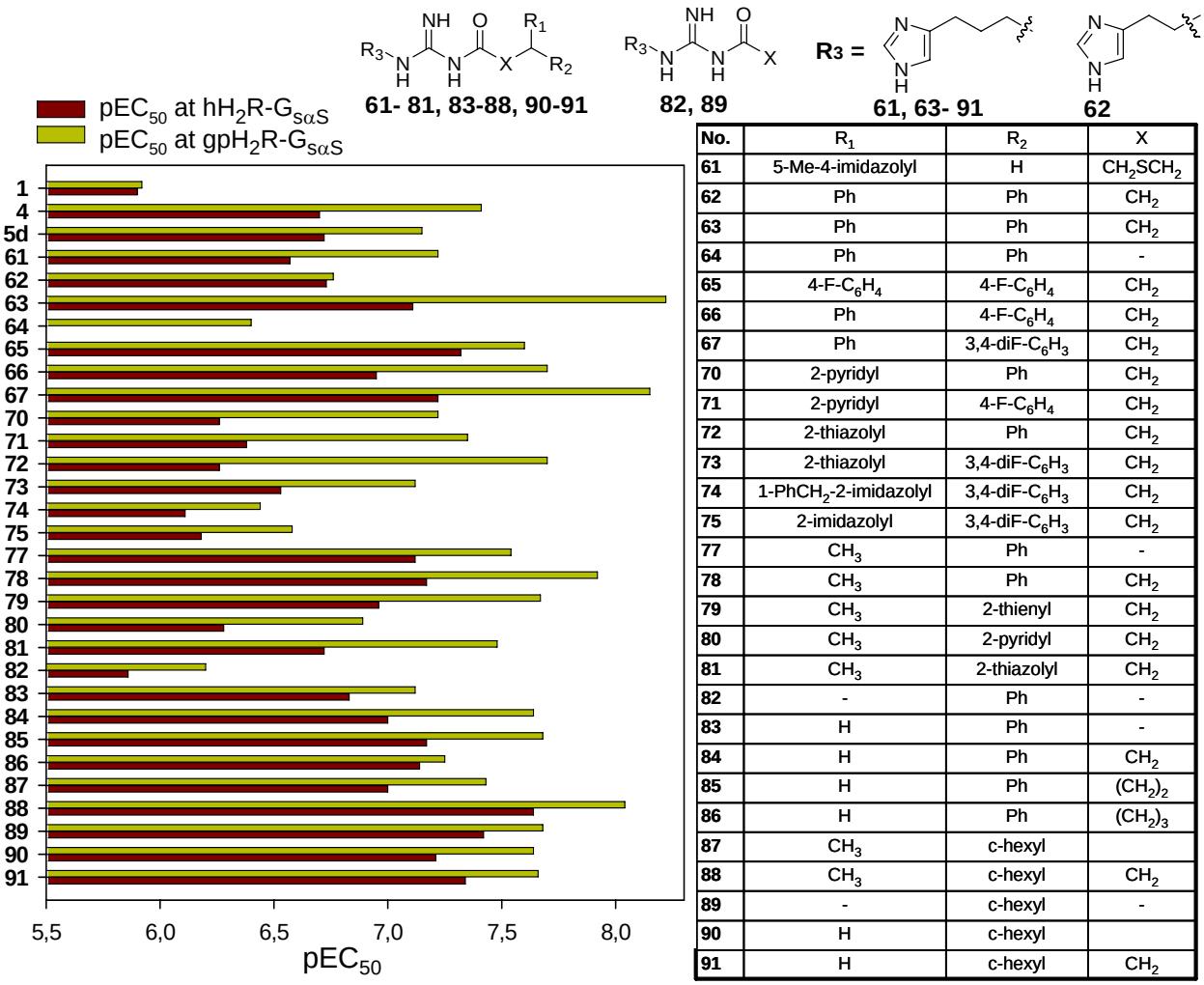


62

No.	R_1	R_2	X
61	5-Me-4-imidazolyl	H	CH_2SCH_2
62	Ph	Ph	CH_2
63	Ph	Ph	CH_2
64	Ph	Ph	-
65	4-F-C_6H_4	4-F-C_6H_4	CH_2
66	Ph	4-F-C_6H_4	CH_2
67	Ph	3,4-diF-C_6H_3	CH_2
70	2-pyridyl	Ph	CH_2
71	2-pyridyl	4-F-C_6H_4	CH_2
72	2-thiazolyl	Ph	CH_2
73	2-thiazolyl	3,4-diF-C_6H_3	CH_2
74	1-PhCH_2-2-imidazolyl	3,4-diF-C_6H_3	CH_2
75	2-imidazolyl	3,4-diF-C_6H_3	CH_2
77	CH_3	Ph	-
78	CH_3	Ph	CH_2
79	CH_3	2-thienyl	CH_2
80	CH_3	2-pyridyl	CH_2
81	CH_3	2-thiazolyl	CH_2
82	-	Ph	-
83	H	Ph	-
84	H	Ph	CH_2
85	H	Ph	$(CH_2)_2$
86	H	Ph	$(CH_2)_3$
87	CH_3	c-hexyl	
88	CH_3	c-hexyl	CH_2
89	-	c-hexyl	-
90	H	c-hexyl	
91	H	c-hexyl	CH_2

Fig. 3.8: Efficacies of acylguanidine-type agonists at gpH_2R-$G_{s\alpha S}$ and hH_2R-$G_{s\alpha S}$

As in the diarylalkylguanidine series of compounds, incorporation of a shorter connecting chain results in a decrease in H_2R agonistic activity (**62** vs. **63**) and the difference in potency at gpH_2R-$G_{s\alpha S}$ and hH_2R-$G_{s\alpha S}$ becomes negligible (**62**). Similarly, in the aryl(heteroaryl)-alkanoylguanidine series (**70-75**), the agonistic potency was much higher at gpH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$. Surprisingly, the most interesting result was found in methyl branched monoaryl, heteroaryl and cyclohexyl analogues (**77-81, 87, 88**). These compounds have nearly the same potency as the diaryl analogues but the difference in activity at guinea pig and human H_2R is considerably lower. This result indicates that methyl branched aryl and cyclohexyl analogues stabilize an active hH_2R-$G_{s\alpha S}$ conformation considerably more efficient for GDP/GTP exchange than the diaryl or aryl-heteroaryl analogues.



No.	R_1	R_2	X
61	5-Me-4-imidazolyl	H	CH_2SCH_2
62	Ph	Ph	CH_2
63	Ph	Ph	CH_2
64	Ph	Ph	-
65	4-F-C_6H_4	4-F-C_6H_4	CH_2
66	Ph	4-F-C_6H_4	CH_2
67	Ph	3,4-diF-C_6H_3	CH_2
70	2-pyridyl	Ph	CH_2
71	2-pyridyl	4-F-C_6H_4	CH_2
72	2-thiazolyl	Ph	CH_2
73	2-thiazolyl	3,4-diF-C_6H_3	CH_2
74	1-PhCH_2-2-imidazolyl	3,4-diF-C_6H_3	CH_2
75	2-imidazolyl	3,4-diF-C_6H_3	CH_2
77	CH_3	Ph	-
78	CH_3	Ph	CH_2
79	CH_3	2-thienyl	CH_2
80	CH_3	2-pyridyl	CH_2
81	CH_3	2-thiazolyl	CH_2
82	-	Ph	-
83	H	Ph	-
84	H	Ph	CH_2
85	H	Ph	$(CH_2)_2$
86	H	Ph	$(CH_2)_3$
87	CH_3	c-hexyl	
88	CH_3	c-hexyl	CH_2
89	-	c-hexyl	-
90	H	c-hexyl	
91	H	c-hexyl	CH_2

Fig 3.9: Potencies (pEC_{50} values) of acylguanidine-type agonists at gpH_2R-$G_{s\alpha S}$ and hH_2R-$G_{s\alpha S}$

Due to the completely different experimental setup the absolute values for agonistic potencies on the isolated guinea pig atrium (positive chronotropic response) and the gpH_2R-$G_{s\alpha S}$ fusion proteins (GTPase assay) are not identical, but the results are essentially comparable. Neveretheless, there are some quantitative differences. On the atrial gpH_2R the 3-phenylbutanoyl-substituted imidazolylpropylguanidin **78** was most active (28 times as potent as histamine), but at the gpH_2R-$G_{s\alpha S}$ the diphenyl analogue **63** was superior to **78** (190 versus 103 times the potency of histamine). Unexpectedly, at hH_2R-$G_{s\alpha S}$ compound **88**, the cyclohexyl analogue of **78**, was found to be most active (54 times the potency of histamine). In **Fig. 3.10** several concentration response curves of acyl guanidines at gpH_2R-$G_{s\alpha S}$ [a), and

c)] and hH_2R-$G_{s\alpha S}$ [b) and d)] are shown. The maximum response is always higher for gpH_2R-$G_{s\alpha S}$ than hH_2R-$G_{s\alpha S}$.

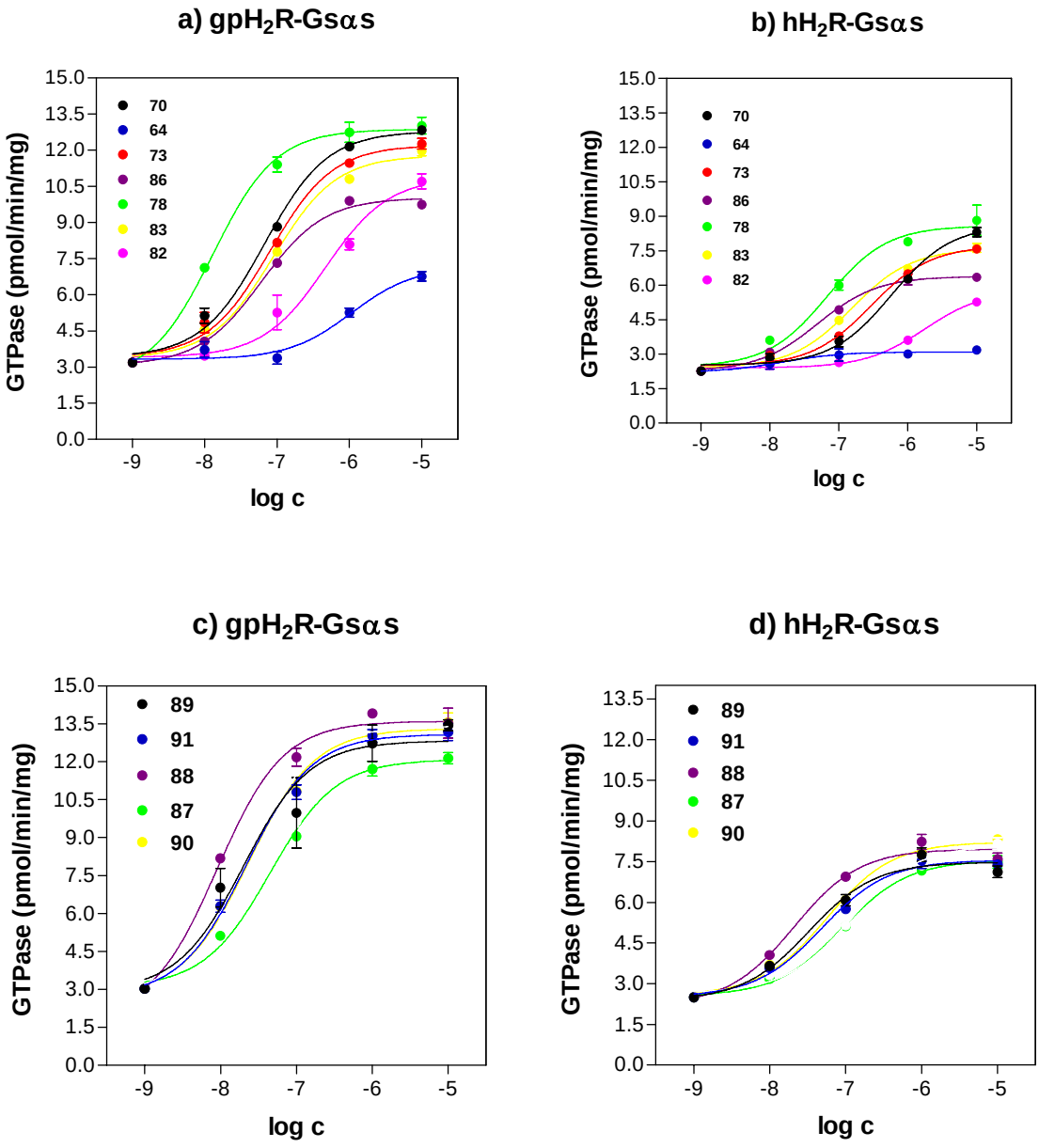


Fig. 3.10: Concentration response curves of acylguanidine-type agonists at gpH_2R-GsαS and hH_2R-GsαS (GTPase assay).

Histamine H_2R binding. Selected compounds were investigated for H_2R binding on gpH_2R-GsαS and hH_2R-GsαS in competition assays using the H_2R antagonist [^{3}H]tiotidine as the radioligand. As an example the displacement curves for compound **71** are shown in **Fig. 3.11.** The K_i values at both human and guinea pig H_2R are in the same range as the potencies found in the GTPase assay.

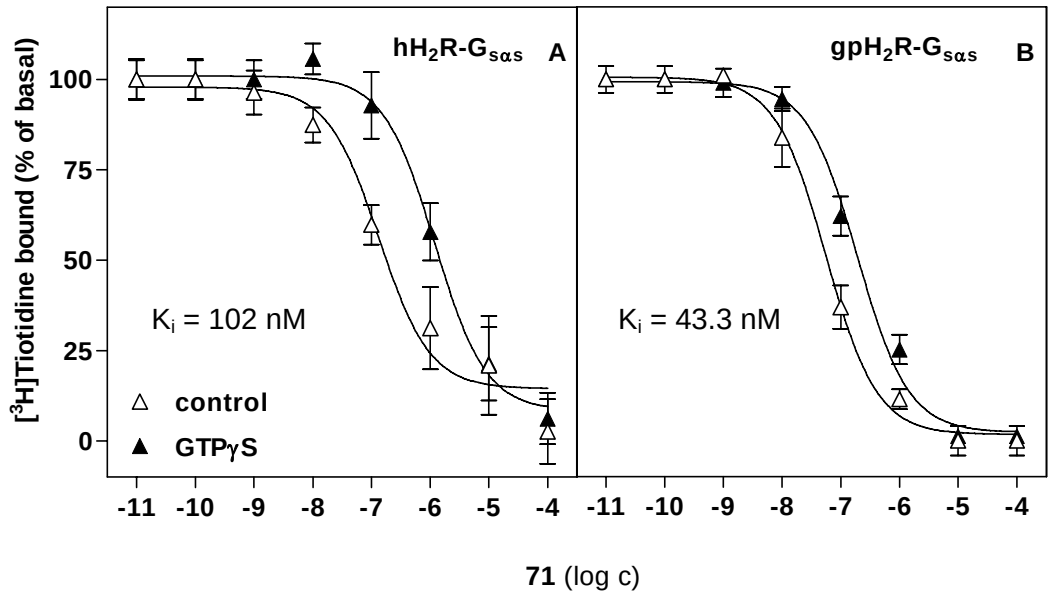


Fig. 3.11. Displacement of the radioligand [^{3}H]tiotidine (10 nM) from gpH_2R-$G_{s\alpha S}$ and hH_2R-$G_{s\alpha S}$ (cell membrane preparations from H_2R-$G_{s\alpha S}$ expressing Sf9 cells) in absence and presence of GTPγS (10 μM). The experiments were performed according to the procedure described.[24]

The pK_a value of acylguanidinium cations is by 4-5 orders of magnitude lower than that of guanidinium ions (guanidinium: $pK_a \approx 12.5$; acetylguanidinium: $pK_a = 7.6$[42]). On one hand, the acylguanidines are still sufficiently basic to form a cation which is supposed to interact with Asp[98] in transmembrane domain 3 of the H_2R by analogy with the binding mode suggested for arpromidine-like agonists. On the other hand, the reduced basicity of acylguanidines results in absorption from the gastrointestinal tract and penetration across the blood brain barrier, as a considerable portion of the substance remains uncharged under physiological conditions. This has been exemplarily proven for compounds **73** and **78** in

mice.[41] Both compounds were detected in plasma after peroral administration and in the brain after intraperitoneal administration.

3.4. Conclusion

Starting from a working hypothesis based on structure-activity relationship of arpromidine-like H_2R agonists structurally related compounds with reduced basicity were prepared in order to obtain agonists with improved pharmacokinetic profile. we were successful to establish a complete route for the synthesis of N^G-acylated imidazolylpropylguanidines. The synthesized compounds were pharmacologically tested on isolated guinea pig tissues (ileum: H_1R, right atrium: H_2R) as well as on cells and membrane preparations of guinea pig and human histamine H_1, H_2, or H_4 receptor expressing cells. The basicity of the acylguanidines is by 4-5 orders of magnitude lower than that of the corresponding guanidines. However, alkanoylguanidines and alkylguanidines appear to be bioisosteres, and it may be speculated that the acylguanidines are capable of interacting with Asp^{98} in transmembrane domain 3 of the H_2R by a charge-assisted hydrogen bond. Compounds having a carbonyl instead of a methylene group adjacent to the guanidine are about equipotent as, for instance, demonstrated for the diphenylpropyl *versus* the diphenylpropanoyl and for impromidine *versus* the oxo-substituted analogue. However, a considerable decrease in activity was found when the methylene group in arpromidine was converted to a carbonyl group. Surprisingly, compounds **77-86,** which have only one phenyl ring are equally or even more potent than the diaryl analogues, whereas in the arpromidine series of histamine H_2R agonists highest potency resides in the 3-phenyl-3-hetarylpropyl-substituted imidazolylpropylguanidines. This may be interpreted as a first hint to different modes of interaction of the affinity-conferring moiety with the receptor protein. On the isolated guinea pig right atrium the most active H_2R agonist, the 3-phenylbutanoyl substituted guanidine **78**, was about 30 times more potent than the natural ligand. In terms of agonistic potency on the guinea pig atrium the new compounds are inferior to the arpromidines. However, the acylated imidazolylpropylguanidines are superior with respect to pharmakokinetic properties. A very important finding is that the compounds are capable of penetrating through the blood-brain barrier (tested by HPLC-MS analysis after administration to mice). Centrally active H_2R agonists will be useful pharmacological tools to study the role of histamine H_2 receptors in the brain.

Meanwhile, imidazolylpropylguanidines and corresponding acylated analogues were found to be useful building blocks beyond the preparation of H_2R agonists, for instance, the synthesis of ligands preferentially binding to other histamine receptor subtypes, like neuropeptide Y Y_1[43] or Y_4 receptors [44]. Thus, there is increasing evidence that in terms of medicinal chemistry the imidazolylpropylguanidine moiety and the acylated analogues may be considered as "privileged structures". An extremely promising perspective results from very recent studies: surprisingly, some of the acylguanidines proved to be rather potent as either agonists or inverse agonists at the recently discovered H_4R (R. Seifert, personal communication; data not shown). The elaboration of the structure-activity relationships based on these results and the design of H_4R selective ligands will be a subject of a new project.

3.5. Experimental section

3.5.1. Chemistry

General procedures

Where indicated, reactions were carried out under a dry, oxygen-free atmosphere of N_2 using Schlenk-technique or under argon atmosphere. Commercially available reagents were used as received. DMF, CH_3CN and CH_2Cl_2 were distilled over P_4O_{10} and stored under N_2 over molecular sieves 3Å. EtOH and MeOH were dried over Mg and stored under N_2. THF, 1,4-dioxane and Et_2O were dried with Na/benzophenone and stored over Na wire under N_2. EtOAc, petroleum ether (60/40), $CHCl_3$, CH_2Cl_2, MeOH and hexane for chromatographic separations were distilled before use. For column chromatography silica gel Geduran 60 (Merck, 0.063-0.200 mm) was used. TLC-analysis was done on silica gel 60 F_{254} (Merck) coated on aluminium sheets. NMR-spectra were recorded on Bruker Avance 300 (^{1}H: 300 MHz, ^{13}C: 75.5 MHz) and Bruker Avance 600 (^{1}H: 600 MHz; ^{13}C: 150.29 MHz) with TMS as internal standard. IR spectroscopy was done on a Mattson Genesis Series FT-IR (sample preparation as indicated). X-ray analysis was performed by the Crystallography Laboratory (University of Regensburg, M. Zabel, S. Stempfhuber). Elemental analysis (Heraeus

Elementar Vario EL III) and mass spectrometry (Finnigan ThermoQuest TSQ 7000) were done by the Central Analytical Laboratory (Universität Regensburg).

The following chromatographic methods were used to assess compound purity:

Preparative chromatography: The pump model was Knauer K-1800 and column was Eurosphere-100 (250 x 32 mm) which was attached to a Knauer-Detector K-2000 UV-detector. UV detection was done at 254 nm. Temperature was 25°C, Flow rate was 40 mL/min. The mobile phase was 0.1% TFA in Millipore water and MeOH. Injection volume was 500 µL.

Analytical chromatography (referred to Table 1): The pump was P4000 (Thermo Separation Products) and the columns were: **Column A**: Nucleodur 100-5 C18 (250 * 4.0, 5 µ), **Column B**: Luna C18 (150 x 4.6, 3µ), which was attached to AS3000 (Thermo Separation Products) Autosampler, UV-VIS Detektor Spectra Focus detector, SN4000 Controller (Thermo Separation Products). The temperature was 30 °C; UV detection was set to 254 and 210 nm. Injection volume was 50 µL of ≈150 µM solution. The mobile phase was 0.05% TFA in Millipore water and CH_3CN.

(*E*)-Methyl 3-(1*H*-imidazol-4-yl)propenoate [10][26]

Urocanic acid (10 g, 72.4 mmol) was added to refluxing absolute methanol (100 mL) and dry HCl gas was bubbled through the mixture, according to the literature procedure. After refluxing for 2 h, the solvent was removed, and the solid residue was recrystallized to obtain the ester as hydrochloride (13.2 g).

Yield 97 %; colorless crystalline solid; $C_7H_8N_2O_2 \cdot HCl$ (188.6); mp 230-231 °C; IR (neat): 3080, 2992, 2716, 2612, 1691, 1583, 1444, 1285, 1234, 1053, 981, 861, 832 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 9.06 (s, 1H, Im-2-***H***), 7.90 (s, 1H, Im-5-***H***), 7.60 (d, 1H, *J* =16.3 Hz, Im-4-C***H***CH), 6.63 (d, 1H, *J* = 16.3 Hz, Im-4-CHC***H***), 3.78 (s, 3H, OC$\boldsymbol{H}_3$); ^{13}C-NMR (DMSO-d_6), δ (ppm): 165.8 (quart, ***C***O), 136.2 (+, Im-***C***-2), 129.3 (+, ***C***H=CHCO), 128.4 (quart, Im-***C***-4), 121.6 (+, CH=***C***HCO), 120.0 (+, Im-***C***-5), 51.7 (+, ***C***H_3); EI-MS, *m/z* (rel. intensity, %): 152 ($M^{+\cdot}$, 50), 120 ($[M\text{-}OMe]^+$, 100).

Methyl 3-(1*H*-imidazol-4-yl)propanoate [11][26]

To a solution of **10** (9.88 g, 52.4 mmol) in 100 mL of MeOH in a beaker (250 mL) was added slowly 1.0 g of Pd/C (10 %) at room temperature and placed in a hydrogenation chamber at 5 bar H_2 pressure for 20 h. After completion of reaction (monitored by TLC; $CHCl_3$: MeOH, 90 : 10 under NH_3 atmosphere) the mixture was filtered through a celite pad, which was rinsed with MeOH, and the solution was concentrated *in vacuo* to get a solid (9.9 g).

Yield 99 %; colorless crystalline solid; $C_7H_{10}N_2O_2 \cdot HCl$ (190.6); mp 107-109 °C; IR (neat): 3128, 3082, 2996, 2736, 2610, 1722, 1619, 1436, 1262, 1244, 1160, 1027, 1002, 837 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 14.7 (br s, 1H, Im-N***H***), 9.03 (s, 1H, Im-2-***H***), 7.43 (s, 1H, Im-5-***H***), 3.60 (s, 3H, OC***H***$_3$), 2.99 (t, 2H, J = 7.1 Hz, Im-4-C***H***$_2$CH$_2$), 2.77 (t, 2H, *J* = 7.1 Hz, Im-4-CH$_2$C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 171.9 (quart, ***C***O), 133.1 (+, Im-***C***-2), 131.9 (quart, Im-***C***-4), 115.5 (+, Im-***C***-5), 51.4 (+, O***C***H$_3$), 31.8 (+, ***C***H$_2$CH$_2$CO), 19.4 (+, CH$_2$***C***H$_2$CO); EI-MS, *m/z* (rel. intensity, %): 154 ($M^{+\cdot}$, 35), 122 ([M-OMe]$^+$, 20), 94 ([M-CO_2Me]$^+$, 100).

N^1,N^2-Bis(benzyloxycarbonyl)guanidine [22][27]

CH_2Cl_2 (80 mL) was added to a solution of guanidine hydrochloride (5, 3.82 g, 40 mmol) and sodium hydroxide (8.0 g, 0.20 mol) in H_2O (40 mL), and the resulting mixture was cooled to 0 °C. Benzyloxycarbonyl chloride (17.1 mL, 120 mmol) was added dropwise with vigorous stirring over a period of 45 min. After the addition was complete, stirring was continued for 20 h at 0 °C. The mixture was diluted with CH_2Cl_2 (100 mL), the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The extracts were combined, washed with water, and dried with magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from methanol. Compound **22** (9.85 g) was obtained as colorless crystals.

Yield 65 %; $C_{17}H_{17}N_3O_4$ (327.3); mp 145-146 °C; IR (neat) 3397, 3228, 2359, 1732, 1652, 1618, 1542, 1440, 1382, 1275, 1209, 949, 750 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.87 (br s, 1H, N***H***); 8.68 (br s, 2H, N***H***), 7.41-7.27 (m, 10H, Ph-***H***), 5.11 (s, 4H, OC***H***$_2$Ph); ^{13}C-NMR ($CDCl_3$), δ (ppm): 159.0 (quart, ***C***O), 135.6 (quart, Ph-***C***-1), 128.5, 128.3, 128.0 (+, Ph-***C***),

67.4 (+, O***C***H_2Ph); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 328 ($[M + H]^+$), 349 ($[M + Na]^+$).

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-trifluoromethanesulfonylguanidine [23][27]

Sodium hydride (400 mg, 60 % dispersion in oil) was added to a solution of N^1,N^2-bis(benzyloxycarbonyl)guanidine (**22**) (1.65 g, 5.0 mmol) in anhydrous chlorobenzene (50 mL) at 0 °C under an argon atmosphere. After being stirred for 1 h at 0 °C, the mixture was cooled to -45 °C. Triflic anhydride (0.84 mL, 5.0 mmol) was added, and the mixture was allowed to warm to room temperature and stir overnight. The solvent was removed under reduced pressure, and the residue was dissolved in a mixture of ethyl acetate (100 mL) and 2M sodium bisulfate (25 mL). The phases were separated, the organic layer was washed with H_2O, brine and dried over magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel (eluent, CH_2Cl_2/ethyl ether, 95 : 5). Compound **23** (1.58 g, 75 %) was obtained as a solid.

Yield: 75 %; $C_{18}H_{16}F_3N_3O_6S$ (459.4); mp 74-75 °C; ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.29 (br, 2H, N***H***), 7.37 (s, 10H, Ar-***H***), 5.24 (s, 2H, Ph-C***H***$_2$O); ^{13}C-NMR ($CDCl_3$), δ (ppm): 150.9 (quart, ***C***O), 150.1 (quart, ***C***=NH), 133.6 (quart, Ph-***C***-1), 129.3, 128.9,128.8 (+, Ph-***C***), 119.2 (quart, q, *J* = 319.9 Hz, ***C***F_3), 69.8 (-, O***C***H_2Ph); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 957 ($[2M + K]^+$), 491 ($[2M + Na]^+$), 936 ($[2M + NH_4]^+$), 477 ($[M + NH_4]^+$), 460 ($[M + H]^+$).

Methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate [24][26]

To a solution of methyl 3-(1*H*-imidazol-4-yl)propanoate hydrochloride **11** (9.5 g, 50 mmol) in 100 mL of acetonitrile and triethylamine (19.6 mL, 0.14 mol) was added dropwise a solution of triphenylmethyl chloride (15.5 g, 55 mmol) in acetonitrile under external cooling with ice. The mixture was allowed to warm up at ambient temperature and was stirred for 12 h. The solvent was evaporated under reduced pressure, and the solid residue was suspended in 300 mL of water. After stirring for 1 h, the slightly yellow solid was isolated and crystallized from dry ethanol (16.1 g).

Yield 77 %; colorless crystalline solid; $C_{26}H_{24}N_2O_2$ (396.5); mp 131-132 °C; IR (neat): 3004, 2359, 1732, 1490, 1444, 1278, 1219, 1121, 748, 701 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 7.32-7.12 (m, 16H, ***H*** of CPh_3 & Im-2-***H***), 6.56 (s, 1H, Im-5-***H***), 3.61 (s, 3H, $OC\boldsymbol{H}_3$), 2.87 (t, 2H, *J* = 7.5 Hz, Im-4-$C\boldsymbol{H}_2CH_2$), 2.66 (t, 2H, *J* = 7.5 Hz, Im-4-$CH_2C\boldsymbol{H}_2$); EI-MS, *m/z* (rel. intensity, %): 396 ($M^{+\cdot}$, 10), 243 ($[Ph_3C]^+$, 100).

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol [25][26]

To a suspension of $LiAlH_4$ (1.9 g, 50 mmol) in 75 mL of freshly distilled THF and 25 mL of dry Et_2O was added **24** (10.0 g, 25 mmol) in portions under cooling with ice. The reaction mixture was refluxed for 2 h. The excess $LiAlH_4$ was decomposed by dropwise addition of a 0.1 N NaOH solution. The solution was extracted with CH_2Cl_2, and the solvent was removed in *vacuo*. The product (7.9 g) was crystallized from Et_2O.

Yield 86 %; colorless crystalline solid; $C_{25}H_{24}N_2O$ (368.5); mp 138 °C; IR (neat): 3255, 2362, 1487, 1443, 1131, 1081, 1069, 995, 748, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.34-7.10 (m, 16H, Im-2-***H*** and Ph-***H***), 6.55 (s, 1H, Im-5-***H***), 3.71 (t, *J* = 6.0 Hz, 2H, $C\boldsymbol{H}_2O$), 2.67 (t, *J* = 7.0 Hz, 2H, Im-4-$C\boldsymbol{H}_2$), 1.85 (m, 2H, $C\boldsymbol{H}_2CH_2O$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 142.2 (quart, Ph-***C***-1), 140.6 (quart, Im-***C***-4), 137.9 (+, Im-***C***-2), 129.7, 129.4, 128.1, 127.8 (+, Ph-***C***H), 118.0 (+, Im-***C***-5), 75.4 (quart, $Ph_3\boldsymbol{C}$), 62.5 (-, $\boldsymbol{C}H_2OH$), 31.9 (-, Im-4-$\boldsymbol{C}H_2$), 25.6 (-, Im-4-$CH_2\boldsymbol{C}H_2$); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 369 ($[M + H]^+$, 60), 242 ($[Ph_3C]^+$, 100); Anal. ($C_{25}H_{24}N_2O$) C, H, N.

***N*^{1},*N*^{2}-Bis(benzyloxycarbonyl)-*N*^{1}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [26]**[27]

A solution of the alcohol **25** (1.0 mmol), di-Cbz protected guanidine **22** (3.0 mmol), and PPh_3 (1.25 mmol) in anhydrous THF (20 mL) was cooled to -5 °C under argon atmosphere. DIAD (1.2 mmol) was added dropwise at a rate such that the reaction mixture was completely colorless before addition of the next drop. After the addition was complete, the reaction mixture was heated at reflux for 15-18 h. The solution was then cooled to room temperature, and the precipitate of excess **22** that formed was collected by filtration and washed with a mixture of THF/hexanes 1 : 1. The filtrate was concentrated in *vacuo*, and the product was isolated by flash column chromatography on silica gel.

Yield: 88 %; colorless crystalline solid; $C_{42}H_{39}N_5O_4$ (677.8); mp 94-96 °C; IR (neat): 3375, 2360, 2341, 1715, 1602, 1510, 1446, 1373, 1253, 1217, 1110, 1086, 1000, 749, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.20-7.48 (m, 11H, Im-2-***H*** & Cbz-Ph), 7.03-7.14 (m, 15H, C***H*** of CPh_3), 6.57 (s, 1H, Im-5-***H***), 5.19 (s, 2H, C***H***$_2$Ph), 5.06 (s, 2H, C***H***$_2$Ph), 4.02 (t, 2H, *J* = 7.3 Hz, Im-CH_2CH_2C***H***$_2$), 2.59 (t, 2H, *J* =7.7 Hz, Im-C***H***$_2CH_2CH_2$), 2.04 (s, 2H, N***H***$_2$), 1.95 (m, 2H, Im-CH_2C***H***$_2CH_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 163.9 (quart, ***C***O), 160.7 (quart, ***C***O), 156.0 (quart, ***C***=N), 142.3 (quart, Ph-***C***-1), 140.3 (quart, Im-***C***-4), 137.9 (quart, Ph-***C***-1), 137.0 (+, Im-***C***-2), 134.8 (quart, Ph-***C***-1), 129.7, 128.7, 128.6, 128.3, 127.8, 127.7, 126.9 (+, Ph-***C***H), 118.0 (+, Im-***C***-5), 75.4 (quart, Ph_3***C***), 68.8, 66.9 (-, O***C***H_2Ph), 44.5 (-, ***C***H_2NH), 28.1 (-, Im-4-***C***H_2), 25.5 (-, Im-4-CH_2***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 678 ($[M + H]^+$, 100); Anal. ($C_{42}H_{39}N_5O_4$) C, H, N.

<u>*N*-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]guanidine [27]</u>

To a solution of **26** (6.59 g, 9.7 mmol) in 150 mL THF: MeOH (1:1) was added 1.2 g of Pd/C (10 %) and then the mixture was stirred under H_2 (5 bar) overnight. The mixture was filtered through a small pad of celite, washed with MeOH, and the solvent was removed to obtain a foam-like solid (3.9 g).

Yield: 98 %; colorless amorphous solid; $C_{26}H_{27}N_5$ (409.5); mp 140-143 °C; IR (neat): 2934, 1635, 1444, 1296, 1234, 1130, 824, 747, 699, 658, cm-1; ^{1}H-NMR ($CDCl_3$ + CD_3OD), δ (ppm): 7.67 (s, 1H, Im-2-***H***), 7.37-7.30 (m, 10H, Ph-***H***), 7.16-7.06 (m, 5H, Ph-***H***), 6.56 (s, 1H, Im-5-***H***), 3.36 (t, *J* = 6.8 Hz, 2H, C***H***$_2$NH), 2.57 (m, 2H, Im-C***H***$_2$), 1.87 (m, 2H, $ImCH_2C$***H***$_2$); ^{13}C-NMR ($CDCl_3$ + CD_3OD), δ (ppm): 161.7 (quart, NH=***C***NH), 142.2 (quart. Ph-***C***), 140.2 (quart, Im-***C***-4), 137.9 (+, Im-***C***-2), 135.0 (+, Ph-***C***), 129.7 (+, Ph-***C***), 128.1, 128.0 (+, Ph-***C***), 118.3 (+, Im-***C***-5), 75.2 (quart, Ph_3***C***), 39.9 (-, ***C***H_2NH), 29.0 (-, Im-4-***C***H_2CH_2), 27.5 (-, Im-4-CH_2***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 410 ($[M + H]^+$).

<u>*N*-Trityl-2-(1-trityl-1*H*-imidazol-4-yl)ethanamine [29]</u>[29]

To a soluton of histamine dihydrochloride (3.7 g, 20 mmol) and Et_3N (8.1 g, 80 mmol) in 50 mL $CHCl_3$ was added dropwise a solution of trityl chloride (14.1 g, 50 mmol) in 50 mL

$CHCl_3$ under external cooling with ice. The mixture was allowed to warm up at ambient temperature and stirred for 20 h. The solvent was evaporated under reduced pressure, and the solid residue was suspended in 100 mL of water. After stirring for 1 h, the product was extracted with $CHCl_3$ (2 x 100 mL), dried over anhydrous sodium sulphate and evaporated to give crystalline solid (7.4 g).

Yield: 62 %; colorless crystalline solid; $C_{43}H_{37}N_3$ (595.8); mp 202-203 °C; IR (neat): 1489, 1445, 1238, 1161, 1127, 1083, 1029, 129 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.42-7.30 (m, 7H, Ph-***H*** & Im-2-***H***), 7.31-7.08 (m, 24H, Ph-***H***), 6.50 (s, 1H, Im-5-***H***), 2.73 (t, *J* = 6.2 Hz, 2H, Im-C***H***$_2$), 2.40 (t, *J* = 6.3 Hz, 2H, C***H***$_2$$NH_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 146.2 (quart, Ph-***C***-1), 142.4 (quart, Ph-***C***-1), 139.9 (quart, Im-***C***-4), 138.2 (+, Im-***C***-2), 129.6, 128.6, 128.1, 127.9, 127.6, 126.3 (+, Ph-***C***), 118.4 (+, Im-***C***-5), 75.0 (quart, Ph_3***C***), 70.7 (quart, Ph_3***C***), 42.7 (-, ***C***H_2NH_2), 29.3 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 596 ($[M + H]^+$).

2-(1-Trityl-1*H*-imidazol-4-yl)ethanamine [30][29]

To a solution of **29** (10 g, 0.017 mol) in 47.5 mL CH_2Cl_2 at 0 °C, 2.5 mL of TFA was added drop by drop. After stirring for 10 min at the same temperature, the mixture was allowed to come to rt and was stirred for additional 45 min. After removing the solvent, the residue was neutralised with saturated sodium bicarbonate solution and then extracted with $CHCl_3$ (4 x 50 mL), washed with, water, brine and dried over anhydrous sodium sulfate. After removing the solvent, the residue was chromatographed with $CHCl_3$/MeOH/TEA (94 : 5 : 1) on silica to obtain a hygroscopic solid ($C_{24}H_{23}N_3$).

Yield: 68 %; yellowish sticky oil; $C_{24}H_{23}N_3$ (353.5); mp 126-128 °C; IR (neat) 1681, 1491, 1444, 1199, 1128, 1036, 829 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40 (s, 1H, Im-2-C***H***), 7.35-7.28 (m, 9H, Ph-***H***), 7.18-7.10 (m, 6H, Ph-***H***), 6.60 (s, 1H, Im-5-C***H***), 2.97 (t, *J* = 6.7 Hz, 2H, C***H***$_2$$NH_2$), 2.70 (t, *J* = 6.7 Hz, 2H, Im-4-C***H***$_2$), 1.78 (s br., 2H, CH_2N***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 142.5 (quart, Ph-***C***-1), 139.4 (quart, Im-***C***-4), 138.6 (+, Im-***C***-2), 129.7 (+, Ph-***C***H), 128.0 (+, Ph-***C***H), 118.6 (+, Im-***C***-5), 75 (quart, ***C***Ph_3), 41.8 (-, ***C***H_2NH_2), 32.3 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 354 ($[M + H]^+$), 243 ($[Ph_3C]^+$).

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine [32][27]

The amine **30** (0.35 g, 1.0 mmol) was added to a solution of **23** (0.41 g, 0.9 mmol) and triethylamine (0.1 g, 1.0 mmol) in CH_2Cl_2 (5 mL, filtered over neutral alumina), and the mixture was allowed to stir at room temperature until **23** was consumed (3 h) as evidenced by TLC. After the reaction was complete, the mixture was diluted with CH_2Cl_2 (6 mL) and washed with 2M sodium bisulfate, saturated sodium bicarbonate, and brine. The organic extract was then dried over sodium sulfate and filtered, and the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography with CH_2Cl_2 to afford material suitable for NMR.

Yield: 92 %; colorless crystalline solid; $C_{41}H_{37}N_5O_4$ (663.8); mp 98 °C; IR (neat): 1729, 1637, 1569, 1444, 1380, 1320, 1261, 1203, 1132, 1055, 804 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 11.70 (s, 1H, N***H***), 8.64 (t, *J* = 5.4 Hz, 1H, N***H***), 7.43 (s, 1H, Im-2-***H***), 7.27-7.40 (m, 19H, Ar-***H***), 7.10-7.16 (m, 6H, Ar-***H***), 6.64 (s, 1H, Im-5-***H***), 5.15 (s, 2H, PhC***H***$_2$O), 5.10 (s, 2H, PhC***H***$_2$O), 3.74 (dd, J_1 = 6.3 Hz, J_2 = 12.0 Hz, 2H, C***H***$_2$NH), 2.81 (t, *J* = 6.4 Hz, Im-4-C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 163.7 (quart, ***C***O), 155.9 (quart, ***C***O), 153.5 (quart, ***C***=NH), 142.3 (quart, Ph-***C***-1), 138.7 (+, Im-***C***-2), 137.8 (quart, Im-***C***-4), 136.8 (quart, benzyl-***C***), 134.7 (quart, benzyl-***C***), 129.8, 128.7, 128.4, 128.1, 128.0, 127.9 (+, Ph-***C***), 118.8 (+, Im-***C***-5), 75.38 (quart, Ph$_3$***C***), 67.9 (-, Ph***C***H$_2$O), 67.1 (-, Ph***C***H$_2$O), 40.6 (-,***C***H$_2$NH), 27.6 (-, Im-4-***C***H$_2$); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 664 ($[M + H]^+$), 242 ($[Ph_3C]^+$).

[2-(1-Trityl-1*H*-imidazol-4-yl)ethyl]guanidine [33]

To a solution of **32** (**3.3** g, **5.0** mmol) in 15 mL THF/MeOH (1 : 1) was added 0.5 g of Pd/C (10 %) and then the mixture was stirred under H_2 (5 bar) overnight. The mixture was filtered through celite bed, washed with MeOH, removed solvent to obtain a hygroscopic semisolid.

Yield 96 %, hygroscopic semisolid; $C_{25}H_{15}N_5$ (395.5); IR (neat): 3140, 1646, 1492, 1444, 1156, 1131, 1085, 1034, 1001, 831 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.51 (s, 1H, N***H***), 7.56 (s, 1H, Im-2-***H***), 7.43-7.01 (m, 15H, Ph-***H***), 6.86 (s, 1H, Im-5-***H***), 3.43 (t, *J* = 7.1 Hz, 2H, C***H***$_2$NH), 3.31 (m, 1H, N***H***), 2.80 (t, *J* = 7.1 Hz, Im-4-C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 158.5 (quart, ***C***=NH), 143.3 (quart, Ph-***C***-1), 139.5 (+, Im-***C***-2), 137.7 (quart, Im-***C***-4), 131.0,

130.0, 129.1 (+, Ph-***C***), 121.0 (+, Im-***C***-5), 77.2 (quart, Ph_3***C***), 42.1 (-, NH***C***H_2), 28.3 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc), *m/z*: 791 ($[2M + H]^+$), 396 ($[M + H]^+$), 242 ($[Ph_3C]^+$).

General procedure for synthesis of the ketones 39a-d

A 250 mL three-necked flask fitted with a magnetic pallet was connected to a sidearm dumper that contained of the pertinent benzonitrile (ArCN) (23 mmol), to a pressure equalizing addition funnel, and to the Schlenk line by a vacuum adapter. The apparatus was purged with N_2 for 30 min, and then 23 mmol of the appropriate heteroarylbromide (ArBr) and 100 mL of Et_2O were added sequentially by syringe to the flask via the addition funnel. Then, 14 mL of 1.6 M solution (23 mmol) of ^{n}BuLi in hexanes and 20 mL of Et_2O were transferred to the addition funnel. The flask was cooled to -78 °C, and the ^{n}BuLi solution was added dropwise to the ethereal ArBr solution. After complete addition, the resulting red solution of 2-lithioaryl was stirred for 20 min, and then ArCN was added in several portions over 5 min. The reaction mixture was stirred at -78 °C for 1 h, the cold bath was removed, and the mixture was allowed to warm to room temperature with stirring over the course of 4 h. The resulting red-violet slurry was poured into 100 mL of cold (0 °C) 3 M HCl. After the mixture had been stirred for 20 min, the organic and aqueous fractions were separated, 3 M NaOH was added to the aqueous fraction until the mixture was slightly basic to litmus, and the aqueous fraction was extracted with CH_2Cl_2 (3 X 100 mL) portions of. The combined organic fractions were dried over $MgSO_4$ and filtered, and solvent was removed by rotary evaporation to leave dark orange oil. The residue was purified by flash column chromatography.

Phenyl(pyridin-2-yl)methanone [39a][30]

Synthesized from 2-bromopyridine and benzonitrile. Yield: 77 %; colorless crystalline solid; $C_{12}H_9NO$ (183.2); mp 42 °C; IR (neat): 1990, 1665, 1579, 1448, 1303, 1281, 1245, 940, 746, 697, 651 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.73 (m, 1H, Pyr-6-***H***), 8.05 (m, 3H, Pyr-***H*** & Ph-***H***), 7.90 (m, 1H, Pyr-***H***), 7.59 (m, 1H, Pyr-***H***), 7.48 (m, 3H, Ph-***H***); ^{13}C-NMR ($CDCl_3$), δ (ppm): 193.9 (quart, ***C***O), 155.0 (quart, Pyr-***C***-2), 148.6 (+, Pyr-***C***-6), 137.1 (+, Pyr-***C***-4), 136.2 (quart, Ph-***C***-1), 132.9 (+), 130.9 (+), 128.2 (+, Ph-***C***), 126.2 (+, Pyr-***C***-3), 124.6 (+,

Pyr-***C***-5); EI-MS, *m/z* (rel. intensity, %): 183 ($M^{+\cdot}$, 63), 182 (73), 156 (12), 155 (86), 154 (29), 105 ([M – Pyr]$^+$, 100), 78 ([Pyr]$^+$, 18), 77 ([Ph]$^+$, 84), 51 (29); Anal. ($C_{12}H_9NO$) C, H, N.

4-Fluorophenyl(pyridin-2-yl)methanone [39b]

Synthesized from 2-bromopyridine and 4-fluorobenzonitrile. Yield 69 %; colorless crystalline solid; $C_{12}H_8FNO$ (201.2); mp 68-69 °C; IR (neat): 1651, 1595, 1581, 1567, 1504, 1433, 1411, 1310, 1288, 1241, 1218, 1153, 1015, 993, 934, 857, 741 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.73 (m, 1H, Pyr-6-***H***), 8.21-8.11 (m, 2H, Ar-***H***), 8.06 (m, 1H, Pyr-***H***), 7.93 (m, 1H, Pyr-***H***), 7.50 (ddd, 1H, J_1 = 1.4 Hz, J_2 = 4.7 Hz, J_3 = 7.7 Hz, Pyr-***H***), 7.22-7.12 (m, 2H, Ar-***H***); ^{13}C-NMR ($CDCl_3$), δ (ppm): 191.8 (quart, ***C***O), 165.7 (quart, d, *J* = 255.1 Hz, Ar-***C***F), 154.6 (quart, Pyr-***C***-2), 148.2 (+, Pyr-***C***-6), 137.4 (+, Pyr-***C***-4), 133.8 (+, d, *J* = 9.9 Hz, 2 Ar-***C***-2,6), 132.4 (quart, d, *J* = 3.0 Hz, Ar-***C***-1), 126.4 (+, Pyr-***C***-3), 124.7 (+, Py-***C***-5), 115.4, (+, d, *J* = 21.9 Hz, Ar-***C***); EI-MS, *m/z* (rel. intensity, %): 201 ($M^{+\cdot}$, 40), 173 ([M – CO]$^+$, 79), 123 ([M – Pyr]$^+$, 100), 95 ([M - Pyr-CO]$^+$, 63), 75 (14), 51 (8); Anal. ($C_{12}H_8FNO$) C, H, N.

Phenyl(thiazol-2-yl)methanone [39c]

Synthesized from 2-bromothiazole and benzonitrile. Yield 86 %; colorless crystalline solid; $C_{10}H_7NOS$ (189.2); mp 35-37 °C; IR (neat): 3120, 1642, 1596, 1385, 1297, 1121, 761, 612 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.50-8.45 (m, 2H, Ph-***H***), 8.10 (d, *J* = 3.0 Hz, 1H, Thiaz-4-***H***), 7.72 (d, *J* = 3.0 Hz, 1H, Thiaz-5-***H***), 7.68-7.6 (m, 1H, Ph-***H***), 7.57-7.49 (m, 2H, Ph-***H***); ^{13}C-NMR ($CDCl_3$), δ (ppm): 181.2 (quart, ***C***O), 154.5 (quart, Thiaz-***C***-2), 145.2 (+, Thiaz-***C***-4), 132.3 (quart, Ph-***C***-1), 131.5 (+) 128.5 (+), 126.7 (+), 125.7 (+, Ph-***C***), 126.5 (+, Thia-***C***-5); EI-MS, *m/z* (rel. intensity, %): 189 ($M^{+\cdot}$, 41), 161 ([M – CO]$^+$, 54), 105 ([M – Thiaz]$^+$, 100), 77 ([M – Thiaz-CO]$^+$, 77), 58 (16), 51 (27); Anal. ($C_{10}H_7NOS$) C, H, N.

3,4-Difluorophenyl(thiazol-2-yl)methanone [39d]

Synthesized from 2-bromothiazole and 3,4-difluorobenzonitrile. Yield 87 %; colorless crystalline solid; $C_{10}H_5F_2NOS$ (225.2); mp 59-60 °C; IR (neat): 3134, 3077, 1650, 1600, 1431, 1386, 1290, 1081, 911, 760, 602 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.09 (d, *J* = 3.0 Hz,

1H, Thiaz-4-***H***), 7.75 (d, *J* = 3.0 Hz, 1H, Thiaz-5-***H***), 7.30 (m, 3H, Ar-***H***), ^{13}C-NMR ($CDCl_3$), δ (ppm): 181.0 (quart, **C**O), 156.3 (quart, Thiaz-***C***-2), 152.4 (quart, dd, J_1 = 12.0 Hz, J_2 = 46.2 Hz, ***C***-F), 149.1 (quart, dd, J_1 = 12.0 Hz, J_2 = 46.2 Hz, ***C***-F), 144.2 (+, Thiaz-***C***-4), 135.5 (quart, Ar-***C***-1), 124.4 (+, Ar-***C***-6), 122.4 (+, Thiaz-***C***-5), 117.5 (+, d, *J* = 17.0 Hz, Ar-***C***-5), 117.1 (+, d, *J* = 17.0 Hz, Ar-***C***-2); EI-MS, *m/z* (rel. intensity, %): 225 ($M^{+\cdot}$, 44), 197 ([M – CO]$^+$, 40), 141 ([M – Thiaz]$^+$, 100), 113 ([M – Thiaz-CO]$^+$, 59), 58 (15); Anal. ($C_{10}H_5F_2NOS$) C, H, N.

General procedure for the synthesis of the ethyl propenoates 40a-d, 44a,b

To a stirred suspension of sodium hydride (0.74 g, 18.5 mmol as 60 % dispersion in oil) in 20 mL of dry THF, triethyl phosphonoacetate (3.95 g, 17.4 mmol) under nitrogen atmosphere was added at a rate such that the reaction temperature was maintained at 30-35 °C. The mixture was stirred at room temperature for 1h and the solution of the pertinent ketone (18 mmol) in 40 mL of THF was added dropwise over 30 min. The mixture was refluxed for 16-24 h and poured into ice water, extracted with diethyl ether, washed with water, dried and concentrated to give a mixture of *E* and *Z* isomeric ethyl esters, as an oil. The residue was separated by flash column chromatography.

Ethyl 3-phenyl-3-pyridin-2-ylpropenoate [40a]

Synthesized from phenyl(pyridin-2-yl)methanone (**39a**). yield 72 %; colorless oil; $C_{16}H_{15}NO_2$ (252.3); IR (neat) 2985, 1716, 1622, 1585, 1468, 1446, 1429, 1367, 1269, 1158, 1030, 994, 876 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.68 (m, 1H, Pyr-6-***H***), 7.72 (m, 1H, Pyr-4-***H***), 7.35-7.25 (m, 7H, 2 Pyr-***H*** and 5 Ph-***H***), 6.48 (s, 1H, C***H***COOEt), 4.05 (q, *J* = 7.2 Hz, 2H, COOC***H***$_2$CH$_3$), 1.11 (t, *J* = 7.2 Hz, 3H, COOCH$_2$C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 165.8 (quart, **C**O), 157.7 (quart, Pyr-***C***-2), 154.6 (quart, Ar$_2$***C***=CH), 149.3 (+, Pyr-***C***-6), 138.9 (quart, Ph-***C***-1), 135.8 (+, Pyr-***C***-4), 129.5 (+, Ph-***C***), 128.5 (+, Ph-***C***), 127.9 (+, Ph-***C***), 124.2 (+, Pyr-***C***-5), 122.6 (+, Pyr-***C***-3), 118.9 (+, Ar$_2$C=***C***H), 60.2 (-, O***C***H$_2$CH$_3$), 14.0 (+, OCH$_2$***C***H$_3$); EI-MS, *m/z* (rel. intensity, %): 254 (10), 253 (65), 252 ($M^{+\cdot}$, 96), 224 ([M – Et]$^+$, 90), 208 ([M – OEt]$^+$, 35), 206 (19), 180 ([M – CO_2Et]$^+$, 100), 167 ([M – CHCOOEt]$^+$, 9).

Ethyl 3-(4-fluorophenyl)-3-pyridin-2-ylpropenoate [40b]

Synthesized from 4-fluorophenyl(pyridin-2-yl)methanone (**39b**). yield 75 %; colorless oil; $C_{16}H_{14}FNO_2$ (241.3); IR (neat): 2983, 1716, 1600, 1564, 1506, 1469, 1431, 1411, 1367, 1270, 1227, 1158, 1095, 1033, 994, 836, 813 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.75 (m, 1H, Pyr-6-***H***), 7.74 (m, 1H, Pyr-4-***H***), 7.30 (m, 4H, 2 Pyr-***H*** and 2 Ph-***H***), 7.01 (m, 2H, 2 Ph-*o*-***H***), 6.42 (s, 1H, C***H***COOEt), 4.04 (q, *J* = 7.2 Hz, 2H, COOC***H***$_2$CH$_3$), 1.11 (t, *J* = 7.2 Hz, 3H, COOCH$_2$C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 165.6 (quart, ***C***O), 165.0 (quart, d, *J* = 250.6 Hz, ***C***F), 157.5 (quart, Pyr-***C***-2), 153.5 (quart, Ar$_2$***C***=CH), 149.4 (+, Pyr-***C***-6), 136.0 (+, Pyr-***C***-4), 135.0 (quart, d, *J* = 3.1 Hz, Ar-***C***-1), 129.9 (+, d, *J* = 10.1 Hz, 2C, Ar-***C***-2,6), 124.2 (+, Pyr-***C***-3), 122.7 (+, Pyr-***C***-5), 118.8 (+, ***C***HCOOEt), 115.6 (+, d, *J* = 21.0 Hz, 2C, Ar-***C***-3,5), 60.2 (-, CO***C***H$_2$CH$_3$), 14.0 (+, COCH$_2$***C***H$_3$); EI-MS, *m/z* (rel. intensity, %): 271 ((M$^{+\cdot}$, 78), 242 ([M – Et]$^+$, 68), 226 ([M – OEt]$^+$, 47), 198 ([M – CO_2Et]$^+$, 100), 170 (10), 120 (6), 78 (14), 51 (3).

Ethyl 3-phenyl-3-thiazol-2-ylpropenoate [40c]

Synthesized from phenyl(thiazol-2-yl)methanone (**39c**). 12 h refluxed; yield 79 %; colorless oil; $C_{14}H_{13}NO_2S$ (259.3); IR (neat): 2981, 1716, 1618, 1489, 1446, 1365, 1334, 1264, 1172, 1096, 1026, 977, 877, 771 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.91 (d, *J* = 3.0 Hz, 1H, Thiaz-4-***H***), 7.47-7.38 (m, 4H, Ph-***H***), 7.37-7.30 (m, 2H, Ph-***H*** and Thiaz-5-***H***), 7.10 (s, 1H, C***H***=CO), 4.04 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$), 1.09 (t, *J* = 7.1 Hz, 3H, OCH$_2$C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 168.9 (quart, ***C***O), 165.3 (quart, Thiaz-***C***-2), 146.7 (quart, ***C***=CHCOOEt), 144.3 (+, Thiaz-***C***-4), 137.0 (quart, Ph-***C***-1), 128.9, 128.6, 128.3, 128.1 (+, Ph-***C***), 122.5 (+, C=***C***HCO), 119.1 (+, Thiaz-***C***-5), 60.3 (-, O***C***H$_2$), 13.9 (+, OCH$_2$***C***H$_3$); EI-MS, *m/z* (rel. intensity, %): 259 (M$^{+\cdot}$, 45), 230 ([M – Et]$^+$, 70), 214 ([M – OEt]$^+$, 40), 186 ([M – CO_2Et]$^+$, 100), 173 ([M – $CHCO_2$Et]$^+$, 3), 128 (11), 102 (6), 77 (4), 58 (6).

Ethyl 3-(3,4-difluorophenyl)-3-thiazol-2-ylpropenoate [40d]

Synthesized from 3,4-difluorophenyl(thiazol-2-yl)methanone (**39d**). 12 h refluxed; yield 68 %; colorless oil; $C_{14}H_{11}F_2NO_2S$ (295.3); IR (neat): 2979, 1716, 1601, 1516, 1268, 1230, 1200, 1163, 1121, 1090, 1029, 871, 819, 776, 729 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.96 (d, *J* = 3.0 Hz, 1H, Thiaz-4-***H***), 7.56 (d, *J* = 3.0 Hz, 1H, Thiaz-5-***H***), 7.26-7.14 (m, 3H, Ar-***H***), 6.56 (s, 1H, C***H***=CO), 4.03 (q, *J* = 7.2 Hz, 2H, COOC***H***$_2$), 1.10 (t, *J* = 7.2 Hz, 3H,

$OCH_2C\boldsymbol{H}_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 164.9 (quart, **C**O), 163.3 (quart, Thiaz-***C***-2), 152.4 (quart, dd, J_1 = 12.0 Hz, J_2 = 46.2 Hz, ***C***-F), 149.4 (quart, dd, J_1 = 12.0 Hz, J_2 = 46.2 Hz, ***C***-F), 144.5 (quart, ***C***=CHCOOEt), 143.2 (+, Thiaz-***C***-4), 135.5 (quart, Ar-***C***-1), 124.4 (+, Ar-***C***-6), 122.6 (+, C=***C***HCO), 121.4 (+, Thiaz-***C***-5), 117.5 (+, d, J = 17.0 Hz, Ar-***C***-5), 117.2 (+, d, J = 17.0 Hz, Ar-***C***-2), 60.8 (-, O***C***H_2), 13.9 (+, $OCH_2\boldsymbol{C}H_3$); EI-MS, *m/z* (rel. intensity, %): 295 ($M^{+\cdot}$, 27), 266 ([M – Et]$^+$, 26), 250 ([M – OEt]$^+$, 49), 222 ([M – CO_2Et]$^+$, 100), 164 (19), 151 (12), 138 (20), 111 (8), 87 (8), 73 (13), 58 (37), 43 (16), 28 (47).

(*E*)-Ethyl 3-pyridin-2-ylbut-2-enoate [44a]

Synthesized from 1-pyridin-2-ylethanone. Yield 89 %; colorless oil; $C_{11}H_{13}NO_2$ (191.2); IR (neat): 2930, 1709, 1633, 1581, 1432, 1341, 1277, 1172, 1093, 1039, 878 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.60 (m, 1H, Pyr-6-***H***), 7.67 (m, 1H, Pyr-4-***H***), 7.53 (m, 1H, Pyr-3-***H***), 7.23 (m, 1H, Pyr-5-***H***), 6.67 (q, J = 1.3 Hz, C***H***CO), 4.19 (q, J = 7.1 Hz, OC$\boldsymbol{H}_2$), 2.59 (d, J = 1.3 Hz, C$\boldsymbol{H}_3$C=CH), 1.28 (t, J = 7.1 Hz, $OCH_2C\boldsymbol{H}_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 166.9 (quart, ***C***O), 157.9 (quart, Pyr-***C***-2), 152.8 (quart, ***C***=CHCO), 149.1 (+, Pyr-***C***-6), 136.8 (+, Pyr-***C***-4), 123.6 (+, Pyr-***C-3***), 120.9 (+, ***C***HCO), 119.4 (+, Pyr-***C-5***), 60.0 (-, O***C***H_2), 16.0 (+, ***C***H_3C=CH), 14.3 (+, $OCH_2\boldsymbol{C}H_3$); EI-MS, *m/z* (rel. intensity, %): 191 ($M^{+\cdot}$, 26), 162 ([M - Et]$^+$, 18), 146 ([M – OEt]$^+$, 43), 117 (([M – CO_2Et]$^+$, 100), 90 (13), 78 (15), 51 (15).

(*E*)-Ethyl 3-thiophen-2-ylbut-2-enoate [44b]

Synthesized from 1-thiophenyl-2-ylethanone; yield 83 %; colorless oil; $C_{10}H_{12}O_2S$ (196.3); IR (neat): 3020, 1703, 1613, 1425, 1366, 1258, 1220, 1154, 1037, 853 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.30 (m, 2H, Thio-3-***H*** and -5-***H***), 7.03 (m, 1H, Thio-4-***H***), 6.25 (d, J = 1.4 Hz, C***H***COO), 4.19 (q, J = 7.1 Hz, OC$\boldsymbol{H}_2$CH$_3$), 2.60 (d, J = 1.4 Hz, 2H, C$\boldsymbol{H}_3$C=CH), 1.30 (t, J = 7.1 Hz, 3H, $OCH_2C\boldsymbol{H}_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 166.7 (quart, ***C***O), 147.7 (quart, ***C***=CH), 145.5 (quart, Thio-***C***-2), 127.9, 127.1, 126.7 (+, Thio-***C***-3,4,5), 114.2 (+, ***C***HCOO), 59.8 (-, O***C***H_2), 17.2 (+, ***C***H_3C=CH), 14.3 (+, $OCH_2\boldsymbol{C}H_3$); EI-MS, *m/z* (rel. intensity, %): 196 ($M^{+\cdot}$, 53), 151 ([M – OEt]$^+$, 100), 124 ([M – CO_2Et]$^+$, 57), 111 ([M – $CHCO_2$Et]$^+$, 15), 97 (18), 79 (25), 71 (26), 57 (52).

General procedure for the synthesis of the ethyl propanoates 41a-d, 45a,b

The ethyl acrylates were dissolved in THF (100 mL) and 10 % Pd/C (1.00 g) was added, and the mixture was stirred at room temperature for 12 h under a hydrogen atmosphere. The catalyst was filtered off through Celite pad and the filtrate was concentrated to obtain colorless oil.

Ethyl 3-phenyl-3-pyridin-2-ylpropanoate [41a]

Synthesized from ethyl 3-phenyl-3-pyridin-2-ylpropenoate (**40a**); yield 96 %; colorless oil; $C_{16}H_{17}NO_2$ (255.3); IR (neat): 2981, 1729, 1589, 1568, 1494, 1472, 1432, 1371, 1311, 1246, 1156,1096, 1021, 996, 962, 847, 805 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.54 (m, 1H, Pyr-6-***H***), 7.52 (m, 1H, Pyr-4-***H***), 7.35- 7.00 (m, 7H, Pyr-3-***H*****,** Pyr-5-***H*** and 5 Ph-***H***), 6.64 (dd, J_1 = 8.8 Hz, J_2 = 6.8 Hz, 1H, C***H***CH_2COOEt), 4.03 (ddq, J = 8.7 and 6.8 Hz, 2H, COOC***H***$_2CH_3$), 3.44 (dd, J_1 = 8.7 Hz, J_2 = 16.0 Hz, 1H, C***H***$_2$COOEt), 2.98 (dd, J_1 = 6.8 Hz, J_2 = 16.0 Hz, 1H, C***H***$_2$COOEt), 1.12 (t, J = 7.1 Hz, 3H, $COOCH_2C$***H***$_3$); ^{13}C-NMR, ($CDCl_3$), δ (ppm): 172.2 (quart, ***C***O), 162.0 (quart, Pyr-***C***-2), 148.9 (+, Pyr-***C***-6), 142.7 (quart, Ph-***C***-1), 136.3 (+, Pyr-***C***-4), 128.6, 128.4, 127.9, 126.7 (+, Ph-***C***), 123.3 (+, Pyr-***C***-5), 121.4 (+, Pyr-***C***-5), 60.2 (-, O***C***H_2CH_3), 49.0 (+, Ar_2***C***HCH_2), 39.6 (-, ***C***H_2CO), 14.0 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 255 ($M^{+\cdot}$, 19), 254 (17), 210 ($[M - OEt]^+$, 20), 182 ($[M - CO_2Et]^+$, 100), 167 ($[M - CH_2CO_2Et]^+$, 60).

Ethyl 3-(4-fluorophenyl)-3-pyridin-2-ylpropanoate [41b]

Synthesized from ethyl 3-(4-fluorophenyl)-3-pyridin-2-ylpropenoate **(40b)**: yield 93 %; colorless oil; $C_{16}H_{16}FNO_2$ (273.3); IR (neat): 2938, 1730, 1686, 1590, 1508, 1472, 1433, 1372, 1307, 1221, 1158, 1098, 1017, 963, 838 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.53 (m, 1H, Pyr-6-***H***), 7.54 (m, 1H, Pyr-4-***H***), 7.33-7.25 (m, 2H, 2 Ph-*o*-***H***), 7.16-7.05 (m, 2H, Pyr-***H***), 7.03-6.89 (m, 2H, 2 Ph-*m*-***H***), 4.62 (ddq, J = 8.2 and 7.1 Hz, 1H, C***H***CH_2COOEt), 4.02 (q, J = 7.1 Hz, 2H, COOC***H***$_2CH_3$), 3.40 (dd, J_1 = 8.2 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOEt), 2.96 (dd, J_1 = 7.1 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOEt), 1.11 (t, J = 7.1 Hz, 3H, $COOCH_2C$***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.0 (quart, ***C***O), 161.6 (quart, d, J = 245.1 Hz, ***C***F), 161.7 (quart, Pyr-***C***-2), 148.9 (+, Py-***C***-6), 138.4 (quart, Ar-***C***-1), 136.6 (+, Pyr-***C***-4), 129.4 (+, d, J = 9.9 Hz, 2C, Ar-***C***-2,6), 123.2 (+, Pyr-***C***-5(3)), 121.6 (+, Pyr-***C***-3(5)), 115.3 (+, d, J = 21.1 Hz, 2C,

Ar-***C***-3,5), 60.3 (-, O***C***H_2), 48.1 (+, ***C***HCH_2CO), 39.8 (-, CH***C***H_2CO), 14.0 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 273 ($M^{+\cdot}$, 19), 228 ([M – OEt]$^+$, 18), 200 ([M – CO_2Et]$^+$, 100), 185 ([M – CH_2CO_2Et]$^+$, 55), 122 (24), 84 (15), 55 (4).

Ethyl 3-phenyl-3-thiazol-2-ylpropanoate [41c]

Synthesized from ethyl 3-phenyl-3-thiazol-2-ylpropenoate (**40c**): Yield 91 %; colorless oil; $C_{14}H_{15}NO_2S$ (261.1); IR (neat): 1730, 1496, 1454, 1373, 1252, 1186, 1031, 963, 722, 699 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.71 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.50-7.20 (m, 5H, Ph-***H***), 7.18 (d, *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 4.90 (ddq, *J* = 7.9 and 7.1 Hz, 1H, C***H***CH_2COOEt), 4.06 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$$CH_3$), 3.45 (dd, J_1 = 7.9 Hz, J_2 = 16.1 Hz, 1H, C***H***$_2$COOEt), 2.96 (dd, J_1 = 7.5 Hz, J_2 = 16.1 Hz, 1H, C***H***$_2$COOEt), 1.14 (t, *J* = 7.1 Hz, 3H, $COOCH_2$C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.8 (quart, ***C***O), 171.2 (quart, Thiaz-***C***-2), 142.1 (+, Thiaz-***C***-4), 141.2 (quart, Ph-***C***-1), 128.8, 128.6, 127.9, 127.5 (+, Ph-***C***H), 119.1 (+, Thiaz-***C***-5), 60.6 (-, O***C***H_2), 45.4 (+, ***C***HCH_2COOEt), 40.5 (-, ***C***H_2COOEt), 14.0 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 261 ($M^{+\cdot}$, 45), 216 ([M – OEt]$^+$, 18), 188 ([M – CO_2Et]$^+$, 100), 173 ([M – CH_2CO_2Et]$^+$, 28), 130 (2), 103 (7), 77 (6), 58 (6).

Ethyl 3-(3,4-difluorophenyl)-3-thiazol-2-ylpropanoate [41d]

Synthesized from ethyl 3-(3,4-difluorophenyl)-3-thiazol-2-ylpropenoate (**40d**), **16** h refluxed; yield 88 %; colorless oil; $C_{14}H_{13}F_2NO_2S$ (297.3); IR (neat): 1730, 1609, 1517, 1434, 1373, 1278, 1174, 1117, 1016, 818, 777, 725 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.72 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.23 (d, *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 7.22-7.04 (m, 3H, Ar-***H***), 4.85 (ddq, *J* = 7.5 and 7.8 Hz, 1H, C***H***CH_2CO), 4.08 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$), 3.41 (dd, J_1 = 7.5 Hz, J_2 = 16.3 Hz, 1H, C***H***$_2$COOEt), 3.01 (dd, J_1 = 7.8 Hz, J_2 = 16.3 Hz, 1H, C***H***$_2$COOEt), 1.17 (t, *J* = 7.1 Hz, 3H, OCH_2C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 171.3 (quart, **C**O), 170.8 (quart, Thiaz-***C***-2), 151.6 (quart, dd, J_1 = 12.6 Hz, J_2 = 249.0 Hz, ***C***-F), 148.3 (quart, dd, J_1 = 12.6 Hz, J_2 = 249.0 Hz, ***C***-F), 142.4 (+, Thiaz-***C***-4), 138.2 (quart, Ar-***C***-1), 124.0 (+, Ar-***C***-6), 119.3 (+, Thiaz-***C***-5), 117.5 (+, d, *J* = 17.0 Hz, Ar-***C***-5), 117.0 (+, d, *J* = 17.0 Hz, Ar-***C***-2), 60.8 (-, O***C***H_2), 44.5 (+, ***C***HCH_2CO), 40.5 (-,CH***C***H_2CO), 14.0 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 297 ($M^{+\cdot}$, 34), 252 ([M – OEt]$^+$, 17), 224 ([M – CO_2Et]$^+$, 100), 209 ([M – CH_2CO_2Et]$^+$, 33), 166 (3), 139 (7), 119 (6), 86 (2), 57 (13), 28 (12).

Ethyl 3-pyridin-2-ylbutanoate [45a]

Yield 92 %; colorless oil; $C_{11}H_{15}NO_2$ (193.2); IR (neat): 2974, 1729, 1592, 1434, 1371, 1166, 1032 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.58 (m, 1H, Pyr-6-***H***), 7.67 (m, 1H, Pyr-4-***H***), 7.25 (m, 1H, Pyr-3-***H***), 7.17 (m, 1H, Pyr-5-***H***), 4.07 (q, *J* = 7.1 Hz, OC***H***$_2$), 3.45 (m, 1H, C***H***CH_2CO), 2.90 (dd, J_1 = 7.9 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2CO$), 2.63 (dd, J_1 = 7.1 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2CO$), 1.36 (d, *J* = 6.9 Hz, 3H, *C****H***$_3CHCH_2CO$), 1.18 (t, *J* = 7.1 Hz, 3H, OCH_2C***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 173.4 (quart, ***C***O), 164.0 (quart, Pyr-***C***-2), 148.5 (+, Pyr-***C***-6), 137.3 (+, Pyr-***C***-4), 122.3 (+, Pyr-***C***-3), 121.7 (+, Pyr-***C***-5), 64.2 (-, O***C***H_2), 40.8 (-, ***C***H_2CO), 37.1 (+, ***C***HCH_2CO), 20.6 (+, ***C***H_3CHCH_2), 14.1 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 193 ($M^{+\cdot}$, 3), 148 ($[M - OEt]^+$, 25), 120 ($[M - CO_2Et]^+$, 100), 106 ($[M - CH_2CO_2Et]^+$, 36), 84 (7), 78 (12), 55 (4), 51 (7).

Ethyl 3-thiophen-2-ylbutanoate [45b]

Yield 96 %; colorless oil; $C_{10}H_{14}O_2S$ (198.3); IR (neat): 2926, 1732, 1616, 1457, 1369, 1345, 1281, 1159, 1030, 849, 825 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.12 (m, 1H, Thio-5-***H***), 6.90 (m, 1H, Thio-3-***H***), 6.83 (m, 1H, Thio-4-***H***), 4.11 (ddq, *J* = 7.1 Hz, OC***H***$_2$), 3.59 (m, 1H, C***H***CH_2CO), 2.68 (dd, J_1 = 6.9 Hz, J_2 = 15.4 Hz, 1H, C***H***$_2CO$), 2.54 (dd, J_1 = 7.9 Hz, J_2 = 15.4 Hz, 1H, C***H***$_2CO$), 1.38 (d, *J* = 6.9 Hz, 3H, C***H***$_3CHCH_2CO$), 1.22 (t, *J* = 7.1 Hz, 3H, $COOCH_2C$***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 171.9 (quart, ***C***O), 147.8 (quart, Thio-***C***-2), 126.5, 122.9, 122.8 (+, Thio-***C***-3,4,5), 59.8 (-, O***C***H_2), 43.9 (-, ***C***H_2CO), 22.6 (-, ***C***H_3CHCH_2), 14.1 (+, OCH_2***C***H_3); EI-MS, *m/z* (rel. intensity, %): 198 ($M^{+\cdot}$, 17), 169 ($[M - Et]^+$, 5), 151 ($[M - OEt]^+$, 23), 124 ($[M - CO_2Et]^+$, 56), 111 ($[M - CH_2CO_2Et]^+$, 100), 97 (15), 84 (16), 71 (10), 57 (14).

General procedure for the synthesis of the propanoic acids 42a-d, 46a,b

To a solution of 5.0 mmol of the pertinent ethyl ester in 20 mL of dimethoxyethane, was added a solution of 7.5 mmol of LiOH in 5 mL of water, and the mixture was stirred at room temperature for 3 h. The pH of the reaction mixture was adjusted with aqueous 1 N HCl to 6.5, and the solution was extracted twice with 300 mL portions of ethyl acetate. The combined extracts were dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to give the desired product as a crystalline solid.

3-Phenyl-3-pyridin-2-ylpropanoic acid [42a]

Synthesized from ethyl 3-phenyl-3-pyridin-2-ylpropanoate **(41a):** yield 96 %; colorless crystalline solid; $C_{14}H_{13}NO_2$ (227.3); mp 105 °C; IR (neat): 2362, 1703, 1593, 1453, 1416, 1369, 1307, 1264, 1209, 1102, 801, 755 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 12.98 (br, 1H, COO***H***), 8.53 (m, 1H, Pyr-6-***H***), 7.61 (m, 1H, Pyr-4-***H***), 7.35-7.10 (m, 7H, 2 Pyr-***H*** and 5 Ph-***H***), 4.64 (ddq, J_1 = 5.2 Hz, J_2 = 8.2 Hz, 1H, C***H***CH_2COOH), 3.37 (dd, J_1 = 8.2 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOH), 3.06 (dd, J_1 = 5.2 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 174.9 (quart, ***C***O), 161.8 (quart, Pyr-***C***-2), 147.7 (+, Pyr-***C***-6), 141.7 (quart, Ph-***C***-1), 137.7 (+, Pyr-***C***-4), 128.8, 128.4 (+, 2 Ph-***C***), 128.0, 127.9 (+, 2 Ph-***C***), 127.0 (+, Ph-***C***), 123.8 (+, Pyr-***C***-5), 122.1 (+, Pyr-***C***-3), 48.1 (-, ***C***HCH_2CO), 40.5 (+, ***C***H_2CO); EI-MS, *m/z* (rel. intensity, %): 227 ($M^{+\cdot}$, 27), 226 (28), 182 ($[M - CO_2H]^+$, 100), 167 ($[M - CH_2CO_2H]^+$, 62).

3-(4-Fluorophenyl)-3-pyridin-2-ylpropanoic acid [42b]

Synthesized from ethyl 3-(4-fluorophenyl)-3-pyridin-2-ylpropanoate (**41b**). yield 97 %; colorless crystalline solid; $C_{14}H_{12}FNO_2$ (245.2); mp 127 °C; IR (neat): 2500 (br.), 1702, 1599, 1508, 1418, 1370, 1265, 1216, 1159, 1104, 1012, 867, 833, 789, 751 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 11.85 (br. 1H, COO***H***), 8.56 (m, 1H, Pyr-6-***H***), 7.66 (m, 1H, Pyr-4-***H***), 7.20 (m, 4H, 2 Pyr-***H*** and 2 Ph-***H***), 6.96 (m, 2H, 2 Ph-***H***), 4.63 (ddq, J_1 = 5.5 Hz, J_2 = 8.2 Hz, 1H, C***H***CH_2COOH), 3.34 (dd, J_1 = 8.2 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOH), 3.07 (dd, J_1 = 5.5 15.9 Hz, J_2 = 15.9 Hz, 1H, C***H***$_2$COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 174.7 (quart, ***C***O), 162.4 (quart, d, J = 145.9 Hz, ***C***F), 160.1 (quart, Pyr-**C**-2), 147.9 (+, Pyr-***C***-6), 137.9 (+, Pyr-***C***-4), 137.4 (quart, Ar-***C***-1), 129.4 (+, d, J = 8.2.1 Hz, 2C, Ar-*o*-***C***), 123.7 (+, Pyr-***C***-3), 122.3 (+, Pyr-***C***-5), 115.6 (+, d, J = 21.4 Hz, 2C, Ar-*m*-***C***H), 47.3 (+, ***C***HCH_2CO), 40.5 (+, ***C***H_2CO); EI-MS, *m/z* (rel. intensity, %): 245 ($M^{+\cdot}$, 32), 227 ($[M - OH]^+$, 5), 200 ($[M - CO_2H]^+$, 100), 185 ($[M - CH_2CO_2H]^+$, 51), 170 (2), 157 (2), 92 (3), 78 (6), 51 (4), 28 (3).

3-Phenyl-3-thiazol-2-ylpropanoic acid [42c]

Synthesized from ethyl 3-phenyl-3-thiazol-2-ylpropanoate (**41c**): Yield: 82 %; colorless crystalline solid; $C_{12}H_{11}NO_2S$ (233.3); mp 208 °C ; IR (in CH_2Cl_2) 2963, 1771, 1496, 1261, 1119 cm^{-1}; ^{1}H-NMR ($CDCl_3$ + CD_3OD), δ (ppm): 7.70 (d, J = 3.4 Hz, 1H, Thiaz-4-***H***), 7.21-7.43 (m, 6H, Ph-***H*** and Thiaz-5-***H***), 4.89 (ddq, J = 7.1 and 8.0 Hz, C***H***CH_2CO), 3.45 (dd, J_1 =

8.0 Hz, J_2 = 16.5 Hz, 1H, C***H***$_2$CO), 3.07 (dd, J_1 = 7.1 Hz, J_2 = 16.5 Hz, 1H, C***H***$_2$CO); ^{13}C-NMR ($CDCl_3$), δ (ppm): 177.7 (quart, ***C***O), 177.4 (quart, Thiaz-***C***-2), 145.3 (+, Thiaz-***C***-4), 144.8 (quart, Ph-***C***-1), 132.8, 131.7, 131.6 (+, Ph-***C***), 123.4 (+, Thiaz-***C***-5), 49.0 (+, ***C***HCH$_2$CO), 44.0 (-, ***C***H$_2$CO); EI-MS, *m/z* (rel. intensity, %): 233 ($M^{+\cdot}$, 51), 215 ($[M - OH]^+$, 7), 188 ($[M - CO_2H]^+$, 100), 173 ($[M - CH_2CO_2H]^+$, 40), 147 (2), 130 (4), 115 (3), 103 (12), 77 (15), 58 (13).

3-(3,4-Difluorophenyl)-3-thiazol-2-ylpropanoic acid [42d]

Synthesized from ethyl 3-(3,4-difluorophenyl)-3-thiazol-2-ylpropanoate (**41d**). Yield: 92 %; colorless crystalline solid; $C_{12}H_9F_2NO_2S$ (269.3); mp 90 °C; IR (neat) 2471, 1709, 1610, 1518, 1436, 1361, 1282, 1222, 1119, 917, 730 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 10.70 (s br, 1H, COO***H***), 7.72 (d, J = 3.3 Hz, 1H, Thiaz-4-***H***), 7.25 (d, J = 3.3 Hz, 1H, Thiaz-5-***H***), 7.20-7.02 (m, 3H, Ar-***H***), 4.84 (t, J = 7.4 Hz, C***H***CH$_2$CO), 3.44 (dd, J_1 = 7.4 Hz, J_2 = 16.7 Hz, 1H, C***H***$_2$CO), 3.05 (dd, J_1 = 7.4 Hz, J_2 = 16.7 Hz, 1H, C***H***$_2$CO); ^{13}C-NMR ($CDCl_3$), δ (ppm): 175.3 (quart, ***C***O), 171.8 (quart, Thiaz-***C***-2), 250.3 (quart, dd, J_1 = 12.7 Hz, J_2 = 249.5 Hz, ***C***-F), 249.8 (quart, dd, J_1 = 12.7 Hz, J = 255.1 Hz, ***C***-F), 142.1 (+, Thiaz-***C***-4), 137.7 (quart, Ph-***C***-1), 123.9 (+, Ar-***C***), 119.5 (+, Thiaz-***C***-5), 117.7 (+, d, J = 17.3 Hz, Ar-***C***), 117.0 (+, d, J = 17.8 Hz, Ar-***C***), 44.1 (-, ***C***H$_2$CO), 40.1 (+, ***C***HCH$_2$CO); EI-MS, *m/z* (rel. intensity, %): 269 ($M^{+\cdot}$, 30), 251 ($[M - OH]^+$, 6), 224 ($[M - CO_2H]^+$, 100), 210 ($[M - CH_2CO_2H]^+$, 24), 191 (3), 166 (4), 143 (3), 139 (7), 119 (7), 111 (10), 86 (3), 58 (16).

3-Pyridin-2-ylbutanoic acid [46a]

Yield 91 %; colorless crystalline solid; $C_9H_{11}NO_2$ (165.2); IR (neat) 2935, 1721, 1662, 1434, 1272, 1174, 1007, 943, 834 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.45 (m, 1H, Pyr-6-***H***), 7.77 (m, 1H, Pyr-4-***H***), 7.36 (m, 1H, Pyr-3-***H***), 7.25 (m, 1H, Pyr-5-***H***), 3.40 (m, 1H, CH$_3$C***H***CH$_2$), 2.80 (dd, J_1 = 7.4 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2$CO), 2.63 (dd, J_1 = 7.1 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2$CO), 1.32 (d, J = 6.9 Hz, 3H, C***H***$_3$CHCH$_2$CO); ^{13}C-NMR ($CDCl_3$), δ (ppm): 174.1 (quart, ***C***O), 163.1 (quart, Pyr-***C***-2), 146.5 (+, Pyr-***C***-6), 137.2 (+, Pyr-***C***-4), 121.5 (+, Pyr-C-3), 121.2 (+, Pyr-***C***-5), 40.1 (+, CH***C***H$_2$CO), 36.1 (+, ***C***HCH$_2$CO), 19.4 (+, CH***C***H$_3$); EI-MS, *m/z* (rel. intensity, %): 165 ($M^{+\cdot}$, 4), 150 ($[M - OH]^+$, 6), 132 ($[M - O_2H]^+$, 12), 120 ($[M - CO_2H]^+$, 100), 106 ($[M - CH_2CO_2H]^+$, 62), 93 (9), 78 (21), 65 (3), 51 (13).

3-Thiophen-2-ylbutanoic acid [46b]

Yield 95 %; colorless crystalline solid; $C_8H_{10}O_2S$ (170.2); IR (neat): 1704, 1533, 1378, 1290, 1225, 1193, 1164, 849, 825, 692 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.13 (m, 1H, Thio-5-***H***), 6.94-6.89 (m, 1H, Thio-4-***H***), 6.86-6.83 (m, 1H, Thio-3-***H***), 3.61 (m, 1H, C***H***CH_2CO), 2.76 (dd, J_1 = 6.6 Hz, J_2 = 15.7 Hz, 1H, C***H***$_2$CO), 2.62 (dd, J_1 = 8.0 Hz, J_2 = 15.7 Hz, 1H, C***H***$_2$CO), 1.43 (d, J = 6.9 Hz, 3H, C***H***$_3CHCH_2CO$), ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.6 (quart, ***C***O), 149.3 (quart, Thio-***C***-2), 126.5, 122.9, 122.8 (+, Thio-***C***-3,4,5), 43.5 (-, ***C***H_2CO), 31.4 (+, ***C***HCH_2CO), 22.6 (-, ***C***H_3CHCH_2); EI-MS, *m/z* (rel. intensity, %): 170 ($M^{+\cdot}$, 44), 154 ($[M - OH]^+$, 6), 125 ($[M - CO_2H]^+$, 8), 111 ($[M - CH_2CO_2H]^+$, 100), 84 (6), 77 (9), 66 (7), 45 (11).

1-Pyridin-2-ylethanol [47a][33]

To an ethereal solution (186 mL) of pyridine-2-carboxaldehyde (2 g, 18.7 mmol) was added methylmagnesium bromide in ether (24.27 mmol) at 0 °C. The mixture was stirred for 2 h, quenched with ice-water (5 mL), and extracted with EtOAc. The organic phase was washed with water, brine and dried over $MgSO_4$. The solvent was removed, and the residue was purified by column chromatography on silica gel eluted with EtOAc to obtain a liquid.

Yield 87 %; colorless oil; C_7H_9NO (123.2), IR (neat) 1594, 1571, 1474, 1435, 1118, 1081, 1015, 999, 903, 785, 749 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.50 (m, 1H, Pyr-6-***H***), 7.69 (m, 1H, Pyr-4-***H***), 7.33 (m, 1H, Pyr-3-***H***), 7.18 (m, 1H, Pyr-5-***H***), 4.90 (quart, J = 6.5 Hz, 1H, PyrC***H***(OH)), 1.50 (d, J = 6.5 Hz, CHC***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 163.4 (quart, Pyr-***C***-2), 148.0 (+, Pyr-***C***-6), 136.9 (+, Pyr-***C***-4), 122.2 (+, Pyr-C-3), 119.8 (+, Pyr-***C***-5), 69.1 (+, ***C***HOH), 24.2 (+, CH(***C***H_3)OH); EI-MS, *m/z* (rel. intensity, %): 123 ($M^{+\cdot}$, 4), 122 (11), 108 ($[M - OH]^+$, 100), 78 ($[Pyr]^+$, 33), 52 (18), 32 (16).

1-Thiazol-2-ylethanol [47b][34]

A solution of *i*-PrMgBr (36 mmol) in THF (0.8 M, 18 mL) was added dropwise over 15 min to a stirred solution of 2-bromothiazole (4.9 g, 30 mmol) in THF (150 mL) at -40 °C under argon. The resulting solution was then stirred for 30 min, and acetaldehyde (3.4 mL, 60 mmol) was added. The reaction mixture was allowed to warm to rt, brine (20 mL) was added,

and the reaction was worked up as usual. The crude residue was purified by column chromatography on silica to give the alcohol **47b** (3.8 g, 98 %).

Yield: 98 %; colorless oil; C_5H_7NOS (129.2); IR (neat): 1721, 1504, 1370, 1246, 1193, 1144, 1106, 1053, 1006, 726 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.58 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.19 (d, , *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 5.07 (q, *J* = 6.5 Hz, 1H, C***H***OH), 1.53 (d, , *J* = 6.5 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 177.4 (quart, Thiaz-***C***-2), 141.8 (+, Thiaz-***C***-4), 118.8 (+, Thiaz-***C***-5), 67.6 (+, ***C***HOH), 23.4 (+, CH***C***H$_3$); EI-MS, *m/z* (rel. intensity, %): 129 ($M^{+\cdot}$, 18), 113 ($[M - OH]^+$, 37), 85 ($[Thiaz]^+$, 100), 58 (35), 43 (50).

General procedure for preparation of the tosylates 48a,b

To a stirred solution of alcohol **47a** or **47b** (29 mmol) and DMAP (7.1 g, 58 mmol) in CH_2Cl_2 (100 mL) was added MsCl (5.6 mL, 72.5 mmol) at 0 °C. The mixture was stirred for 10 min at the same temperature and for an additional time (see below) at rt. An ice water was added to the reaction mixture and the mixture was extracted with CH_2Cl_2. The organic layer was washed with water, brine and dried over anhydrous sodium sulfate. After removal of the solvent, the residual oil was purified by column chromatography on flash silica gel (elution with a mixture of EtOAc and hexane).

1-Pyridin-2-ylethyl methanesulfonate [48a][35]

Stirred for 1 h; Yield: 96 %; colorless oil; $C_8H_{11}NO_3S$ (201.2); IR (neat): 1624, 1463, 1208, 1155, 1035, 769 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.52 (m, 1H, Pyr-6-***H***), 7.69 (m, 1H, Pyr-4-***H***), 7.40 (m, 1H, Pyr-3-***H***), 7.25 (m, 1H, Pyr-5-***H***), 5.71 (quart, *J* = 6.6 Hz, 1H, PyrC***H***(OTs)), 2.86 (s, 3H, SO_2C***H***$_3$), 1.68 (d, *J* = 6.6 Hz, CHC***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 158.1 (quart, Pyr-***C***-2), 149.4 (+, Pyr-***C***-6), 137.3 (+, Pyr-***C***-4), 123.6 (+, Pyr-***C***-3), 120.9 (+, Pyr-***C***-5), 80.6 (+, ***C***HOTs), 38.7 (+, SO_2***C***H$_3$), 21.7 (+, CH(***C***H$_3$)OTs); CI-MS (NH_3), *m/z* (rel. intensity, %): 202 ($[M + H]^+$).

1-Thiazol-2-ylethyl methanesulfonate [48b]

Stirred for 16 h; Yield: 96 %; colorless oil; $C_6H_9NO_3S_2$ (207.3); IR (neat): 1498, 1443, 1375, 1230, 1142, 1042, 955 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.72 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***),

7.34 (d, *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 5.36 (q, , *J* = 6.8 Hz, 1H, C***H***OTs), 3.66 (s, 3H, $SO_2C\boldsymbol{H}_3$), 1.96 (d, , *J* = 6.8 Hz, 3H, $CHC\boldsymbol{H}_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 171.7 (quart, Thiaz-***C***-2), 142.4 (+, Thiaz-***C***-4), 120.3 (+, Thiaz-***C***-5), 54.0 (+, ***C***HOTs), 52.5 (+, $SO_2\boldsymbol{C}H_3$), 25.5 (+, $CH\boldsymbol{C}H_3$); CI-MS (NH_3), *m/z* (rel. intensity, %): 208 ($[M + H]^+$).

General procedure for preparation of the 2-substituted dimethyl malonates 49a,b

To a suspension of sodium hydride (72 mg, 3.0 mmol) in THF (6.0 ml) was added the dimethyl malonate (3.22 mmol) at 0 °C. After the hydrogen was ceased at room temperature, the mixture was added to a solution of 1-pyridin-2ylethyl methanesulfonate (**48a**) or 1-thiazol-2ylethyl methanesulfonate (**48b**) (1.0 mmol) in DMSO (4.0 ml). The reaction mixture was warmed to 60 °C and stirred until the reaction was completed (2h). After cooling, EtOAc and water were added. The organic phase was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water, brine and dried over $MgSO_4$. After the solvent was removed, the residue was purified by flash column chromatography on silica gel (eluent: mixture of EtOAc and hexane) to give the products.

Dimethyl 2-(1-pyridin-2-ylethyl)malonate [49a][36]

Yield 82 %; colorless oil; $C_{12}H_{15}NO_4$ (237.3); IR (neat): 2954, 1736, 1593, 1570, 1435 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.49 (m, 1H, Pyr-6-***H***), 7.61 (m, 1H, Pyr-4-***H***), 7.23 (m, 1H, Pyr-3-***H***), 7.11 (m, 1H, Pyr-5-***H***), 4.17 (d, *J* = 10.4 Hz, $C\boldsymbol{H}(CO_2Me)_2$), 3.77 (s, 3H, $COOC\boldsymbol{H}_3$), 3.70 (dq, J_1 = 7.0 Hz, J_2 = 10.4 Hz, 1H, $C\boldsymbol{H}CH_3$), 3.54 (s, 3H, $COOC\boldsymbol{H}_3$), 1.23 (d, *J* = 7.0 Hz, 3H, $CH\boldsymbol{C}H_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 169.4, 168.9 (quart, ***C***O), 162.6 (quart, Pyr-***C***-2), 149.0 (+, Pyr-***C***-6), 136.5 (+, Pyr-***C***-4), 122.9 (+, Pyr-C-3), 121.7 (+, Pyr-***C***-5), 56.6 (+, $\boldsymbol{C}H(CO_2Me)_2$), 52.5, 52.2 (+, $COO\boldsymbol{C}H_3$), 41.2 (+, $\boldsymbol{C}HCH_3$), 19.2 (+, $CH(\boldsymbol{C}H_3)$); CI-MS (NH_3), *m/z* (rel. intensity, %): 238.0 ($[M + H]^+$, 100).

Dimethyl 2-(1-thiazol-2-ylethyl)malonate [49b]

Yield 87 %; colorless oil; $C_{10}H_{13}NO_4S$ (243.3); IR (neat) 1732, 1500, 1434, 1275, 1198, 1147, 1006 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.59 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.16 (d, , *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 3.98 (d, *J* = 10.3 Hz, $C\boldsymbol{H}(CO_2Me)_2$), 3.93 (dq, J_1 = 6.7 Hz, J_2 = 10.3 Hz, 1H, $C\boldsymbol{H}CH_3$), 3.68 (s, 3H, $COOC\boldsymbol{H}_3$), 3.56 (s, 3H, $COOC\boldsymbol{H}_3$), 1.38 (d, *J* = 6.7 Hz,

3H, CH***C***H_3); ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.3 (quart, Thiaz-***C***-2), 169.0 (quart, 2 ***C***O), 142.0 (+, Thiaz-***C***-4), 118.6 (+, Thiaz-***C***-5), 56.9 (+, ***C***H($CO_2Me)_2$), 52.6, 52.5 (+, COO***C***H_3), 37.5 (+, ***C***HCH_3), 19.3 (+, CH(***C***H_3)); EI-MS, *m/z* (rel. intensity, %): 243 ($M^{+\cdot}$, 9), 212 ([M – 2 x Me]$^+$, 17), 184 ([M – $CO_2Me]^+$, 100), 180 ([M – 2 x OMe]$^+$, 21), 152 ([$C_7H_7NOS]^+$, 97), 124 ([M – 2 x $CO_2Me]^+$, 29), 112 ([M – CH($CO_2Me)_2]^+$, 38).

General procedure for preparation of the 3-heteroarylbutanoic acids 50a,b

A solution of diester **49a** or **49b** (1 mmol) in MeOH (3 mL) was treated with NaOH (2.2 N, 1.8 mL) in water, the resulting mixture was heated to reflux, and the reaction was monitored by TLC. When no starting material remained (12-18 h), the reaction mixture was allowed to come to room temperature. Volatiles were removed *in vacuo* and the crude dicarboxylic acid was taken up in AcOH (6 mL) and heated to reflux. The reaction was monitored by TLC, and when no starting material remained (12-18 h), the solution was concentrated *in vacuo*. The resultant crude acid was dissolved in water (5 mL), extracted with EtOAc (3 x 3 mL), and the combined extract was dried over MgSO4 and concentrated to give product.

3-Pyridin-2-ylbutanoic acid [50a]

Refluxed for 15 h each time; yield 87 %; colorless crystalline solid; $C_9H_{11}NO_2$ (165.2); IR (neat) 2935, 1721, 1662, 1434, 1272, 1174, 1007, 943, 834 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.45 (m, 1H, Pyr-6-***H***), 7.77 (m, 1H, Pyr-4-***H***), 7.36 (m, 1H, Pyr-3-***H***), 7.25 (m, 1H, Pyr-5-***H***), 3.40 (m, 1H, CH_3C***H***CH_2), 2.80 (dd, J_1 = 7.4 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2$CO), 2.63 (dd, J_1 = 7.1 Hz, J_2 = 15.6 Hz, 1H, C***H***$_2$CO), 1.32 (d, J = 6.9 Hz, 3H, C***H***$_3$$CHCH_2CO$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 174.1 (quart, ***C***O), 163.1 (quart, Pyr-***C***-2), 146.5 (+, Pyr-***C***-6), 137.2 (+, Pyr-***C***-4), 121.5 (+, Pyr-C-3), 121.2 (+, Pyr-***C***-5), 40.1 (+, CH***C***H_2CO), 36.1 (+, ***C***HCH_2CO), 19.4 (+, CH***C***H_3); EI-MS, *m/z* (rel. intensity, %): 165 ($M^{+\cdot}$, 4), 150 ([M – OH]$^+$, 6), 132 ([M – $O_2H]^+$, 12), 120 ([M – $CO_2H]^+$, 100), 106 ([M – $CH_2CO_2H]^+$, 62), 93 (9), 78 (21), 65 (3), 51 (13).

3-Thiazol-2-ylbutanoic acid [50b]

Refluxed for 15 h each time; yield 91 %; colorless oil; $C_7H_9NO_2S$ (171.2); IR (neat): 2969, 1707, 1503, 1366, 1276, 1051, 875 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 12.42 (s, COOH), 7.71

(d, J = 3.3 Hz, 1H, Thiaz-4-***H***), 7.22 (d, , J = 3.3 Hz, 1H, Thiaz-5-***H***), 3.75 (m, 1H, C***H***CH_2CO_2H), 2.90 (dd, J_1 = 6.9 Hz, J_2 = 16.0 Hz, 1H, CHC***H***$_2CO_2H$), 2.65 (dd, J_1 = 7.3 Hz, J_2 = 16.0 Hz, 1H, CHC***H***$_2CO_2H$), 1.45 (d, J = 7.0 Hz, 3H, C***H***$_3CH$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 176.9 (quart, CO), 176.7 (quart, Thiaz-***C***-2), 141.7 (+, Thiaz-***C***-4), 118.2 (+, Thiaz-***C***-5), 42.0 (+, ***C***H_2COOH), 34.4 (+, ***C***HCH_3), 21.1 (+, CH***C***H_3); EI-MS, *m/z* (rel. intensity, %): 171 ($M^{+\cdot}$, 21), 156 ($[M - Me]^+$, 7), 126 ($[M - CO_2H]^+$, 100), 112 ($[M - CH_2CO_2H]^+$, 23), 99 (2), 86 (4), 58 (15).

(5-Methyl-1*H*-imidazol-4-ylmethylsulfanyl)acetic acid [52]

A soluton of (5-methyl-1*H*-imidazol-4-yl**)**methanol (4 g, 21.7 mmol) and sulfanylacetic acid (1.51 mL, 21.7 mmol) in 50 ml acetic acid was refluxed for 24 h. After removing the solvent in *vacuo*, the residue was neutralised by sodium carbonate. The precipitate was filtered off and washed with water, dried and recrystallized.

Yield: 82 %; colorless crystalline solid; $C_7H_{10}N_2O_2S$ (186.2); mp 170 °C; IR (neat) 1640, 1543, 1414, 1370, 1253, 1193, 1146, 868, 824 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 14.49 (br s, 1H, COO***H***), 8.93 (s, 1H, Im-2-***H***), 3.89 (s, 2H, Im-4-C***H***$_2$S), 3.24 (s, 2H, C***H***$_2$COOH), 2.25 (s, 3H, C***H***$_3$); ^{13}C-NMR (DMSO-d_6), δ (ppm): 170.9 (quart, ***C***O), 132.9 (+, Im-***C***-2), 126.3 (quart, Im-***C***-4), 124.9 (quart, Im-***C***-5), 32.7 (-, S***C***H_2COOH), 23.5 (-, Im-4-***C***H_2S), 8.3 (-, CH_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): *m/z* 187 ($[M + H]^+$).

General procedure for the synthesis of the propenoic acids 54a-c

The ketones **53a**, **53b** or **53c** (8.7 mmol) and potassium *tert*-butoxide (2.25 g, 20 mmol) were taken in a 250 mL flask with *tert*-butanol (100 mL) and triethyl phosphonacetate (1.95 g, 8.7 mmol) was added. The mixture was refluxed for overnight. After cooling, the solvent was removed on a rotatory evaporator. After addition of 100 mL 1N NaOH and 75 mL MeOH the mixture was refluxed for 24 h, concentrated *in vacuo* to remove MeOH, diluted with water (300 mL) and extracted with $CHCl_3$ (3 x 75 mL). The aqueous layer was acidified by concentrated hydrochloric acid, extracted again with $CHCl_3$, washed with brine and water, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the remaining solid was recrystallized from EtOAc/n-hexane.

3,3-Bis(4-fluorophenyl)propenoic acid [54a]

Synthesized from bis(4-fluorophenyl)methanone (**53a**); yield 91 %; colorless crystalline solid; $C_{15}H_{10}F_2O_2$ (260.2); mp 97-98 °C; IR (neat): 2959, 1688, 1598, 1504, 1418, 1287, 1208, 1156, 900, 837, 804 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.3-7.15 (m, 4H, Ph-***H***), 7.1-6.99 (m, 4H, Ph-***H***), 6.52 (s,1H, Ar_2CC***H***); ^{13}C-NMR ($CDCl_3$), δ (ppm): 170.3 (quart, ***C***O), 163.7 (quart, d, *J* = 251.2 Hz, ***C***-F), 162.9 (quart, d, *J* = 248.5 Hz, ***C***-F), 157.0 (quart, ***C***=CHCO), 136.8 (quart, d, *J* = 3.4 Hz, Ar-***C***-1), 134.0 (quart, d, *J* = 3.4 Hz, Ar-***C***-1), 131.1 (+, d, *J* = 8.3 Hz, 2C, Ar-***C***-2,6), 130.4 (+, d, *J* = 8.3 Hz, 2C, Ar-***C***-2,6), 116.2 (+, ***C***HCO), 115.5 (+, d, *J* = 21.8 Hz, 2C, Ar-***C***-3,5), 115.1 (+, d, *J* = 21.8 Hz, 2C, Ar-***C***-3,5); EI-MS, *m/z* (rel. intensity, %): 260 ($M^{+\cdot}$, 100), 259 (43), 243 ($[M - OH]^+$, 20), 214 ($[M - CO_2H]^+$, 61), 201 ($[M - CHCO_2H]^+$, 30), 194 (18),165 (11), 123 (18), 107 (18), 28 (54); Anal. ($C_{15}H_{10}F_2O_2$) C, H, N.

3-(4-Fluorophenyl)-3-phenylpropenoic acid [54b]

Synthesized from (4-fluorophenyl)phenylmethanone, a mixture of *E* and *Z* (**53b**); yield 85 %; colorless crystalline solid; $C_{15}H_{11}FO_2$ (242.2); mp 97-98 °C; IR (neat): 2947, 1689, 1598, 1505, 1415, 1353, 1269, 1210, 1156, 924, 879, 837, 744 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 11.00 (s br, 0.8H, COO***H***), 7.45-6.95 (m, 9H, Ph-***H***), 6.28 (s, 1H, C***H***COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 171.1 (quart, ***C***O), 163.6 (quart, d, *J* = 250.6 Hz, ***C***F), 162.8 (quart, d, *J* = 248.2 Hz, ***C***F), 158.2, 157.0 (quart, for 2 ***C***H=CHCOOH), 140.7, 138.2 (quart, 2 Ph-***C***-1), 136.9, 134.2 (quart, d each, *J* = 3.4 Hz, Ar-***C***-1), 131.2 (+, d, *J* = 8.3 Hz, Ar-***C***-2,6) 130.4 (+, d, *J* = 8.3 Hz, Ar-***C***-2,6), 129.9, 128.6 (+, Ph-***C***-4), 129.2, 128.5, 128.4, 128.0 (+, Ph-***C***-2,6,3,5), 116.6, 116.1 (+, *C*H=***C***HCOOH), 115.5 (+, d, *J* = 21.6 Hz, Ar-***C***-3,5), 115.1 (+, d, *J* = 8.3 Hz, Ar-***C***-3,5); EI-MS, *m/z* (rel. intensity, %): 242 ($M^{+\cdot}$, 100), 225 ($[M - OH]^+$, 16), 196 ($[M - CO_2H]^+$, 53), 183 ($[M - CHCO_2H]^+$, 16), 170 (7), 165 (5), 147 (2), 120 (5), 98 (8), 51 (5), 28 (4); Anal. ($C_{15}H_{11}FO_2$) C, H, N.

3-(3,4-Difluorophenyl)-3-phenylpropenoic acid [54c]

Synthesized from (3,4-difluorophenyl)phenylmethanone (**53c**); yield 89 %; colorless crystalline solid; $C_{15}H_{10}F_2O_2$ (260.2); mp 134-136 °C; IR (neat): 2943, 1692, 1583, 1504, 1484, 1439, 1277, 1225, 1201, 1157, 1124, 886, 837, 805, 784, 729, 691 cm^{-1}; ^{1}H-NMR (

$CDCl_3$), δ (ppm): 7.40-7.15 (m, 3H, Ph-***H***), 7.14-6.80 (m, 5H, Ph-***H***), 6.30 (s, 1H, C***H***COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 170.2 (quart, ***C***O), 163.7 (quart, d, *J* = 251.4 Hz, CF), 162.0 (quart, d, *J* = 246.5 Hz, CF), 157.4 (quart, ***C***=CH), 140.7 (quart, Ph-***C***-1), 136.1, (quart, d, *J* = 3.2 Hz, Ar-***C***-1), 130.3 (+, Ph -***C***-2,6), 129.7 (+, d, *J* =- 8.1 Hz, Ar-***C***-6), 124.9 (+, Ph-***C***-4), 116.6 (+, ***C***HCO), 116.1 (+, d, *J* = 22.3 Hz, Ar-***C***-5), 115.5 (+, d, *J* = 21.0 Hz, Ar-***C***-2), 115.8, 115.5 (+, Ph-C-3,5); EI-MS, *m/z* (rel. intensity, %): 260 ($M^{+\cdot}$, 100), 243 ([M – OH]$^+$, 21), 214 ([M – CO_2H]$^+$, 70), 194 (20), 183 (13), 165 (13), 120 (17), 107 (22), 94 (15), 75 (14), 28 (12); Anal. ($C_{15}H_{10}F_2O_2$) C, H, N.

General procedure for the synthesis of the propanoic acids 55a-c

0.05 g of Pd/C (10 %) was added to solution of the pertinent acrylic acid (**54a-c**, 8 mmol) in methanol (100 mL). The mixture was hydrogenated with constant stirring at 5 bar H_2 pressure overnight, filtered through celite pad, washed with methanol, and the solvent was removed in *vacuo*. The remaining crude solid was recrystallized from chloroform/n-hexane.

3,3-Bis(4-fluorophenyl)propanoic acid [55a]

Synthesized from 3,3-bis(4-fluorophenyl)propenoic acid (**54a**), yield 94 %; colorless crystalline solid; $C_{15}H_{12}F_2O_2$ (262.3); mp 97-98 °C; IR (neat): 3083, 1698, 1602, 1509, 1410, 1303, 1265, 1229, 1212, 1159, 1104, 865, 829 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.2-7.1 (m, 4H, Ph-***H***), 7.05-6.80 (m, 4H, Ph-***H***), 4.48 (t, *J* = 7.9 Hz, 1H, Ar_2C***H***), 3.02 (d, *J* = 7.9 Hz, 2H, C***H***$_2$COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 177.4 (quart, ***C***O), 161.5 (quart, d, *J* = 245.4 Hz, ***C***F), 138.7 (quart, d, *J* = 3.2 Hz, Ar-**C**-1), 129.0 (+, d, *J* = 8.3 Hz, Ar-***C***-2,6), 115.5 (+, d, *J* = 8.3 Hz, Ar-***C***-3,5), 45.1 (+, ***C***HCH_2CO), 40.6 (-, ***C***H_2CO); EI-MS, *m/z* (rel. intensity, %): 262 ($M^{+\cdot}$, 22), 203 ([M – CH_2CO_2H]$^+$, 100), 183 (([M – FCH_2CO_2H]$^+$, 11), 121 (3), 101 (4); Anal. ($C_{15}H_{10}F_2O_2$) C, H, N.

3-(4-Fluorophenyl)-3-phenylpropanoic acid [55b]

Synthesized from (4-fluorophenyl)phenylpropenoic acid (**54b**); yield 96 %; colorless crystalline solid; $C_{15}H_{13}FO_2$ (244.3); mp 108-110 °C; IR (neat) 2909, 1701, 1602, 1507, 1411, 1308, 1266, 1209, 1161, 1102, 1014, 918, 836, 799, 716 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.3-7.1 (m, 7H, Ph-***H***), 6.9-7.0 (m, 2H, Ph-***H***), 4.5 (t, *J* = 8.0 Hz, 1H, Ar_2C***H***), 3.0 (d, *J* = 8.0

Hz, 2H, C***H***$_2$COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.0 (quart, ***C***O), 161.5 (quart, d, *J* = 245.0, Hz, ***C***F), 142.9 (quart, Ph-***C***-1), 138.9 (quart, d, *J* = 3.0 Hz, Ar-***C***-1), 129.0 (+, d, *J* = 8.0 Hz, 2C, Ar-***C***-2,6), 128.7 (+, 2C, Ph-***C***-2,6), 127.5 (+, 2C, Ph-***C***-2,6), 126.8 (+, Ph-***C***-4), 115.3 (+, d, *J* = 21.3 Hz, 2C, Ar-***C***-3,5), 45.8 (+, ***C***HCH$_2$CO), 40.5 (-, ***C***H$_2$CO); EI-MS, *m/z* (rel. intensity, %): 244 ($M^{+\cdot}$, 19), 185 ([M – CH_2CO_2H]$^+$, 100), 165 ([M – FCH_2CO_2H]$^+$, 25), 28 (5); Anal. ($C_{15}H_{10}F_2O_2$) C, H, N.

3-(3,4-Difluorophenyl)-3-phenylpropanoic acid [55c]

Synthesized from (3,4-difluorophenyl)phenylpropenoic acid (**54c**); yield 92 %; colorless crystalline solid; $C_{15}H_{12}F_2O_2$ (262.3); mp 105-106 °C; IR (neat): 2981, 1697, 1588, 1508, 1487, 1445, 1408, 1310, 1226, 1209, 837, 790, 715 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.1-7.3 (m, 3H, Ph-***H***), 7.0-6.8 (m, 5H, Ph-***H***), 4.48 (t, *J* = 7.9 Hz, 1H, Ar$_2$C***H***), 3.02 (d, *J* = 7.9 Hz, 2H, C***H***$_2$COOH); ^{13}C-NMR ($CDCl_3$), δ (ppm): 177.7 (quart, ***C***O), 162.9 (quart, d, *J* = 246.5 Hz, *m*-***C***F), 161.6 (quart, d, *J* = 245.5 Hz, *p*-***C***F), 145.5 (quart, Ph-***C***-1), 138.1 (quart, d, *J* = 3.3 Hz, Ar-***C***-1), 130.2, 130.1 (+, Ph-***C***), 129.1, 129.0 (+, Ph-***C***-3,5), 123.1 (+, d, *J* = 3.0 Hz, Ar-***C***-6), 115.7, 115.4 (+, Ph-***C***-2,6), 114.5 (+, d, *J* = 21.7 Hz, Ar-***C***-5), 113.7 (+, d, *J* = 21.7 Hz, Ar-***C***-2), 45.5 (+, ***C***HCH$_2$CO), 40.3 (-, ***C***H$_2$CO); EI-MS, *m/z* (rel. intensity, %): 262 ($M^{+\cdot}$, 20), 216 (([M – CO_2H]$^+$, 11), 203 (([M – CH_2CO_2H]$^+$, 100), 183 (([M – FCH_2CO_2H]$^+$, 34), 101 (12), 75 (5), 28 (81); Anal. ($C_{15}H_{10}F_2O_2$) C, H, N.

***N*^{1}-[-2-(5-Methyl-1*H*-imidazol-4-ylmethylsulfanyl)acetyl]-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [61-Tr]**

(1*H*-Imidazol-4-ylmethylsulfanyl)acetic acid (0.372 g, 2 mmol) and 1,1'-carbonyldiimidazol (0.356 g, 2.2 mmol) in 2 mL of DMF were stirred for 4 h at 30 °C. On the other hand, **27** (0.818 g, 2 mmol) and sodium hydride (160 mg, 60 % dispersion in oil) in 2 ml of DMF were stirred for 45 min at 50 °C. After cooling the two solutions were mixed together at rt and stirred for 18 h. The DMF was removed under reduced pressure and after addition of water (10 mL) the mixture was extracted with $CHCl_3$, washed with saturated sodium bicarbonate, and brine. After removing the solvent, residue was purified by a Chromatotron™ using $CHCl_3$/MeOH/TEA (90: 8 : 2). Yield 65 %

Yield 65 %; colorless foam; $C_{33}H_{35}N_7OS$ (577.7); IR (neat): 1660, 1430, 1179, 1124, 831 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.41 (s, 1H, Im-2-***H***), 7.33 (m, 10H, Ph-***H*** and Im-2-***H***), 7.16-7.05 (m, 6H, Ph-***H***), 6.57 (s, 1H, Im-5-***H***), 3.73 (s, 2H, Im-4-C***H***$_2$S), 3.41 (m, 2H, C***H***$_2$NH), 3.12 (s, 2H, C***H***$_2$CO), 2.58 (m, 2H, Im-4-C***H***$_2$), 2.17 (s, 3H, C***H***$_3$), 1.89 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 181.7 (quart, ***C***O), 162.6 (quart, ***C***=NH), 142.2 (quart, Ph-***C***-1), 140.1 (quart, Im-***C***-4), 138.0 (+, Im-***C***-2), 133.1 (+, Im-***C***-2), 129.7 (+, Ph-***C***), 127.3 (quart, Im-***C***-4), 128.0 (quart, Im-***C***-5), 128.1 (+, Ph-***C***), 118.3 (+, Im-***C***-5), 75.3 (quart, Ph_3***C***), 40.3 (-, NH***C***H_2), 38.6 (-, S***C***H_2CO), 32.5 (-, Im-4-*C*H_2S), 29.1 (-, Im-4-CH_2***C***H_2), 23.1 (-, Im-4-***C***H_2CH_2), 11.8 (-, CH_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 578 ([M + H]$^+$).

General procedure for the preparation of the N^1-acyl-N^2-[ω-(1-trityl-1*H*-imidazol-4-yl)-alkyl]guanidines [(62 – 81)-Tr]

Under argon atmosphere, the pertinent carboxylic acid (1.0 mmol) was added to a solution of CDI (0.162 g, 1.0 mmol) in DMF or THF (3 mL), and the mixture was stirred at rt for 1 h. At the same time, under argon atmosphere and stirring, sodium hydride (2 mmol, 80 mg of a 60 % dispersion in oil) was added to a solution of an imidazolylalkylguanidine (1 mmol) in DMF or THF (5 mL), and the mixture was heated at 50 °C for 20 min and then allowed to cool to rt. The two mixtures were combined and stirred at rt for 2-16 h, then the solution was poured on water (10 mL) and extracted with EtOAc. The organic phase was washed with a saturated sodium bicarbonate and brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by a Chromatotron™ under NH_3 atmosphere with $CHCl_3$/MeOH as solvent.

N^1-(3,3-Diphenylpropanoyl)-N^2-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine [62-Tr]

Usual procedure for coupling, mixture stirred overnight. Purification by Chromatotron™: eluent MeOH/$CHCl_3$, 3 : 97, under NH_3 atmosphere); yield 62 %; colorless foam; $C_{40}H_{37}N_5O$ (603.8); IR (neat): 1592, 1492, 1445, 1370, 1129, 1033 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.36-7.05 (m, 26H, Ph-***H*** and Im-2-***H***), 6.59 (s, 1H, Im-5-***H***), 4.65 (t, *J* = 7.8 Hz, 1H, Ph_2C***H***), 3.84 (t, *J* = 6.1 Hz, 2H, C***H***$_2$NH), 3.05 (d, *J* = 7.7 Hz, 2H, C***H***$_2$CO), 2.71 (t, *J* = 6.0 Hz, 2H, Im-4-C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 182.8 (quart, ***C***O), 160.8 (quart, ***C***=NH), 144.8,

142.1 (quart, Ph-***C***-1), 138.8 (+, Im-***C***-2), 137.5 (quart, Im-***C***-4), 129.6, 129.4, 128.5, 128.3, 128.1, 127.8, 127.6, 126.2, 125.9 (+, Ph-***C***), 119.5 (+, Im-***C***-5), 75.3 (quart, Ph_3***C***), 47.1 (+, ***C***HCH_2CO), 46.1 (-, CO***C***H_2), 40.5 (-, ***C***H_2NH_2), 28.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 604 ($[M + H]^+$), 1207 ($[2M + H]^+$).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [63-Tr]

Yield: 55 %; colorless foam-like solid; $C_{41}H_{39}N_5O$ (617.8); IR (neat): 3029, 1588, 1492, 1445, 1375, 1275, 1130, 748 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.45-7.05 (m, 26 H, Ph-***H*** and Im-2-***H***), 6.54 (s, 1H, Im-5-***H***), 4.65 (t, *J* = 7.8 Hz, 1H, Ph_2C***H***), 3.30 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 3.06 (d, *J* = 7.7 Hz, 2H, COC***H***$_2$), 2.53 (m, 2H, Im-4-C***H***$_2$), 1.82 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.0 (quart, ***C***O), 145.1, 142.3 (quart, Ph-***C***-1), 140.3 (quart, Im-***C***-4), 138.0 (+, Im-***C***-2), 129.7, 128.3, 128.1, 128.0, 125.9 (+, Ph-***C***), 118.3 (+, Im-***C***-5), 75.3 (quart, Ph_3***C***), 47.3 (+, Ph_2***C***H), 46.3 (-, ***C***H_2CO), 40.1 (-, ***C***H_2NH), 29.1 (-, Im-4-CH_2***C***H_2), 23.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 618 ($[M + H]^+$), 1235 ($[2M + H]^+$).

N^1-(2,2-Diphenylacetyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [64-Tr]

Yield 55 %; colorless foam-like solid; $C_{40}H_{37}N_5O$ (603.8); IR (neat): 3055, 1736, 1690, 1590, 1492, 1442, 1370, 1127, 832, 759, 746, 728 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.39-7.01 (m, 26H, Ph-***H*** and Im-2-***H***), 6.54 (s, 1H, Im-5-***H***), 4.98 (s, 1H, Ph_2C***H***), 3.24 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 2.54 (t, *J* = 6.0 Hz, 2H, Im-4-C***H***$_2$), 1.81 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 184.3 (quart, ***C***O), 162.8 (quart, ***C***=NH), 142.3, 141.6 (quart, Ph-***C***), 140.4 (quart, Im-***C***-4), 138.0 (+, Im-***C***-2), 129.7, 129.0, 128.1, 126.2 (+, Ph-***C***), 118.2 (+, Im-***C***-5), 75.2 (quart, Ph_3***C***), 62.34 (+, Ph_2***C***H), 40.2 (-, ***C***H_2NH), 29.1 (-, Im-4-CH_2***C***H_2), 23.5 (-, Im-4-***C***H_2);; MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 604 ($[M + H]^+$), 1207 ($[2M + H]^+$).

N^1-[3,3-Bis(4-fluorophenyl)propanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine [65-Tr]

Yield 61 %; colorless foam-like solid; $C_{41}H_{37}F_2N_5O$ (653.8); IR (neat): 1690, 1601, 1505, 1444, 1221, 1157, 1130, 826, 747, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40-6.80 (m, 24H, Ar-***H*** and Im-2-***H***), 6.58 (s, 1H, Im-5-***H***), 4.68 (t, *J* = 8.0 Hz, 1H, Ar_2C***H***), 3.50 (m, 2H,

C***H***$_2$NH), 3.22 (d, *J* = 8.0 Hz, 2H, COC***H***$_2$), 2.50 (m, 2H, Im-4-C***H***$_2$), 1.85 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.9 (quart, ***C***O), 161.6 (quart, d, *J* = 245.2 Hz, ***C***F), 155.7 (quart, ***C***=NH), 142.1 (quart, Ph-***C***-1), 140.2 (quart, Im-***C***-4), 138.3 (quart, d, *J* = 3.0 Hz, Ar-***C***-1), 137.6 (+, Im-***C***-2), 129.7 (+, Ph-C), 129.2 (+, d, *J* = 8.0 Hz, Ar-***C***-2,6) 128.3, 128.2, 128.0 (+, Ph-***C***), 118.6 (+, Im-***C***-5), 115.6 (+, d, *J* = 21.3 Hz, Ar-***C***-3,5), 75.6 (quart, Ph_3***C***), 45.0 (+, Ar_2***C***H), 43.3 (-, CO***C***H_2), 40.1 (-, NH***C***H_2), 28.4 (-, Im-4-CH_2***C***H_2), 22.1 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 654 ($[M + H]^+$).

<u>*N*^{1}-[3-(4-Fluorophenyl)-3-phenylpropanoyl]-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine [66-Tr]</u>

Yield 53 %; colorless foam like solid; $C_{41}H_{38}FN_5O$ (635.8); IR (neat) 3061, 1589, 1488, 1442, 1400, 1365, 1230, 1134, 977, 952, 834, 803, 745 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.42-6.73 (m, 25H, Ar-***H*** and Im-2-***H***), 6.54 (s, 1H, Im-5-***H***), 4.62 (t, *J* = 7.8 Hz, 1H, Ar_2C***H***), 3.33 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 3.00 (d, *J* = 7.8 Hz, 2H, C***H***$_2$CO), 2.53 (m, 2H, Im-4-C***H***$_2$), 1.82 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.0 (quart, ***C***O), 161.7 (quart, ***C***=NH), 161.1 (quart, d, *J* = 245.2 Hz, ***C***F), 142.3, 140.2 (quart, 2C, Ph-***C***-1), 138.5 (quart, d, *J* = 3.3, Ar-***C***-1), 138.3 (quart, Im-***C***-4), 137.9 (+, Im-***C***-2), 129.7 (+, Ph-C), 129.2 (+, d, *J* = 8.0 Hz, 2C, Ar-***C***-2,6), 128.1 (+, Ph-C), 128.0 (+, Ar-***C***), 126.0, 123.4 (+, Ph-***C***), 118.2 (+, Im-***C***-5), 115.2 (+, d, *J* = 21.0 Hz, 2C, Ar-***C***-3,5), 75.4 (quart, Ph_3***C***), 46.3 (+, Ph_2***C***H), 46.3 (-, CO***C***H_2), 40.0 (-, NH***C***H_2), 28.1 (-, Im-4-CH_2***C***H_2), 23.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 636 ($[M + H]^+$), 1273 ($[2M + H]^+$).

<u>*N*^{1}-[3-(3,4-Difluorophenyl)-3-phenylpropanoyl]-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine [67-Tr]</u>

Yield 55 %; colorless foam-like solid; $C_{41}H_{37}F_2N_5O$ (653.8); IR (neat) 3060, 1586, 1490, 1444, 1401, 1360, 1310, 1230, 1134, 1011, 977, 952, 905, 834, 803, 745 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40-6.76 (m, 24H, Ar-***H*** and Im-2-***H***), 6.55 (s, 1H, Im-5-***H***), 4.63 (t, *J* = 7.7 Hz, 1H, Ar_2C***H***), 3.34 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 3.01 (d, *J* = 7.9 Hz, 2H, COC***H***$_2$), 2.54 (m, 2H, Im-C***H***$_2$), 1.84 (m, 2H, Im-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.0 (quart, ***C***O), 162.9 (quart, d, *J* = 246.6 Hz, 3-***C***F), 161.6 (quart, d, *J* = 245.8 Hz, 4-***C***F), 161.7 (quart, ***C***=NH), 142.3 (quart, Ph-***C***-1), 140.2 (quart, Ph-***C***-1), 140.1 (quart, d, *J* = 3.3 Hz, Ar-***C***-1),

138.9 (quart, Im-***C***-4), 137.9 (+, Im-***C***-2), 129.8, 129.7 (+, 2C, Ph-***C***), 129.3, 129.2 (+, 2C, Ph-***C***-3,5), 123.4 (+, d, *J* = 3.3 Hz, Ar-***C***-6), 118.3 (+, Im-***C***-5), 115.3, 115.0 (+, 2C, Ph-C-2,6), 114.8 (+, d, *J* = 21.3 Hz, Ar-***C***-5), 113.0 (+, d, *J* = 21.1 Hz, Ar-***C***-2), 75.3 (quart, Ph_3***C***), 46.3 (+, Ar_2***C***HCO), 45.9 (-, CO***C***H_2), 40.1 (-, NH***C***H_2), 29.2 (-, Im-4-CH_2***C***H_2), 22.7 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 654 ($[M + H]^+$).

N^1-(3-Phenyl-3-pyridin-2-ylpropanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine [70-Tr]

Yield 50 %; colorless foam-like solid; $C_{40}H_{38}N_6O$ (618.8); IR (neat) 1732, 1651, 1568, 1492, 1472, 1186, 1156, 1035, 870 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.52 (m, 1H, Pyr-6-***H***), 7.56-7.49 (m, 1H, Pyr-4-***H***), 7.40-7.00 (m, 23H, Pyr-3-***H***, -5-***H,*** Im-2-***H*** and 20 Ph-***H***), 6.56 (s, 1H, Im-5-***H***), 4.80 (ddq, *J* = 8.5 and 6.7 Hz, 1H, Ar_2C***H***), 3.60 (dd, J_1 = 8.5 Hz, J_2 = 15.7 Hz, 1H, C***H***$_2$CO), 3.45 (t, *J* = 6.8 Hz, 2H, NHC***H***$_2$), 3.16 (dd, J_1 = 6.7 Hz, J_2 = 15.7 Hz, 1H, C***H***$_2$CO), 2.53 (m, 2H, Im-4-C***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 175.1 (quart, ***C***O), 163.6 (quart, Py-***C***-2), 155.1 (quart, ***C***=NH), 149.0 (+, Pyr-***C***-6), 143.8, 142.3 (quart, Ph-***C***-1), 140.3 (quart, Im-***C***-4), 138.0 (+, Im-***C***-2), 136.3 (+, Pyr-C-4), 129.7, 128.4, 128.1, 128.0, 126.2 (+, Ph-***C***), 122.9 (+, Pyr-***C***-3), 121.0 (+, Pyr-***C***-5), 118.3 (+, Im-***C***-5), 75.2 (quart, Ph_3***C***), 49.6 (+, $COCH_2$***C***H), 45.3 (-, CO***C***H_2CH), 40.0 (-, NH***C***H_2), 28.9 (-, Im-4-CH_2***C***H_2), 23.1 (-, Im-4-***C***H_2CH_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 619 ($[M + H]^+$), 1237 ($[2M - H]^+$).

N^1-[3-(4-Fluorophenyl)-3-pyridin-2-ylpropanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine [71-Tr]

Yield 49 %; $C_{40}H_{37}FN_6O$ (636.8); IR (in CH_2Cl_2) 2993, 1590, 1507, 1445, 1376, 1269, 1157, 1131, 701 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.50 (dm, 1H, Pyr-6-***H***), 7.56 (dt, J_1 = 1.8 Hz, J_2 = 7.7 Hz,1H, Pyr-4-***H***), 7.41-6.85 (m, 22H, Pyr-3-, -5-***H***, Im-2-***H*** and 19 Ph-***H***), 6.54 (s, 1H, Im-5-***H***), 4.73 (dd, *J* = 7.2 and 7.8 Hz, 1H, Ar_2C***H***), 3.30 (m, 3H, one H of C***H***$_2$CO and C***H***$_2NH_2$), 2.97 (dd, J_1 = 7.2 Hz, J_2 = 15.6 Hz, 1H, one H of C***H***$_2$CO), 2.54 (m, 2H, Im-4-C***H***$_2$), 1.84 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 182.9 (quart, ***C***O), 163.5 (quart, Py-***C***-2), 161.3 (quart, d, *J* = 143.9 Hz, ***C***F), 161.4 (quart, ***C***=NH), 149.2 (+, Pyr-***C***-

6), 142.3 (quart, Ph-***C***-1), 140.2 (quart, Ph-***C***-1), 139.5 (quart, Im-***C***-4), 138.0 (+, Im-***C***-2), 136.3 (+, Pyr-***C***-4), 131.1 (+, d, *J* = 8.2 Hz, 2C, Ar-*C*-2/-6), 129.7, 129.6, 129.5, 128.1 (+, Ph-***C***), 122.9 (+, Pyr-***C***-3), 121.0 (+, Pyr-***C***-5), 118.3 (+, Im-***C***-5), 115.2 (+**,** d, *J* = 21.1 Hz, 2C**,** Ar-***C***-3/-***C***-5), 75.2 (quart, Ph_3***C***), 48.9 (+, $COCH_2$***C***H), 45.5 (-, CO***C***H_2CH), 40.1 (-, NH***C***H_2), 29.1 (-, Im-4-CH_2***C***H_2), 23.3 (-, Im-4-***C***H_2CH_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 637 ($[M + H]^+$).

<u>*N*[1]-(3-Phenyl-3-thiazol-2-ylpropanoyl)-*N*[2]-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine [72-Tr]</u>

Yield 46 %; colorless foam-like solid; $C_{38}H_{36}N_6OS$ (624.8); IR (in CH_2Cl_2): 1597, 1493, 1445, 1392, 1265, 1235, 1130, 1036, 732, 698 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.67 (d, *J* = 3.4 Hz, 1H, Thiaz-4-***H***), 7.40-6.98 (m, 22H, Ph-***H***, Thia-5-***H,*** Im-2-***H***), 6.55 (s, 1H, Im-5-***H***), 4.99 (ddq, *J* = 7.7 Hz, 1H, C***H***CH_2CO), 3.51 (dd, J_1 = 7.7 Hz, J_2 = 15.9 Hz, 1H, one H of C***H***$_2$CO), 3.40 (t, 2H, NHC***H***$_2$), 2.98 (dd, J_1 = 7.7 Hz, J_2 = 15.9 Hz, 1H, one H of C***H***$_2$CO), 2.54 (m, 2H, Im-4-C***H***$_2$), 1.84 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 180.2 (quart, ***C***O), 173.9 (quart, Thiaz-***C***-2), 160.3 (quart, ***C***=NH), 142.2 (+, Thia-***C***-4), 142.1 (quart, Ph-***C***-1), 140.0 (quart, Im-***C***-4*)*, 138.0 (+, Im-***C***-2), 129.7, 128.6, 128.0 (+, Ph-***C***), 118.5 (+, Thia-***C***-5), 118.3 (+, Im-***C***-5), 75.3 (quart, Ph_3***C***), 45.7 (+, Ar_2***C***H), 45.5 (-, ***C***H_2CO), 40.1 (-, ***C***H_2NH), 28.9 (-, Im-4-CH_2***C***H_2), 23.1 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 625 ($[M + H]^+$).

<u>*N*[1]-[3-(3,4-Difluorophenyl)-3-thiazol-2-ylpropanoyl]-*N*[2]-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine [73-Tr]</u>

Yield 52 %; colorless foam-like solid; $C_{38}H_{34}F_2N_6OS$ (660.8); IR (neat) 1592, 1515, 1493, 1443, 1378, 1130, 700 659 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.95 (s br, H, N***H***), 7.64 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.40-6.98 (m, 20H, Ph-***H***, Thiaz-5-***H,*** Im-2-***H***), 6.55 (s, 1H, Im-5-***H***), 4.96 (m, 1H, C***H***CH_2CO), 3.32 (m, 3H, C***H***$_2$NH and one H of COC***H***$_2$), 2.98 (dd, J_1 = 7.7 Hz, J_2 = 15.9 Hz, 1H, one H of C***H***$_2$CO), 2.54 (m, 2H, Im-4-C***H***$_2$), 1.84 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 181.7 (quart, ***C***O), 173.2 (quart, Thiaz-***C***-2), 162.1

(quart, ***C***=NH), 150.0 (quart, dd, J_1 = 12.4 Hz, J_2 = 247.6 Hz, CF), 149.2 (quart, dd, J_1 = 12.0 Hz, J_2 = 247.6 Hz, CF), 142.4 (+, Thiaz-***C***-4), 142.3 (quart, Ph-***C***-1), 140.2 (quart, Im-***C***-4), 139.4 (quart, Ar-***C***-1), 138.0 (+, Im-***C***-2), 129.7, 128.0 (+, Ph-***C***), 124.0 (+, Ar-***C***-6), 118.7 (+, Thia-***C***-5), 118.6 (+, Im-***C***-5), 118.4 (+, d, J = 17.0 Hz, *Ar*-***C***-5), 117.1 (+, dd, J_1 = 3.4 Hz, J_2 = 17.0 Hz, Ar-***C***-2), 75.2 (quart, Ph_3***C***), 46.3 (-, ***C***H_2CO), 45.2 (+, Ar_2***C***H), 40.1 (-, ***C***H_2NH), 29.1 (-, Im-4-CH_2***C***H_2), 23.3 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 242 ($[M\text{-}CPh_3]^+$), 661 ($[M + H]^+$), 1321 ($[2M + H]^+$), 1343 ($[2M + Na]^+$).

<u>*N*[1]-[3-(1-Benzyl-1*H*-imidazol-2-yl)-3-(3,4-difluorophenyl)propanoyl]-*N*[2]-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [74-Tr]</u>

Yield 55 %; colorless foam-like solid; $C_{45}H_{41}F_2N_7O$ (733.9); IR (in CH_2Cl_2): 1667, 1519, 1496, 1444, 1266, 1182, 1128, 700, 665 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 10.53 (s, 1H), 9.03 (s, 1H), 8.08 (s, 1H), 7.51-6.96 (m, 24H, Im-***H***, Ph-H), 5.30 (m, 2H, PhC***H***$_2$), 4.80 (m, 1H, C***H***CH_2CO), 3.78 (dd, J_1 = 10.6 Hz, J_2 = 16.8 Hz, 1H, one H of COC***H***$_2$), 3.36 (m, 2H, C***H***$_2$NH), 3.23 (dd, J_1 = 5.2 Hz, J_2 = 16.8 Hz, 1H, one H of COC***H***$_2$), 2.83 (t, J = 7.3 Hz, 2H, Im-4-C***H***$_2$), 2.00 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 173.0 (quart, ***C***O), 154.1 (quart, ***C***=NH), 150.3 (quart, dd, J_1 = 12.3 Hz, J_2 = 247.6 Hz, CF), 150.0 (quart, dd, J_1 = 12.1 Hz, J_2 = 247.6 Hz, CF) 146.6 (quart, Benzyl-Im-***C***-2), 139.7 (quart, Ph-***C***-1), 135.0 (+, Im-***C***-2), 134.5 (quart, Im-***C***-4), 133.1 (quart, Ph-***C***-1), 132.9 (quart, Ar-C-1), 129.4, 129.3, 129.0, 128.9, 127.3, 127.2 (+, Ph-***C***), 124.1 (+, Ar-C-6), 121.8 (+, Benzyl-Im-***C***-4), 120.3 (+, Benzyl-Im-***C***-5), 118.5 (+, Im-***C***-5)), 118.2 (+, d, J = 17.6 Hz, Ar-*C*-5), 117.1 (+, d, J = 17.9 Hz, Ar-*C*-2), 78.6 (quart, Ph_3***C***), 51.2 (-, Ph***C***H_2), 40.4 (-, CO***C***H_2), 41.0 (-, NH***C***H_2), 37.3 (+, $COCH_2$***C***H), 28.0 (-, Im-4-CH_2***C***H_2), 21.9 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 734 ($[M + H]^+$).

<u>*N*[1]-(3-Thiophen-2-ylbutanoyl)-*N*[2]-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [79-Tr]</u>

Yield 47 %; colorless foam-like solid; $C_{34}H_{35}N_5OS$ (561.7); IR (neat): 1652, 1490, 1442, 1325, 1233, 1178, 1131, 1033, 994, 840 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.41-7.29 (m, 10H, Ph-***H)***, 7.52-7.05 (m, 7H, Ph-***H***, Im-2-***H*** and Thio-5-***H***), 6.91-6.83 (m, 2H, Thio-***H***) 6.57 (s, 1H, Im-5-***H***), 3.67 (m, 1H, $COCH_2$C***H***), 3.58 (m, 2H, NHC***H***$_2$), 2.79 (dd, J_1 = 6.6 Hz,

J_2 = 15.1 Hz, 1H, one H of C***H***$_2$CO), 2.61 (dd, J_1 = 8.1 Hz, J_2 = 15.1 Hz, 1H, one H of C***H***$_2$CO), 2.58 (m, 2H, Im-4-***C***H$_2$), 2.00 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.36 (d, J = 7.0 Hz, 3H, ***C***H$_3$); ^{13}C-NMR (CD$_3$OD), δ (ppm): 180.3 (quart, ***C***O), 159.6 (quart, ***C***=NH), 150.6 (quart, Thio-***C***-2), 142.5 (quart, Ph-***C***-1), 139.9 (quart, Im-***C***-4), 137.9 (+, Im-***C***-2), 129.7, 129.4, 128.2, 128.0, 127.9 (+, Ph-C), 127.5 (+, Thio-***C***-5), 122.7, 122.6 (+, Thio-***C***-3,4), 118.4 (+, Im-***C***-5), 69.9 (quart, Ph$_3$***C***), 48.6 (-, CH***C***H$_2$CO), 40.1 (-, ***C***H$_2$NH), 32.0 (+, ***C***H***C***H$_2$CO), 28.9 (-, Im-4-CH$_2$***C***H$_2$), 22.8 (+, ***C***H$_3$), 22.0 (-, Im-4-***C***H$_2$); MS (ESI, CH$_2$Cl$_2$/MeOH + 10 mM NH$_4$OAc): 562 ([M + H]$^+$).

<u>*N*^{1}-(3-Pyridin-2-ylbutanoyl)-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [80-Tr]</u>

Yield 52 %; colorless foam-like solid; C$_{35}$H$_{36}$N$_6$O (556.7); IR (neat): 1655, 1445, 1287, 1236, 1155, 1131, 1063, 700 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm): 8.57 (m, 1H, Pyr-6-***H***), 7.52 (m, 1H, Pyr-4-***H***), 7.63-7.00 (m, 18H, Pyr-3-***H***, -5-***H,*** Im-2-***H*** and Ph-***H***), 6.55 (s, 1H, Im-***H***-5), 3.62 (m, 1H, C***H***CH$_2$CO), 3.45 (m, 2H, C***H***$_2$NH), 3.00 (dd, J_1 = 8.3 Hz, J_2 = 16.6 Hz, 1H, one H of C***H***$_2$CO), 2.90 (dd, J_1 = 6.2 Hz, J_2 = 16.6 Hz, 1H, one H of C***H***$_2$CO), 2.83 (t, J = 7.6 Hz, 2H, Im-4-C***H***$_2$), 2.01 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.41 (d, J = 7.0 Hz, 3H, C***H***$_3$CH); ^{13}C-NMR (CDCl$_3$), δ (ppm): 175.4 (quart, ***C***O), 163.3 (quart, Py-***C***-2), 155.2 (quart, ***C***=NH), 149.7 (+, Pyr-***C***-6), 142.1 (quart, Ph-***C***-1), 134.5 (+, Im-2-***C***H), 138.3 (quart, Im-***C***-4), 129.8, 129.7, 128.2, 128.0 (+, Ph-***C***), 125.4 (+, Pyr-***C***-3), 124.8 (+, Pyr-***C***-5), 117.3 (+, Im-***C***-5), 75.4 (quart, Ph$_3$***C***), 43.3 (-, CO***C***H$_2$CH), 41.1 (-, ***C***H$_2$NH), 37.5 (+, COCH$_2$***C***H), 27.6 (-, Im-4-CH$_2$***C***H$_2$), 22.6 (-, Im-4-***C***H$_2$), 20.9 (+, ***C***H$_3$); MS (ESI, CH$_2$Cl$_2$/MeOH + 10 mM NH$_4$OAc): *m/z* 557 ([M + H]$^+$).

<u>*N*^{1}-(3-Thiazol-2-ylbutanoyl)-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine [81-Tr]</u>

Yield 55 %; colorless foam-like solid; C$_{33}$H$_{34}$N$_6$OS (562.7); IR (in CH$_2$Cl$_2$): 1692, 1493, 1445, 1265, 1130, 731, 699 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm): 7.65 (d, J = 3.3 Hz, 1H, Thiaz-4-***H***), 7.41-7.05 (m, 17H, Ph-***H***, Thiaz-5-***H*** and Im-2-***H***), 6.58 (s, 1H, Im-5-***H***), 3.85 (m, 1H, C***H***CH$_2$CO), 3.51 (t, J = 6.5 Hz, 2H, NHC***H***$_2$), 3.11 (dd, J_1 = 7.3 Hz, J_2 = 16.0 Hz, 1H, one H of COC***H***$_2$), 2.81 (dd, J_1 = 6.9 Hz, J_2 = 16.0 Hz, 1H, one H of COC***H***$_2$), 2.57 (m, 2H, Im-4-C***H***$_2$), 1.89 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.46 (d, J = 7.0 Hz, 3H, ***C***H$_3$); ^{13}C-NMR (CDCl$_3$), δ (ppm): 175.1 (quart, Thiaz-***C***-2), 174.6 (quart, ***C***O), 155.2 (quart, ***C***=NH), 142.2 (+, Thiaz-***C***-

4), 142.1 (quart, Ph-***C***-1), 139.5 (quart, Im-***C***-4), 137.8 (+, Im-***C***-2), 129.8, 129.7, 128.2, 128.0 (+, Ph-***C***), 118.5 (+, Thiaz-***C***-5), 118.0 (+, Im-***C***-5), 75.5 (quart, Ph_3***C***), 45.0 (-, CH***C***H_2CO), 40.3 (-, ***C***H_2NH), 34.4 (+, ***C***HCH_2CO), 28.7 (-, Im-4-CH_2***C***H_2), 22.4 (-, Im-4-***C***H_2), 21.1 (+, ***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): *m/z* 563 ($[M + H]^+$).

General procedure for deprotection of trityl group

The trityl protected acylguanidines (1 mmol) were dissolved in dry CH_2Cl_2 (8 mL), added 2 mL of TFA drop by drop with constant stirring for 15 min at rt, and continued to stirred till the starting material was completely disappeared. After removing the solvent in *vacuo*, the residue was purified by preparative HPLC, using mobile phase 0.1 % TFA in water and MeOH (experimental setup is given at General conditions). And the compound purity was checked by analytical HPLC (experimental setup is given at General conditions).

N^1-[-2-(1*H*-Imidazol-4-ylmethylsulfanyl)acetyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]-guanidine [61]

Stirred for 10 h; yield 83 %; sticky oil; $C_{14}H_{21}N_7OS \cdot 3$ TFA (678.0); IR (neat) 2863, 1660, 1430, 1179, 1124, 831 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.81 (s, 1H, Im-2-***H***), 8.75 (s, 1H, Im-***H***-2), 7.37 (s, 1H, Im-***H***-5), 3.95 (s, 2H, SC***H***$_2$CO), 3.41-3.33 (m, 2H, NHC***H***$_2$), 3.31 (s, 2H, Im-4-C***H***$_2$S), 2.90-2.80 (m, 2H, Im-4-C***H***$_2$), 2.36 (s, 3H, C***H***$_3$), 2.04 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 173.4 (quart, ***C***O), 155.4 (quart, ***C***=NH), 134.9 (+, Im-***C***-2), 134.3 (quart, Im-***C***-4), 134.2 (+, Im-***C***-2), 128.8 (quart, Im-***C***-4), 126.5 (quart, Im-***C***-5), 117.1 (+, Im-***C***-5), 41.6 (-, ***C***H_2NH), 35.7 (-, S***C***H_2CO), 27.9 (-, Im-4-CH_2***C***H_2), 25.0 (-, Im-4-***C***H_2S), 22.5 (-, Im-4-***C***H_2), 8.9 (+, ***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 336 ($[M + H]^+$); HRMS [FAB(glycerin)]: *m/z*, calculated for $[C_{14}H_{21}N_7OS + H]^+$ 336.1601, found: 336.1607;

Analytical HPLC: R_t 8.75 min (Column A; 0 min: 0.05% TFA/CH_3CN 97/3, 20 min: 85/15); purity: 93 %.

N^1-(3,3-Diphenylpropanoyl)-N^2-[2-(1*H*-imidazol-4-yl)ethyl]guanidine [62]

Stirred for 10 h; yield 92 %; sticky colorless oil; $C_{21}H_{23}N_5O \cdot 2$ TFA (589.8); IR (neat) 3026, 1662, 1494, 1433, 1183, 1128, 832 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.81 (s, 1H, Im-2-***H***),

7.35 (s, 1H, Im-***H***-5), 7.32-7.12 (m, 10H, Ph-***H***), 4.57 (t, *J* = 8.0 Hz, 1H, C***H***CH_2CO), 3.58 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 3.31 (m, 1H, N***H***), 3.25 (d, *J* = 8.0 Hz, 2H, COC***H***$_2$), 3.03 (t, *J* = 6.7 Hz, 2H, Im-4-C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 175.5 (quart, ***C***O), 162.6 (quart, ***C***=NH), 144.4 (quart, Ph-***C***-1), 135.2 (+, Im-***C***-2), 131.4 (quart, Im-***C***-4), 129.6, 128.8, 127.8 (+, Ph-***C***), 118.1 (+, Im-***C***-5), 48.0 (+, ***C***HCH_2CO), 43.7 (-, CO***C***H_2), 41.0 (-, ***C***H_2NH_2), 28.3 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 361 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for ($C_{21}H_{23}N_5O$) 361.1903, found: 361.1901;
Analytical HPLC: R_t 14.66 min (Column A; 0 min: 0.05% TFA/CH_3CN 95/5, 20 min: 50/50); purity: 97 %.

N^1-[3,3-Diphenylpropanoyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine [63]

Stirred for 10 h; yield: 92 %; colorless sticky oil; $C_{22}H_{25}N_5O \cdot 2$ TFA (603.9); IR (neat) 3083, 1599, 1508, 1390, 1221, 1158, 1100, 820 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 7.40 (s, 1H, Im-2-***H***), 7.10-7.35 (m, 10H, Ph-***H***), 4.65 (t, *J* = 7.7 Hz, 1H, Ph_2C***H***), 3.26 (t, *J* = 6.5 Hz, 2H, C***H***$_2$NH), 3.06 (d, *J* = 7.7 Hz, 2H, C***H***$_2$CO), 2.59 (m, 2H, Im-4-C***H***$_2$), 1.82 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 183.5 (quart, ***C***O), 161.7 (quart, ***C***=NH), 145.0 (quart, Ph-***C***-1), 134.6 (quart, Im-***C***-4), 134.2 (+, Im-***C***-2), 128.4, 128.0, 126.0 (+, Ph-***C***), 47.4 (+, Ph_2***C***H), 46.5 (-, CO***C***H_2), 40.0 (-, NH***C***H_2), 29.1 (-, Im-4-CH_2***C***H_2), 22.9 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 376.2 ($[M + H]^+$); HRMS [FAB (CH_2Cl_2/MeOH)]: *m/z*, calculated for $[C_{21}H_{23}N_5O + H]^+$ 376.2132, found: 376.2137;
Analytical HPLC: R_t 16.51 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 64/36); purity: 100 %.

N^1-(2,2-Diphenylacetyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine [64]

Stirred for 10 h; yield: 92 %; colorless sticky oil; $C_{21}H_{23}N_5O \cdot 2$ TFA (589.8); IR (neat) 1663, 1495, 1184, 1128, 1029, 798, 720, 698, 628 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.74 (s, 1H, Im-2-***H***), 7.86 (s, 1H, Im-5-***H***), 7.40-7.10 (m, 10H, Ph-***H***), 5.27 (s, 1H, Ph_2C***H***), 3.35 (t, *J* = 6.7 Hz, 2H, C***H***$_2$NH), 2.80 (t, *J* = 7.6 Hz, 2H, Im-4-C***H***$_2$), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 176.1 (quart, ***C***O), 155.4 (quart, ***C***=NH), 139.0 (quart, Ph-***C***-1), 134.8 (quart, Im-***C***-4), 134.2 (+, Im-***C***-2), 129.9, 129.7, 128.7 (+, Ph-***C***), 117.1 (+, Im-***C***-5), 59.4 (+, Ph_2***C***H), 41.6 (-, ***C***H_2NH_2), 27.8 (-, Im-4-CH_2***C***H_2) 22.5 (-, Im-4-***C***H_2); MS (ESI,

CH_2Cl_2/MeOH + 10 mM NH_4OAc): 362 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for $[C_{21}H_{23}N_5O]$ 361.1903, found: 361.1903;
Analytical HPLC: R_t 14.72 min (Column B; 0 min: 0.05% TFA/CH_3CN 90/10, 20 min: 75/25); purity: 100%.

N^1-[3,3-Bis(4-fluorophenyl)propanoyl]- N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine [65]

Stirred for 10 h; yield: 85 %; colorless sticky oil; $C_{22}H_{23}F_2N_5O$ (639.8); IR (neat) 2922, 1587, 1505, 1377, 1220, 1157, 823, 749 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.42 (s, 1H, Im-2-***H***), 7.12-7.22 (m, 4H, Ar-***H***), 6.86-6.97 (m, 4H, Ar-***H***), 6.73 (s, 1H, Im-5-***H***), 4.62 (t, *J* = 7.9 Hz, 1H, Ar_2C***H***), 3.28 (t, *J* = 6.7 Hz, 2H, NHC***H***$_2$), 2.98 (d, *J* = 7.9 Hz, 2H, C***H***$_2$CO), 2.58 (m, 2H, Im-4-C***H***$_2$), 1.83 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 183.2 (quart, ***C***O), 161.2 (quart, d, *J* = 244.2 Hz, 2C, ***C***F), 161.9 (quart, ***C***=NH), 140.0 (quart, Ar-***C***-1), 134.6 (quart, Im-***C***-4), 134.1 (+, Im-***C***-2), 129.1 (+, d, *J* = 8.0 Hz, 4C, Ar-***C***-2,6), 117.1 (+, Im-***C***-5), 115.2 (+, d, *J* = 21.1 Hz, 4C, Ar-***C***-3,5), 46.8 (-, ***C***H_2CO), 45.9 (+, Ar_2***C***H), 39.9 (-, ***C***H_2NH), 29.3 (-, Im-4-CH_2***C***H_2), 22.9 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 412 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for ($C_{22}H_{23}F_2N_5O$) 411.1871, found: 411.1866;
Analytical HPLC: R_t 19.50 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 64/36); purity: 95 %.

N^1-[3-(4-Fluorophenyl)-3-phenylpropanoyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine [66]

Stirred for 10 h; yield: 91 %; colorless sticky oil; $C_{22}H_{24}FN_5O$ (621.8); IR (neat) 2941, 1601, 1508, 1390, 1220, 1158, 819 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.41 (s, 1H, Im-2-***H***), 7.10-7.30 (m, 7H, Ar-***H***), 6.87-6.97 (m, 2H, Ar-***H***), 6.72 (s, 1H, Im-5-***H***), 4.64 (t, *J* = 7.9 Hz, 1H, Ar_2C***H***), 3.28 (t, *J* = 6.5 Hz, 2H, C***H***$_2$NH), 3.03 (d, *J* = 7.9 Hz, 2H, C***H***$_2$CO), 2.60 (m, 2H, Im-4-C***H***$_2$), 1.83 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 183.3 (quart, ***C***O), 161.2 (quart, d, *J* = 243.8 Hz, Ar-***C***F), 161.8 (quart, ***C***=NH), 144.8 (quart, Ph-***C***-1), 140.6 (quart, d, *J* = 3.3 Hz, Ar-***C***-1), 134.8 (quart, Im-***C***-4), 134.1 (+, Im-***C***-2), 129.2 (+, d, *J* = 8.0 Hz, 2C, Ar-***C***-2,6), 128.4 (+, 2C, Ph-***C***-2,6), 127.8 (+, 2C, Ph-***C***-3,5), 126.1 (+, Ph-***C***-4), 118.0 (+, Im-***C***-5), 115.1 (+, d, *J* = 21.1 Hz, 2C, Ar-***C***-3,5), 46.6 (-, Ar_2***C***H), 45.6 (+,

CO***C***H_2), 39.9 (-, NH***C***H_2), 29.3 (-, Im-4-CH_2***C***H_2), 22.9 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 394.3 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for [$C_{22}H_{24}FN_5O$] 393.1965, found: 393.1964;

Analytical HPLC: R_t 18.18 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 64/36); purity: 85 %.

N^1-[3-(3,4-Difluorophenyl)-3-phenylpropanoyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]-guanidine [67]

Stirred for 10 h; yield: 91 %; colorless sticky oil; $C_{22}H_{23}F_2N_5O \cdot 2$ TFA (639.8); IR (neat) 3124, 1586, 1507, 1380, 1221, 1134, 783 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.50 (s, 1H, Im-2-***H***), 7.11-7.27 (m, 3H, Ar-***H***), 6.78-7.06 (m, 5H, Ar-***H***), 6.76 (s, 1H, Im-5-***H***), 4.64 (t, *J* = 7.9 *Hz*, 1H, Ar_2C***H***), 3.33 (t, *J* = 6.7 Hz, 2H, NHC***H***$_2$), 3.03 (d, *J* = 7.9 Hz, 2H, C***H***$_2$CO), 2.65 (m, 2H, Im-4-C***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 183.1 (quart, ***C***O), 162.9 (quart, d, *J* = 246.8 Hz, 3-***C***F), 161.6 (quart, d, *J* = 245.3 Hz, 4-***C***F), 161.8 (quart, ***C***=NH), 144.6 (quart, Ph-***C***-1), 140.0 (quart, d, *J* = 3.3 Hz, Ar-***C***-1), 139.8 (quart, Im-***C***-4), 134.1 (+, Im-***C***-2), 129.9 (+, Ph-***C***-4), 129.3, 129.4 (+, 2C, Ph-***C***-3,5), 123.5 (+, d, *J* = 3.3 Hz, Ar-***C***-6), 118.1 (+, Im-***C***-5), 115.4, 115.1 (+, 2C, Ph-C-2,6), 114.7 (+, d, *J* = 21.3 Hz, Ar-***C***-5), 113.0 (+, d, *J* = 21.1 Hz, Ar-***C***-2), 46.6 (+, Ar_2***C***HCO), 45.9 (-, CO***C***H_2), 39.9 (-, NH***C***H_2), 29.3 (-, Im-4-CH_2***C***H_2), 22.9 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 412 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for [$C_{22}H_{23}F_2N_5O$] 411.1871, found: 411.1866;

Analytical HPLC: R_t 18.59 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 68/32); purity: 97 %.

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-[3-phenyl-3-(pyridin-2-yl)propanoyl]guanidine [70]

Stirred for 10 h; Yield: 85 %; colorless sticky oil; $C_{21}H_{24}N_6O \cdot 3$ TFA (719.0); IR (neat) 1664, 1180, 1125, 831, 798, 719, 700 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.80 (m, 1H, Pyr-6-***H***), 8.57 (m, 1H, Pyr-4-***H***), 7.99 (m, 1H, Pyr-5-***H***), 7.58 (d, *J* = 8.3 Hz, 1H, Pyr-3-***H***), 7.46 (m, 1H, Ph-***H***), 7.21-7.39 (m, 6H, Ph-***H*** *and* Im-2-***H***), 4.84 (t overlap with H_2O, 1H, Ar_2C***H***), 3.61 (dd, J_1 = 8.8 Hz J_2 = 16.7 Hz, 1H, C***H***$_2$CO), 3.40-3.31 (m, 3H, C***H***$_2$CO and NHC***H***$_2$), 2.80 (t, *J* = 7.8 Hz, 2H, Im-4-C***H***$_2$), 1.99 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ

(ppm): 175.1 (quart, ***C***O), 161.7 (quart, Py-***C***-2), 155.1 (quart, ***C***=NH), 147.4 (+, Pyr-***C***-6), 142.0 (quart, Ph-***C***-1), 141.8 (+, Im-***C***-2), 134.9 (+, Pyr-***C***-4), 134.5 (quart, Im-***C***-4), 130.1, 129.0, 128.6 (+, Ph-***C***), 125.6 (+, Pyr-***C***-3), 124.4 (+, Pyr-***C***-5), 117.1 (+, Im-***C***-5), 48.1 (+, CO***C***H_2CH), 42.2 (-, CO***C***H_2), 41.5 (-, NHC***H***$_2$), 27.8 (-, Im-4-CH_2***C***H_2), 22.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 377 ($[M + H]^+$) ; HRMS [EI-MS]: *m/z*, calculated for ($C_{21}H_{24}N_6O$) 376.2012, found: 376.2002;

Analytical HPLC: R_t 14.27 min (Column B; 0 min: 0.05% TFA/CH_3CN 90/10, 20 min: 80/20); purity: 86 %.

<u>*N*^{1}-[3-(4-Fluorophenyl)-3-(pyridin-2-yl)propanoyl]-*N*^{2}-[3-(1*H*-imidazol-4-yl)propyl]-guanidine [71]</u>

Stirred for 10 h; yield: 79 %; colorless sticky oil; $C_{21}H_{23}FN_6O \cdot 3$ TFA (737.0); IR (neat) 1663, 1510, 1181, 1125, 831, 787, 719 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.78 (s, 1H, Im-2-***H***), 8.71 (d, *J* = 5.2 Hz,1H, Pyr-4-***H***), 8.57 (m, 1H, Pyr-***H***), 8.38 (t, *J* = 7.8 Hz, 1H, Pyr-C), 8.00 (d, *J* = 8.2 Hz, 1H, Pyr-***H***), 7.78 (t, *J* = 6.7 Hz, 1H, Ph-***H***), 7.50-7.40 (m, 2H, Ph-***H*** and Im-***H***), 7.09 (t, *J* = 7.8 Hz, 2H, Ph-***H***), 4.84 (t overlap with H_2O, 1H, Ar_2C***H***), 3.68 (dd, J_1 = 9.0 Hz, J_2 = 17.3 Hz, 1H, one of C***H***$_2$CO), 3.43 (dd, J_1 = 6.6 Hz, J_2 = 17.3 Hz, 1H, one of C***H***$_2$CO), 3.35-3.30 (m, 2H, NHC***H***$_2$), 2.82 (t, *J* = 7.5 Hz, 2H, Im-4-C***H***$_2$), 2.00 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 174.4 (quart, ***C***O), 163.7 (quart, d, *J* = 246.1 Hz, ***C***F), 159.8 (quart, Py-***C***-2), 155.1 (quart, ***C***=NH), 146.2 (+, Py-***C***-6), 144.5 (+, Im-***C***-2), 136.3 (quart, Ar-***C***-1), 134.9 (+, Pyr-***C***-4), 134.2 (quart, Im-***C***-4), 131.1 (+, d, *J* = 8.2 Hz, 2C, Ar-***C***-2/-6), 126.6 (+, Pyr-***C***-3), 125.9 (+, Pyr-***C***-5), 117.1 (+, d, *J* = 21.1 Hz, 2C, Ar-***C***-3/-5), 117.0 (+, Im-***C***-5), 45.9 (+, COCH_2***C***H), 41.8 (-, CO***C***H_2), 41.6 (-, NH***C***H_2), 28.0 (-, Im-4-CH_2***C***H_2), 22.4 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 395 ($[M + H]^+$);

HRMS [EI-MS]: *m/z*, calculated for ($M^{+\cdot}$,) 394.1917, found 394.1917;

Analytical HPLC: R_t 17.51 min (Column B; 0 min: 0.05% TFA/CH_3CN 90/10, 20 min: 80/20); purity: 90 %.

<u>*N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl-*N*^{2}-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine [72]</u>

Stirred for 10 h; Yield: 85 %; colorless sticky oil; $C_{19}H_{22}N_6OS \cdot 2$ TFA (610.8); IR (neat) 1663, 1179, 1128, 832, 798, 720, 702 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.81 (s, 1H, Im-***H***-

2), 7.70 (d, J = 3.3 Hz, 1H, Thiaz-4-***H***), 7.49 (d, J = 3.3 Hz, 1H, Thiaz-5-***H***), 7.35-7.15 (m, 6H, Ph-***H*** and Im-5-***H***), 5.04 (t, J = 7.7 Hz, 1H, $COCH_2C\boldsymbol{H}$), 3.59 (dd, J_1 = 8.2 Hz, J_2 = 16.5 Hz, 1H, one of $COC\boldsymbol{H}_2$), 3.38 (m, 3H, $C\boldsymbol{H}_2NH$ and one of $COC\boldsymbol{H}_2$), 2.84 (t, J = 7.7 Hz, Im-$C\boldsymbol{H}_2$), 2.00 (m, 2H, Im-$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 174.8 (quart, ***C***O), 174.4 (quart, Thiaz-***C***-2), 155.5 (quart, ***C***=NH), 142.7 (+, Thiaz-***C***-4), 142.2 (quart, Ph-***C***-1), 134.8 (+, Im-***C***-2), 134.2 (quart, Im-***C***-4), 130.0, 129.8, 129.3, 128.9 (+, Ph-***C***), 121.2 (+, Thiaz-***C***-5), 117.0 (+, Im-***C***-5), 45.2 (+, ***C***HCH_2CO), 43.4 (-, ***C***H_2CO), 41.5 (-, ***C***H_2NH), 27.9 (-, Im$CH_2\boldsymbol{C}H_2$), 22.5 (-, Im-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 383 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for ($C_{19}H_{22}N_6OS$) 382.1576, found 382.1576;

Analytical HPLC: R_t 10.91 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 70/30); purity: 95 %.

N^1-[3-(3,4-Difluorophenyl)-3-(thiazol-2-yl)propanoyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]-guanidine [73]

Stirred for 10 h; yield: 86 %; colorless sticky oil; $C_{19}H_{20}F_2N_6OS \cdot 2$ TFA (646.8); IR (neat) 1664, 1518, 1181, 1129, 798, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.80 (s, 1H, Im-2-***H***), 7.73 (d, J = 3.3 Hz, 1H, Thiaz-4-***H***), 7.48 (d, J = 3.3 Hz, 1H, Thiaz-5-***H***), 7.35 (s, 1H, Im-5-***H***), 7.35-7.15 (m, 3H, ArC), 5.02 (ddq, J = 8.2 and 6.6 Hz, 1H, C***H***CH_2CO), 3.59 (dd, J_1 = 8.2 Hz, J_2 = 16.5 Hz, 1H, one of C***H***$_2$CO), 3.38 (t, J = 6.8 Hz, C***H***$_2$NH), 3.27 (dd, J_1 = 6.6 Hz, J_2 = 16.5 Hz, 1H, one of $COC\boldsymbol{H}_2$), 2.84 (t, J = 7.7 Hz, Im-4-C***H***$_2$), 2.03 (m, Im-4-$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 174.5 (quart, Thiaz-***C***-2), 173.1 (quart, ***C***O), 155.1 (quart, ***C***=NH), 151.3 (quart, dd, J_1 = 12.5 Hz, J_2 = 247.6 Hz, CF), 150.8 (quart, dd, J_1 = 12.6 Hz, J_2 = 247.1 Hz, CF), 143.1 (+, Thiaz-***C***-4), 139.6 (quart, Ar-***C***-1), 134.8 (+, Im-***C***-2), 134.2 (quart, Im-***C***-4), 125.7 (+, Ar-***C***-6), 121.3 (+, Thiaz-***C***-5), 118.7 (+, d, J = 17.2 Hz, Ar-***C***-5), 118.2 (+, d, J = 17.2, Hz, Ar-***C***-2), 117.1 (+, Im-***C***-5), 44.8 (+, ***C***HCH_2CO), 43.3 (-, ***C***H_2CO), 41.5 (-, ***C***H_2NH), 27.9 (-, Im-4-$CH_2\boldsymbol{C}H_2$), 22.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 419 ($[M + H]^+$); HRMS [EI-MS]: *m/z*, calculated for ($C_{19}H_{20}F_2N_6OS$) 418.1387, found 418.1387;

Analytical HPLC: R_t 12.62 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 62/38); purity: 100%.

N^1-[3-(Benzyl-1*H*-imidazol-2-yl)-3-(3,4-difluorophenyl)propanoyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine [74]

Stirred for 10 h; yield: 87 %; colorless sticky oil; $C_{26}H_{27}F_2N_7O \cdot 2$ TFA (719.8); IR (neat) 1661, 1519, 1180, 1122, 829, 797, 719 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.79 (s, 1H, Im-2-***H***), 7.65 (dd, J_1 = 1.9 Hz, J_2 = 5.8 Hz, Benzyl-Im-4-***H*** and -5-***H***), 7.35 (s, 1H, Im-5-***H***), 7.33-7.24 (m, 3H, Ar-***H***), 7.20-7.00 (m, 4H, Ph-***H***), 6.90 (m, 1H, Ph-***H***), 5.51 (s, 2H, PhC***H***$_2$), 5.13 (t overlap with H_2O, 1H, C***H***CH_2CO), 3.62 (AMX, J_1 = 9.6 Hz, J_2 = 17.6 Hz, C***H***$_2CO$), 3.45-3.32 (AMX and m overlap, 3H, C***H***$_2CO$ and C***H***$_2NH$), 2.83 (t, J = 7.8 Hz, 2H, Im-4-C***H***$_2$), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 173.5 (quart, ***C***O), 163.0 (quart, ***C***=NH), 154.9 (quart, ***C***=NH), (quart, Benzyl-Im-***C***-2), 151.5 (quart, dd, J_1 = 12.9 Hz, J_2 = 248.9 Hz, CF), 151.3 (quart, dd, J_1 = 12.6 Hz, J_2 = 248.9 Hz, CF), 148.1 (quart, Benzyl-Im-***C***-2), 135.1 (quart, Im-***C***-4), 134.9 (+, Im-***C***-2), 134.4 (quart, Ar-***C***-1), 134.3 (quart, Ph-***C***-1), 130.2, 129.9, 128.6 (+, Ph-***C***), 125.5 (+, Ar-***C***-6), 124.8 (+, Benzyl-Im-***C***-4), 120.6 (+, Benzyl-Im-***C***-5), 119.2 (+, d, J = 17.7 Hz, Ar-***C***-5), 118.4 (+, d, J = 18.7 Hz, Ar-***C***-2), 117.1 (+, Im-***C***-5), 52.4 (-, Ph***C***H_2), 41.6 (-, CO***C***H_2), 41.5 (-, NH***C***H_2), 37.6 (+, ***C***HCH_2CO), 27.9 (-, Im-4-CH_2***C***H_2), 22.4 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 419 ([M + H]$^+$; HRMS (EI-MS): *m/z* calculated for [$C_{26}H_{27}F_2N_7O$], 491.2245, found 491.2234;

Analytical HPLC: R_t 13.78 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 70/30); purity: 98 %.

N^1-[3-(3,4-Difluorophenyl)-3-(1*H*-imidazol-2-yl)propanoyl]-N^2-[3-(1*H*-imidazol-4-yl)-propyl]guanidine [75]

Stirred for 10 h; yield: 88 %; colorless sticky oil; $C_{19}H_{21}F_2N_7O \cdot 3$ TFA (744.0); IR (neat) 1659, 1624, 1520, 1182, 1129, 832, 797, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.79 (s, 1H, 1*H*-Im-2-***H***), 7.94 (s, 2H, *2H*-Im-3-***H*** and -4-***H***), 7.47 (s, 1H, 1*H*-Im-5-***H***), 7.41-7.10 (m, 4H, Ar-***H*** and N***H***), 5.03 (t overlap with H_2O, 1H, C***H***CH_2CO), 3.61 (dd, J_1 = 8.2 Hz, J_2 = 17.2 Hz, C***H***$_2CO$), 3.44 (dd, J_1 = 6.9 Hz, J_2 = 17.2 Hz, COC***H***$_2$), 3.30 (m, 2H, C***H***$_2NH$), 2.81 (t, J = 7.8 Hz, 2H, 1*H*-Im-4-C***H***$_2$), 2.01 (m, 2H, 1*H*-Im-4-CH_2C***H***$_2$). ^{13}C-NMR ($CDCl_3$), δ (ppm): 173.4 (quart, ***C***O), 155.0 (quart, C=NH), 151.8 (quart, dd, J_1 = 12.5 Hz, J_2 = 249.0 Hz, CF), 151.4 (quart, dd, J_1 = 12.6 Hz, J_2 = 248.9 Hz, CF), 148.8 (quart, 2*H*-Im-***C***-2), 135.5 (quart,

Ar-***C***-1), 134.9 (+, 1*H*-Im-***C***-2), 134.2 (quart, 1*H*-Im-***C***-4), 125.6 (+, Ar-***C***-6), 120.6 (+, 2*H*-Im-***C***-5), 120.4 (+, 2*H*-Im-***C***-4), 119.4 (+, d, *J* = 17.7 Hz, Ar-C-5), 118.3 (+, d, *J* = 18.1 Hz, Ar-C-2), 117.1 (+, 1*H*-Im-***C***-5), 41.6 (-, ***C***H_2CO), 40.4 (-, ***C***H_2NH), 39.1 (+, ***C***HCH_2CO), 27.8 (-, 1*H*-Im-4-CH_2***C***H_2), 22.5 (-, 1*H*-Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 402 ($[M + H]^+$); HRMS: FAB-MS: *m/z* for ($[C_{19}H_{21}F_2N_7O + H]^+$) calcd. 402.1854, found 402.1842;

Analytical HPLC: R_t 9.12 min (Column B; 0 min: 0.05% TFA/CH_3CN 90/10, 20 min: 75/25); purity: 80 %.

<u>N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N'-[3-(thiophen-2-yl)butanoyl]guanidine [79]</u>

Stirred for 10 h; yield: 92 %; colorless sticky oil; $C_{15}H_{21}N_5OS \cdot 2$ TFA (547.8); IR (neat) 2873, 1663, 1433, 1180, 1127, 832, 798, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.84 (s, 1H, Im-2-***H***), 7.48 (s, 1H, Im-5-***H***), 7.30 (m, 1H, Thio-***H***), 6.93 (m, 2H, Thio-***H***), 3.60 (m, 1H, $COCH_2C$***H***), 3.32 (m, 2H, NHC***H***$_2$), 2.92 (m, 4H, Im-4-C***H***$_2$ and one of COC***H***$_2$), 2.00 (m, 2H, Im-4-CH_2C***H***$_2$), 1.42 (d, *J* = 7.0 Hz, 3H, ***C***H_3); ^{13}C-NMR (CD_3OD), δ (ppm): 175.6 (quart, ***C***O), 155.2 (quart, ***C***=NH), 150.0 (+, Thio-***C***-2), 134.9 (+, Im-***C***-2), 134.3 (quart, Im-***C***-4), 127.7 (+, Thio-***C***-5), 126.3, 124.4 (+, Thio-***C***-3,4), 117.1 (+, Im-***C***-5), 47.0 (-, CH***C***H_2CO), 41.6 (-, ***C***H_2NH), 32.9 (+, ***C***H***C***H_2CO), 27.9 (-, Im-4-CH_2***C***H_2), 23.2 (+, ***C***H_3), 22.5 (-, Im-4-***C***H_2); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): *m/z* 320 ($[M + H]^+$);

HRMS: EI-MS: *m/z* for ($C_{15}H_{21}N_5OS$) calcd. 319.1467, found 319.1469;

Analytical HPLC: R_t 9.03 min (Column B; 0 min: 0.05% TFA/CH_3CN 80/20, 20 min: 70/30); purity: 95 %.

<u>N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-[3-(pyridin-2-yl)butanoyl]guanidine [80]</u>

Stirred for 10 h; yield: 89 %; colorless sticky oil; $C_{16}H_{22}N_6O \cdot 2$ TFA (657.0); IR (neat) 3138, 1665, 1434, 1181, 1129, 833, 798, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.79 (s, 1H, Im-2-***H***), 8.57 (d, *J* = 8.3 Hz, 1H, Pyr-6-***H***), 8.09 (dt, J_1 = 1.7 Hz, J_2 = 7.8 Hz, 1H, Pyr-4-***H***), 7.67 (d, *J* = 8.0 Hz, 1H, Pyr-3-***H***), 7.53 (ddd, J_1 = 1.0 Hz, J_2 = 5.3 Hz, J_3 = 6.4 Hz, 1H, Pyr-5-***H***), 7.53 (s, 1H, Im-5-***H***), 3.60 (m, 1H, Ar_2C***H***), 3.35 (m, 2H, C***H***$_2$NH), 3.09 (dd, J_1 = 8.3 Hz, J_2 = 16.6 Hz, 1H, C***H***$_2$CO), 2.92 (dd, J_1 = 6.2 Hz, J_2 = 16.6 Hz, 1H, C***H***$_2$CO), 2.82 (t, *J* = 7.6 Hz, 2H, Im-4-C***H***$_2$), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 1.40 (d, *J* = 7.0 Hz, 3H, C***H***$_3$CH); ^{13}C-NMR

(CD_3OD), δ (ppm): 175.4 (quart, ***C***O), 163.7 (quart, Py-***C***-2), 155.2 (quart, ***C***=NH), 146.7 (+, Pyr-***C***-6), 142.5 (+, Pyr-***C***-4), 134.9 (+, Im-***C***-2), 134.3 (+, Im-***C***-4), 125.4 (+, Pyr-***C***-3), 124.8 (+, Pyr-***C***-5), 117.1 (+, Im-***C***-5), 43.4 (-, CO***C***H_2CH), 41.5 (-, ***C***H_2NH), 37.4 (+, COCH$_2$***C***H), 27.9 (-, Im-4-CH$_2$***C***H_2), 22.5 (-, Im-4-***C***H_2), 20.7 (+, ***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 315 ($[M + H]^+$);
HRMS: EI-MS: *m/z* for ($C_{16}H_{22}N_6O$) calcd. 314.1855, found 314.1863;
Analytical HPLC: R_t 13.00 min (Column B; 0 min: 0.05% TFA/CH_3CN 98/2, 20 min: 85/15); purity: 95 %.

<u>*N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl)-*N*^{2}-[3-(thiazol-2-yl)butanoyl]guanidine [81]</u>

Stirred for 10 h; yield: 84 %; colorless sticky oil; $C_{14}H_{20}N_6OS \cdot 2$ TFA (548.8); IR (neat) 3131, 1663, 1431, 1180, 1128, 832, 798, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.84 (s, 1H, Im-2-***H***), 7.70 (d, *J* = 3.3 Hz, 1H, Thiaz-4-***H***), 7.49 (d, *J* = 3.3 Hz, 1H, Thiaz-5-***H***), 7.28 (s, 1H, Im-***H***-5), 3.90 (m, 1H, C***H***CH_2CO),), 3.38 (m, 2H, C***H***$_2$NH), 3.12 (dd, J_1 = 8.2 Hz, J_2 = 16.5 Hz, 1H, one of COC***H***$_2$), 2.92 (m, 3H, Im-4-C***H***$_2$ and one of COC***H***$_2$), 2.00 (m, 2H, Im-4-CH_2C***H***$_2$), 1.42 (d, *J* = 7.0 Hz, 3H, ***C***H_3); ^{13}C-NMR (CD_3OD), δ (ppm): 176.4 (quart, ***C***O), 175.1 (quart, Thiaz-***C***-2), 155.2 (quart, ***C***=NH), 142.7 (+, Thiaz-***C***-4), 134.9 (+, Im-***C***-2), 134.3 (quart, Im-***C***-4), 120.2 (+, Thiaz-***C***-5), 117.1 (+, Im-***C***-5), 44.3 (-, CH***C***H_2CO), 41.6 (-, ***C***H_2NH), 35.1 (+, ***C***HCH_2CO), 27.9 (-, Im-4-CH$_2$***C***H_2), 22.5 (-, Im-4-***C***H_2), 21.7 (+, ***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 321.5 ($[M + H]^+$); HRMS: FAB-MS: *m/z* for ($[C_{14}H_{20}N_6OS + H]^+$) calcd. 321.1498, found 321.1508;
Analytical HPLC: R_t 11.95 min (Column B; 0 min: 0.05% TFA/CH_3CN 97/3, 20 min: 65/35); purity: 100 %.

3.5.2. Pharmacological methods

Data handling and pharmacological parameters: Data presented as mean ± *SEM* or *SE* or with 95% confidence limits (cl) unless otherwise indicated. Agonist potencies are given in percent or are expressed as pEC_{50} values (negative decadic logarithm of the molar concentration of the agonist producing 50% of the maximal response) and were corrected according to the long term mean value of the reference agonist histamine in our laboratory

(guinea pig atrium (H_2): pEC_{50} = 6.00 for histamine; guinea pig ileum (H_1): pEC_{50} = 6.70 for histamine). Maximal responses are expressed as E_{max} values (percentage of the maximal response to a reference compound). Antagonist affinities are expressed as either an apparent pA_2 or a full pA_2 value. The apparent pA_2 value was calculated from the following equation: pA_2 = –log $c(B)$ + log(r – 1), where $c(B)$ is the molar concentration of antagonist and r the ratio of agonist EC_{50} measured in the presence and absence of antagonist.[45] The full pA_2 value was determined according to the method of Arunlakshana and Schild[46] using antagonist concentrations over 1-2 log units. Noncompetitive antagonists are characterized by estimation of a pD'_2 value according to the equation: pD'_2 = –log $c(B)$ + log(100/E_{max} – 1).[47] Where appropriate, differences between means were determined by Student's t-test, after checking the homogeneity of the variances; P values < 0.05 were considered to indicate a significant difference between the mean values being compared.

Histamine H_1 receptor assay on the isolated guinea pig ileum: Guinea pigs of either sex (250-500 g) were stunned by a blow on the neck and exsanguinated. The ileum was rapidly removed, rinsed and cut into segments of 1.5-2 cm length. The tissues were mounted isotonically (preload of 5 mN) in a jacketed 20-mL organ bath that was filled with Tyrode's solution of the following composition [mM]: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, and glucose 5.0. The solution additionally contained atropine to block cholinergic M receptors at a concentration not affecting H_1 receptors (0.1 μM). The solution was aerated with 95% O_2-5% CO_2 and warmed to a constant temperature of 37 °C. During an equilibration period of 80 min, the tissues were stimulated three times with histamine (1 μM, then 10 μM) followed by washout. *Antagonists:* Up to four cumulative concentration-response curves were determined on each tissue: a first to histamine (0.01-30 μM), and the 2^{nd} – 4^{th} to histamine in the presence of increasing concentrations of antagonist (incubation time 10–15 min). pEC_{50} differences were not corrected since four successive curves for histamine were superimposable (n > 10).

Histamine H_2 receptor assay on isolated guinea pig right atrium (spontaneously beating): Hearts were rapidly removed from guinea pigs used for studies on the ileum (see above). The right atrium was quickly dissected and set up isometrically in Krebs-Henseleit

solution under a diastolic resting force of 5 mN in a jacketed 20 mL-organ bath of 32.5 °C as previously described.[6] The bath fluid (composition [mM]: NaCl 118.1, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.64, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.0, sodium pyruvate 2.0) was gassed with 95% O_2-5% CO_2 and additionally contained (*RS*)-propranolol (0.3 μM) and mepyramine (1 μM). Experiments were started after 30 min of continuous washing and an additional equilibration period of 15 min. *Antagonists:* Two successive concentration-frequency curves to histamine (0.1-30 μM) were established, the first in the absence and the second in the presence of the compound under study (incubation time 30 min). pEC_{50} differences were not corrected since two successive curves for histamine were virtually superimposable ($n > 10$). *Agonists:* Two successive concentration-frequency curves were established, the first to histamine (0.1-30 μM) and the second for the agonist under study in the absence or presence of cimetidine (30 μM, 30 min incubation time). Furthermore, the sensitivity to 30 μM cimetidine was routinely checked at the end of each H_2 agonist concentration-effect curve established in the absence of an H_2 receptor antagonist, and a significant reduction of frequency was always observed after 15–30 min.

Calcium assays with U-373 MG cells

Cells and culture conditions: The human U-373 MG (HTB 17) glioblastoma/astrocytoma cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). Cell banking and quality control were performed according to the "seed stock concept". Cells were grown in Eagle´s minimum essential medium containing L-glutamine, 2.2 g/l NaHCO3, 110 mg/l sodium pyruvate (Sigma, Deisenhofen, FRG), and 10% FCS (GIBCO, Eggenstein, FRG The cells were cultured in a water-saturated atmosphere of 95% air and 5% carbon dioxide at 37 °C in 75-cm² culture flasks (Costar, Tecnomara, Fernwald, FRG) and were serially passaged following trypsinisation using trypsin (0.05%) / EDTA (0.02%) (Roche Diagnostics, Mannheim, GER).

Preparation of the cells: Adherently growing U-373 MG cells were trypsinized and transferred from a 75-cm^2 flask to 175-cm^2 flask (Nunclon, 178883, Nunc, Wiesbaden, and Germany) 5-6 days before the experiment. At approx. 80% confluence cells were trypsinized, the suspension (2-4·10^5 cells per ml), was centrifuged for 10 min at 200 g and room

temperature. After resuspension in 10 ml of loading buffer (25 mM HEPES (Sigma, Deisenhofen, GER), 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose), pH 7.4, cell number was determined with a hemocytometer (Neubauer, improved), and the cells were adjusted to a density of $1.3 \cdot 10^6$ per ml by addition of an appropriate volume of loading buffer.

Loading of the cells with Ca^{2+} indicator Fura-2/AM: To three volumes of the prepared cell suspension, one volume of loading dispersion was added, before the cells were incubated in the dark at room temperature for 30 min. The loading dispersion was freshly made by mixing 10 ml of loading buffer, containing 2 % bovine serum albumin (BSA), with 50 µl of Pluronic-F-127 (Molecular Probes, Eugene, Oregon, USA) (20 % in DMSO), and 40 µl of Fura-2/AM (Molecular Probes, Eugene, Oregon, USA) (1 mM in anhydrous DMSO).
Cells were centrifuged (200 g, 7 min), resuspended in fresh loading buffer and allowed to stand for another 30 min at room temperature in the dark. After two washing/centrifugation cycles (loading buffer, 200 g, 7 min) and adjustment of the cell number to a value of 10^6 /ml, cells were incubated for at least 15 min at 20 °C in the dark.

Fluorimetric determination of intracellular [Ca^{2+}]: 1 ml-aliquots of loading buffer were filled into disposable acrylic cuvettes (Sarstedt, No. 67.755, Nuembrecht, GER), which were thermostatted at 30 ° C in an incubator hood (Infors AG, Bottmingen, CH). Immediately after addition of 1 ml of the Fura-2/AM loaded cell suspension and a magnetic stirrer, the cuvette was placed into the thermostatted (25 °C) stirred cell holder of a LS 50 B Luminescence Spectrometer (Perkin Elmer, Ueberlingen, GER), equipped with a fast filter accessory. Fluorescence signals were registered (instrument settings: excitation 340/380 nm, emission 510 nm, slits 10 nm, resolution 0.1, stirrer low) for 300 s after addition of 10 µl of 6 mM histamine dihydrochloride dissolved in millipore water.

Calculation of Ca^{2+} concentrations: Calcium concentrations were calculated from dual wavelength fluorescence intensities according to the Grynkiewicz equation:

$$\left[Ca^{2+}\right] = K_d \cdot \frac{(R - R_{\min})}{(R_{\max} - R)} \cdot SFB$$

were K_d (224 nM) is the dissociation constant of the Fura-2-Ca^{2+}-complex, R is the experimental fluorescence ratio value (F_{340} / F_{380}), R_{min} and R_{max} are the fluorescence value ratios (F_{340} / F_{380}) under Ca^{2+}-free and Ca^{2+}-saturation conditions, respectively, and SFB is the ratio of fluorescence intensities for Ca^{2+}-free/Ca^{2+}-bound indicator, measured at 380 nm. R_{min}, R_{max} and SFB were determined by calibration experiments, performed in every test series.
To measure R_{max}, 10 µl of an aqueous solution of 2 % digitonin (Sigma, Deisenhofen, GER), were pipetted into the cuvette, wheras R_{min} was determined after subsequent addition of 50 µl of a 0.6 M EGTA solution (in 1 M Tris/HCl, pH 8.7).

Determination of histamine H_1 receptor antagonists on U-373 MG cells:[48]

To determine the histamine H_1 receptor antagonism on U-373 MG cells the inhibition of the (submaximal) increase in $[Ca^{2+}]_i$ elicited by 30 µM histamine was measured. Prior to stimulation with histamine the cells were incubated with the putative antagonists for 15 min in the dark with stirring. The inhibition (%) was calculated as follows:

$$\text{Inhibition [\%]} = \left(100 - \frac{[Ca^{2+}]_i\text{-increase (antagonist + 30 µM hist.) [µM]}}{[Ca^{2+}]_i\text{-increase (control) [µM]}}\right) \cdot 100$$

At least seven concentrations of the antagonists were used and at least two independent experiments were carried out on different days. The mean values of the resulting inhibition curves were used to calculate IC_{50} and pK_B values, respectively.

Calculation of IC_{50} values: IC_{50}- values were calculated from at least two antagonist concentrations [B], inhibiting the agonist-stimulated increase in intracellular $[Ca^{2+}]$ between 20 and 80 %. The mean percentual inhibition values P with SEM < 10 %, determined from at least 3 independent experiments, performed on different days, were logit transformed, according to the equation

$$\text{logit (P)} = \log \frac{P}{100 - P}$$

and IC_{50} values (logit P = 0) were determined from the plot logit (P) versus log [B] with the slope n according to

$$\log \frac{P}{100 - P} = n \cdot \log [B] - n \cdot \log IC_{50}$$

by linear regression with Fig. P (Biosoft, Cambridge, UK).

Steady-sstate GTPase activity assay and receptor ligand binding assay on guinea pig and human histamine H_2 receptor $G_{s\alpha S}$ fusion proteins: These investigations were performed according to the procedure described by Kelley *et al*.[24]

3.6. References:

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Chapter 4

Synthesis and Pharmacological Activity of *N*-Acyl-*N*'-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines: Towards Improved H_2 Receptor Selectivity

Only such substances can be anchored at any particular part of the organism, as fit into the molecules of the recipient complex like a piece of mosaic finds its place in the pattern.

Paul Ehrlich, 1956

4.1. Introduction

The clinical use of compounds active on the histamine H_2 receptor (H_2R) is mainly restricted to antagonists, which are useful in the treatment of peptic ulcers. However, some efforts have been made with histamine H_2R agonists in the treatment of patients suffering from congestive heart failure.[1,2] Histamine H_2 receptor agonists can be divided in two structural classes of compounds: histamine analogues and dimaprit[3,4] (containing amine), and impromidine analogues (containing guanidine)[5,6] including arpromidine and related imidazolylpropylguanidines[7]. Histamine and dimaprit both show moderate histamine H_2R agonistic activity at the guinea pig right atrium, however, the H_1-receptor agonistic potency of dimaprit on the isolated guinea pig ileum (H_1) proved to be less than 0.0001% of that of histamine.[8]

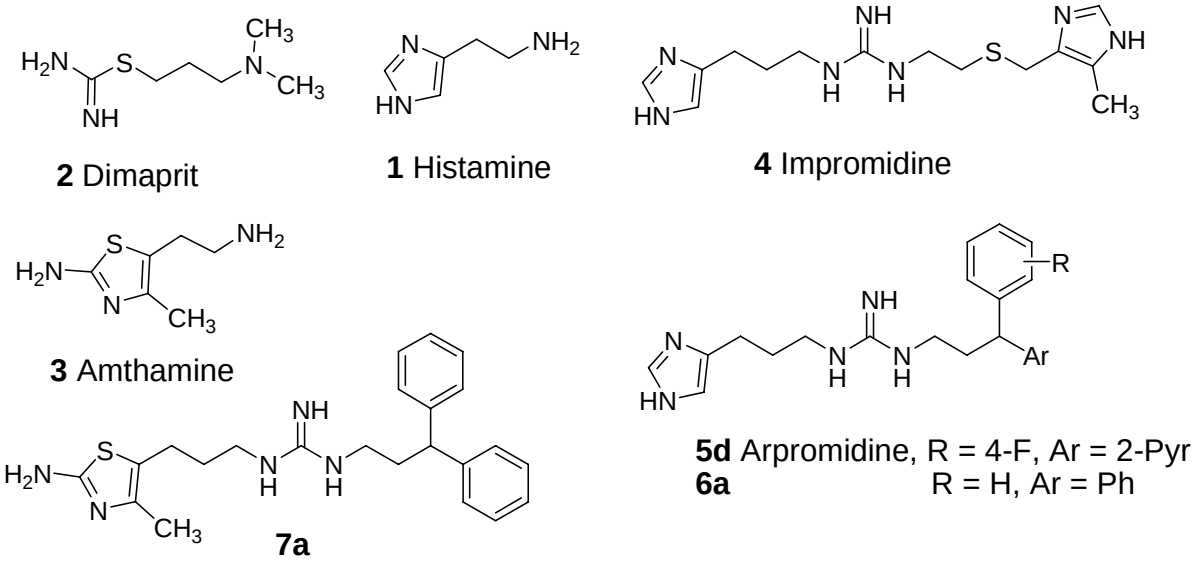


Fig. 4.1. Structure of some histamine H_2 receptor agonists.

Analogues of impromidine as described by Sterk[5] and Buschauer[7] are potent histamine H_2R agonists, and some of them show considerable H_1- and H_3-receptor antagonistic properties[9].

Histamine, impromidine and all its analogues contain a 4-(ω-aminoalkyl)imidazole fragment. The imidazole moiety is supposed to trigger the histamine H_2 receptor via a l,3-prototropic shift[10] (**Fig. 4.2**) involving one proton from a proton donating group at the histamine H_2 receptor and the N^{tele} proton of the imidazole nucleus. Amino acids Asp-186 and Tyr-182 in transmembrane domain 5 are considered to be involved in this process (see receptor models in **Chapter 4.1**). The mechanism for dimaprit is less clear.

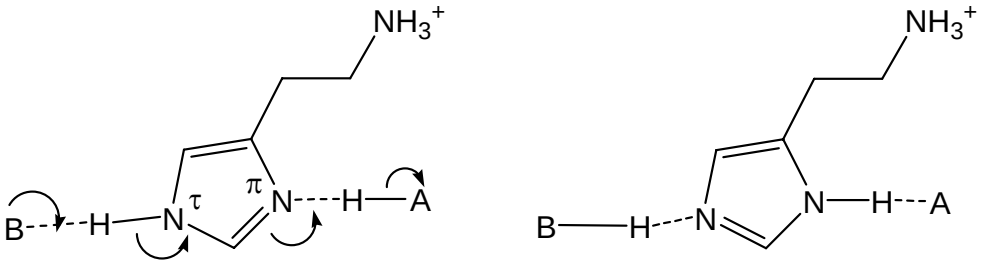


Fig. 4.2. Interaction of the histamine monocation with the H_2 receptor (1,3-prototropic shift)

The structure-activity relationship studies on the H_2 receptor agonism of dimaprit have revealed that the dimethylamino group has the same function as the amino group of histamine, while the isothiourea group of dimaprit and the imidazole group of histamine are regarded as bioisosteric groups[3].

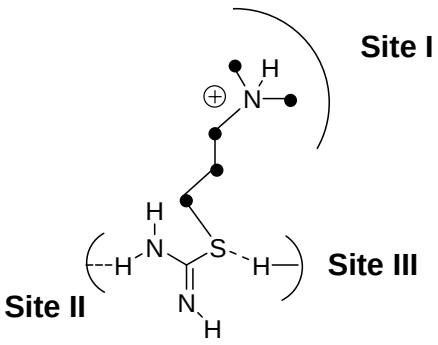


Fig. 4.3a. Interaction of dimaprit with the histamine H_2 receptor (S-fit)

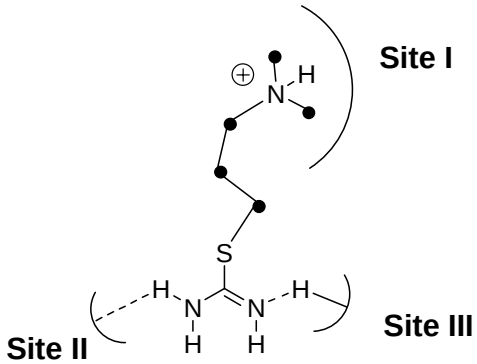


Fig. 4.3b. Interaction of dimaprit with the histamine H_2 receptor (N-fit)

Green *et al.*[11] suggested the formation of a hydrogen bond between a proton of the histamine H_2 receptor and the sulphur atom of dimaprit in the so-called S-fit (**Fig. 4.3a**) and the formation of a second hydrogen bond between a proton of one of the nitrogen atoms of the isothiourea group and another site of the same receptor. As with histamine two protons are involved, one from the receptor and one from the agonist, however, in contrast to histamine a tautomeric proton shift is impossible in the same region of the active site of the receptor. Durant *et al*[3] considered a second possibility for the interaction of dimaprit with the histamine H_2 receptor, so-called N-fit, placing the two nitrogen atoms of the isothiourea moiety of dimaprit in the same position as the two imidazole nitrogen atoms of histamine. As can be seen in **Fig. 4.3b**, this mechanism supposes an interaction between the receptor proton (**site III**) and a nitrogen atom of the isothiourea group and a proton of the second nitrogen atom of this same group and **site II** of the receptor, thus leaving the possibility for a 1,3-prototropic shift as proposed for the mechanism of histamine. However, quantum chemical calculations suggest that the S-fit, which includes the interaction of the NH-group and the S-atom of the isothiourea moity with the H_2 receptor, is favourable. Donné-Op den Kelder *et al.*[12] performed fit procedures on both the N- and S-fit of dimaprit, showing that the conformation of the N-fit is not likely to be the "active" conformation.

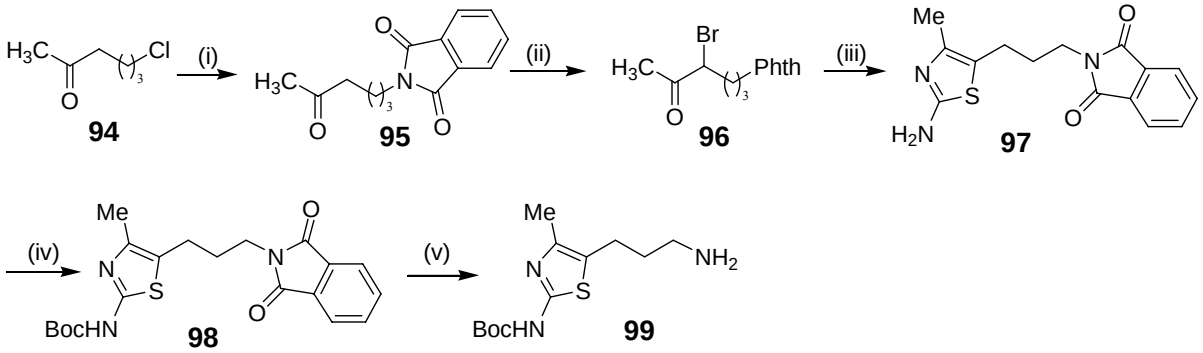
On the basis of that, Impicciatore *et al.*[13] first recognized the possibility of H_2R agonistic activity of 2-amino-5-(2-aminoethyl)thiazole, being a rigid analogue of dimaprit. Several substituted 2-aminothiazoles have indeed been shown to be active at the histamine H_2 receptor; amthamine[14,15] **3** (**Fig 4.1**) is the most potent H_2 agonist in this series. These compounds show moderate to strong H_2 receptor agonistic activity compared to histamine. Moreover, these thiazole analogues of histamine have very less effects on H_1 and H_3 receptors.

Realizing that 4-methyl-2-aminothiazoles are capable of stimulating the H_2 receptor, a series of impromidine and arpromidine analogues, such as **7a**[16], was synthesized by replacing the 3-[(1*H*-imidazolyl-4-yl)propyl] group with a 3-(4-methyl-2-aminothiazol-5-yl)propyl moiety. Such compounds are reported to be selective agonists for the H_2 receptor, in particular *versus* the H_3 receptor[17], i. e. the imidazoles and the aminothiazoles may be considered bioisosteres at histamine H_2 but not at H_3 receptors.

The strongly basic guanidino group (pK_a value about 13), which is supposed to interact with Asp-98 in transmembrane domain 3, is essential for the agonistic activity of impromidine and analogues, but it is also the main reason for very low oral bioavailability, non-H_2R-mediated effects and lack of penetration across the blood-brain barrier[18]. A major advancement was achieved by replacing the guanidino group by an acylguanidine moiety (see **Chapter 3**) resulting in potent H_2R agonists with reduced basicity and improved pharmacokinetic properties. Therefore, we decided to synthesise a series of *N*-acyl-*N*'-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines. According to this concept the title compounds should combine advantages of the acylguanidines with improved H_2R selectivity, in particular the histamine H_3 receptor antagonistic activity was expected to be considerably reduced.

4.2. Chemistry

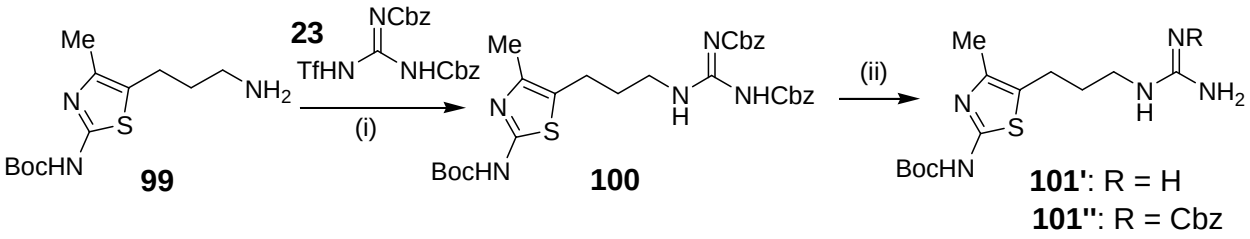
The 2-[3-(2-amino-4-methylthiazol-5-yl)propyl]isoindoline-1,3-dione (**97**) (**Scheme 4.1**) was synthesized according to Eriks[19-22] from the chloroketone **94** via phthalimide protection, regioselective bromination, and ring closure of 3-bromo-6-(phthalimido)hexan-2-one (**96**) with thiourea as presented in Scheme 1. Subsequently, the 2-amino group was Boc-protected and, finally, the phthalimide was deprotected by hydroginolysis.



Reagents: (i) K_2CO_3, phthalimide, DMF, 80 °C, 24 h, 88 %; (ii) Br_2, CCl_4, rt, 1h, 90 %; (iii) thiourea, DMF, 100 °C, 3 h, 49%; (iv) $(Boc)_2O$, Et_3N, DMAP, $CHCl_3$, rt, 6 h, 86 %; (v) $NH_2NH_2 \cdot H_2O$, EtOH, rt, 16 h, 82 %.

Scheme 4.1: Synthesis of Boc-protected homoamthamine

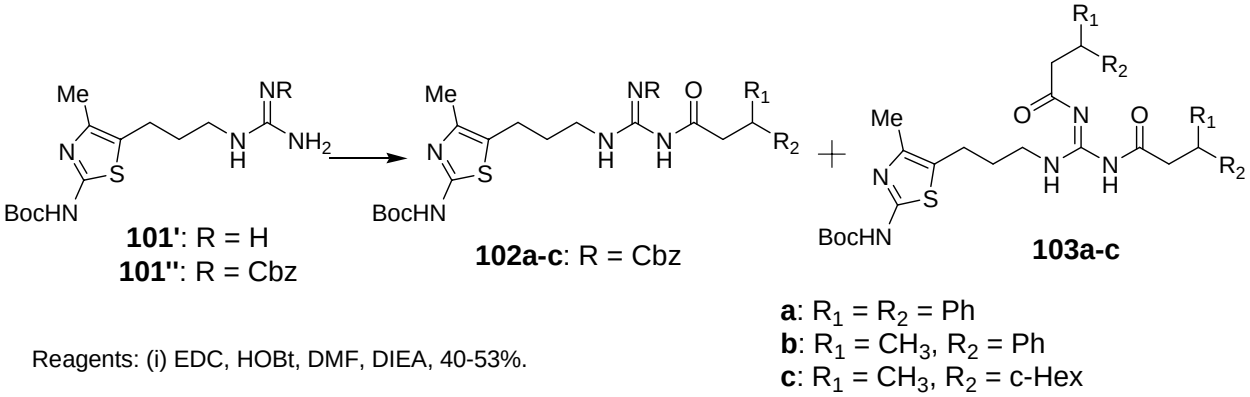
The guanidino group was attached to the propyl chain by treating the amine **99** with di-Cbz-triflate-protected guanidine (**23**, **Chapter 3**, **Scheme 3.5**), followed by deprotection of the Cbz-groups by hydrogenolysis (**Scheme 4.2**).



Reagents: (i) **23**, Et_3N, $CHCl_3$, rt, 93 %; (ii) H_2 / Pd-C, MeOH : THF (1:1), rt, 4 h

Scheme 4.2: Introduction of the guanidino group

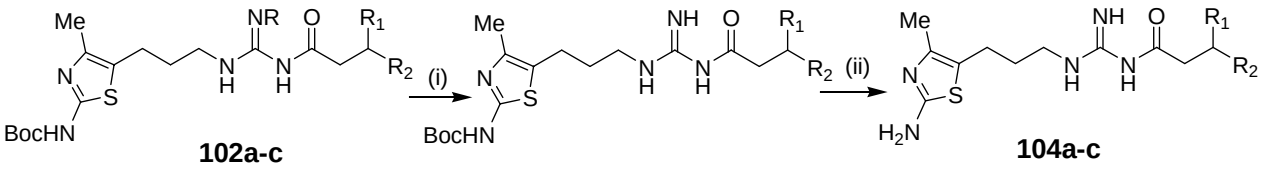
The acylation of compound **101´** was very interesting. Under similar reaction condition as described for acylation of imidazolylpropylguanidine (**Chapter 3**), **101´** gave the diacylated product **102** instead of the monoacylated one (**102**). But with the mixture of mono Cbz protected (**101´´**) and deprotected guanidines (**101´**), two products were obtained as shown in **Scheme 4.3**.



Reagents: (i) EDC, HOBt, DMF, DIEA, 40-53%.

Scheme 4.3: Coupling of protected guanidine with acids

The two products were separated by column chromatography. Subsequently, the Cbz-substituted acylguanidines (**102a-c**) were hydrogenolyzed to deprotect the Cbz group and then treated with 50 % TFA to remove the Boc protecting group (**Scheme 4.4**).



Reagents: (i) H_2/Pd-Cs, MeOH, rt, 8-12 h; (ii) 20 % TFA, CH_2Cl_2, rt, 90-96 %.

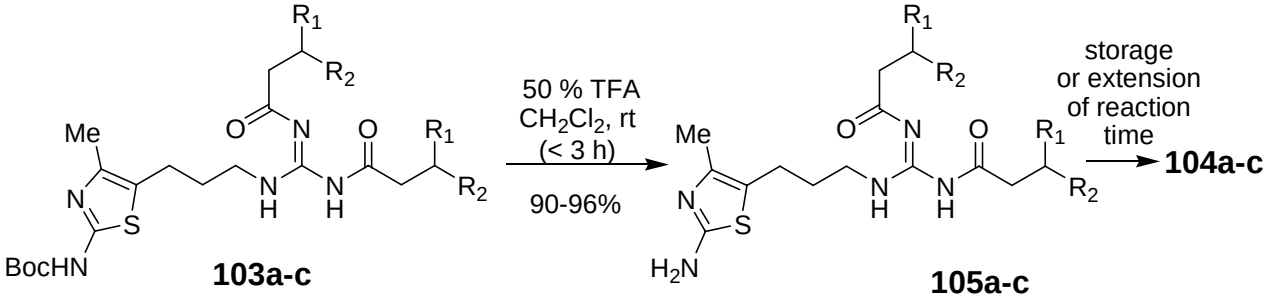
Scheme 4.4: Deprotection of Cbz and Boc groups

Table 4.1: Yields, formulas and analytical data of compounds **104a-c**

No.	R_1	R_2	Yield (%)[a]	Formula[b]	HRMS: *m/z* Found (calcd.)[c]	R_t[d]
104a	Ph	Ph	83	$C_{23}H_{27}N_5OS$ (2 TFA)	421.1935 (421.1936)	13.50
104b	CH_3	Ph	92	$C_{18}H_{25}N_5OS$ (2 TFA)	359.1778 (359.1779)	11.75
104c	CH_3	c-Hex	92	$C_{18}H_{31}N_5OS$ (2 TFA)	365.2250 (365.2249)	13.62

[a] After preparative HPLC; [b] isolated as trifluoroacetic acid salt; [c] Techniques for HRMS is described in experimental section of respective compounds; [d] retention time in minutes; the solvent system is presented in experimental section.

On the other hand, the Boc-protected di-acylated products (**103a-c**) were deprotected to the corresponding aminothiazoles **105a-c** by treating with 50 % TFA for short time (2-3 h) and isolated by preparative HPLC (**Scheme 5**). However, stirring the reaction for a longer period of time (e.g. overnight) or storage of **105a-c** · 2 TFA for a few days, led to slow decomposition of the corresponding monoacylated products **104a-c** (analyzed by HPLC).



Scheme 5.5: Deprotection of Boc group

4.3. Pharmacological Results and Discussion

As for the imidazolylpropylguanidines in section **3.3** (**Chapter 3)**, synthesized compounds were tested for histamine H_2R activity on the isolated spontaneously beating guinea pig right atrium

(positive chronotropic response) and were also investigated for H_1 antagonism on the isolated guinea pig ileum and on U-373 MG human cells. The results are summarized in **Table 4.3**. Moreover, the compounds were investigated for activity in the GTPase assay using guinea pig and human H_2R expressing Sf9 insect cells (co-operation with Prof. Seifert, Institute of Pharmacy, University of Regensburg) in **Table 4.4**. The compounds were investigated for receptor specificity on H_3 receptor antagonism on the isolated guinea pig ileum. The results are summarized in **Table 4.5**.

Table 4.3: Histamine H_2 receptor agonism on guinea pig atrium, H_1 receptor antagonism on guinea pig ileum and on U-373MG cells (Ca^{2+}-assay)

	Histamine H_2 receptor agonism				Histamine H_1 receptor antagonism		
	Guinea pig atrium				Guinea pig ileum		U-373MG cells (Ca^{2+}-assay)
No.	pEC_{50}[a] ± SEM[i]	Rel. pot[b]	E_{max} (%)[c]	N[d]	pA_2[a,c] ± SEM [or pD'_2[a,c] ± SEM][e]	N[d]	IC_{50} [μM][h]
HIS	6.00 ± 0.10	100	100 ± 2	>50	6.70 ±		-
63	7.22 ± 0.09	1650	85 ± 3	5	5.93 ± 0.05[f] [5.00 ± 0.09]	10 18	-
78	7.45 ± 0.07	2710	99 ± 2	4	6.31 ± 0.06	2	19.5
88	6.81 ± 0.07	641	101 ± 3	3	5.43 ± 0.009	5	0.95
104a	7.14 ± 0.11	1370	71 ± 4	4	5.64 ± 0.05[f] [5.48 ± 0.12]	8 8	13
104b	7.18 ± 0.17	1520	89 ± 3	4	5.48 ± 0.10[g]	6	18
104c	7.03 ± 0.06	1070	74 ± 8	3	6.36 ± 0.04[g]	11	10

[a] Explanation of abbreviations see appendix; [b] Potency relative to histamine = 100 %, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine.[d] Number of experiments; [e] pD'_2 values given in brackets for compounds producing a significant, concentration-dependent reduction of histamine's maximal response; [f] non-surmountable; [g] competitive/non-surmountable; [h] IC_{50} values for the inhibition of the histamine (30 μM) induced increase in intracellular calcium; mean of at least 3 independent experiments; SEM < 10 %; [i] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by the equation: $pEC_{50} = 6.00 + \Delta pEC_{50}$. The *SEM* given for pEC_{50} is the *SEM* calculated for ΔpEC_{50};

On replacement of the 3-(imidazol-4-yl)propyl moiety of acyl guanidines (**63, 78** and **88**) by 3-(2-amino-5-methylthiazol-4-yl)propyl (in **104a-c** respectively), the synthesized compounds are

found to be partial agonists (intrinsic activities 70 – 90 %) on spontaneously beating guinea pig right atrium (H_2 receptor). On the guinea pig ileum as well as on human U373 cells all tested compounds proved to be devoid of histamine H_1 receptor agonistic activity and to have only weak H_1R antagonistic activity.

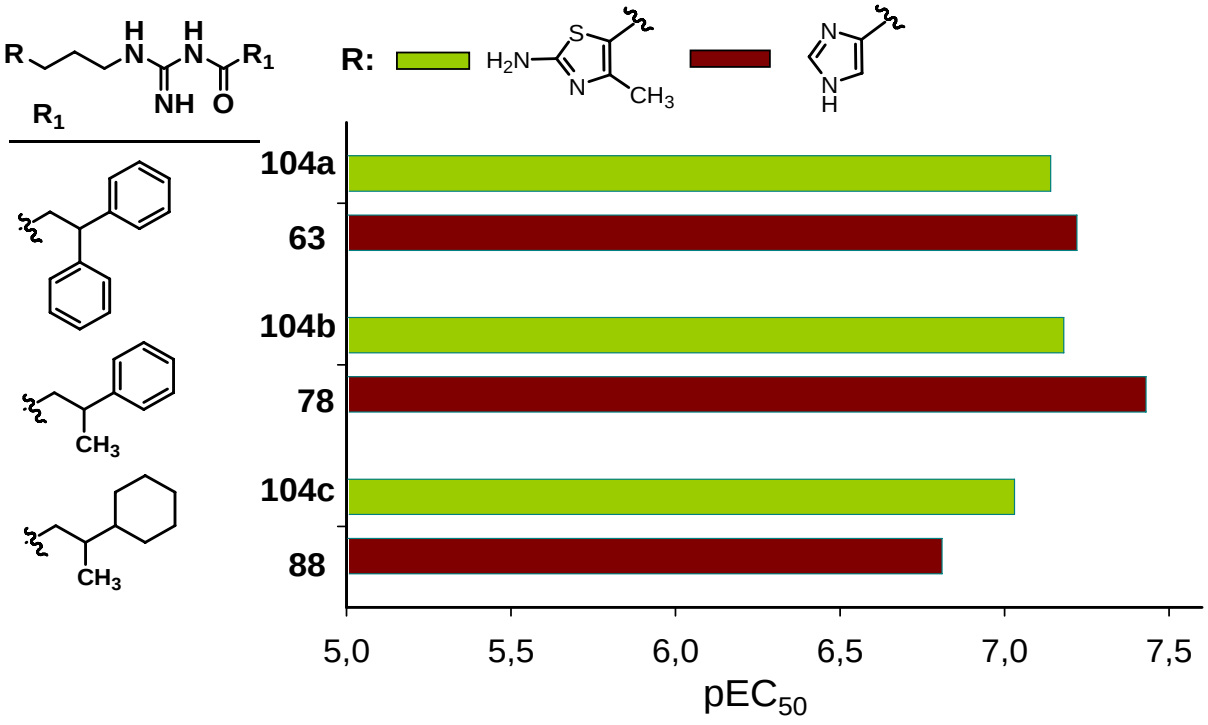


Fig. 4.4: Comparison of potency (pEC_{50} values) on guinea pig right atrium of imidazolyl (in dark) and 2-amino-4-methyl-thiazolyl (in light) guanidines (from **Table 4.3**)

Histamine H_2R agonism on the isolated guinea pig right atrium

As shown in **Fig. 4.4**, in case of the diphenylpropanoylguanidines the compound with imidazolylpropyl moiety and the corresponding aminothiazolylpropyl analogue are about equipotent on the guinea pig atrium (**63** vs. **104a**; difference in pEC_{50} = 0.08), whereas for the two 3-phenylbutanoylguanidines there is a small difference (**78** vs. **104b**: ΔpEC_{50} = 0.27). Comparing the 3-cyclohexylbutanoylguanidines **88** and **104c**, the aminothiazole **104c** was found to be slightly more active than the corresponding imidazole **88**. However, the intrinsic activities (*Emax*) of the aminothiazoles **104a-c** were by 10 - 30 % lower than those of the corresponding imidazoles (**Table 4.3**).

Species selectivity - agonism on guinea pig and human H_2R-$G_{s\alpha}$ fusion proteins

The synthesized compounds were also tested for potency and efficacy on hH_2R-$G_{s\alpha s}$ and gpH_2R-$G_{s\alpha s}$ fusion proteins expressed in Sf9 cells (investigations on cell membrane preparations according to the procedure reported by Kelley *et al*.)[23]. The results are summarized in **Table 4.4**. Compared to imidazolylpropylguanidines (**63**, **78** and **88**), the corresponding aminothiazolyl-propyl analogues (**104a-c**) are found to have little less efficacy at both hH_2R-$G_{s\alpha s}$ and gpH_2R-$G_{s\alpha s}$ (in **Table 4.4**), but the difference of efficacy between hH_2R-$G_{s\alpha s}$ and gpH_2R-$G_{s\alpha s}$ is about the same (0.15- 0.25 logarithm units).

Table 4.4: Agonistic activity on the human and guinea pig histamine H_2 receptor (GTPase assay*; gpH_2R-$G_{s\alpha s}$ fusion proteins and hH_2R-$G_{s\alpha s}$ fusion proteins)

	hH_2R-$G_{s\alpha s}$			**gpH_2R-$G_{s\alpha s}$**			**EC_{50} hH_2R-$G_{s\alpha s}$ / EC_{50} gpH_2R-$G_{s\alpha s}$**
No	Efficacy	pEC_{50}	Rel. Pot.	Efficacy	pEC_{50}	Rel. Pot.	
HIS	1,00	5.90	100	1,00	5.92	100	1,05
63	0.69	7.11	1600	0.93	8.22	19000	12.1
104a	0.61	6.93	1071	0.86	7.59	4677	4.37
78	0.87	7.17	1880	1.03	7.92	10300	5.58
104b	0.82	7.32	2630	0.98	7.65	5370	2.04
88	0.87	7.64	5480	1.11	8.04	2920	2.68
104c	0.56	8.04	13804	0.81	8.15	16982	1.23

***** GTP hydrolysis was determined according to the procedure described by Kelley *et al*. using cell membrane preparations of Sf9 cells expressing the respective H_2R $G_{s\alpha s}$ fusion proteins. For details see ref.[23] and footnote to **Table 3.3** in **Chapter 3**.

The order of potency (pEC_{50}) for the 2-amino-4-methylthiazolyl series (**104a-c**) on gpH_2R-$G_{s\alpha s}$ assay was in agreement with the atrium values. For both series of agonists, thiazoles and imidazoles, the potency is higher at gpH_2R than at hH_2R. But in case of imidazolyl series (**63**, **78** and **88**), the ratios of EC_{50} values between hH_2R-$G_{s\alpha s}$ and gpH_2R-$G_{s\alpha s}$ are 12.1, 5.6 and 2.7, whereas for 2-amino-4-methyl-thiazolyl series (**104a-c**) the ratios are 4.4, 2.1 and 1.2 respectively. This result indicates a tendency towards higher activity of 3-(2-amino-4-methyl-thiazolylpropyl)guanidines compared to the corresponding imidazoles at the human H_2 receptor.

If we compare the pEC_{50} values of imidazolyl analogues (**63**, **78** and **88**) and 2-amino-4-methyl-thiazolyl series (**104a-c**) for $hH_2R\text{-}G_{s\alpha s}$ (graphical presentation in **Fig. 5.5**), the 3-cyclohexylbutanoylguanidine is more favoured by human H_2 receptor compared to guinea pig.

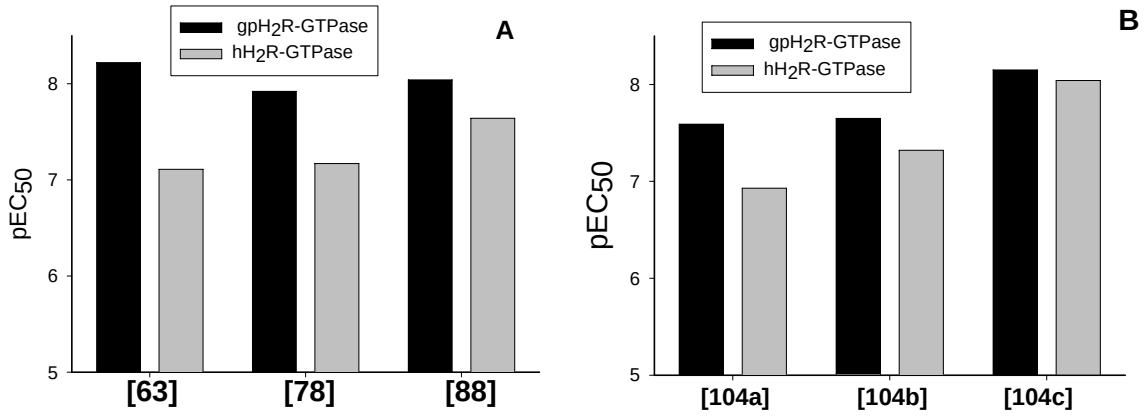


Fig. 4.5: H_2R agonistic potencies (pEC_{50} values) of imidazolylpropyl (**A**) and 2-amino-4-methyl-thiazolylpropyl (**B**) guanidines on guinea pig and human $H_2R\text{-}G_{s\alpha}$ fusion proteins (from **Table 4.4**)

Histamine H_3R antagonism on the guinea pig ileum: receptor selectivity

The first identified histamine H_3 receptor antagonists were mono-substituted imidazoles. Meanwhile numerous imidazole-type H_3R antagonists are described.[24] Imidazolylpropylguanidine-type histamine H_2R agonists such as arpromidine or impromidine are also known to be H_3R antagonists. Therefore, a selection of the synthesized H_2R agonists was investigated for activity at H_3 receptors. The field stimulated guinea pig ileum longitudinal muscle was used as a model (data see **Table 5.5**).[a]

As expected the imidazolylpropylguanidine-type H_2R agonists (**61**, **63**, **71**, **78**, **88**), proved to be moderate to potent H_3R antagonists on the guinea pig ileum. The maximal response of the H_3R agonist (*R*)-α-methylhistamine was not significantly affected. The lowest pA_2 value was found for "oxo-impromidine" (**61**), whereas compound **78** (pA_2 8.33) was the most potent H_3R antagonist in this series. However, the aminothiazole analogues **104a-c** were inactive at the

[a] Prior to these investigations the compounds were tested for antagonistic activity on muscarinic M_3 receptors (guinea pig ileum; see experimental section). All test compounds proved to be only very weak M_3 antagonists ($pA_2 < 6$). In order to avoid measurement artefacts the investigations for H_3R antagonism were generally performed with antagonist concentrations at least half an order of magnitude below the K_B value for M_3R antagonism.

concentrations tested, confirming the working hypothesis that the aminothiazole and the imidazole moiety are bioisoteric groups at H_2R but not at H_3R. By this approach the histamine H_2R selectivity (vs. H_3R) can be considerably increased.

Table 5.5: H_3R antagonistic activity of selected compounds
(guinea pig ileum: field stimulated longitudinal muscle with adhering myenteric plexus)

No	N[a]	c(antagonist) [nM]	$pA_2 \pm SEM$[b]	$E_{max} \pm SEM$[c] [%]	$E_{max} \pm SEM$[d] [%]	N[e]
61	4	1000	6.86 ± 0.06	106 ± 15	110 ± 1	3
63	4	100	7.81 ± 0.09	93 ± 10	91 ± 8	7
71	4	100	7.90 ± 0.05	95 ± 15	93 ± 10	4
78	4	100	8.33 ± 0.08	75 ± 7	84 ± 6	4
88	4	100	7.64 ± 0.11	72 ± 7	87 ± 8	6
104a	3	500	[–0.18 ± 0.07]	79 ± 5	85 ± 9	4
104b	3	500	[0.06 ± 0.12]	85 ± 1	85 ± 9	4
104c	3	500	[0.14 ± 0.02]	88 ± 7	85 ± 9	4

[a] Number of experiments (tissues from 2 – 4 animals). [b] Corrected ΔpEC_{50} values given in brackets for compounds producing no relevant shift by the concentration tested. For these compounds, pA_2 was < 6.3. [c] E_{max} of (*R*)-α-methylhistamine in the presence of the compound. [d] E_{max} of (*R*)-α-methylhistamine in untreated control tissues (second concentration-effect curve). [e] Number of untreated control tissues (from 2 – 4 animals).

As expected the imidazolylpropylguanidine-type H_2R agonists (**61**, **63**, **71**, **78**, **88**), proved to be moderate to potent H_3R antagonists on the guinea pig ileum. The maximal response of the H_3R agonist (*R*)-α-methylhistamine was not significantly affected. The lowest pA_2 value was found for "oxo-impromidine" (**61**), whereas compound **78** (pA_2 8.33) was the most potent H_3R antagonist in this series. However, the aminothiazole analogues **104a-c** were inactive at the concentrations tested, confirming the working hypothesis that the aminothiazole and the imidazole moiety are bioisoteric groups at H_2R but not at H_3R. By this approach the histamine H_2R selectivity (vs. H_3R) can be considerably increased.

4.4. Conclusion

The bioisosteric replacement of the imidazolyl moiety in N^G-acylated guanidine-type histamine H_2 receptor agonists by a 2-amino-4-methylthiazol-5-yl group resulted in about the same H_2R

agonistic potency in the isolated guinea pig right atrium as well as in GTPase assays performed with both guinea pig and human H_2R-$G_{s\alpha S}$ fusion proteins. However, the maximal response induced by the thiazoles was reduced, i. e. these compounds were partial agonists in the used pharmacological models. Interestingly, in the GTPase assay the thiazolyl analogue with cyclohexylbutanoyl substituent was favoured compared to the corresponding phenylbutanoyl-guanidine on the human H_2R. This could provide a hint that a certain degree of selectivity for the human H_2R may be achieved by structural variation of both the acyl and the heterocyclylpropyl group. Moreover, the aminothiazoles proved to be devoid of H_3R antagonistic activity. Thus, the selectivity for H_2R versus H_3R can be considerably improved.

4.5. Experimental section

4.5.1. Chemistry:

General procedures

For detailed description of the general procedures and equipments, see section **3.6.1** of **Chapter 3**.

2-(5-Oxohexyl)isoindoline-1,3-dione [95][25]

A mixture of phthalimide (6.3 g, 0.043 mol), 6-chloro-2-hexanone (11.5 g, 0.086 mol), potassium carbonate (9.0 g, 0.064 mol) and N,N-dimethylformamide (100 mL) was heated to 80°C for 24 h. After cooling to room temperature, the mixture was added to ice cold water and extracted with chloroform. The organic solvent was removed under reduced pressure to get a viscous reaction mixture. Column chromatography of the residue gave a solid.

Yield 88 %; colorless crystalline solid; $C_{14}H_{15}NO_3$ (245.3); mp 57°C; IR (neat): 1768, 1706, 1437, 1392, 1357, 1257, 1163, 1122, 1045, 890, 793, 720 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.66-7.90 (m, 4H, Phth-***H***), 3.70 (t, *J* = 6.3 Hz, 2H, COC***H***$_2$), 2.52 (t, *J* = 6.8 Hz, 2H, Phth-C***H***$_2$), 2.14 (s, 3H, C***H***$_3$CO), 1.48-1.80 (m, 4H, MeCOCH$_2$C***H***$_2$C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 208.3 (quart, CH$_3$***C***O), 168.4 (quart, ***C***(O)N), 133.9 (+, Phth-***C***H), 132.1 (quart, Phth-***C***), 123.2 (+, Phth-***C***H), 42.8, 37.5 (-, ***C***H$_2$), 29.9 (+, ***C***H$_3$), 27.9, 20.7 (-, ***C***H$_2$); EI-MS, *m/z* (rel. intensity, %): 245 ([M]$^{+\cdot}$, 17), 202 ([M-CH$_3$CO]$^+$, 12), 188 ([M-CH$_3$COCH$_2$]$^+$,66), 174 ([Phth-CH$_2$CH$_2$]$^+$, 23), 160

([Phth-CH_2]$^+$, 100), 148 (6), 133 (10), 130 (18), 104 (3), 77 (20), 43 (51): Anal. ($C_{14}H_{15}NO_3$) C, H, N.

2-(4-Bromo-5-oxohexyl)isoindoline-1,3-dione [96][25]

Bromine (14.5 g (0.09 mole) was cautiously added to a solution of 6-phthalimidohexane-2-one (**95**) (23.0 g, 0.09 mol) in carbontetrachloride (150 mL) with vigorous stirring at room temperature. After decoloration of the reaction mixture the stirring was continued at room temperature for 2 h. Subsequently, chloroform (100 mL) and water (300 mL) were added and the mixture was stirred for 30 minutes. The water phase was removed and the organic phase was washed with water until neutralized. The organic phase was subsequently dried on anhydrous sodium sulphate, filtered and vacuum concentrated. The residue (viscous oil) was used without any purification for the preparation of 2-amino-4-methyl-5-(3-phthalimidopropyl)thiazole (**97**).

Yield 92 %; colorless viscous oil; $C_{14}H_{14}BrNO_3$ (324.2); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.60-7.95 (m, 4H, Phth-***H***), 4.36 (t, *J* = 7.2 Hz, 1H, C***H***Br), 3.46-3.90 (m, 2H, C***H***$_2$N), 2.36 (s, 3H, C***H***$_3$CO), 1.74-2.22 (m, 4H, CHBrC***H***$_2$C***H***$_2$).

2-[3-(2-Amino-4-methylthiazol-5-yl)propyl]isoindoline-1,3-dione [97][25]

To a solution of crude 2-(4-bromo-5-oxohexyl)isoindoline-1,3-dione (**96**) (20.0 g, 0.06 mol) in anhydrous DMF (50 mL) a solution of thiourea (4.5 g, 0.06 mol) in 50 mL of dry DMF was added with stirring. After the exothermic reaction (the temperature may rise to 100°C), the mixture is heated at 100°C for 3h. After cooling the reaction mixture is high vacuum concentrated, then to the residue an ethylacetate/methanol mixture (1:1, v/v) is added. After stirring for 30 minutes the precipitate is filtered off, washed subsequently with ethyl acetate and diethyl ether and thereafter dried *in vacuo*.

Yield 49 %; colorless solid; $C_{15}H_{15}N_3O_2S$ (301.4); mp 242 ^{o}C; IR (neat): 1769, 1711, 1623, 1464, 1438, 1398, 1337, 1293, 1270, 1120, 1067, 1039, 872, 719 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 9.23 (broad s, 2H, N***H***$_2$), 7.88 (s, 4H, Ar-***H***), 3.64 (t, *J* = 6.6 Hz, 2H, Thiaz-C***H***$_2$), 2.70 (t, *J* = 8.1 Hz, 2H, PhthN-C***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.62-2.04 (m, 2H, Thiaz-CH_2C***H***$_2$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 167.9 (quart, ***C***O), 167.5 (quart, Thiaz-C-2), 134.2 (+, Phth-***C***), 131.6 (quart,

Phth-C), 130.8 (quart, Thiaz-***C***-4), 122.8 (+, Phth-***C***), 116.7 (quart, Thiaz-C-5), 36.5 (-, PhthN-***C***), 28.6 (-, Thiaz-***C***), 22.3 (-, Thiaz-CH_2***C***H_2), 11.2 (+, Thiaz-4-***C***H_3); EI-MS *m/z,* 301 ($[M]^{+\cdot}$, 24), 169 ($[C_7H_{11}N_3S]^+$, 21), 160 (8), 141 (15), 127 ($[C_5H_7N_2S]^+$, 100), 100 (19), 77 (8), 28 (37); Anal. ($C_{15}H_{15}N_3O_2S$) C, H, N.

***tert*-Butyl 4-methyl-5-[3-(1,3-dioxoisoindolin-2-yl)propyl]thiazol-2-ylcarbamate [98]**

2-[3-(2-Amino-4-methylthiazol-5-yl)propyl]isoindoline-1,3-dione (**97**) (6 g, 20 mmol) was dissolved in $CHCl_3$ (50 mL), di-*tert*-butyl dicarbonate (4.8 g, 22 mmol, 1.1 eqiuv.), triethylamine (3.4 mL, 24 mmol, 1.2 equiv) and 4-dimethylaminopyridine (DMAP, 10 mg) were added, and the mixture was stirred for 6h at room temperature. Subsequently, the mixture was diluted with dichloromethane (100 mL), washed with 0.1N HCl, brine, and then water and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography to give a foam-like solid.

Yield 86 %; colorless foam-like solid; $C_{20}H_{23}N_3O_4S$ (401.5); mp 71 ^oC; IR (neat): 1771, 1708, 1553, 1437, 1394, 1368, 1296, 1244, 1153, 1069, 1038, 870, 791, 716 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.89-7.79 (m, 2H, Phth-***H***), 7.77-7.66 (m, 2H, Phth-***H***), 3.75 (t, *J* = 6.9 Hz, 2H, Thiaz-C***H***$_2$), 2.72 (t, *J* = 7.8 Hz, 2H, Phth-NC***H***$_2$), 2.24 (s, 3H, Thiaz-4-C***H***$_3$), 1.99 (m, 2H, Thiaz-CH_2C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 168.2 (quart, Phth-***C***O), 158.5 (quart, Thia-***C***-2), 152.4 (quart, tBuO-***CO***), 140.2 (quart, Thiaz-***C***-4), 134.0 (+, Phth-***C***), 132.0 (quart, Phth-C), 123.2 (+, Phth-***C***), 122.9 (quart, Thiaz-***C***-5), 82.7 (quart, ***C***$(CH_3)_3$), 37.4 (-, Phth-***C***H_2), 30.0 (-, Thiaz-5-***C***H_2), 28.2 {+, C(***C***H_3)$_3$}, 23.5 (-, Thiaz-5-CH_2***C***H_2), 14.0 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 402 ($[M+H]^+$); Anal. ($C_{20}H_{23}N_3O_4S$) C, H, N.

***tert*-Butyl 5-(3-aminopropyl)-4-methylthiazol-2-ylcarbamate [99]**

To a suspension of **98** (6.7 g, 16.7 mmol) in ethanol (60 mL) was added hydrazine monohydrated (4.0 mL, 83.5 mmol). After 30 min of stirring at room temperature, the solid was dissolved and the solution was further stirred overnight at same temperature. After the mixture was cooled in ice bath the precipitated was removed by filtration and the filtrate was concentrated to dryness.

The residue was purified by column chromatography on silica by using chloroform and methanol with 1 % of triethylamine.

Yield 82 %; grey colored sticky oil; $C_{12}H_{21}N_3O_2S$ (271.4); IR (neat): 1713, 1555, 1452, 1367, 1297, 1244, 1154, 1068, 871, 764, 690 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 5.9 (br, N***H***$_2$), 2.80 (t, *J* = 7.1 Hz, 2H, C***H***$_2$NH$_2$), 2.77-2.63 (m, 2H, Thiaz-5-C***H***$_2$), 2.26 (s, 3H, Thiaz-4-C***H***$_3$), 1.82 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.51 (s, 9H, C(C***H***$_3$)$_3$; ^{13}C-NMR ($CDCl_3$) δ: 157.9 (quart, Thia-***C***-2), 153.0 (quart, Boc-***CO***), 141.5 (quart, Thia-***C***-4), 123.5 (quart, Thia-***C***-5), 81.9 {quart, ***C***(CH$_3$)$_3$}, 40.9 (-, ***C***H$_2$NH$_2$), 34.3 (-, Thiaz-5-***C***H$_2$), 28.2 {+, C(***C***H$_3$)$_3$}, 23.3 (-, Thiaz-5-CH$_2$***C***H$_2$), 14.4 (+, Thiaz-4-***C***H$_3$); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 215 ([(M-C_4H_8) + H]$^+$), 272 ([M+H]$^+$), 543 ([2M + H]$^+$).

<u>*tert*-Butyl 5-[3-[*N*^{2},*N*^{3}-bis(benzyloxycarbonyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate [100]</u>

The amine **99** (3.98 g, 14.7 mmol) was dissolved in 40 mL of $CHCl_3$ and added to a solution of *N*^{1},*N*^{2}-bis(benzyloxycarbonyl)-*N*^{3}-trifluoromethanesulphonylguanidine (**23**, 6.14 g, 13.4 mmol) and triethylamine (1.49 g, 14.74 mmol) in $CHCl_3$ (40 mL) and the mixture was allowed to stir at rt until the triprotected guanidine **23** was consumed (4 h) as evidenced by TLC. After the reaction was complete, the mixture was diluted with $CHCl_3$ (100 mL) and washed with 2 M sodium bisulphate, saturated sodium bicarbonate, and brine. The organic extract was then dried over sodium sulphate and filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography to afford a colorless solid.

Yield **93** %; colorless solid; $C_{29}H_{35}N_5O_6S$ (581.7); mp 110°C; IR (in CH_2Cl_2): 1726, 1638, 1572, 1427, 1382, 1302, 1263, 1203, 1156, 1044, 731, 698 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 11.74 (br., 1H, N***H***), 8.36 (t, *J* = 5.3 Hz, 1H, CH$_2$N***H***), 7.43-7.23 (m, 10H, Ph-***H***), 5.17 (s, 2H, Ph-C***H***$_2$O), 5.12 (s, 2H, Ph-C***H***$_2$O), 3.47 (m, 2H, C***H***$_2$NH), 2.70 (t, *J* = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.23 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 {s, 9H, C(C***H***$_3$)$_3$}; ^{13}C-NMR ($CDCl_3$), δ (ppm): 163.6 (quart, Thiaz-***C***-2), 157.9 (quart, PhCH$_2$O-***C***O), 153.8 (quart, tBuO-***C***O), 152.6 (quart, PhCH$_2$O-***C***O), 141.5 (quart, Thiaz-***C***-4), 136.7 (quart, Ph-***C***-1), 134.5 (quart, Ph-***C***-1), 128.8, 128.6, 128.5, 128.4, 128.1, 127.9 (+, Ph-***C***), 122.9 (quart, Thia-***C***-5), 82.4 {quart, ***C***(CH$_3$)$_3$}, 68.1, 67.1 (-, Ph***C***H$_2$), 40.1 (-, ***C***H$_2$NH), 30.6 (-, Thiaz-5-***C***H$_2$), 28.2 {+, C(***C***H$_3$)$_3$},

23.2 (-, Thiaz-5-CH_2***C***H_2), 14.3 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 582 ($[M+H]^+$), 1163 ($[2M+H]^+$), 1185 ($[2M+Na]^+$); Anal. ($C_{29}H_{35}N_5O_6S$) C, H, N.

***tert*-Butyl 5-(3-guanidinopropyl)-4-methylthiazol-2-ylcarbamate [101']**

To a solution of **100** (10 mmol) in a mixture of 150 mL THF and MeOH (1:1) was added 0.5 g of Pd/C (10 %) and then the mixture was stirred under H_2 (filled with balloon) overnight. The catalyst was removed by filtration through celite and washed with MeOH. The solvent was evaporated *in vacuo* to obtain a foam-like solid. However, when the reaction mixture was stirred for only 6h, there was a partial deprotection and the remaoval of solvent after filtration gave a mixture of **101'** and **101''** (40:60).

Yield 96 %; colorless solid; $C_{13}H_{23}N_5O_2S$ (313.4); mp 147 ^oC; IR (neat): 1622, 1555, 1451, 1366, 1294, 1246, 1154, 1053, 869, 784, 689 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 3.19 (t, *J* = 6.8 Hz, 2H, C***H***$_2$NH), 2.69 (t, *J* = 7.4 Hz, 2H, Thiaz-C***H***$_2$), 2.11 (s, 3H, Thiaz-4-C***H***$_3$), 1.83 (m, 2H, CH_2C***H***$_2$$CH_2$NH), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ^{13}C-NMR (CD_3OD), δ (ppm): 163.7 (quart, Thiaz-***C***-2), 158.8 (quart, tBuO-***C***O), 158.2 (quart, ***C***=NH), 142.3 (quart, Thiaz-***C***-4), 122.5 (quart, Thiaz-***C***-5), 80.7 (quart, Boc-***C***), 41.4 (-, ***C***H_2NH), 31.6 (-, Thiaz-5-***C***H_2), 28.8 {+, C(***C***H_3)$_3$}, 23.9 (-, Thiaz-5-CH_2***C***H_2), 14.4 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 314 ($[M+H]^+$).

General procedure for the preparation of the acylguanidines 102a-c and 103a-c

To a solution of 3,3-diphenylpropanoic acid, (*R*,*S*)-3-phenylbutanoic acid or (*R*,*S*)-3-cyclohexylbutanoic acid (1 equivalent), EDC (1 equivalent) and HOBt (1 equivalent) in CH_2Cl_2 (3 mL) under argon, was added DIEA (1 mmol) and stirred for 10 min. To this mixture a solution of 1 mmol of **101'** and **101''** (used as mixture of both, ratio = 40:60) in CH_2Cl_2 (2 mL) was added dropwise, and stirred overnight (12-16 h) at rt. After removing the solvent, the residue was dissolved in EtOAc, 5 mL of water was added, the organic layer was separated, and the aqueous phase extracted with EtOAc. After drying over anhydrous sodium sulphate, the solvent was removed *in vacuo* and the residue was purified to give monoacylated (**102a-c**) and diacylated (**103a-c**) products.

***tert*-Butyl 5-[3-[*N*^{2}-benzyloxycarbonyl-*N*^{3}-(3,3-diphenylpropanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate [102a]**

Stirring for 12h; yield 48 %; colorless sticky liquid; $C_{36}H_{41}N_5O_5S$ (655.8); IR (neat): 1714, 1643, 1560, 1451, 1368, 1300, 1245 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 12.32 (s. br, 1H, N***H***), 8.94 (m, 1H, N***H***CH_2), 7.43-7.09 (m, 15H, Ph-***H***), 5.14 (s, 2H, OC***H***$_2$Ph), 4.57 (t, *J* = 8.0 Hz, 1H, Ph_2C***H***), 3.40 (m, 2H, C***H***$_2$NH), 3.14 (d, *J* = 7.9 Hz, 2H, COC***H***$_2$), 2.63 (t, *J* = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.51 {s, 9H, (C***H***$_3$)$_3$ of Boc}; ^{13}C-NMR (CD_3OD), δ (ppm): 173.0 (quart, ***C***OCH_2), 163.7 (quart, Thiaz-***C***-2), 158.2 (quart, ***C***(=O)OBn), 156.2 (quart, ***C***(=O)O-^{t}Bu), 152.2 (quart, ***C***(=N)), 144.5 (quart, Thiaz-***C***-4), 142.6, 136.6 (quart, Ph-***C***-1), 128.7, 128.4, 128.1, 127.9, 127.8, 127.5, 126.8, 126.3, 126.1, 125.8 (+, ***C*** of Ph), 123.1 (quart, Thiaz-***C***-5), 82.9 (quart, ***C*** of Boc), 67.3 (-, C***H***$_2$Ph), 46.5 (+, Ph_2***C***), 44.1 (-, CO***C***H_2), 39.8 (-, ***C***H_2NH), 30.3 (-, Thiaz-5-***C***H_2), 28.2 {+, (***C***H_3)$_3$ of Boc}, 23.1 (-, Thiaz-5-CH_2***C***H_2), 13.9 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 656 ($[M + H]^+$), 556 {$[(M\text{-Boc}) + H]^+$}.

***tert*-Butyl 5-[3-[*N*^{2},*N*^{3}-bis(3,3-diphenylpropanoyl)guanidino]propyl]-4-methylthiazol-2-yl-carbamate [103a]**

Yield 30 %; colorless sticky liquid; $C_{43}H_{47}N_5O_4S$ (729.9); IR (neat): 1714, 1618, 1556, 1493, 1450, 1368, 1301, 1264, 1246 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 13.09 (s. br, 1H, N***H***), 8.91 (m, 1H, N***H***CH_2), 7.31-7.10 (m, 20H, Ph-***H***), 4.60 (t, *J* = 7.9 Hz, 1H, Ph_2C***H***), 4.52 (t, *J* = 7.9 Hz, 1H, Ph_2C***H***), 3.34 (m, 2H, C***H***$_2$NH), 3.09 (m, 4H, COC***H***$_2$), 2.61 (t, *J* = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.19 (s, 3H, Thiaz-4-C***H***$_3$), 1.79 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.51{s, 9H, (C***H***$_3$)$_3$ of Boc}; ^{13}C-NMR (CD_3OD), δ (ppm): 173.4 (quart, ***C***OCH_2), 158.2 (quart, Thiaz-2-***C***), 155.4 (quart, ***C***(=O)O-^{t}Bu), 152.3 {quart, ***C***(=N)}, 144.5 (quart, Thiaz-***C***-4), 142.7, 133.3 (quart, Ph-***C***-1), 128.9, 128.7, 128.4, 127.9, 127.8, 127.5, 126.8, 126.3, 126.1 (+, ***C*** of Ph), 123.1 (quart, Thiaz-5-***C***), 82.7 (quart, ***C*** of Boc), 47.6, 46.5 (+, Ph_2***C***H), 47.2, 44.0 (-, CO***C***H_2), 40.0 (-, ***C***H_2NH), 30.4 (-, Thiaz-5-***C***H_2), 28.2 {+, (***C***H_3)$_3$ of Boc}, 23.1 (-, Thiaz-5-CH_2***C***H_2), 14.2 (+, Thiaz-4-***C***H_3); MS (ESI, MeOH + 10 mM NH_4OAc): 730 ($[M + H]^+$), 752 ($[M + Na]^+$).

***tert*-Butyl 5-[3-[N^2-benzyloxycarbonyl-N^3-(3-phenylbutanoyl)guanidino]propyl]-4-methyl-thiazol-2-ylcarbamate [102b]**

Stirring for 15h; yield 52 %; colorless liquid; $C_{31}H_{39}N_5O_5S$ (593.7); IR (in CH_2Cl_2): 1715, 1642, 1558, 1495, 1452, 1368, 1299, 1243, 1155, 1123, 1068, 803, 734, 698 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 12.21 (br. S, 1H, N***H***), 9.03 (br. s, 1H, N***H***), 7.45-7.12 (m, 10H, Ph-***H***), 5.13 (s, 2H, OC***H***$_2$Ph), 3.52-3.15 {m, 3H, C***H***$_2$NH & C***H***(CH_3)Ph}, 2.82-2.52 (m, 4H, COC***H***$_2$ & Thiaz-5-C***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.95-1.74 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 {s, 9H, CH(C***H***$_3$)$_3$}, 1.33 {d, *J* = 6.9 Hz, 3H, Ph(C***H***$_3$)CH}; ^{13}C-NMR ($CDCl_3$), δ (ppm): 174.2 (quart, ***C***O), 163.7 (quart, ***C***O), 157.9 (quart, Thiaz-***C***-2), 156.3 (quart, Boc-***C***O), 152.4 (quart, Thiaz-***C***-4), 144.6, 136.8 (quart, Ph-***C***-1), 128.7, 128.5, 128.4, 128.3, 128.1, 127.9, 126.8, 126.7, 126.6, 125.9 (+, Ph-***C***), 123.0 (quart, Thiaz-***C***-5), 82.7 (quart, Me_3***C***), 67.2 (-, ***C***H_2Ph), 46.4 (-, CO***C***H_2), 39.9 (-, ***C***H_2NH), 36.3 {+, Ph(CH_3)***C***H}, 30.4 (-, Thiaz-5-***C***H_2), 28.2 {+, C(***C***H_3)}, 23.2 (-, Thiaz-5-CH_2***C***H_2), 14.2 {+, Ph(***C***H_3)CH}, 14.17 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 594 ($[M + H]^+$), 494{[(M-Boc) + H]$^+$}.

***tert*-Butyl 5-[3-[N^2,N^3-bis(3-phenylbutanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate [103b]**

Yield 32 %; colorless liquid; $C_{33}H_{43}N_5O_4S$ (605.8); IR (CH_2Cl_2): 1714, 1616, 1556, 1451, 1366, 1301, 1245, 1154, 1069, 762, 734, 698 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.33-7.11 (m, 10H, Ph-***H***), 3.46-3.20 (m, 4H, CH_3C***H*** & C***H***$_2$NH), 2.51-2.75 (m, 6H, COC***H***$_2$ & Thiaz-5-C***H***$_2$), 2.23 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, Boc-C***H***$_3$), 1.30 (d, *J* = 6.9 Hz, 3H, COCHC***H***$_3$), 1.27 (d, *J* = 6.9 Hz, 3H, COCHC***H***$_3$); ^{13}C-NMR (CD_3OD), δ (ppm): 186.7 (quart, ***C***OCH_2), 174.1 (quart, ***C***OCH_2), 157.9 (quart, Thiaz-2-***C***), 155.4 (quart, Boc-***C***O), 152.6 (quart, ***C***=NH), 146.9, 144.7 (quart, Ph-***C***-1), 141.5 (quart, Thiaz-***C***-4), 133.2, 133.1, 128.8, 128.6, 128.5, 128.3, 128.1, 126.8, 125.9 (+, Ph-***C***), 122.9 (quart, Thiaz-***C***-5), 82.3 (quart, Boc-***C***), 49.5, 46.4 (-, CO***C***H_2CH), 40.0 (-, ***C***H_2NH), 36.9, 36.3 (+, COCH_2***C***H), 30.5 (-, Thiaz-5-***C***H_2), 28.2 (+, Boc-***C***H_3), 23.2 (-, Thiaz-5-CH_2***C***H_2), 22.2, 21.9 (+, CH***C***H_3), 14.3 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 606 ($[M + H]^+$), 506 {[(M-Boc) + H]$^+$}.

***tert*-Butyl 5-[3-[N^2-benzyloxycarbonyl-N^3-(3-cyclohexylbutanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate [102c]**

Stirring for 16h; yield 46 %; colorless sticky liquid; $C_{31}H_{45}N_5O_5S$ (599.8); ^{1}H-NMR ($CDCl_3$), δ (ppm): 12.21 (br, N***H***), 9.17 (br. t, *J* = 5.3 Hz, 1H, N***H***CH_2), 7.45-7.20 (m, 5H, Ph-***H***), 5.15 (s, 2H, PhC***H***$_2$), 3.47 (m, 2H, NHC***H***$_2$), 2.70 (t, *J* = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.45 (m 1H, one of COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 2.11 {m, 2H, one of COC***H***$_2$ & C***H***(c-Hex)}, 2.01-1.59 (m, c-Hex), 1.52 (s, 9H, Boc), 1.42-0.71 (m, c-Hex), 0.92 (d, *J* = 6.9 Hz, 3H, $COCH_2CHC\boldsymbol{H}_3$); ^{13}C-NMR ($CD_3OD$), δ (ppm): 179.2 (quart, ***C***=N), 175.4 (quart, ***C***OCH_2), 163.8 (quart, ***C***(=O)), 158.3 (quart, Thiaz-***C***-2), 156.5 (quart, Boc-***C***O), 152.5 (quart, Thiaz-***C***-4), 136.7 (quart, Ph-***C***-1), 128.4, 128.1, 127.9 (+, Ph-***H***), 82.5 (quart, Boc-***C***), 67.2 (-, PhC***H***$_2$O), 42.8 (-, CO***C***H_2), 42.5 (+, Cy-***C***-1), 39.9 (-, ***C***H_2NH), 35.3 (+, $COCH_2\boldsymbol{C}H$), 30.5, 30.3, 29.7 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$, c-Hex-***C***H_2), 28.9 (+, Boc ***C***H_3), 26.7, 26.6 (-, c-Hex-***C***H_2), 16.5 (+, c-Hex-CH***C***H_3), 14.1 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 600.3 ($[M + H]^+$), 500{$[(M-Boc) + H]^+$}, 1199 ($[2M + H]^+$)

***tert*-Butyl 5-[3-[N^2,N^3-bis(3-cyclohexylbutanoyl)guanidino]propyl]-4-methylthiazol-2-yl-carbamate [103c]**

Yield 28 %; colorless sticky liquid; $C_{33}H_{55}N_5O_4S$ (617.9); ; ^{1}H-NMR (CD_3OD), δ (ppm): 12.21 (br, N***H***), 9.17 (br. m, 1H, N***H***CH_2), 3.46 (m, 2H, C***H***$_2$NH), 2.69 (t, *J* = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.45 (d, *J* = 4.9 Hz, 2H, COC***H***$_2$), 2.39 (d, *J* = 4.9 Hz, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 2.15-1.50 (m, H), 1.54 {s, 9H, (C***H***$_3$)$_3$ of Boc}, 1.30-1.00 (m, H), 0.93 (d, *J* = 6.8 Hz, 6H, $COCH_2CHC\boldsymbol{H}_3$), ^{13}C-NMR ($CD_3OD$), δ (ppm): 188.9 (quart, $CH_2\boldsymbol{C}O$), 179.8 (quart, $CH_2\boldsymbol{C}O$), 176.0 {quart, ***C***(=N)}, 158.9 (quart, Thiaz-***C***-2), 155.6 (quart, Boc-***C***O), 152.5 (quart, Thiaz-***C***-4), 122.7 (quart, Thiaz-***C***-5), 82.5 (quart, Boc-***C***), 42.8 (-, CO***C***H_2), 42.5 (+, Cy-***C***-1), 39.9 (-, ***C***H_2NH), 35.3 (+, $COCH_2\boldsymbol{C}H$), 30.5, 30.3, 29.7 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$, c-Hex-***C***H_2), 28.9 (+, Boc-***C***H_3), 26.7, 26.6 (-, c-Hex-***C***H_2), 16.5 (+, c-HexCH***C***H_3), 14.1 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 618 ($[M + H]^+$).

General procedure for the preparation of the N^2-monoacylated 3-(2-amino-4-methylthiazol-5-yl)propylguanidines 104a-c

To a solution of corresponding Boc- and Cbz-compound (**102a-c**) (1 mmol) in 5 mL MeOH was added 20 mg of Pd/C (10 %) and then the mixture was stirred under H_2 (baloon) overnight. The catalyst was filtered off through celite, washed with MeOH, and then the solvent was removed in *vacuo*. To the residue 5 mL of 50 % TFA in CH_2Cl_2 was added and stirred at rt till the Boc group was completely removed (3 - 4 h). The solvent was then removed *in vacuo* and the residue was purified by preparative HPLC (Instrumental setup described at General condition in section **3.6.1.** of **Chapter 3**).

N^1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N^2-(3,3-diphenylpropanoyl)guanidine [104a]

Reaction time: 3 h; yield 88 %; colorless sticky liquid; $C_{23}H_{27}N_5OS$ (421.5); IR (neat) 1639, 1596, 1493, 1433, 1392, 1200, 1131, 827, 799, 754, 720, 706, 626 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 7.34-7.11 (m, 10H, Ph-***H***), 4.59 (t, *J* = 8.0 Hz, 1H, $Ph_2C\boldsymbol{H}$), 3.28 (m, 4H, $COC\boldsymbol{H}_2$ & $C\boldsymbol{H}_2NH$), 2.65 (t, *J* = 7.6 Hz, 2H, Thiaz-5-$C\boldsymbol{H}_2$), 2.17 (s, 3H, Thiaz-4-$C\boldsymbol{H}_3$), 1.85 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD), δ (ppm): 175.5 (quart, ***C***OCH_2), 170.4 (quart, Thiaz-***C***-2), 155.1 {quart, ***C***(=N)}, 144.5 (quart, Ph-***C***-1), 132.6 (quart, Thiaz-***C***-4), 129.7, 128.8, 127.8 (+, ***C*** of Ph), 118.4 (quart, Thiaz-***C***-5), 48.1 (+, $Ph_2\boldsymbol{C}$), 43.8 (-, $CO\boldsymbol{C}H_2$), 41.5 (-, $\boldsymbol{C}H_2NH$), 29.6 (-, Thiaz-5-$\boldsymbol{C}H_2$), 23.5 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 11.4 (+, Thiaz-4-$\boldsymbol{C}H_3$); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 422 ($[M + H]^+$).

Analytical HPLC: R_t 13.5 min (Column B; 0 min: 97/3, 24 min: 85/15); purity: 95 %.

N^1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine [104b]

Reaction time: 2.5 h; yield 92 %; colorless sticky liquid; $C_{18}H_{25}N_5OS$ (359.5); IR (in CH_2Cl_2) 1664, 1600, 1432, 1180, 1130, 1024, 832, 798, 762, 720, 700 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 7.33-7.13 (m, 5H, Ph-***H***), 4.15 {m, 1H, $Ph(CH_3)C\boldsymbol{H}$}, 3.34 (m, 2H, $C\boldsymbol{H}_2NH$), 2.76 (dd, J_1 = 3.3 Hz, J_2 = 15.4 Hz, 2H, $COC\boldsymbol{H}_2$), 2.67 (t, *J* = 7.5 Hz, 2H, Thiaz-5-$C\boldsymbol{H}_2$), 2.15 (s, 3H, Thiaz-4-$C\boldsymbol{H}_3$), 1.88 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.31 {d, *J* = 7.1 Hz, 3H, $Ph(C\boldsymbol{H}_3)CH$}; ^{13}C-NMR (CD_3OD), δ (ppm): 176.1 (quart, ***C***O), 170.3 (quart, Thiaz-1-***C***), 155.1 {quart, ***C***(=NH)}, 146.4 (quart, Thiaz-4-***C***), 132.6 (quart, Ph-1-***C***), 129.6, 127.9, 127.7 (+, Ph-***C***), 118.3 (quart, Thiaz-***C***-5), 46.1 (-, $CO\boldsymbol{C}H_2$), 41.5 (-, $\boldsymbol{C}H_2NH$), 37.6 {+, ***C***$H(CH_3)Ph$}, 29.6 (-, Thiaz-5-$\boldsymbol{C}H_2$), 23.5 (-, Thiaz-5-

$CH_2\boldsymbol{C}H_2$), 22.3 (+, Thiaz-4-$\boldsymbol{C}H_3$), 11.4 {+, CH($\boldsymbol{C}H_3$)Ph}; MS (ESI, H_2O/AcCN + 0.0059% TFA): 360 ($[M + H]^+$);

Analytical HPLC: R_t 11.75 min (Column B; 0 min: 97/3, 24 min: 85/15); purity: >90 %.

N^1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N^2-(3-cyclohexylbutanoyl)guanidine [104c]

Reaction time: 3 h; yield 92 %; colorless sticky liquid; $C_{18}H_{31}N_5OS$ (365.5); IR (neat): 1788, 1663, 1433, 1179, 1130, 835, 798, 720 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 3.53 (t, J = 6.9 Hz, 2H, C$\boldsymbol{H}_2$NH$_2$), 2.72 (t, J = 7.5 Hz, 2H, Thiaz-5-C$\boldsymbol{H}_2$), 2.57 (dd, J_1 = 5.2 Hz, J_2 = 14.8 Hz, 1H, one of COC$\boldsymbol{H}_2$), 2.23 (dd, J_1 = 5.2 Hz, J_2 = 14.8 Hz, 1H, one of COC$\boldsymbol{H}_2$), 2.16 (s, 3H, Thiaz-4-C$\boldsymbol{H_3}$), 2.10-1.58 {m, 8H, C$\boldsymbol{H}_2$ of c-Hex & C$\boldsymbol{H}$(c-Hex)}, 1.40-0.96 (m, 6H, C$\boldsymbol{H}_2$ of c-Hex), 0.92 (d, J = 6.9 Hz, 3H, COCH$_2$CHC$\boldsymbol{H}_3$); ^{13}C-NMR (CD_3OD), δ (ppm): 177.5 (quart, $\boldsymbol{C}$OCH$_2$), 170.4 (quart, Thiaz-$\boldsymbol{C}$-2), 155.3 {quart, $\boldsymbol{C}$(=N)}, 132.6 (quart, Thiaz-$\boldsymbol{C}$-4), 118.4 (quart, Thiaz-$\boldsymbol{C}$-5), 43.9 (+,Cy-$\boldsymbol{C}$-1), 42.7 (-, CO$\boldsymbol{C}$H$_2$), 41.6 (-, $\boldsymbol{C}$H$_2$NH), 36.4 (+, c-Hex$\boldsymbol{C}$HCH$_3$), 31.5 (-, Thiaz-5-$\boldsymbol{C}$H$_2$), 30.0 (-, Thiaz-5-CH$_2\boldsymbol{C}$H$_2$), 29.7 (-, $\boldsymbol{C}$H$_2$ of c-Hex), 27.8, 27.7, 23.6 (-, $\boldsymbol{C}$H$_2$ of c-Hex), 16.6 (+, c-HexCH$\boldsymbol{C}$H$_3$), 11.4 (+, Thiaz-4-$\boldsymbol{C}$H$_3$); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 366 ($[M + H]^+$).

Analytical HPLC: R_t 13.60 min (Column B; 0 min: 97/3, 24 min: 85/15); purity: **90** %.

General procedure for the preparation of the diacylated guanidines 105a-c

1 mmol of the Boc-proteceted diacylated guanidines (**103a-c**) in 5 mL of 50 % TFA in CH_2Cl_2 was stirred at rt till the starting material completely disappeared (< 3 h). The solvent was then removed *in vacuo* and the residue was purified by preparative HPLC.

N^1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N^1,N^2-bis(3-phenylbutanoyl)guanidine [105b]

Reaction time: 2.5 h; yield 73 %; colorless sticky oil; $C_{28}H_{35}N_5O_2S$ (505.7); IR (neat) 1685, 1619, 1493, 1366, 1199, 1134, 1050, 1024, 1005, 823, 799, 761, 720, 699 cm^{-1}; ^{1}H-NMR (DMSO-d_6), δ (ppm): 10.86 (br, N$\boldsymbol{H}$), 9.44 (br, N$\boldsymbol{H}$), 9.25 (br, 2H, N$\boldsymbol{H}_2$), 7.69-7.10 (m, 10H, Ph-$\boldsymbol{H}$), 3.40-3.05 (m, 4H, CH$_3$C$\boldsymbol{H}$ & C$\boldsymbol{H}_2$NH), 2.90-2.40 (m, 6H, COC$\boldsymbol{H}_2$ & Thiaz-5-C$\boldsymbol{H}_2$), 2.12-1.98 (m, 3H, Thiaz-4-C$\boldsymbol{H}_3$), 1.81-1.61 (m, 2H, Thiaz-5-CH$_2$C$\boldsymbol{H}_2$), 1.30-1.10 (m, 6H, COCHC$\boldsymbol{H}_3$); ^{13}C-NMR (DMSO-d_6), δ (ppm): 173.8 (quart, $\boldsymbol{C}$OCH$_2$), 167.8 (quart, $\boldsymbol{C}$OCH$_2$), 159.1 (quart, Thiaz-$\boldsymbol{C}$-2), 153.8 (quart, $\boldsymbol{C}$=NH), 146.4, 145.1 (quart, Ph-$\boldsymbol{C}$-1), 141.5 (quart, Thiaz-$\boldsymbol{C}$-4), 133.2, 133.1, 128.8,

128.6, 128.5, 128.3, 128.1, 126.8, 125.9 (+, Ph-***C***), 116.5 (quart, Thiaz-***C***-5), 44.8, 41.9 (-, CO***C***H_2CH), 39.6 (-, ***C***H_2NH), 36.2, 35.6 (+, COCH$_2$***C***H), 28.9 (-, Thiaz-5-***C***H_2), 22.0 (-, Thiaz-5-CH$_2$***C***H_2), 22.2, 21.9 (+, CH***C***H_3), 11.1 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 506 ($[M + H]^+$);

However, this compounds are not stable enough, decomposed slowly even storing at -27°C for few days.

<u>*N*^{1}-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N*^{2},*N*^{3}-bis(3-cyclohexylbutanoyl)guanidine [105c]</u>

Reaction time: 2.5 h; yield **65** %; colorless sticky liquid; $C_{28}H_{47}N_5O_2S$ (517.8); IR (neat) 1668, 1597, 1384, 1198, 1181, 1134, 1026, 720 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 12.21 (br, N***H***), 9.17 (br. m, 1H, N***H***CH$_2$), 3.41 (m, 2H, ***C***H_2NH), 2.53-2.29 (m, 4H), 2.22-0.52 (m, 41H); ^{13}C-NMR (CD_3OD), δ (ppm): 188.9 (quart, ***C***(=O)CH$_2$), 179.8 (quart, ***C***OCH$_2$), 176.0 (quart, ***C***(=N)), 158.9 (quart, Thiaz-***C***-2), 152.5 (quart, Thiaz-***C***-4), 122.7 (quart, Thiaz-***C***-5), 42.8 (-, CO***C***H_2), 42.5 (+, Cy-***C***-1), 39.9 (-, ***C***H_2NH), 35.3 (+, COCH$_2$***C***H), 30.5, 30.3, 29.7 (-, Thiaz-5-CH$_2$***C***H_2, c-Hex-***C***H_2), 26.7, 26.6 (-,c-Hex-***C***H_2), 16.5 (+, c-HexCH***C***H_3), 14.1 (+, Thiaz-4-***C***H_3); MS (ESI, CH_2Cl_2/MeOH + 10 mM NH_4OAc): 518 ($[M + H]^+$).

However, this compounds are not stable enough, decomposed slowly even storing at -27°C for few days.

4.5.2. Pharmacological Methods

Data handling and pharmacological parameters, histamine H_1 receptor assay on the isolated guinea pig ileum, histamine H_2 receptor assay on isolated guinea pig right atrium (spontaneously beating), calcium cssays with U-373 MG cells, and steady-state GTPase activity assay was performed similar method as described in section **3.6.2** in **Chapter 3**.

Acetylcholine M_3 receptor assay on the isolated guinea pig ileum. Guinea pigs of either sex were stunned by a blow on the head and exsanguinated. The ileum was removed, and whole segments (1.5-2 cm) were mounted isotonically (preload 0.5 g) at 37 °C in Tyrode's solution,[26] aerated with 95% O_2-5% CO_2, in the continuous presence of 1–3 μM mepyramine, a concentration not affecting M_3 receptors. During an equilibration period of *ca.* 80 min, the organs

were stimulated three times with carbachol (1 and 10 μM) followed by washout. Each preparation was used to establish a cumulative concentration-effect curve for carbachol (0.003-10 μM) followed by up to three curves for carbachol in the presence of increasing concentrations of antagonist (incubation time 10-15 min). The pEC_{50} difference was not corrected since four successive curves for carbachol were superimposable ($n > 10$).

Histamine H_3 receptor assay on electrically stimulated guinea pig ileum longitudinal muscle with adhering myenteric plexus. Strips of guinea pig ileal longitudinal muscle, with adhering myenteric plexus of approximately 2 cm length and proximal to the ileocaecal junction, were prepared as previously described.[27,28] The strips were mounted isometrically under an initial tension of approximately 7.5 mN in a jacketed 20-mL organ bath of filled with modified Krebs-Henseleit solution of the following composition [mM]: NaCl 117.9, KCl 5.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.3, $NaHCO_3$ 25.0, glucose 5.5, and choline chloride 0.001. The solution was aerated with 95% O_2-5% CO_2 and warmed to a constant temperature of 37 °C. After an equilibration period of 1 h with washings every 15 min, the preparations were stimulated for 30 min with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. Viability of the muscle strips was monitored by addition of the histamine H_3 receptor agonist (*R*)-α-methylhistamine (100 nM). The agonist caused a relaxation of the twitch response of more than 50% up to 100%. After washout, reequilibration and 30 min field-stimulation, a cumulative concentration-response curve to (*R*)-α-methylhistamine (1-1000 nM) was constructed. Subsequently, the preparations were washed intensively and reequilibrated for 20-30 min. During the incubation period of the antagonist under study, the strips were stimulated continuously for 30 min. Finally, a second concentration-response curve to (*R*)-α-methylhistamine was obtained. The rightward displacement of the curve to the histamine H_3 receptor agonist evoked by the antagonist under study was corrected with the mean shift monitored by daily control preparations in the absence of antagonist. New antagonists were tested at concentrations that did not block ileal cholinergic M_3 receptors. Mepyramine (1–3 μM) and cimetidine (30 μM) were present throughout the experiments to block H_1 and H_2 receptors, respectively.

4.6. References

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Chapter 5

Synthesis and Pharmacological Activity of Chiral N^G-Acylated Heteroarylpropylguanidines

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories instead of theories to suit facts. **Conan Doyle**

5.1. Introduction

Information on the stereochemical features of the ligands is necessary with respect to the potential therapeutic use as well as for theoretical considerations, e. g. for molecular modelling and for the development of a three-dimensional model of the receptor. Arpromidine (**5d**) and related imidazolylpropylguanidines, the most potent histamine H_2 receptor agonists described in literature,[1] are promising new cardiovascular agents which may be useful for the treatment of severe congestive heart failure.[2] The pharmacological investigation of stereoisomers, e.g. of the 3,4-difluorinated (**5e**) and 3,4-dichlorinated (**5g**) analogues, revealed that the (*S*)-configured compounds are more active enantiomers with eudismic ratios of up to 40[3,4]. According to a model for the interaction of agonists with the histamine H_2 receptor, supported by site-directed mutagenesis and molecular modelling investigations, the guanidino group is supposed to interact with Asp-98 in transmembrane domain 3 (TM3), the imidazole ring is assumed to interact with Asp-186 and Tyr-182 in TM5 and the (*S*)-configured phenyl(pyridyl)alkyl group is probably oriented towards amino acids in TMs 6 and 7. As the strongly basic guanidino group is the main reason for very low oral bioavailability, non-H_2R-mediated effects and lack of penetration across the blood-brain barrier the diarylalkyl group was replaced by diaryl-, monoaryl- or cyclohexylalkanoyl residues, i. e. the strong basicity was reduced by 4-5 orders of magnitude (**Chapters 3** and **4**). A diaryl group is not required: compounds such as the acylated guanidines **78** and **80** (**Fig. 5.1**) proved to be potent histamine H_2 receptor agonists which possess up to 30 times higher potency than the natural ligand and have more favourable pharmacokinetic properties than the corresponding alkylated guanidines. Moreover, the replacement of imidazolyl ring with a 2-amino-4-methylthiazol-5-yl group

(e.g. see compound **104b** in **Fig. 5.1**) resulted in increased selectivity for histamine H_2 over H_3 receptors.

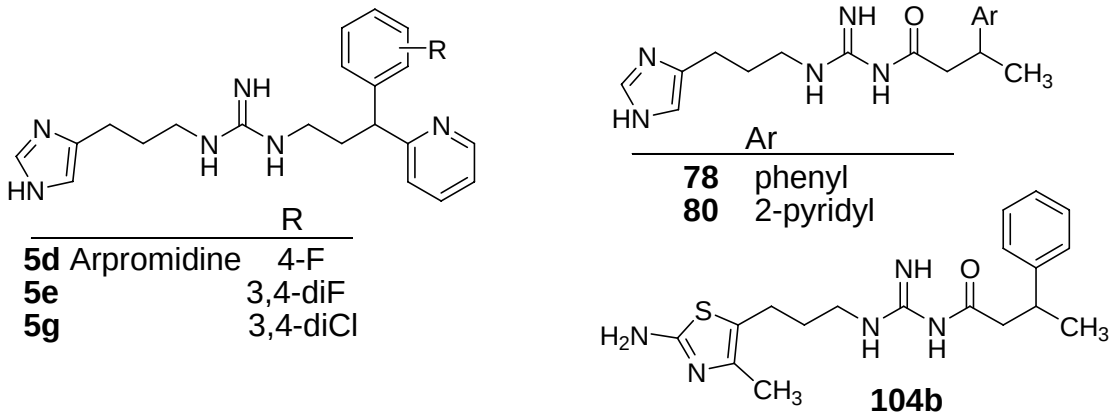
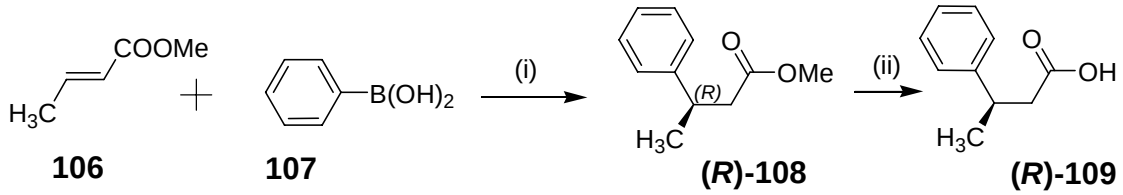


Fig. 5.1: Structure of some histamine H_2 receptor agonists

Enantiomers of the aforementioned acylguanidines are required to compare the stereo-chemical requirements with those of the arpromidine series and to further improve the receptor model. In this chapter the synthesis of the enantiomers of **78** as well as the corresponding pyridine (**80**) and aminothiazole analogues (**104b**) via asymmetric synthesis of 3-arylbutanoic acid is described.

5.2. Chemistry

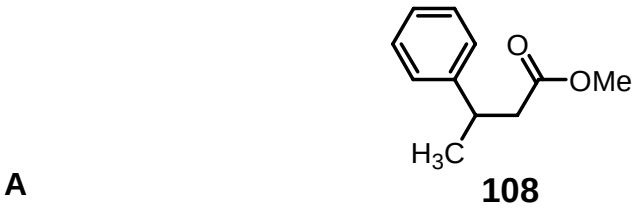
For the synthesis of the target molecules according to the method reported in the previous **Chapters 3 and 4**, the enantiomers of the chiral acids were required. The building block (*R*)-3-phenylbutanoic acid ((*R*)-**109**) was synthesized from the achiral precursor, methyl (*E*)-but-2-enoate (**106**), via asymmetric addition of phenylboronic acid (**107**) as reported by Hayashi *et al.*[5,6], using a catalytic amount of rhodium catalyst and (*S*)-binap ligand, followed by hydrolysis of the methyl ester (*R*)-**108**. As the enantiomeric excess (ee) of this reaction did not exceed about 80 %, the acid was recrystallized to obtain a pure enantiomer (99 % ee) which was hydrolysed to give the pertinent acid (*R*)-**109** (**Scheme 5.1**).



106 **107** **(*R*)-108** **(*R*)-109**

Reagents: (i) $[Rh(acac)(C_2H_4)_2]$ / (*S*)-binap, dioxane / H_2O (10/1), 100 °C, 16 h; (ii) LiOH, THF, rt.

Scheme 5.1: Synthesis of (*R*)-3-phenylbutanoic acid ((*R*)-**109**)



A **108** **B**

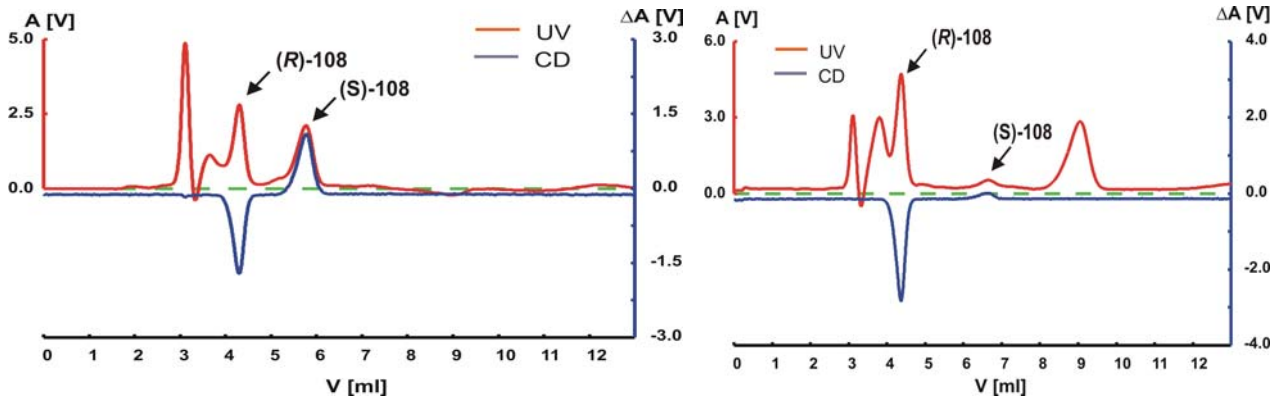
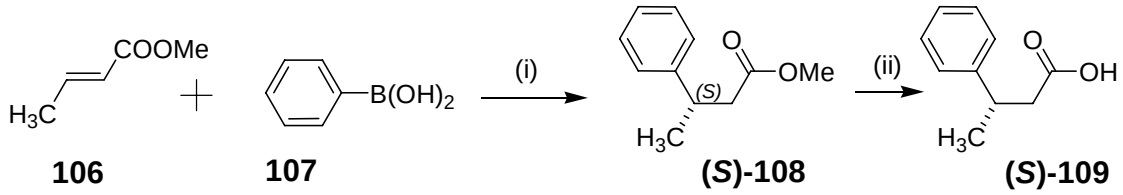


Fig 5.2: UV detection and optical rotation (CD) signal at 240 nm for the chiral separation of the 3-phenylbutanoic acid methyl ester, racemic (**A**) and (*R*)-**108** (**B**), by HPLC on a Chiralcel OD column. Injection volume: 50 µL; analyte concentration: 0.5 mg/mL; mobile phase: n-hexane-isopropanol (99:1); temperature: 22 °C; flow: 0.5 mL/min.



106 **107** **(*S*)-108** **(*S*)-109**

Reagents: (i) $[Rh(acac)(C_2H_4)_2]$ / (*R*)-binap, dioxane / H_2O (10/1), 100 °C, 16 h; (ii) LiOH, THF, rt.

Scheme 5.2: Synthesis of (*S*)-3-phenylbutanoic acid ((*S*)-**109**)

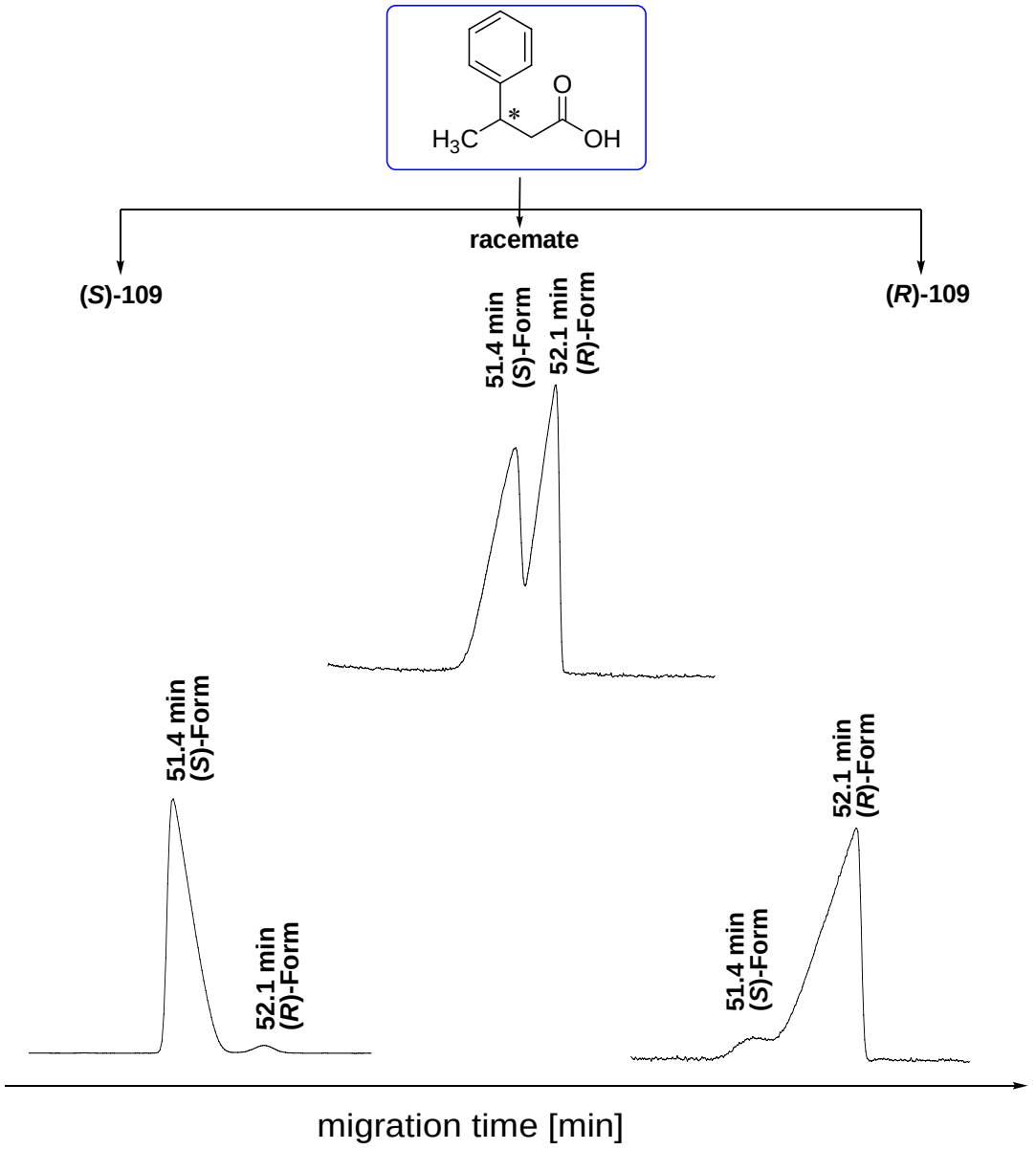
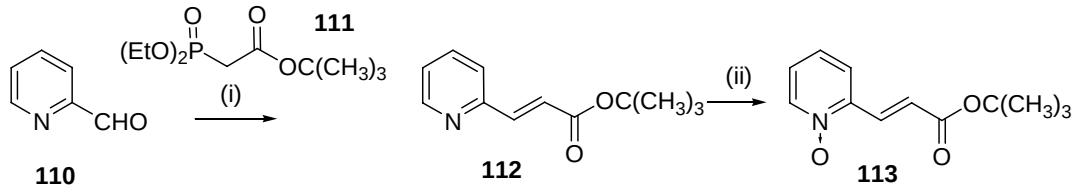


Fig 5.3: Representative electropherogram of the chiral separation of acids, racemate, (*R*)- and (*S*)-**109** by capillary electrophoresis using (2-hydroxypropyl)-β-CD as chiral selector. Analyte concentration: 500 µM; capillary temperature 30 °C; running buffer 30 mM (2-hydroxypropyl)-β-CD in 5 M urea and 125 mM sodium phosphate buffer pH 6.00; UV-detection: 210 nm.

(*S*)-3-Phenylbutanoic acid was prepared according to the same procedure except for using (*R*)-binap instead of (*S*)-binap as the chiral ligand (**Scheme 5.2**). The methyl esters **(*R*)-**

and **(*S*)-108** could be separated by chiral HPLC (see **Fig. 5.2**). The enantiomeric purities of the acids **(*R*)-** and **(*S*)-109** were determined by means of capillary electrophoresis using (2-hydroxypropyl-)-β-cyclodextrin as chiral selector (**Fig. 5.3**).

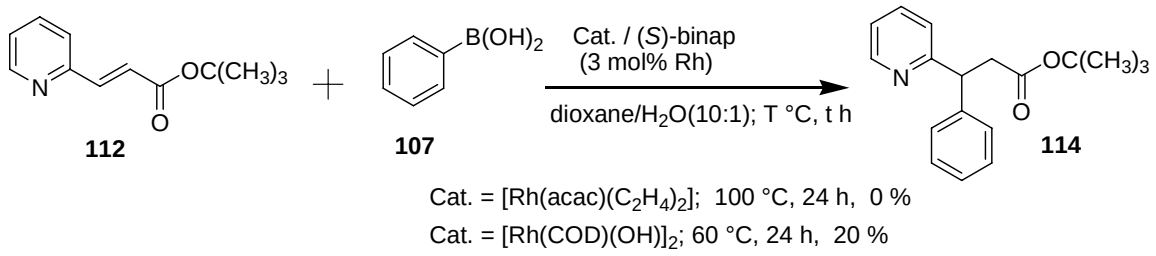
For the synthesis of chiral 2-pyridyl analogues according to the Hayashi *et al.*[5,6], 2-pyridylboronic acid was required. As this compound is not commercially available and a procedure for the synthesis is not described, we thought of preparing 3-phenyl-3-(2-pyridyl)propanoic acid by conjugate addition of phenylboronic acid (**107**) with t-butyl 3-pyridin-2-ylpropenoate (**112**). The acrylate **112** (**Scheme 5.3**) was prepared from the corresponding aldehyde (**110**) via Witting-Horner reaction, which gave mainly the *trans* form. To block the lone pair of the pyridine-N in acrylate **112**, the compound was converted to its N-oxide (**113**) by using *m*-chloroperoxybenzoic acid (*m*-CPBA).



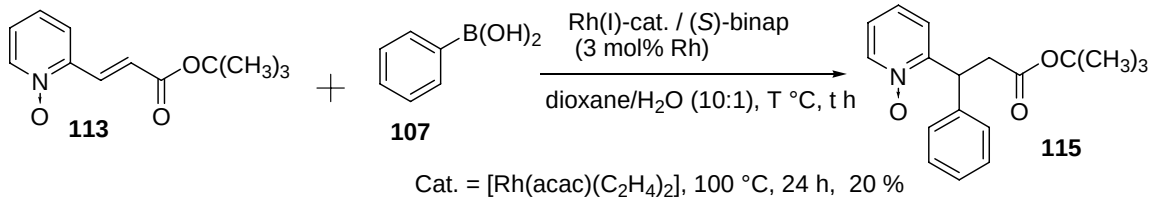
Reagents: (i) n-BuLi, THF, rt; (ii) *m*-CPBA, CH_2Cl_2, rt

Scheme 5.3: Synthesis of 2-pyridyl and 2-pyridyl-N-oxide acrylate

The conjugate addition reaction of phenylboronic acid with the tertiary butyl ester of 3-pyridin-2-ylacrylic acid (**112**) in presence of $[Rh(acac)(C_2H_4)_2]$ catalyst and (*S*)-binap was unsuccessful (**Scheme 5.4**). Replacing the catalyst with $[Rh(COD)(OH)]_2$ led to a little advance of reaction. As it was conceivable that the nucleophilic pyridine caused the problem, we used the N-oxide **113**. However, the conjugate addition reaction of **113** at the same reaction condition was not satisfactory (**Scheme 5.5**).

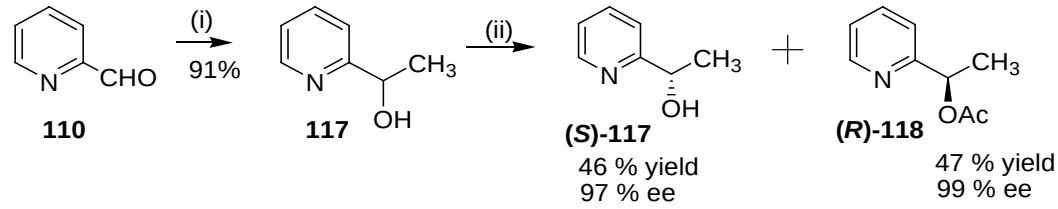


Scheme 5.4: Conjugate addition of phenylboronic acid to t-butyl 3-pyridin-2-ylpropenoate



Scheme 5.5: Conjugate addition using the pyridine-N-oxide **113** as starting material

In search for a better synthetic route an enzyme-catalysed reaction described in the literature was selected as the most promising alternative: the lipase catalysed enantioselective acetylation of racemic 1-(2-pyridyl)ethanol (**116**) with vinyl acetate is reported to give the (*R*)-configured ester, (*R*)-**118**, with high yield and excellent enantiomeric excess[7] (**Scheme 5.6**). Accordingly, we prepared the racemic alcohol (**116**), converted it to (*R*)-**118** with high yield, and separated (*S*)-1-(2-pyridyl)ethanol ((*S*)-**117**).



Reagents: (i) MeMgBr, Et_2O, 0°C, 2h; (ii) Lipase, Vinyl acetate, *iso*-Pr_2O, Molecular sieves 4A, 4h.

Scheme 5.6: Enantioselective acylation of 1-(2-pyridyl) ethanol using vinyl acetate *Candida antarctica* lipase (CAL).

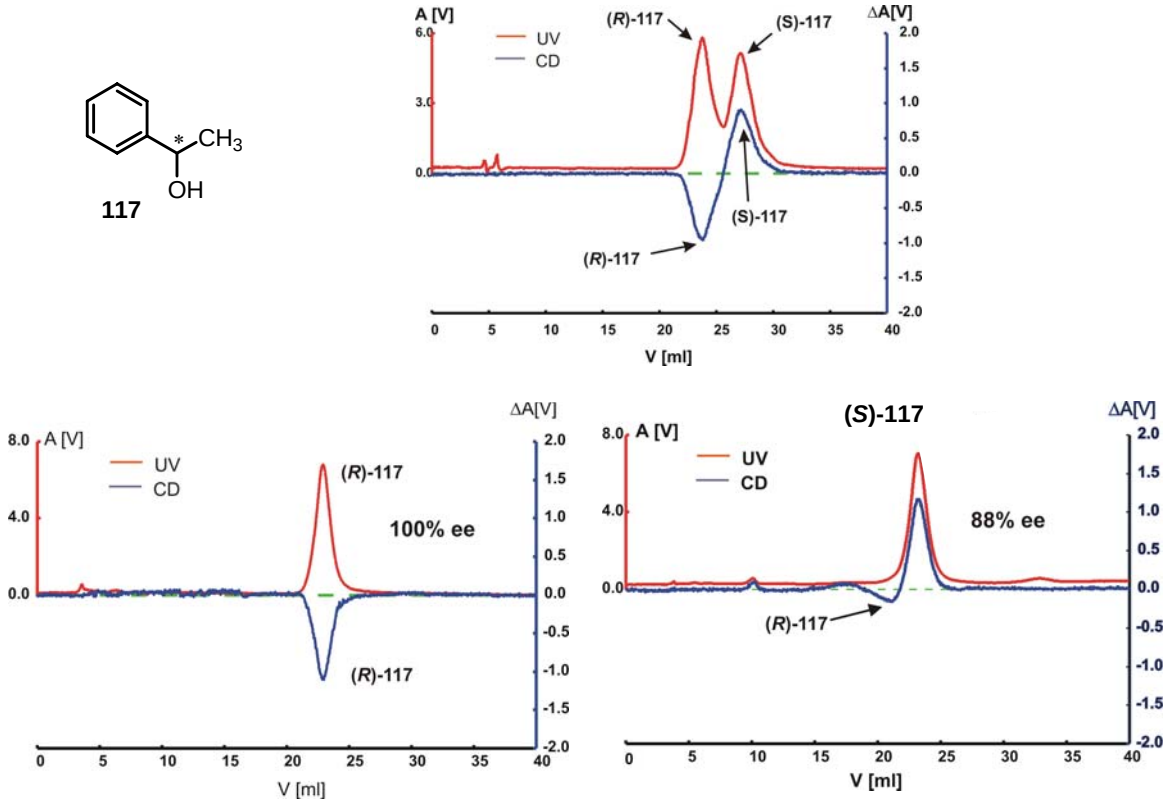
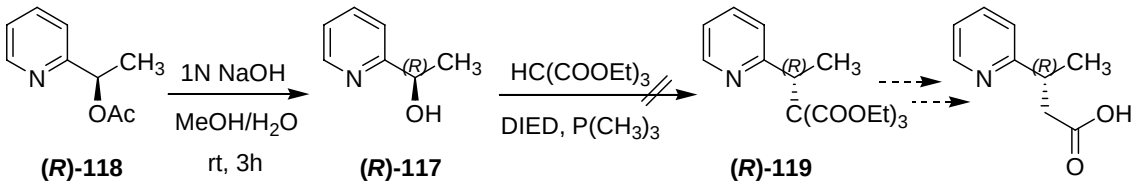


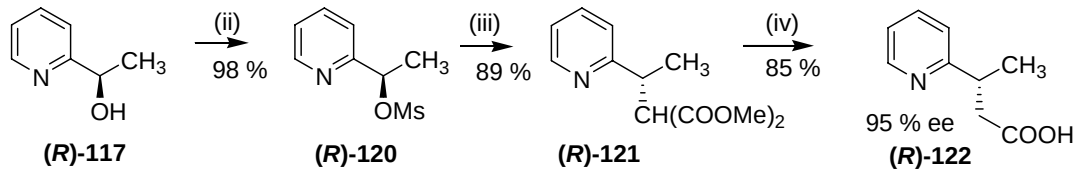
Fig 5.4: UV detection and optical rotation signal at 245 nm for the chiral separation of the 1-(2-pyridyl)ethanol racemic and (*R*)- and (*S*)- **117** on a Chiralcel OD column. Injection volume: 40 µL; analyte concentration: 0.5 mg/mL; mobile phase: n-heptane-isopropanol (99:1); temperature: 22°C; flow: 0.5 mL/min.

The acetyl protected compound (*R*)-**118** was then hydrolyzed to alcohol (*R*)-**117** by treating with 1N NaOH solution. All attempts to stereoselectively displace the OH group of the chiral secondary alcohol (*R*)-**117** by a CH-acidic compound [$HC(COOEt)_3$] under Mitsunobu conditions[8,9] (DEAD, PMe_3) failed (**Scheme 5.7**).



Scheme 5.7: Stereoselective Mitsunobu reaction to synthesize (*R*)-**119** was unsuccessful

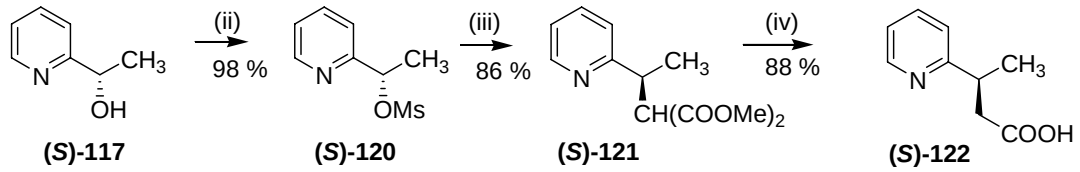
So, the alcohol functionality of (*R*)-1-pyridin-2-ylethanol was first converted to the methanesulfonate[10] (*R*)-**120** (**Scheme 5.8**) to make a better leaving group and then treated with the sodium salt of dimethyl malonate [$CH_2(COOMe)_2$] to give dimethyl (*R*)-(1-pyridin-2-ylethyl)malonate[11] (*R*)-**121** stereospecifically. Subsequent saponification and decarboxylation yielded (*R*)-3-phenyl-3-pyridin-2ylpropanoic acid ((*R*)-**122**).



Reagents: (i) MsCl, Et_3N, DMAP, CH_2Cl_2, rt; (ii) DMM, NaH, DMSO, rt, 12h; (iii) a. NaOH, reflux, 12h; b. HCl, reflux, 12h.

Scheme 5.8: Synthesis of (*R*)-3-phenyl-3-pyridin-2-ylpropanoic acid *via* S_N2 displacement of (*R*)-1-pyridin-2-ylethyl methanesulfonate.

Similarly, the (*S*)-enantiomer (*S*)-**122** was prepared from (*S*)-**117** *via* synthesis of the mesyl (Ms) ester, S_N2 displacement with $H_2C(COOMe)_2$, hydrolysis of the methyl ester groups and decarboxylation (**Scheme 5.9**).



Reagents: (i) MsCl, Et_3N, DMAP, CH_2Cl_2, rt; (ii) DMM, NaH, DMSO, rt, 12h; (iii) a. NaOH, reflux, 12h; b. HCl, reflux, 12h.

Scheme 5.9: Synthesis of (*S*)-3-phenyl-3-pyridin-2-ylpropanoic acid *via* S_N2 displacement of (*S*)-1-pyridin-2-ylethyl methanesulfonate.

Unfortunately, the HPLC and CE methods which were successfully used to separate the stereoisomers of 3-phenylbutanoic acid and its methyl ester (see above) were not appli-

cable to compound **122**. However, the circular dichroism spectra confirm that the optical antipodes of 3-pyridin-2-ylbutanoic acid, **(*R*)-** and **(*S*)-122**, were obtained (**Fig. 5.5**).

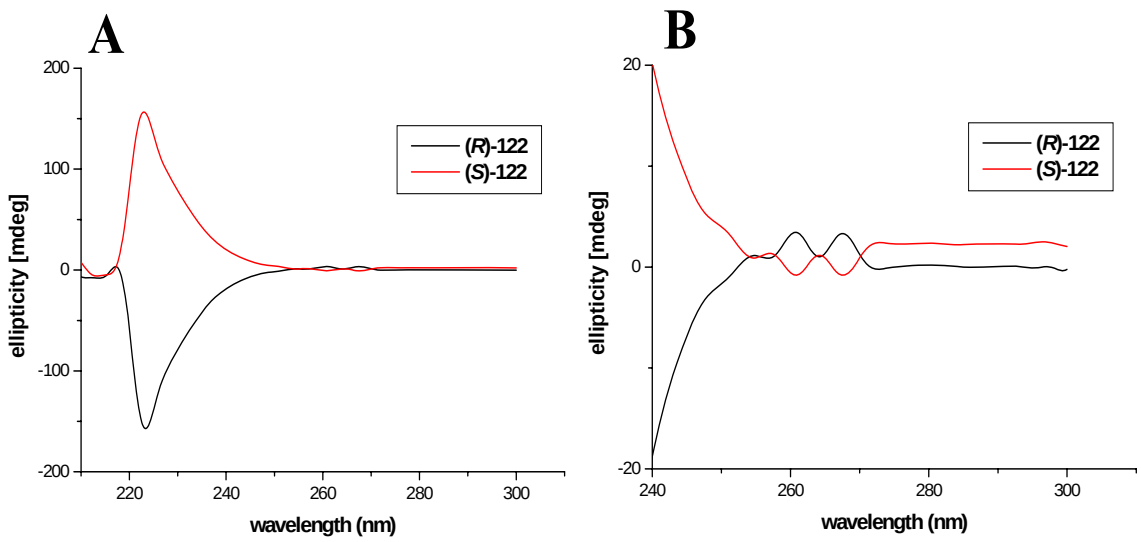
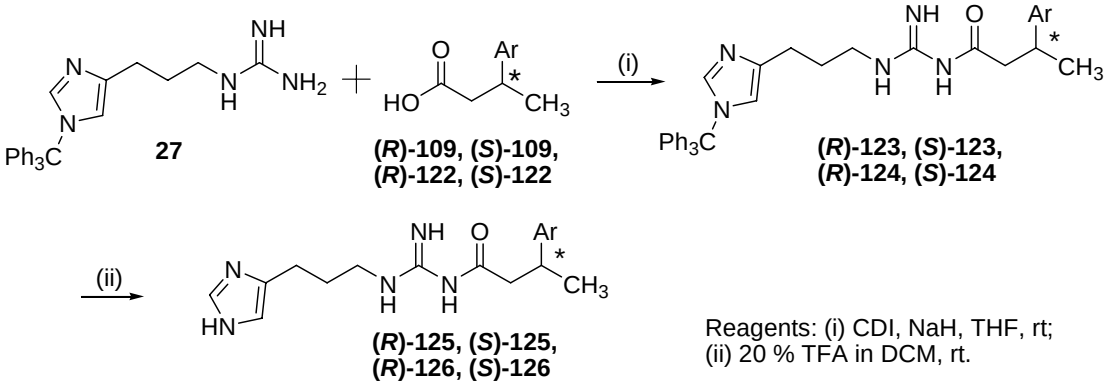


Fig 5.5: Circular dichroism spectra of the two enantiomers of the carboxylic acid **122**. **A**: spectrum from 200 to 330 nm wavelength; **B** (magnification): 240 - 300 nm. The analytes were dissolved in methanol. The analyte concentrations were 10 mM.

The coupling of the chiral acids (*R*)-**109**, (*S*)-**109**, (*R*)-**122** and (*S*)-**122** with the imidazolylpropylguanidine building block **27** according to the general procedure described in **Chapter 3** followed by detritylation with TFA gave the corresponding chiral N^G-acylated products (**Scheme 5.10**).



Scheme 5.10: Coupling of chiral acids with imidazolylpropylguanidin

The enantiomeric purities of *(R)*- and *(S)*-**125**, 98.3 and 95.5 %, respectively, were determined by capillary electrophoresis (**Fig. 5.6**).

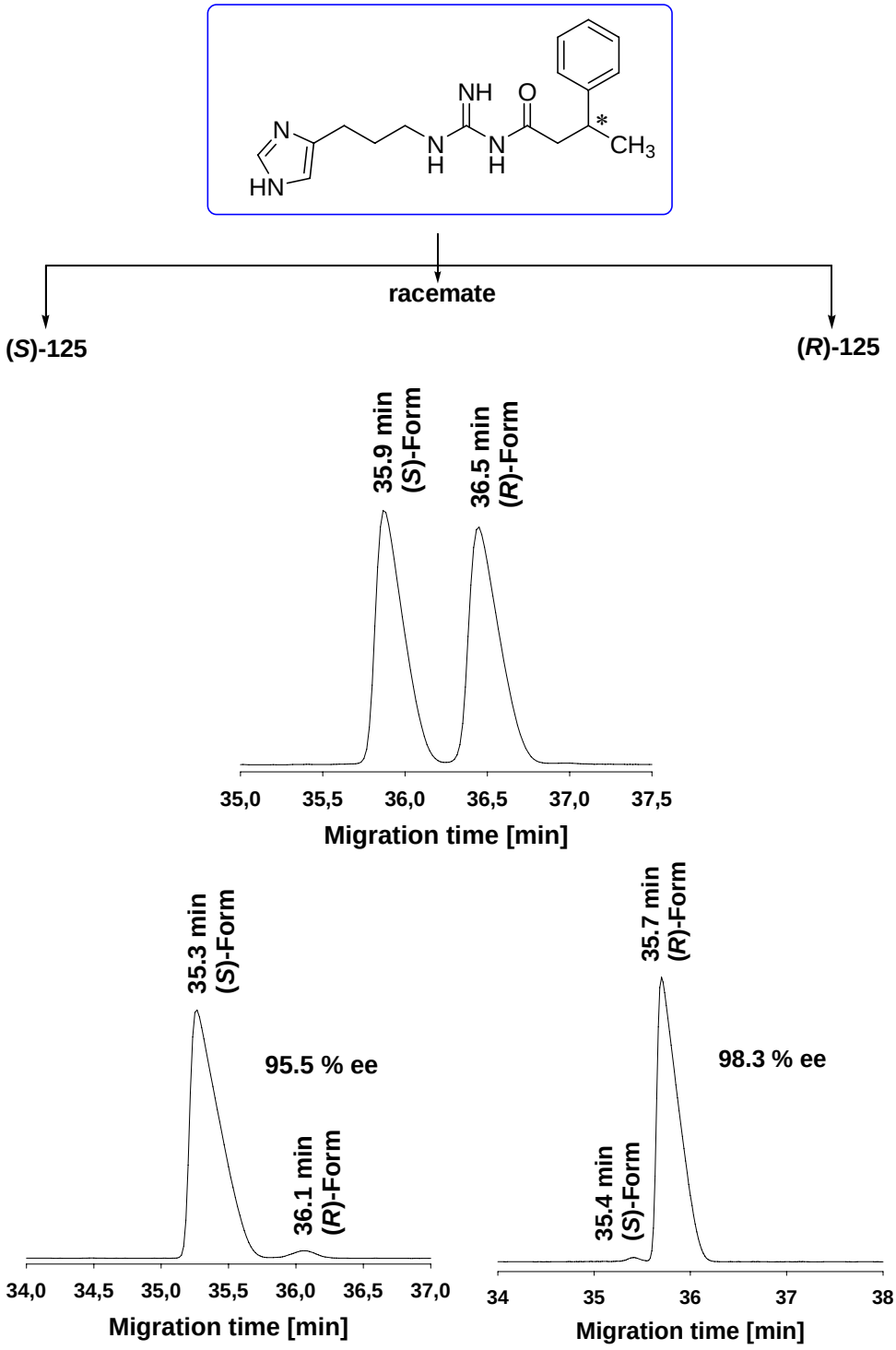
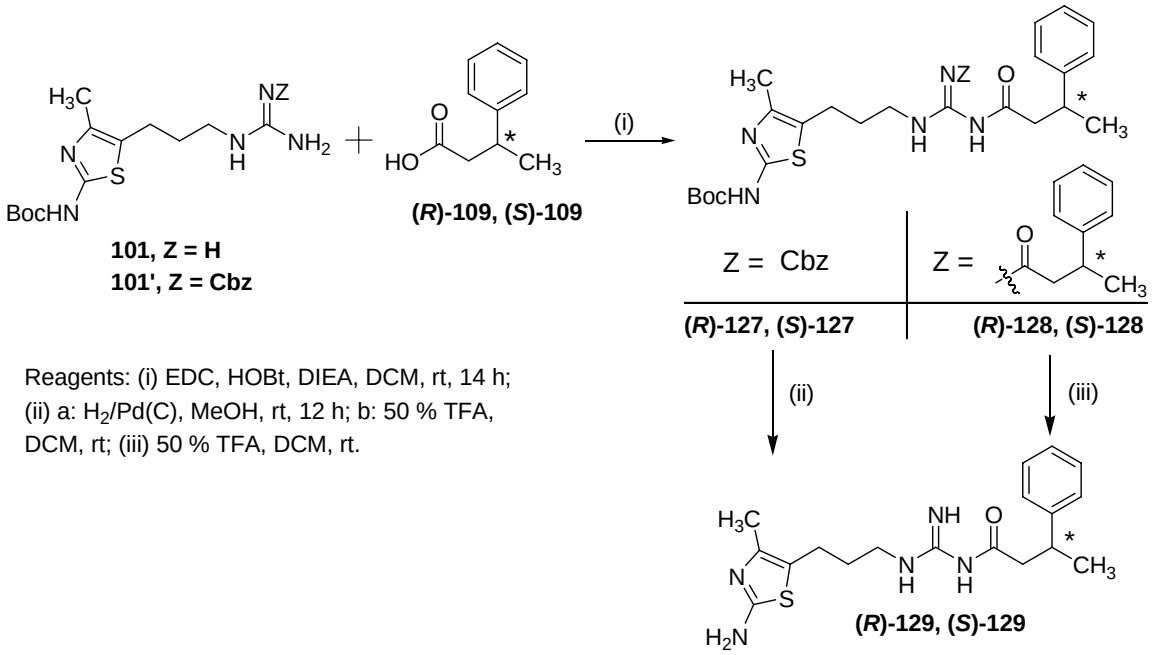


Fig 5.6: Representative electropherogram of the chiral separation of acids, racemate (**78**), (*R*)- and (*S*)-**125** using (2-hydroxypropyl)-β-CD as chiral selector. Analyte concentration:

500 µM; capillary temperature 30 °C; running buffer 30 mM/l (2-hydroxypropyl)-β-CD in 5M urea and 125 mM sodium phosphate buffer pH 2.50; UV-detection: 210 nm.

The corresponding 2-aminothiazole analogue was prepared from the building blocks **101** and **101'**, and the chiral acids (*R*)-**109** and (*S*)-**109** according to the general coupling procedure described in **Chapter 4**, followed by cleavage of the Boc protecting group (**Scheme 5.11**).



Scheme 5.11: Coupling of chiral acids with 3-(2-amino-4-methylthiazol-5-yl)propyl-guanidine

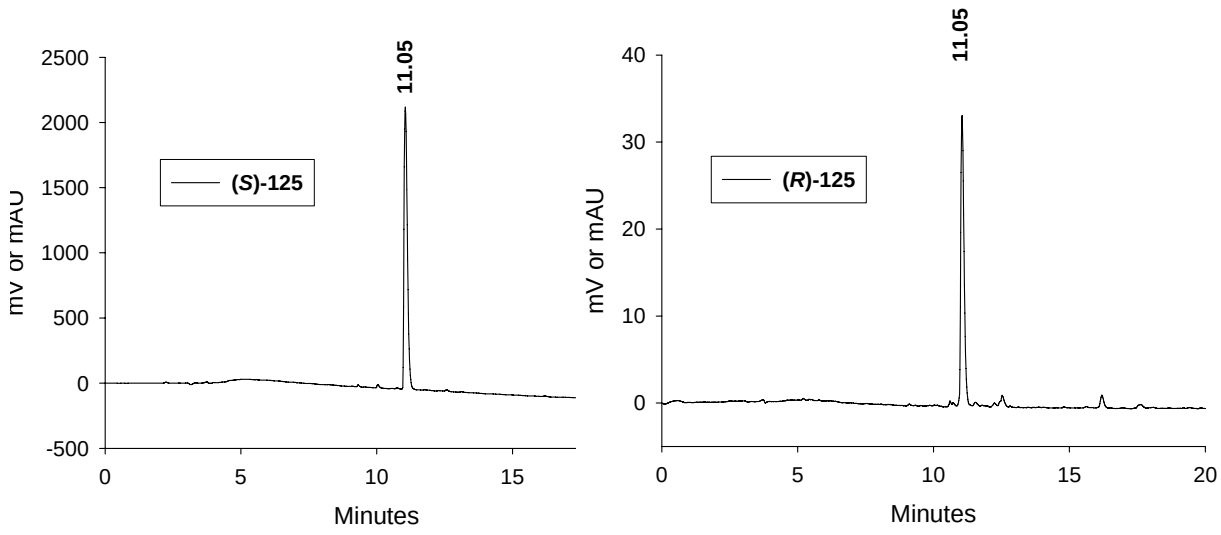


Fig. 5.7: Representative analytical HPLC chromatograms of **(*S*)-125** (Rt: 11.05 min; UV detection at 210 nm) and **(*R*)-125** (Rt: 11.05 min; UV detection at 254 nm). Detailed description is given in experimental section.

The purity of the synthesised compounds was checked by HPLC analysis as shown for (*R*)- and (S)-**125** in **Fig. 5.7**.

Table 5.1: Analytical data of the chiral N^G-acylated heteroarylpropylguanidines **125, 126** and **129**

Enantiomer[a]	**% ee**[b,]	**Formula**[c]	**HRMS**[d] **m/z found (calculated)**	**Rt (min)**[e]
(*R*)-125	98.3	$C_{17}H_{23}N_5O$ (2 TFA)	313.1906 (313.1902)	11.0
(*S*)-125	95.5	$C_{17}H_{23}N_5O$ (2 TFA)	313.1905 (313.1902)	11.0
(*R*)-126	n.d.	$C_{16}H_{22}N_6O$ (3 TFA)	314.1863 (314.1855)	9.4
(*S*)-126	n.d.	$C_{16}H_{22}N_6O$ (3 TFA)	314.1863 (314.1855)	9.4
(*R*)-129	98.3	$C_{18}H_{25}N_5OS$ (2 TFA)	359.1778 (359.1779)	11.7
(*S*)-129	95.5	$C_{18}H_{25}N_5OS$ (2 TFA)	359.1780 (359.1779)	11.7

[a] Purified by preparative HPLC; b techniques for determination of ee is given in **Fig. 5.6** and experimental section [c] isolated as trifluoroacetic acid salt; [d]Techniques for HRMS is described in experimental section of respective compounds; [e] retention time in minutes; the solvent system (0.05 % TFA / acetonitrile; gradient)

is presented in experimental section and the experimental setup is in the experimental section; representative chromatogram in **Fig. 5.7;** nd = not determined.

5.3. Pharmacological Results and Discussion

The synthesised compounds were tested for histamine H_2 receptor (H_2R) agonistic activity on the isolated spontaneously beating guinea pig right atrium (positive chronotropic response). Most of the acylguanidines were also investigated for H_1 antagonism on the isolated guinea pig ileum and on U-373 MG human cells. The results are summarized in **Table 5.2**. Moreover, the agonistic activity was studied in the GTPase assay using guinea pig and human H_2R $G_{s\alpha S}$ fusion protein expressing Sf9 insect cells (see **Table 5.3**).

Table 5.2: Histamine H_2 receptor agonism on guinea pig right atrium, H_1 receptor antagonism on guinea pig ileum and on U-373 MG human cells (Ca^{2+}-assay)

No.	Histamine H_2 receptor agonism on the isolated guinea pig right atrium				Histamine H_1 receptor antagonism		
					guinea pig ileum		U-373 MG cells
	pEC_{50}[a,f] ± SEM[f]	relative potency[b]	E_{max} (%)[c]	N[d]	pA_2[a,c,g] ± SEM	N[d]	IC_{50} [µM][e]
1, HIS	6.00 ± 0.10	100	100 ± 2	>50			
78[h]	7.45 ± 0.07	2710	99 ± 2	4	6.31 ± 0.06	2	19.5
(*R*)-125	7.47 ± 0.08	2930 (108 % relative to **78**)	102 ± 2	3	5.59 ± 0.04	14	32
(*S*)-125	6.90 ± 0.12	800 (30 % relative to **78**)	93 ± 1	3	5.85 ± 0.03	16	37
gpH_2R: **(*R*)-125 : (*S*)-125 = 3.5 : 1**					gpH_1R: **(*R*)-125 : (*S*)-125 = 1 : 1.8**		
80[i]	7.29 ± 0.06	1940	98 ± 2	3	n.d.		49
(*R*)-126	7.15 ± 0.12	1400	101 ± 3	3	n.d.	-	35
(*S*)-126	7.15 ± 0.07	1400	101 ± 1	3	n.d.	-	66
104b[j]	7.18 ± 0.17	1520	89 ± 3	4	5.48 ± 0.10	6	18
(*R*)-129	6.68 ± 0.10	730 (48 % rel. to **(±)-129**)	73 ± 4	3	n.d.	-	17

(*S*)-129	7.27 ± 0.11	1840 (120 % rel. to **(±)-129**)	87 ± 3	4	n.d.	-	25
gpH$_2$R: **(*R*)-129 : (*S*)-129 = 1 : 2.5**							

[a] Explanation of abbreviations see appendix; [b] Potency relative to histamine = 100 %, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; [d] Number of experiments; [e] IC_{50} values for the inhibition of the histamine (30 µM) induced increase in intracellular calcium; mean of at least 3 independent experiments; SEM < 10 %; [f] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by the equation: $pEC_{50} = 6.00 + \Delta pEC_{50}$. The *SEM* given for pEC_{50} is the *SEM* calculated for ΔpEC_{50}; [g] Antagonist (pA_2); n.d. = not determined; [h] racemic **78** (see **Chapter 3**) corresponds to **(±)-125;** [i] racemic **80** (see **Chapter 3**) corresponds to **(±)-126**; [j] racemic **104b** (see **Chapter 4**) corresponds to **(±)-129**.

On the spontaneously beating right atrium (*R*)-3-phenylbutanoylguandine **(*R*)-125** was found to be slightly more active (108 %) than the corresponding racemate (compd. **78** (see **Chapter 3**) corresponds to **(±)-125**), whereas **(*S*)-125** was only 30 % as potent as **78**, i.e. the (R)-enantiomer is the eutomer with an eudismic ratio of 3.5. **(*R*)-125** was found to be full agonist whereas the intrinsic activity of **(*S*)-125** was slightly reduced (partial agonist with i. a. = 0.93) (**Table 5.2**). On the guinea pig ileum there was only a very low difference in antagonistic activities in favour of the (*S*)-enantiomer.

No difference in agonistic activity on the atrium was found for the enantiomeric 3-pyridin-2-ylpropanoylguanidines. In this case, the chiral separation of the acylguanidines by means of capillary electrophoresis or HPLC was unsuccessful. As racemisation during the coupling reaction cannot be precluded there is still some doubt concerning the enantiomeric purities of **(*R*)-** and **(*S*)-126**. Further analytical investigations are required for clarification.

Compared to **(*R*)-125** the situation was other way round for the corresponding 2-amino-thiazole analogues (graphical presentation in **Fig. 5.7**): **(*S*)-129**, was found to be more active than **(*R*)-129** by a factor of 2.5. This may be interpreted as a hint to a different binding mode of thiazoles and corresponding imidazoles. As the difference in stereoselection is rather small, overestimation of these results should be prevented, however, the result is in accordance with the data from the GTPase assay (see **Table 5.3**).

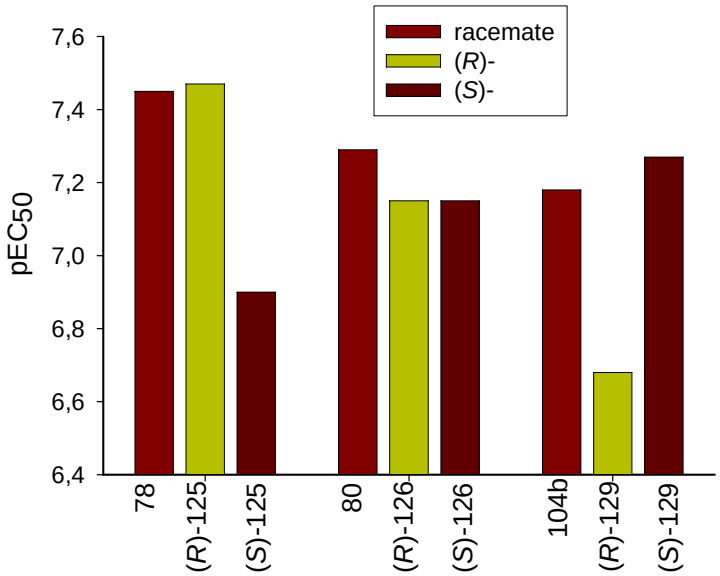


Fig. 5.7: H_2R agonistic potencies of racemates (**78**, **80**, **104b**) and corresponding enantiomers on the isolated spontaneously beating guinea pig right atrium (pEC_{50} values).

The pharmacological data obtained from the guinea pig right atrium (**Table 5.2**) was supported by the results obtained from GTPase assay on guinea pig H_2R-$G_{s\alpha}$ fusion proteins; here also the **(*R*)-125** was found to be 3.2 times more potent than corresponding **(*S*)**-enantiomer. Moreover, similar to the results from guinea pig right atrium, the (*S*)-configured 2-aminothiazole analogue **(*S*)-129** was 2 times more active than its optical antipode. The efficacy was also higher for the (*S*)-enantiomer. By analogy with the results from the guinea pig right atrium no significant difference was found for **(*R*)-** vs. **(*S*)-126** (R. Seifert, personal communication, data not shown). On the human H_2R-$G_{s\alpha s}$ neither enantiomeric pair produced significantly different agonistic effects.

Table 5.3: Activity on the human and guinea pig receptor (GTPase assay* on human and guinea pig H_2R-$G_{s\alpha}$ fusion proteins)

	hH_2R-$G_{s\alpha S}$			**gpH_2R-$G_{s\alpha S}$**			$\frac{EC_{50}\ hH_2R\text{-}G_{s\alpha S}}{EC_{50}\ gpH_2R\text{-}G_{s\alpha S}}$
No.	Efficacy	pEC_{50}	Rel. Pot.	Efficacy	pEC_{50}	Rel. Pot.	
HIS[a]	1.00	5.90	100	1.00	5.92	100	1.05
5d[a]	0.79 ± 0.07	6.72	659	1.02 ± 0.04	7.15	1600	2.71
78[b]	0.87 ± 0.01	7.17	1880	1.03 ± 0.06	7.92	10300	5.58
(*R*)-125	1.07 ± 0.17	7.10	1575	1.19 ± 0.17	7.62	5000	3.33

(*S*)-125	1.01 ± 0.16	6.92	1050	1.01 ± 0.10	7.12	1600	1.6
104b[c]	0.82	7.32	2630	0.98	7.65	5370	2.04
(*R*)-129	0.64	7.27	2333	0.86	7.81	8000	3.6
(*S*)-129	0.87	7.27	2333	1.00	8.12	15000	6.75

[a] data from ref.[16] [b] from **Table 3.3** in **Chapter 3**; [c] from **Table 4.4** in **Chapter 4**.

***** Potencies and efficacies of ligands at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were determined in the GTPase assay. GTP hydrolysis was determined as described in literature. Reaction mixtures contained membranes of Sf9 cells expressing fusion proteins and agonists at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Curves were analyzed by nonlinear regression. Typical basal GTPase activities ranged between ~1 and 2 pmol/mg/min, and typical GTPase activities stimulated by HIS (100 µM) ranged between ~4 and 8 pmol/mg/min. To calculate agonist efficacies, the maximum stimulatory effect of HIS was set at 1.00, and the stimulatory effects of other agonists were referred to this value. Data shown are the means ± SD of four to six experiments performed in duplicate. The relative potency (Rel. Pot.) of HIS was set at 100, and the potencies of other agonists were referred to this value to facilitate comparison of agonist potencies. Efficacies and potencies, respectively, of ligands at hH_2R-$G_{s\alpha S}$ were compared with the corresponding parameters at gpH_2R-$G_{s\alpha S}$ using the t test.

5.4. Conclusion

Previously, in the arpromidine series of H_2R agonists eudismic ratios up to 40 were found in favour of the (*S*)-enantiomer. In the new series of acylguanidine-type histamine H_2 receptor agonists described in this thesis N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-(3-phenyl-butanoyl)guanidine was discovered as a potent H_2 histaminergic agent and a promising candidate for further structural optimisation. Analogues having a heterocyclyl instead of the phenyl rings were equipotent. Moreover, replacement of the imidazolyl ring with a bioisosteric 2-amino-4-methylthiazol-5-yl group resulted about the same H_2R agonistic potency. Due to the chirality of the aforementioned acylguanidines special attention had to be paid to the stereoselective synthesis and pharmacological investigation of a few representative enantiomeric H_2R agonists. The pertinent intermediate, 3-phenylbutanoic acid, was prepared *via* enantioselective addition of phenylboronic acid to (*E*)-butenoic acid ester. The pyridyl analogue was accessible by enantioselective enzymatic prepar-ation of 1-pyridin-2-ylethyl acetate followed by conversion of the enantiopure alcohol to the required building block. Finally, the chiral acids were coupled to imidazolylpropyl-guanidine. With the exception of the pyridyl analogue, the enantiomeric purity of the syn-thesised acylguanidines could be determined by CE. The enantiomeric excess were in the range of 95-99 %, which were considered to be sufficient for pharmacological investigations.

Compared to the arpromidine series the acylguanidine-type H_2R agonists showed rather low eudismic ratios in the range of 2 - 3.5. The data from the guinea pig atrium were consistent with those determined in the GTPase assay using H_2R-$G_{s\alpha s}$ fusion proteins. Interestingly, the preferred stereoisomers were conversely configured in case of the imidazolyl compound (R > S) and its aminothiazolyl analogue (S > R), respectively. This information is of particular interest with respect to the further refinement of receptor models, as the binding mode of imidazoles and aminothiazoles appears to be different. Although the alternate exchange of both heterocyclic rings was tolerated without noteworthy changes in H_2R agonistic activity, the concept of bioisosterism may be questioned. Additional enantiomeric pairs are required to study this phenomenon in more detail. As suggested by molecular modelling the replacement of the methyl group in the 3-phenylbutanoyl portion of the molecule, especially the variation of substituent size and functionality, will be useful to interpret the H_2R binding on the molecular level and to identify potential interaction sites of the acyl group.

5.5. Experimental Section

5.5.1. Chemistry

General procedure

For detailed description of the general procedures and equipments, and preparative and analytical HPLC, see section **3.6.1** of **Chapter 3**.

The chiral HPLC and CD spectra referred to this chapter consisted of a L-6000A pump (MERCK-HITACHI), a CD 1595 (Jasco) detector, a Chiralcel OD column. Injection volume was 40-50 µL, analyte concentration was 0.5 mg/mL; mobile phase was either n-heptane-isopropanol or n-hexane-isopropanol (99:1) as mentioned later; temperature: 22°C; Flow: 0.5 mL/min.

Circular dichroism spectrometry

Circular dichroism measurements were made with a Model J-710 CD Spectropolarimeter from Jasco (Groß-Umstadt, Germany) using a 5 mm quartz cell at room temperature. In order to avoid errors due to racemization, the sample solutions were prepared by dilution

of methanolic stock solutions (analyte concentration: about 10 mM) immediately before recording the spectra.

Capillary electrophoresis

Electrophoretic experiments were performed with a Biofocus 3000 capillary electrophoresis system (Bio-Rad, Munich, Germany). Chiral separations were carried out in a 75 (effective length 70.4 cm)×50 μm I.D. fused-silica capillary (Microquarz, Gesellschaft für Quarzglaskomponenten, Munich, Germany). The voltage (positive mode) was set at 20 kV, resulting in a field strength of 266.7 V/cm. In order to obtain reproducible operating conditions with respect to the capillary surface, before hydrodynamical injection of the samples at a constant pressure–time integral of 20 p.s.i.·s (1 p.s.i.=6894.76 Pa), the capillary was flushed with 0.1 M NaOH for 5 min, with water for 2 min and finally with running buffer for 10 min. The UV detector was set to 210 nm.

For the preparation of the running buffers 125 mM $NaH_2PO_4 \cdot H_2O$ and 5 M urea were dissolved in water, and the pH was adjusted by adding appropriate amounts of 85% H_3PO_4 and NaOH (conc.), respectively. The CDs were dissolved in these sodium phosphate buffers. CD concentrations were calculated as $c^* = n(\text{CD})/v(\text{solvent})$ (mM). The high urea concentration was necessary to increase the water solubility of the CDs, in particular of β-CD. Urea slowly decomposes in water to CO_2 and NH_3. To preclude changes of the buffer pH, the buffers were made freshly every few days. To reach the required high concentrations of α- and β-CD in the sodium phosphate buffers, the solutions were sonicated and subsequently carefully heated on a water bath. For dissolving γ-CD at the required high concentrations, sonication alone was sufficient. Because of the high viscosity of the running buffers, these solutions were used without final filtration.

General procedure for the preparation of (*R*)- and (*S*)-methyl 3-phenylbutanoate [(*R*)-and (*S*)-108] [6]

Rh(I) catalysed conjugate 1,4-addition of phenylboronic acid to methyl alkenoates: Rhodium complex (0.03 mmol), ligand (0.045 mmol), and arylboronic acid (2 mmol)

were added to the flask containing a magnetic stirring bar, a septum inlet, and a reflux condenser. The flask was charged with 1,4-dioxane (6 mL) and water (1 mL). After being stirred for 30 min, the pertinent α,β-unsaturated ester (1.0 mmol) was added. The mixture was then stirred for 16 h (temperature see below). The product was extracted with benzene, washed with brine, and dried over anhydrous magnesium sulfate. Chromatography over silica gel gave the desired product.

(*R*)-Methyl 3-phenylbutanoate [(*R*)-108][5]

At 100 °C; yield 80 %; 88 % ee (HPLC, OD-H: hexane/2-propanol; colourless oil; $C_{11}H_{14}O_2$ (178.2); $[\alpha]^{20}_D$ -18.9° (c 0.72, $CHCl_3$) {lit.[12] $[\alpha]^{33}_D$ -29.4° (c 1.02, $CHCl_3$)}; IR (neat): 3062, 3029, 2965, 1738, 1604 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.78 (m, 5H, Ar-***H***), 3.71 (s, 3H, OC***H***$_3$), 3.38 (m, 1H, C***H***CH_3), 2.66 (dd, J = 6.9 Hz, J_2 = 15.1 Hz, 1H, one H of C***H*****$_2$**$CHCH_3$), 2.58 (dd, J = 8.2 Hz, J_2 = 15.1 Hz, 1H, one H of C***H*****$_2$**$CHCH_3$), 1.40 (d, J = 7.0 Hz, 3H, CHC***H***$_3$); 13C-NMR ($CDCl_3$), δ (ppm): 172.7 (quart, **C**=O), 145.5 (quart, Ph-C-1), 128.3, 126.5, 126.2 (+, Ph-C), 51.3 (+, $COCH_3$), 42.5 (-, ***C***H_2CO); 36.3 (+, ***C***HCH_3), 21.6 (+, CH***C***H_3); MS (ESI,), 201 $[M + Na]^+$

(*S*)-Methyl 3-phenylbutanoate [(*S*)-108][5]

At 100 °C; yield 82 %; 86 % ee (HPLC, OD-H: hexane/2-propanol; colourless oil; $C_{11}H_{14}O_2$ (178.2); $[\alpha]^{20}_D$ +19.5° (c, 0.70, $CHCl_3$); IR, NMR and MS data is the same as for (*R*)-**108**.

Preparation of (*R*)- and (*S*)-3-phenylbutanoic acid [(*R*)- and (*S*)-109]

General procedure for the hydrolysis of the methyl esters **(*R*)-** and **(*S*)-108:** To a solution of ester (0.178 g, 1.0 mmol) in THF (8 ml) was added 1N LiOH aqueous solution (1.5 mL). After being stirred at room temperature for 6 h, the solution was acidified to pH 2 with 1N aqueous HCl. It was diluted with EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. To increase the enantiomeric excess the acids were recrystallized from a mixture of EtOAc and n-hexane.

(*R*)-3-Phenylbutanoic acid [(*R*)-109]

Yield 89 %; 98 % ee (CE); colourless oil; $C_{10}H_{12}O_2$ (164.2); $[\alpha]^{20}_D$ -42.5° (c 0.77, benzene) {Lit.[13] $[\alpha]_D$ -45.8° (c 0.77, benzene)} ; IR (neat): 3400-2500, 1705 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.19-7.32 (m, 5H, Ph-***H***), 3.27 (ABq, J_1 = 6.8, 8.3 Hz, J_2 = 15.6 Hz 1H, Ph-C***H***CH_3), 2.67 (AB, J_1 = 6.8 Hz, J_2 = 15.6 Hz, 1H, one H of C***H***$_2$CO), 2.58 (AB, J_1 = 8.3 Hz, J_2 = 15.6 Hz, 1H, one H of C***H***$_2$CO), 1.32 (d, J = 6.8 Hz, 3H, Ph-CHC***H***$_3$); ^{13}C-NMR ($CDCl_3$), δ (ppm): 178.3 (quart, ***C***O), 145.4 (quart, Ph-***C***-1), 128.5, 127.7, 126.5 (+, Ph-***C***), 42.5 (+, Ph-***C***HCH_3), 36.1 (-, ***C***H$_2$CO), 21.8 (+, ***C***H$_3$); EI-MS: *m/z* (rel. intensity, %): 164 ($M^{+\cdot}$, 4), 147 ($[M - OH]^+$, 6), 110 ($[M - CO_2H]^+$, 100).

(*S*)-3-Phenylbutanoic acid [(*S*)-109]

Yield 86 %; 95 % ee (CE); colourless oil; $C_{10}H_{12}O_2$ (164.2); $[\alpha]^{20}_D$ +46.5° (c 0.77, benzene) {Lit.[14] $[\alpha]^{20}_D$ +50.0° (c 1.12, benzene, 87 % ee)}. The analytical data (IR, NMR and MS) is the same as for (*R*)-**109**.

Synthesis of (*R*)- and (*S*)-1-pyridin-2-ylethanol [(*R*)- and (*S*)-117][7]

General procedure for the stereoselective acetylation of (*RS*)-1-pyridin-2-ylethanol (117): A mixture of (*RS*)-**117** (100 mg), lipase {*Candida antarctica* lipase (CAL)} (30 mg), and vinyl acetate (0.2 mL) in anhydrous diisopropyl ether (20 mL) was stirred vigorously at rt for 4 h in the presence of molecular sieves 4A (100 mg). The reaction mixture was filtered through a filtering paper. The filtrate was condensed, and the residue was chromatographed on silica gel to give the ester (*R*)-**122** and recovered alcohol (*S*)-**121**. The enantiomeric purities of the products were determined by HPLC using a chiral Chiralcel OD column (**Fig. 5.4**). Physical properties including specific rotation values and spectroscopic data for the (*R*)-acetates and physical data and specific rotation values for the recovered (*S*)-alcohol is as follows:

(*S*)-1-Pyridin-2-ylethanol [(*S*)-117]

Yield 46 %; colourless oil; $C_9H_{11}NO_2$ (165.2); $[\alpha]^{20}_D$ -29.0° (c 2.0, $CHCl_3$) {Lit.[7] $[\alpha]^{29}_D$ -28.1° (c 2.23, $CHCl_3$)} ; the analytical data (IR, NMR, MS) are all the same as for compound **47a** (**Chapter 3**).

(*R*)-1-Pyridin-2-ylethyl acetate [(*R*)-118]

Yield 46 %; colourless oil; $C_9H_{11}NO_2$ (165.2); $[\alpha]^{20}_D$ +94.1° (c 2.1, $CHCl_3$) {Lit.[7] $[\alpha]^{28}_D$ +97.4° (c 2.03, $CHCl_3$)}; ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.59 (m, 1H, Pyr-6-***H***), 7.69 (m, 1H, Pyr-4-***H***), 7.33 (m, 1H, Pyr-5-***H***), 7.23 (m,1H, Pyr-3-***H***), 5.91 (q, *J* = 6.7 Hz, 1H, Pyr-2-C***H***CH_3), 2.13 (s, 3H, C***H***$_3$CO), 1.60 (d, *J* = 6.7 Hz, 3H, Pyr-2-C***H***CH_3); ^{13}C-NMR ($CDCl_3$), δ (ppm): 170.3 (quart, CO), 160.2 (quart, Pyr-***C***-2), 149.3 (+, Pyr-***C***-6), 136.8 (+, Pyr-***C***-4), 122.7 (+, Pyr-***C***-5), 120.5 (+, Pyr-***C***-3), 73.0 (+, Pyr-2-***C***HCH_3), 21.3 (+, ***C***H_3), 20.6 (+, ***C***H_3); CI-MS [NH_3], *m/z* (rel. intensity, %): 166 ($[M + H]^+$);

(*R*)-1-Pyridin-2-ylethanol [(*R*)-117]

Procedure for chemical hydrolysis of (*R*)-118: To a solution of **(*R*)-118** (3.0 mmol) in methanol (40 mL) and water (10 mL), a 1N solution of NaOH (3.0 mL, 3.0 mmol) was added. The mixture was stirred at rt 3 h. The solution was extracted with chloroform, dried with anhydrous Na_2SO_4 and evaporated to give the alcohol (*R*)-**117**, which was purified with column chromatography on silica gel eluting with ethyl acetate.

Yield 46 %; colourless oil; $C_9H_{11}NO_2$ (165.2); $[\alpha]^{20}_D$ + 22.4° (c 2.00, $CHCl_3$). The analytical data are the same as for **47a** (**Chapter 3**).

Synthesis of (*R*)- and (*S*)-1-pyridin-2-ylethyl methanesulfonate [(*R*)- and (*S*)-120]

The procedure for the preparation of methanesulfonates (*R*)- and (*S*)-**120** is the same as that described for the racemic compound in **chapter 3** (compound **48a**):

(*S*)-1-Pyridin-2-ylethyl methanesulfonate [(*S*)-120]: Yield 92 %; colourless oil; $C_8H_{11}NO_3S$ (201.2); $[\alpha]^{20}_D$ -95.3° (c, 1.50, $CHCl_3$) {Lit.[15] $[\alpha]^{27}_D$ -90.3° (c, 1.63, $CHCl_3$)}; the analytical data (IR, NMR, MS) are all the same as for **48a** (**Chapter 3**).

(*R*)-1-Pyridin-2-ylethyl methanesulfonate [(*R*)-120]: Yield 95 %; colourless oil; $C_8H_{11}NO_3S$ (201.2); $[\alpha]^{20}_D$ + 96.8 (c, 1.53, $CHCl_3$); the analytical data (IR, NMR, MS) are all the same as for **48a** (**Chapter 3**).

Synthesis of (*R*)- and (*S*)-dimethyl 2-(1-pyridin-2-ylethyl)malonate [(*R*)- and (*S*)-121]
The procedure for the preparation of **(*R*)-** and **(*S*)-121** is the same as that described for the racemate in **Chapter 3** (compound **49a**).

Dimethyl 2-((*S*)-1-pyridin-2-ylethyl)malonate [(*S*)-121]: Yield 81 %; colourless oil; $C_{12}H_{15}NO_4$ (237.3); $[\alpha]^{20}_D$ -27.9° (c, 1.20, $CHCl_3$); the analytical data (IR, NMR, MS) are all the same as for **49a** in **Chapter 3**.

Dimethyl 2-((*R*)-1-pyridin-2-ylethyl)malonate [(*R*)-121]: Yield 83 %; colourless oil; $C_{12}H_{15}NO_4$ (237.3); $[\alpha]^{20}_D$ +20.12° (c, 1.24, $CHCl_3$) {Lit.[11] $[\alpha]^{25}_D$ +23.0 ° (c, 1.29, $CHCl_3$)}; the analytical data (IR, NMR, MS) are all the same as for **49a** in **Chapter 3**.

Synthesis of (*R*)- and (*S*)-3-pyridin-2-ylbutanoic acid [(*R*)- and (*S*)-122]
The procedure for the preparation of (*R*)- and (*S*)-**122** is the same as that described for the racemate **50a** in **Chapter 3**.

(*S*)-3-Pyridin-2-ylbutanoic acid [(*S*)-122]: Yield 86 %; colourless oil; $C_9H_{11}NO_2$ (165.2); $[\alpha]^{20}_D$ -35.1° (c, 1.02, $CHCl_3$); the analytical data (IR, NMR, MS) are all the same as for **50a** (**Chapter 3**).

(*R*)-3-Pyridin-2-ylbutanoic acid [(*R*)-122]: Yield 84 %; colourless oil; $C_9H_{11}NO_2$ (165.2); $[\alpha]^{20}_D$ +41.6° (c, 1.06, $CHCl_3$); the analytical data (IR, NMR, MS) are all the same as for **50a** (**Chapter 3**).

Synthesis of N^1-(3-arylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidines (*R*)- and (*S*)-125 and -126
The trityl protected acylguanidines **(*R*)-123**, **(*S*)-123**, **(*R*)-124** and **(*S*)-124** were prepared by analogy with the general procedure for the coupling of racemic carboxylic acids with 3-(1-trityl-1*H*-imidazol-4-yl)propylguanidine described in **Chapter 3** {Compounds **(61-81)a**}. The crude products were purified by a chromatotron under ammonia atmosphere and then treated with 20 % TFA solution as described in **Chapter 3** for the synthesis of

compounds **78 and 80**. Purification of **125** and **126** was done by preparative HPLC as described in **Chapter 3**.

(*R*)-N^1-[3-(1*H*-Imidazol-4-yl)propyl)-N^2-(3-phenylbutanoyl)guanidine [(*R*)-125]

Yield 65 % in two steps; colourless oil; $C_{17}H_{23}N_5O \cdot 2TFA$ (541.8); 95.5 % ee (CE); $[\alpha]^{20}_D$ -14.14° (c, 1.57, MeOH); IR (neat): 3207, 2877, 1667, 1570, 1365, 1277, 1119, 728 cm^{-1}; ^{1}H-NMR (CD_3OD), δ (ppm): 8.76 (s, 1H, Im-2-***H***), 7.34 (s, 1H, Im-5-***H***), 7.30-7.10 (m, 5H, Ph-***H***), 3.32 (m, 3H, Ph-C***H***CH_3 and NHC***H***$_2$), 2.78 (m, 4H, C***H***$_2$CO and Im-4-CH_2C***H***$_2$), 2.00 (m, 2H, Im-C***H***$_2$), 1.30 (d, J = 7.1 Hz, 3H, Ph-CHC***H***$_3$); ^{13}C-NMR (CD_3OD), δ (ppm): 176.1 (quart, CO), 155.2 (quart, C=N), 146.4 (quart, Im-C-4), 134.8 (+, Im-C-2), 134.2 (quart, Ph-C-1), 129.6, 128.1, 127.6 (+, Ph-C), 117.1 (+, Im-C-5), 46.1 (-, ***C***H_2CO), 41.4 (-, ***C***H_2NH), 37.6 (+, Ph-***C***H), 28.1 (-, Im-4-CH_2***C***H_2), 22.5 (+, Im-4-***C***H_2CH_2), 22.3 (+, CH_3); MS (ESI, CH_3CN + 0.1% TFA): 314 ($[M + H]^+$), 627 ($[2M + H]^+$); HRMS: EI-MS: calculated for ($C_{17}H_{23}N_5O$) 313.1903, found 313.1906;

Analytical HPLC: R_t 11.05 min (Column B; 0 min: 0.05% TFA/CH_3CN 97/3, 24 min: 85/15); purity: 99 %.

(*S*)-N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine [(*S*)-125]

Yield 61 % in two steps; colourless oil; $C_{17}H_{23}N_5O \cdot 2TFA$ (541.8); 98.3 % ee (CE); $[\alpha]^{20}_D$ -14.71° (c, 1.45, MeOH); the analytical spectra are all the same as those of **(*R*)-125**.

Analytical HPLC: R_t 11.05 min (Column B; 0 min: 0.05% TFA/CH_3CN 97/3, 24 min: 85/15); purity: 99 %.

(*R*)-N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-(3-pyridin-2-ylbutanoyl)guanidine [(*R*)-126]

Yield 55 % for two steps; colourless sticky oil; $C_{16}H_{22}N_6O \cdot 3TFA$ (656.4); $[\alpha]^{20}_D$ +23.0° (c, 1.35, MeOH); the analytical spectra are all the same as those of the racemic compound **80** (**Chapter 3**).

Analytical HPLC: R_t 9.39 min (Column B; 0 min: 0.05% TFA/CH_3CN 97/3, 20 min: 85/15); purity: 97 %.

(*S*)-N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-(3-pyridin-2-ylbutanoyl)guanidine [(*S*)-126]

Yield 53 % for two steps; colourless sticky oil; $C_{16}H_{22}N_6O \cdot 3TFA$ (656.4); $[\alpha]^{20}_D$ -28.6° (c, 1.40, MeOH); the analytical spectra are all the same as those of the racemic compound **80** (**Chapter 3**).

Analytical HPLC: R_t 9.39 min (Column B; 0 min: 0.05% TFA/CH_3CN 97/3, 20 min: 85/15); purity: 80 %.

Synthesis of (*R*)- and (*S*)-N^1-[3-(1-amino-4-methylthiazol-5-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine [(*R*)-129 and (*S*)-129]

The preparation of the intermediates **(*R*)-127**/(*R*)-1**28** and **(*S*)-127**/**(*S*)-128** (structures see **Scheme 5.11**) was performed by analogy with the procedure described for the coupling of racemic carboxylic acids with Boc-protected-3-(2-amino-4-methylthiazol-5-yl)propylguanidine (**101**) in **Chapter 4** {Compounds }: The crude products were purified by column chromatography to give the enantiomers of **127** and **128**, respectively. The products, **(*R*)-127** and **(*S*)-127** was hydrogenated, followed by deprotection of the Boc group to give **(*R*)-129** and **(*S*)-129**, respectively (cf. racemic compound, **Chapter 4**). The Boc-protected intermediates **(*R*)-128** and **(*S*)-128** were likewise converted to **(*R*)-129** and **(*S*)-129**, respectively, by treatment with 50 % TFA solution (see **Chapter 3**) for a longer period of time (overnight).

(*R*)-N^1-[3-(1-Amino-4-methylthiazol-5-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine [(*R*)-129]

Yield 62 % overall; colourless sticky oil; $C_{28}H_{35}N_5O_2S.2TFA$ (733.7); 95.5 % ee (CE); $[\alpha]^{20}_D$ -11.92° (c, 1.34, MeOH); the analytical spectra are all the same as those of **105b** in **Chapter 4**.

Analytical HPLC: R_t 11.79 min (Column B; 0 min: 97/3, 24 min: 85/15); purity: 85 %.

(*S*)-N^1-[3-(1-Amino-4-methylthiazol-5-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine [(*S*)-129]

Yield 59 % overall; colourless sticky oil; $C_{28}H_{35}N_5O_2S.2TFA$ (733.7); 98.3 % ee (CE); $[\alpha]^{20}_D$ + 14.27° (c, 1.10, MeOH); the analytical spectra are all the same as those of **105b** in **Chapter 4**.

Analytical HPLC: R_t 11.74 min (Column B; 0 min: 97/3, 24 min: 85/15); purity: 100 %.

5.5.2. Pharmacological methods

Described in section **3.6.2** in **Chapter 3**.

5.6. References

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Chapter 6

Summary

Since the characterization of the histamine H_2 receptor by Black et al, several H_2 receptor (H_2R) antagonist such as cimetidine, ranitidine, famotidine, nizatidine and roxatidine acetate have been developed as antiulcer drugs. From the therapeutic point of view the interest in drugs acting on histamine H_2 receptors is focused on antagonists. Nevertheless, there has been increasing evidence that histamine receptor stimulation might be an interesting aspect for the development of future drugs as well. The first highly potent H_2R agonist impromidine resulted from histamine, by replacing the amine functionality with the strongly basic guanidine group and combining the structure with the 2-[(5-methyl-1*H*-imidazole-4-yl)methylthio]ethyl moiety known from the H_2R antagonist cimetidine. Later on, Buschauer and co-workers produced arpromidine and analogues by replacing the "cimetidine-like" part with lipophilic H_2R nonspecific structures. Arpromidine and related imidazolylpropyl-guanidines with a N^G-phenyl(pyridyl)alkyl substituent are the most potent H_2R agonists on the isolated guinea pig right atrium, a standard model for the pharmacological investigation of histamine H_2 receptor ligands. However, the arpromidines are less potent or act as partial agonists at human (hH_2R) compared to guinea pig H_2 receptors (gpH_2R). The strongly basic guanidino group, which is supposed to interact with Asp-98 in transmembrane domain 3 (TM3) of the H_2R, is essential for the agonistic activity but it is also responsible for very low oral bioavailability, non-H_2R-mediated effects and lack of penetration across the blood-brain barrier. Therefore, the objective of this work was to design, synthesize and characterize histamine H_2 receptor agonists, structurally related to arpromidine, but with lower basicity in order to achieve more favourable pharmacokinetic properties, in particular, oral bioavailability and penetration across the blood-brain barrier.

The first part of this project was to develop a general synthetic route for the synthesis of N^G-acylated imidazolylpropylguanidines, i. e. a methylene had to be replaced with a carbonyl group in the connecting chain between the diaryl and guanidine group. In **Chapter 3**, the development of a very convenient and straight forward synthetic route is described for the

synthesis of the central building block, trityl-protected imidazolylpropylguanidine, from the commercially available starting material, urocanic acid and guanidine. Then, the N^G-acylated guanidines were synthesised via coupling of this building block with corresponding acids, followed by deprotection of resulting products.

The synthesised compounds were pharmacologically tested on isolated guinea pig tissues (ileum: H_1R, H_3R; right atrium: H_2R), on human H_1R expressing cells (U373MG) and on membrane preparations of guinea pig and human histamine H_2 or H_4 receptor expressing Sf9 cells. The basicity of the acylguanidines is by 4-5 orders of magnitude lower than that of the corresponding guanidines. However, alkanoylguanidines and alkylguanidines appear to be bioisosteres, and it may be speculated that the acylguanidines are capable of interacting with Asp-98 in transmembrane domain 3 of the H_2R by a charge-assisted hydrogen bond. Compounds having a carbonyl instead of a methylene group adjacent to the guanidine are about equipotent as, for instance, demonstrated for the diphenylpropyl *versus* the diphenylpropanoyl and for impromidine *versus* the oxo-substituted analogue. However, a considerable decrease in activity was found when the methylene group in arpromidine was converted to a carbonyl group. Surprisingly, compounds with only one phenyl ring are equally or even more potent than the diaryl analogues, whereas in the arpromidine series of histamine H_2R agonists highest potency resides in the 3-phenyl-3-hetarylpropyl-substituted imidazolylpropylguanidines. This may be interpreted a first hint to different modes of interaction of the affinity-conferring moiety with the H_2R protein. On the isolated guinea pig right atrium the most active H_2R agonist, the 3-phenylbutanoyl substituted guanidine was about 30 times more potent than the natural ligand. In terms of agonistic potency on the guinea pig atrium the new compounds are inferior to the arpromidines. However, the acylated imidazolylpropylguanidines are superior with respect to pharmacokinetic properties. A very important finding is that the compounds are absorbed from the gastrointestinal tract and are capable of penetrating through the blood-brain barrier (tested by HPLC-MS analysis after administration to mice). Centrally active H_2R agonists will be useful pharmacological tools to study the role histamine H_2 receptors in the brain.

In **Chapter 4**, a general method is described for the synthesis of *N*-acyl-*N*'-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines from a simple starting material. The 3-(2-amino-4-

methylthiazol-5-yl)propylguanidine side chain was prepared first by following a reported method, then the guanidine group was introduced by treating with di-Cbz-triflate-protected guanidine with the amine, followed by deprotection of the Cbz-groups with hydrogenolysis. Then the *N*-acyl-*N*'-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines were prepared via acylation of Boc-protected-3-(2-amino-4-methylthiazol-5-yl)propylguanidine with the corresponding acid, followed by deprotection of the Boc group.

The bioisosteric replacement of the imidazolyl moiety in N^G-acylated guanidine-type histamine H_2 receptor agonists by a 2-amino-4-methylthiazol-5-yl group resulted in about the same H_2R agonistic potency on the isolated guinea pig right atrium as well as in GTPase assays performed with both guinea pig and human H_2R-$G_{s\alpha S}$ fusion proteins. However, the maximal response induced by the thiazoles was slightly reduced, i. e. these compounds were partial agonists in the used pharmacological models. Interestingly, in the GTPase assay on the human H_2R the thiazolyl analogue with cyclohexylbutanoyl substituent was favoured compared to the corresponding phenylbutanoylguanidine. This may be interpreted a hint that a certain degree of selectivity for the human H_2R may be achieved by structural variation of both the acyl and the heterocyclylpropyl group. Moreover, the aminothiazoles proved to be devoid of H_3R antagonistic activity. Thus, the selectivity for H_2R versus H_3R can be considerably improved.

With respect to development of models for the ligand-receptor interactions on the molecular level enantiomerically pure agonists of defined 3D structure are valuable tools to explain the species dependent potency and to optimise the structure of the substances. In **Chapter 5**, the stereoselective preparation of enantiomers of several chiral new H_2R agonists is described. For the synthesis of the target molecules according to the method reported in the previous chapters, the enantiomers of the chiral acids were required. The building block (*R* and *S*)-3-phenylbutanoic acid was synthesized from the achiral precursor, methyl (*E*)-but-2-enoate, via asymmetric addition of phenylboronic acid as reported by Hayashi et al., using a catalytic amount of rhodium catalyst and chiral binap ligand, followed by hydrolysis of the methyl ester. The corresponding chiral pyridyl propanoic acids were prepared by the lipase catalysed enantioselective acetylation of racemic 1-(2-pyridyl)ethanol, then protection of alcohol with methanesulfonate, followed by S_N2 displacement with sodium salt of dimethyl

malonate, and finally the decarboxylation of saponified product. Finally, the chiral acids were coupled to imidazolylpropylguanidine. With the exception of the pyridyl analogue, the enantiomeric purity of the synthesised acylguanidines could be determined by CE. The enantiomeric excess was in the range of 95-99 %, which was considered sufficient for pharmacological investigations.

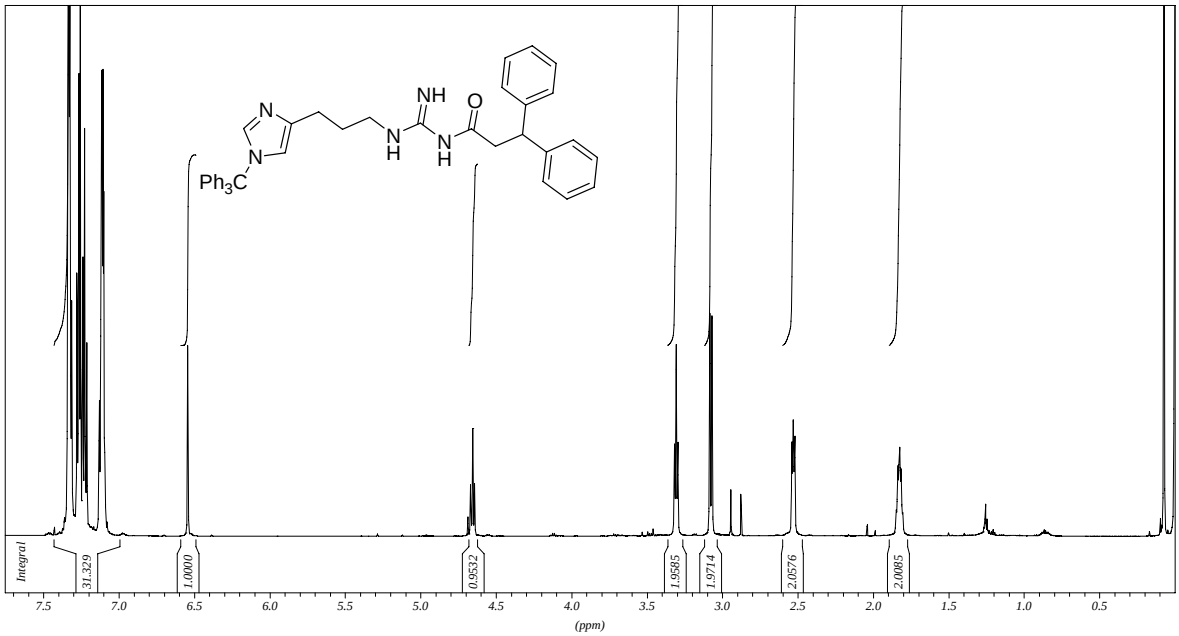
Compared to the arpromidine series the acylguanidine-type H_2R agonists showed rather low eudismic ratios in the range of 2 - 3.5. The data from the guinea pig atrium were consistent with those determined in the GTPase assay using H_2R-$G_{s\alpha s}$ fusion proteins. Interestingly, the preferred stereoisomers were conversely configured in case of the imidazolyl compound (R > S) and its aminothiazolyl analogue (S > R), respectively. This information is of particular interest with respect to the further refinement of receptor models, as the binding mode of imidazoles and aminothiazoles appears to be different. Although the alternate exchange of both heterocyclic rings was tolerated without noteworthy changes in H_2R agonistic activity, the concept of bioisosterism may be questioned. Additional enantiomeric pairs are required to study this phenomenon in more detail. As suggested by molecular modelling the replacement of the methyl group in the 3-phenylbutanoyl portion of the molecule, especially the variation of substituent size and functionality, will be useful to interprete the H_2R binding on the molecular level and to identify potential interaction sites of the acyl group.

Meanwhile imidazolylpropylguanidines and corresponding acylated analogues were found to be useful building blocks beyond the preparation of H_2R agonists, for instance for the synthesis of ligands preferentially binding to other histamine receptor subtypes, to neuropeptide Y Y_1 or Y_4 receptors. Thus, there is increasing evidence that in terms of medicinal chemistry the imidazolylpropylguanidine moiety and the acylated analogues may be considered “privileged structures”. An extremely promising perspective results from very recent studies: surprisingly, some of the acylguanidines proved to be rather potent as either agonists or inverse agonists at the recently discovered H_4R. The elaboration of the structure-activity relationships based on these results and the design of H_4R selective ligands will be subject of a new project.

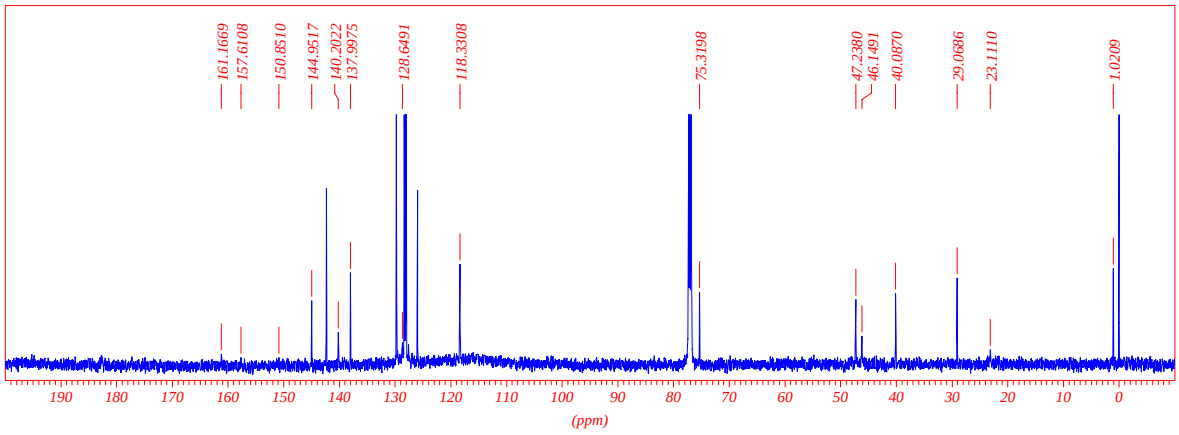
Appendix

1. NMR spectra of 63a:

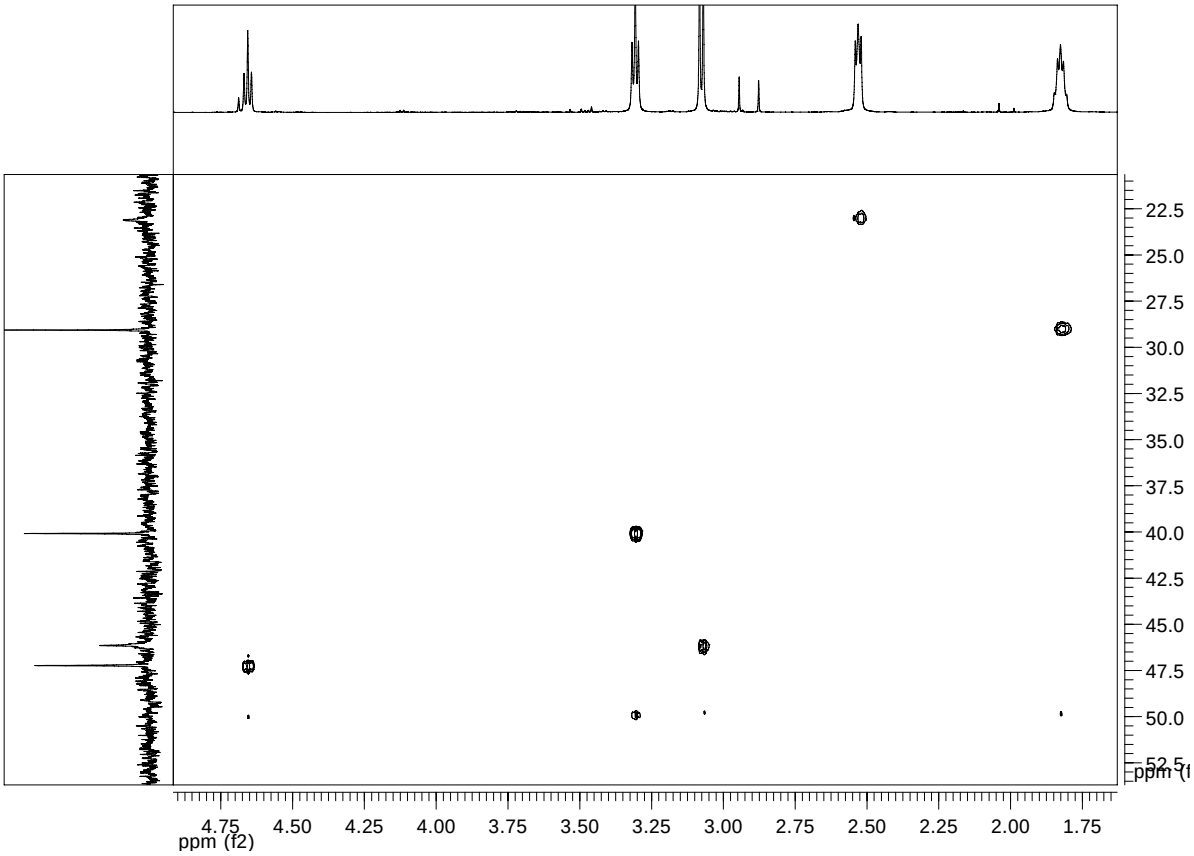
^{1}H-NMR ($CDCl_3$, 600 MHz):



^{13}C-NMR ($CDCl_3$, MHz):

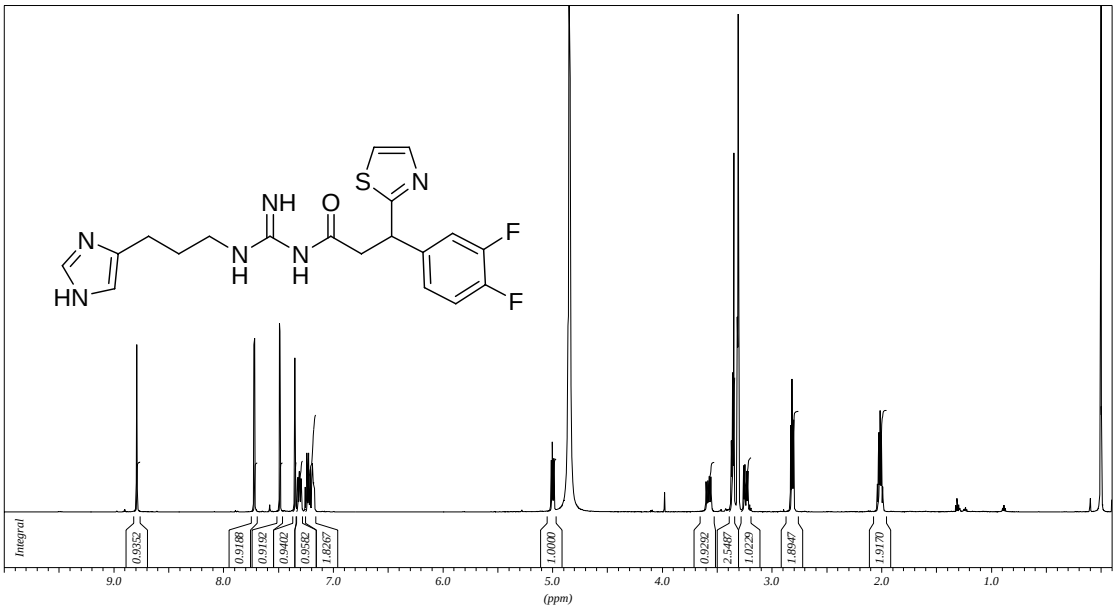


HSQC NMR ($CDCl_3$, 151 MHz) spectra of 63a:

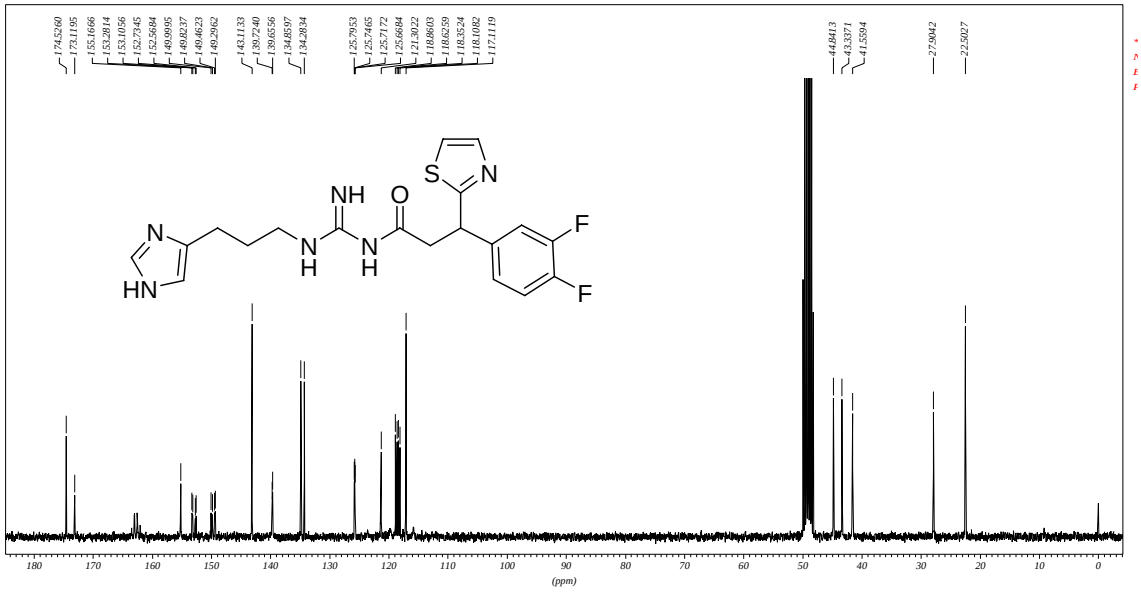


2. NMR spectra of 73:

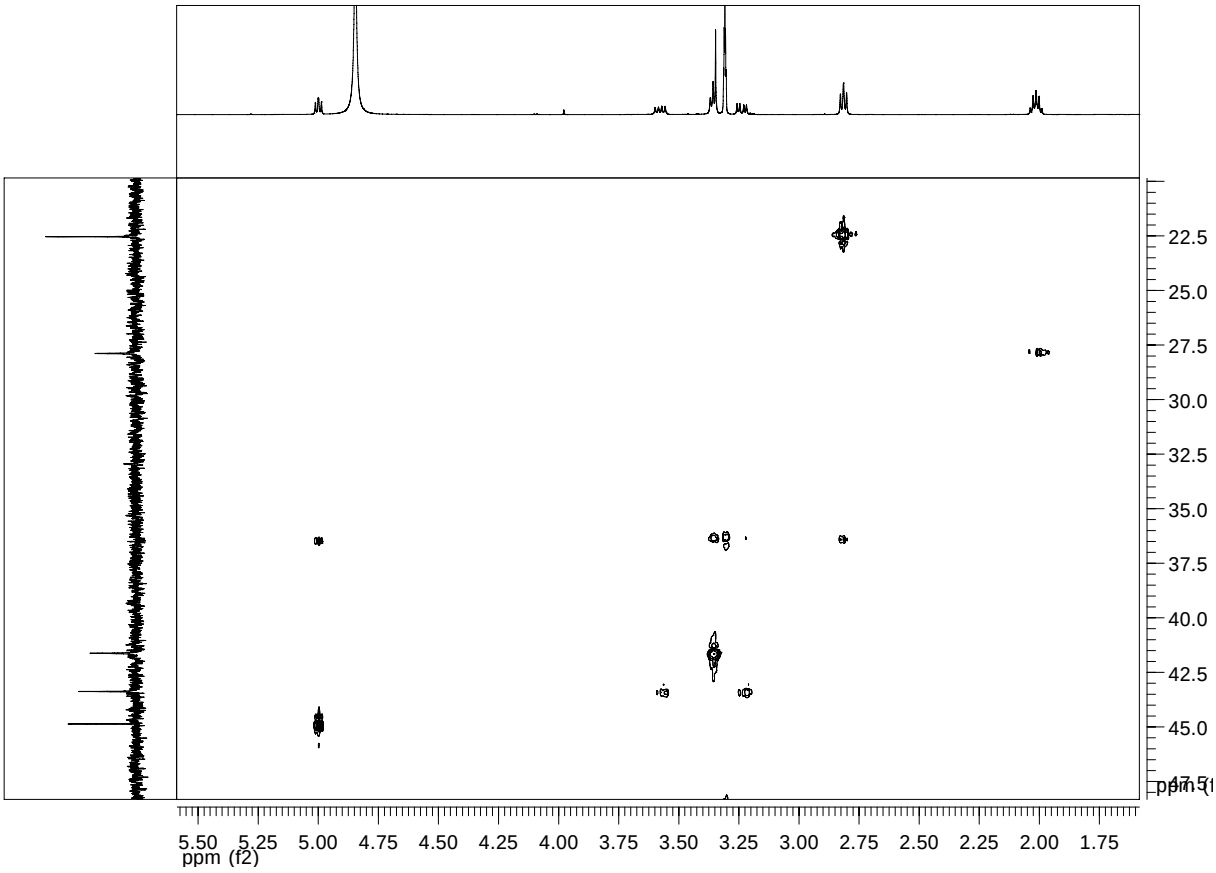
^{1}H-NMR ($CDCl_3$, 600 MHz):



^{13}C-NMR ($CDCl_3$, 151 MHz):



HSQC NMR ($CDCl_3$, 151 MHz) spectra of 63a:



Publications and Abstracts

Publications:

Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and Pharmacological Activity of N^G-Acylated Imidazolylpropylguanidines. (in preparation).

Ghorai, P.; Elz, S.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Buschauer A. Synthesis and Pharmacological Activity of Chiral N^G-Acylated Heteroarylpropylguanidines. (in preparation).

Ghorai, P.; Elz, S.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Buschauer A. Synthesis and Pharmacological Activity of *N*-Acyl-*N*'-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines: Towards Improved H_2 Receptor Selectivity (in preparation).

2003

Posters Presentations:

On **'Tag der Naturwissenschaften'2003** at University of Regensburg, Germany**:**
Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists

On **Jahrestagung der Deutschen Pharmazeutischen Gesellschaft**, October, 8-11, **2003**, University of Wuerzburg, Germany**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. 'Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists'

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Role of Chemical and Biological Sciences in 21st century, December**, 2003,** Agra, India**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A.Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists

2004

Posters Presentations:

On **Frontiers in Medicinal Chemistry**, March **2004**, Erlangen, Germany**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists

On '**2nd Summer School "Medicinal Chemistry**" October 5-7, **2004**' at University of Regensburg, Germany**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists.

On "**Jahrestagung - Joint Meeting 2004**" (Annual Meeting of the German Pharmaceutical Society/Joint Meeting with Austrian and Czech Pharmaceutical Society) at University of Regensburg, Germany**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and pharmacological activity of N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists.

2005

Posters Presentations:

On '**7th CRSI NATIONAL SYMPOSIUM IN CHEMISTRY**', 4-6 February, **2005**, Jadavpur, Kolkata, India**:** Ghorai, P.; Kraus, A.; Götte, C.; Bernhardt, G.; Dove, S.; Reiser, O.; Seifert, R.; Elz, S.; Buschauer A. Synthesis and pharmacological activity of N^G-Acylated aminothiazolylpropyl guanidines: a novel class of histamine H_2 receptor agonists.

On **Frontiers in Medicinal Chemistry**, March **2005**, Leipzig, Germany**:** Kraus, A., Ghorai, P., Keller, M., Bernhardt, G., Dove, S., Reiser, O., Seifert, R., Elz, S., Buschauer, A., N^G-Acylated imidazolylpropylguanidines: a novel class of histamine H_2 receptor agonists

On '**Jahrestagung der Deutschen Gesellschaft für Experimentelle und Klinische Pharmakologie und Toxikologie'**, 15-17 March, **2005**, Mainz, Germany**:** Seifert, R., Xie, S.-X., Ghorai, P., Kraus, A., Elz, S., Buschauer, A., Species-selective histamine H_1-receptor activation by N^G-acylated imidazolylpropyl guanidines, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 371, Suppl. 1, R21 (2005).

Curriculum Vitae

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Date of birth: **28th January, 1978**

Research

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Studies

07/2000-05/2002	M. Sc. In Organic Chemistry, Department of Chemistry, Indian Institute of Technology, Bombay (IITB), India.
07/2000-05/2002	B. Sc. (Hons.) in Chemistry, Kharagpur College, Vidyasagar University, Midnapur, India.