4-(4-Guanidinobenzoyl)-2-imidazolones and Related Compounds: Phosphodiesterase Inhibitors and Novel Cardiotonics with Combined Histamine H_2 Receptor Agonist and PDE III Inhibitor Activity ☆

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Key Words: *cardiotonics, PDE III inhibitors, histamine H_2 receptor agonists, guanidines, imidazolones*

Summary

A series of new positive inotropic agents was synthesized with the aim of combining the pharmacophores of the imidazolone-type phosphodiesterase (PDE) inhibitor enoximone and guanidine-type histamine H_2 receptor agonists such as arpromidine. All compounds are *para*-substituted 4-benzoyl-5-alkyl-2-imidazolones. H_2 agonism was incorporated by *p*-(hetero)arylalkyl substituents, in particular by an imidazolylpropyl guanidine group. In addition analogous ureas, cyanoguanidines, alkyl guanidine carboxylates, and amides were prepared. These functional groups were either directly attached to the phenyl ring or linked by an appropriate spacer. The compounds were screened for positive inotropic activity in the isolated electrically stimulated guinea pig papillary muscle and for inhibition of PDE III (cGMP-inhibited cAMP PDE, isolated from guinea pig heart). The cardiotonics obtained proved to be either PDE III inhibitors, some of them surmounting up to 3-fold the potency of enoximone, or pharmacological hybrids combining both PDE III inhibitor and histamine H_2 receptor agonist activities. These hybrids were the most potent positive inotropic substances at the papillary muscle, probably due to their synergistic mechanism of action. The participation of histamine H_2 receptors could be demonstrated in the papillary muscle preparation by pretreatment with the H_2 antagonist famotidine (10 μM) as well as by further pharmacological experiments using isolated perfused hearts of guinea pigs and rats, isolated guinea pig right atria, adenylyl cyclase and H_2 receptor binding assays. At equieffective concentrations the moderate PDE III inhibitor and histamine H_2 agonist N^1-{4-[(1,3-dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)-carbonyl]phenyl}-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine **65** and the 5-ethyl homologue **66** were about 2 and 10 times more potent than enoximone at the papillary muscle. Moreover, both compounds produced a 2.5-fold higher maximal response than the reference compound.

Introduction

The search for non-steroidal non-adrenergic cardiotonics as digitalis replacement in the therapy of congestive heart failure (CHF) has led to the development of a huge number of positive inotropic agents with different mechanisms of action[1]. Among these compounds inhibitors of the cGMP-inhibited cAMP phosphodiesterase (phosphodiesterase III, PDE III; nomenclature cf. ref.[2]) such as amrinone, milrinone, or enoximone (Scheme 1) represent a class of drugs combining positive inotropic and vasodilatory activities ('inodilators'). Regardless of short-term benefit in acute CHF, long-term treatment with PDE inhibitors appears to be associated with severe adverse reactions and increased mortality as described for milrinone[3]. PDE inhibition combined with additional mechanisms of action, for instance calcium sensitization of contractile proteins[4] or special electrophysiological effects, may possibly be superior to PDE inhibition alone (e.g., cf. pimobendan[5,6], EMD 53998[7], vesnarinone[8]). The stimulation of cardiovascular histamine H_2 receptors, combining both inotropic support and vasodilation, represents another promising approach in acute treatment of catecholamine-insensitive CHF[9]. The H_2 receptor agonist impromidine produced very impressive beneficial hemodynamic effects in severely ill patients refractory to conventional therapy[9]. New highly potent histamine H_2 agonists, such as arpromidine and related guanidines[10,11] (Scheme 1), with a more beneficial hemodynamic profile than that of impromidine were developed as inotropic vasodilators, in particular for potential use in intensive care. In CHF not only the β_1-adrenergic response is reduced as a result of receptor down-regulation[12,13] but also the cAMP signalling pathway in general appears to be impaired owing to up-regulation of G_i protein α-subunits[14] reducing adenylyl cyclase activity. Therefore, the effectiveness of PDE inhibitors may be also reduced in CHF[15]. On the other hand, as shown both *in vitro* and *in vivo*, the increase in contractility achieved by stimulation of myocardial membrane receptors such as β-adrenoceptors or histamine H_2 receptors may be enhanced by inhibition of PDE III and *vice versa*[16,17,18]. For example, the dose of a single drug may be reduced by administration of H_2 agonist plus PDE inhibitor[18] and it may be speculated if H_2 receptor down-regulation can be reduced during treatment over a longer period of time.

In this study synthesis and *in vitro* pharmacological screening of new cardiotonics with benzoylimidazolone partial structure are described. Starting from H_2 receptor agonists and structural modifications around the PDE III inhibitor enoximone[19,20] we tried to obtain novel inotropic agents with both qualities of action by a symbiotic approach, *i.e. by* combining the pharmacophoric features of imidazolone-type PDE inhibitors and guanidine-type histamine H_2 receptor agonists.

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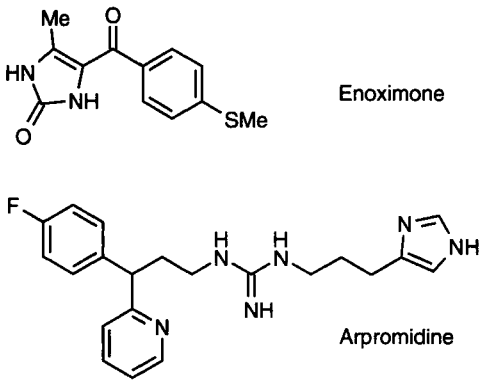
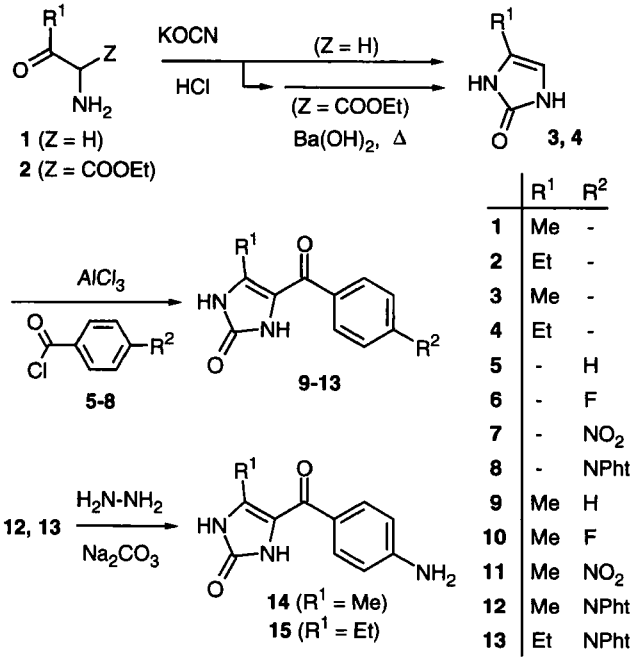


Scheme 1

Chemistry

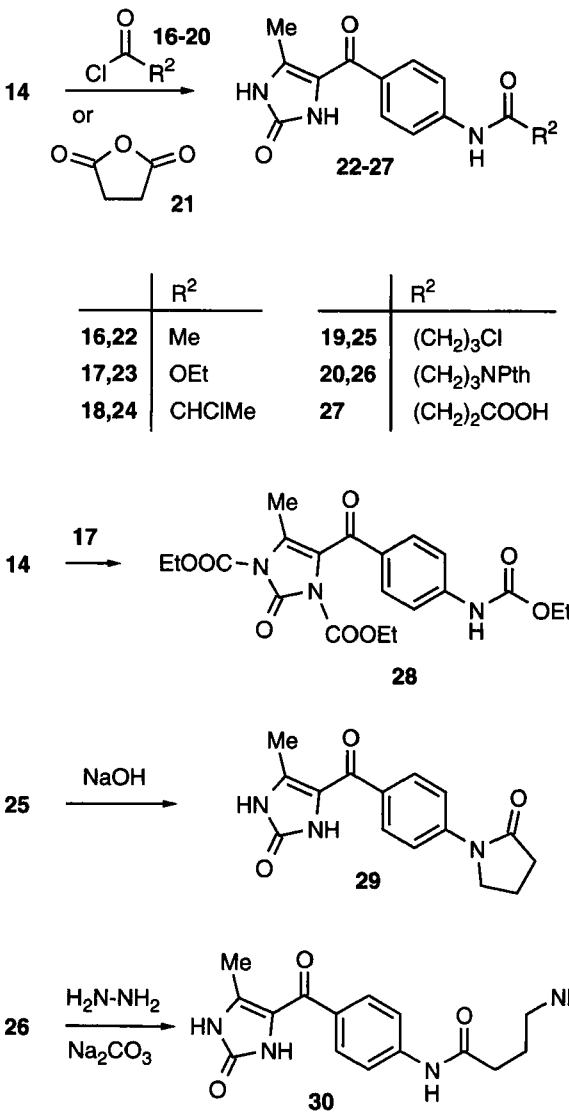
The imidazolones **3, 4**[21,22] were prepared according to known procedures from aminoacetone (**1**) or ethyl 2-amino-3-oxopentanoate (**2**) by cyclization with cyanic acid followed by hydrolysis and decarboxylation in case of the ester (Scheme 2). Subsequently, **3** and **4** were acylated with *p*-substituted benzoylchlorides **5–8** under Friedel-Crafts conditions[19] affording the benzoylimidazolones **9–13**. The phthalimides **12, 13** were converted into the amines **14, 15** which were used as central building blocks for further syntheses.



	R^1	R^2
1	Me	-
2	Et	-
3	Me	-
4	Et	-
5	-	H
6	-	F
7	-	NO_2
8	-	NPht
9	Me	H
10	Me	F
11	Me	NO_2
12	Me	NPht
13	Et	NPht

Scheme 2

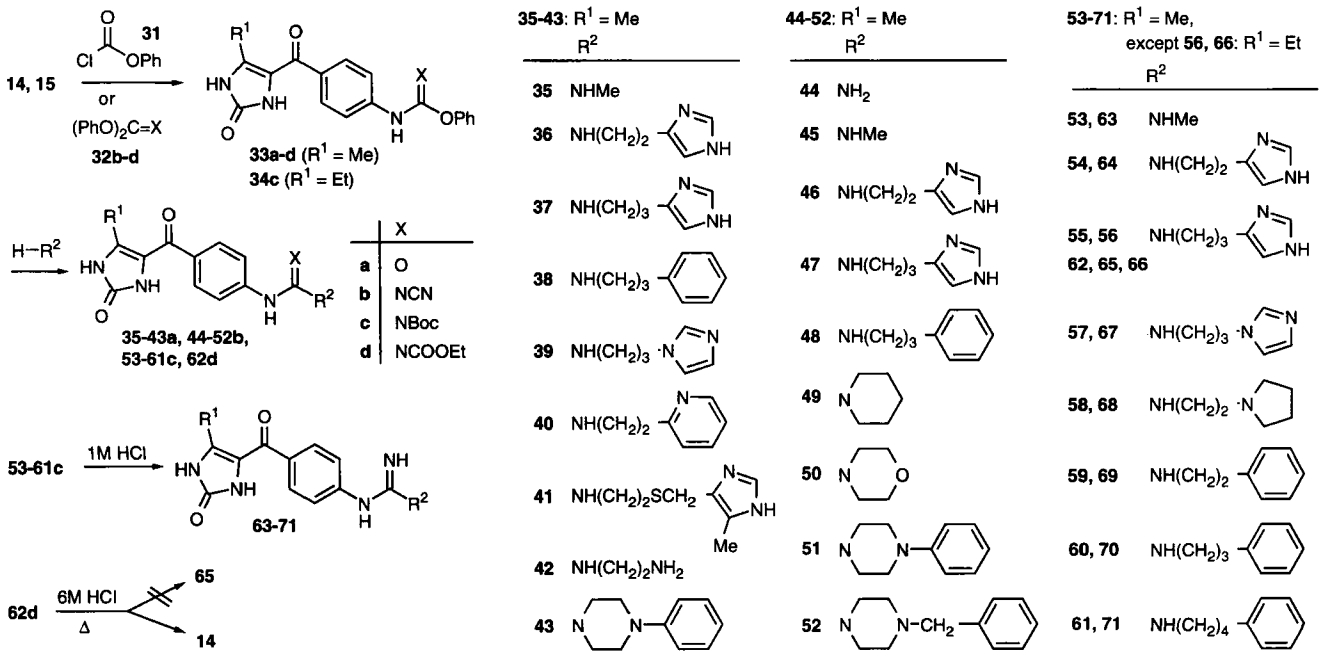
The aminobenzoylimidazolone **14** was *N*-acylated with acyl chlorides **16–20** or succinic anhydride **21** affording the amides **22–27** (Scheme 3). Using an excess of ethyl chloroformate **17** led to further two-fold acylation of the imidazolone ring (**28**). Alkali treatment of the 4-chlorobutanamide **25** resulted in the lactam **29**. Phthalimide **26** could be converted into the primary amine **30** by hydrazinolysis.



	R^2		R^2
16,22	Me	**19,25**	$(CH_2)_3Cl$
17,23	OEt	**20,26**	$(CH_2)_3NPth$
18,24	CHClMe	**27**	$(CH_2)_2COOH$

Scheme 3

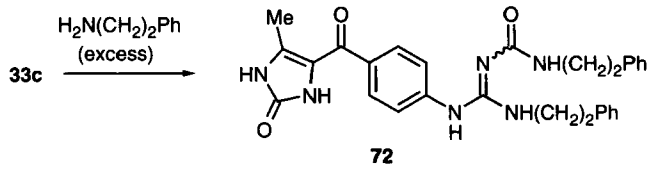
The aminobenzoylimidazolones **14, 15** were converted into phenyl carbamate **33a** by reaction with **31** or were treated with the corresponding diphenyl carbonimidates **32b–d**[23,24] forming the *N*-substituted phenyl isoureas **33b–d, 34c** (Scheme 4). Subsequently, **33a–d, 34c** were allowed to react with a series of amines yielding the ureas **35–43a**, cyanoguanidines **44–52b** and the guanidine carboxylates **53–61c, 62d**. Usually, the preparation of aliphatic *N,N'*-disubstituted cyanoguanidines or alkyl guanidine carboxylates according to the same synthetic pathway requires either refluxing of the corresponding *O*-phenyl isourea with an amine in an inert solvent for several hours or using an excess of the amine component when stirring at ambient temperature. By contrast, the aminolysis of **33b–d, 34c** can be carried out at room temperature with one equivalent of amine using pyridine or acetonitrile as the solvent. This may be interpreted as a consequence of a more pronounced tendency towards elimination of phenol favoured by the electron-withdrawing phenyl nucleus or the phenylogeous amide structure, respectively. Whereas aliphatic alkyl guanidine



Scheme 4

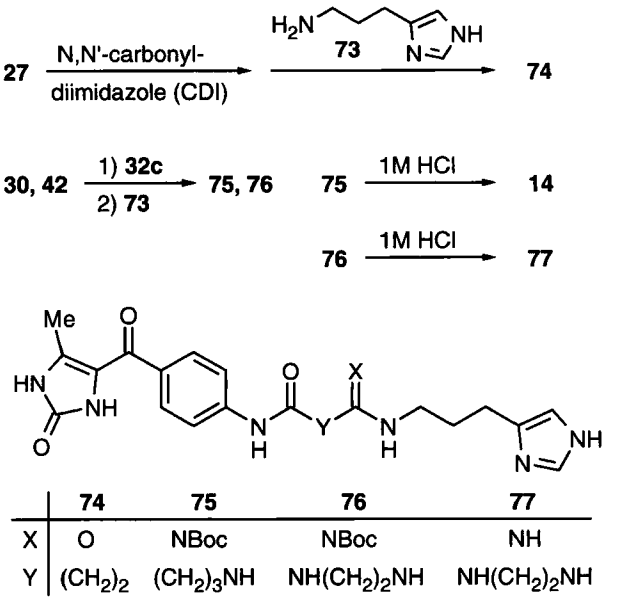
carboxylates may be converted into guanidines by acid hydrolysis without formation of by-products worthwile to be noticed, a considerable decomposition occurs when treating aryl substituted ethyl guanidine carboxylates (for several hours) with hydrochloric acid at elevated temperature. Compound **14** was identified in the reaction mixture of **62d** in 6M HCl. By contrast, the Boc group in **53–61c** could be easily removed under mild conditions in 1M HCl resulting in the guanidines **63–71**.

The ester groups in guanidine carboxylates may also be attacked by nucleophiles. For example, with an excess of phenylethylamine instead of an equimolar amount **33c** was aminolysed at both functional groups the *O*-phenyl isourea and the *tert*-butyl ester resulting in amidino urea **72** (Scheme 5).



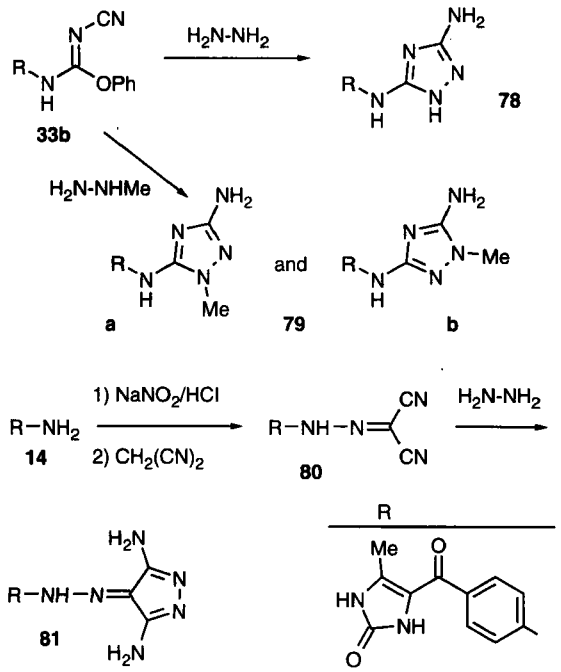
Scheme 5

A further variation of linking partial structures of H_2 agonists with the benzoylimidazolone moiety was realized by introducing a spacer. The succinic acid monoamide **27** was allowed to react consecutively with CDI and imidazolepropanamine **73** affording amide **74** (Scheme 6). The primary amines **30, 42** were converted into the Boc-protected guanidines **75, 76** by reaction with **32c** followed by aminolysis with **73**. Compound **76** could be deprotected with 1M HCl affording guanidine **77** whereas **75** decomposed under the same conditions to give **14**.



Scheme 6

In addition to the syntheses around hybrid molecules some new PDE inhibitors were synthesized starting from **14** or **33b**, respectively (Scheme 7). The *N*-cyano-*O*-phenyl isourea **33b** was either treated with hydrazine affording the diaminotriazole **78** or with methylhydrazine yielding a 92 : 8 mixture of the isomeric triazoles **79a** and **79b**. Diazotisation of **14** followed by coupling with malonitrile resulted in the hydrazonodinitrile **80**, which could be cyclized by reaction with hydrazine affording the diaminopyrazole **81**.

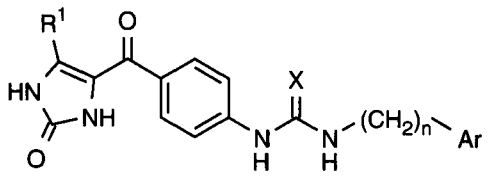


Scheme 7

Data of the synthesized compounds cf. Table 1 and experimental section.

Pharmacological Results and Discussion

Table 1 also summarizes the results of the pharmacological screening of the compounds for (1) positive inotropic activity using isolated electrically stimulated guinea pig papillary muscle preparations and (2) PDE III inhibitory potency using the PDE III isoenzyme (cGMP-inhibited cAMP phosphodiesterase) isolated from guinea pig heart. For comparison inotropic activity (EC_{50}) is also given for enoximone and histamine as well as the IC_{50} of enoximone for PDE III inhibition.



PDE III inhibitory activity

R^1: Et ≈ Me
X: NCN > NBoc > O ≥ NH
Ar: Ph > 4-imidazolyl > 1-imidazolyl

Positive inotropic potency (papillary muscle)

R^1: Et > Me
X: NH > NBoc > NCN > O
Ar: 4-imidazolyl > Ph > 1-imidazolyl

Fig. 1. Order of potency of (hetero)arylalkyl-substituted carbonic acid derivatives in the PDE III activity assay and in the guinea pig papillary muscle.

The PDE III inhibitory and positive inotropic potency of compounds with a (hetero)arylalkyl-substituted carbonic acid derived substructure directly attached to the benzoylimidazolone moiety depend on the substituents Ar, R^1, and X, as depicted in Fig. 1. A more or less pronounced decrease in PDE inhibitor activity can be noted when the cyanoguanidine, *tert*-butyl guanidine carboxylate or the urea group is replaced by a basic guanidine system. This is in accordance with structure activity relationships in enoximone-type PDE inhibitor as, generally, an electron-rich partial structure appears to be required in *p*-position of the phenyl ring[19, 25]. On the other hand, the strongly basic (imidazolylpropyl substituted) guanidino group is characteristic of histamine H_2 receptor agonists[26]. Thus, both mechanisms of action appear to require contrary structural features. However, regardless of their moderate PDE inhibitor activity (**65**: IC_{50} 51.6 μM, **66**: 50.1 μM) compared to enoximone (IC_{50} 2.6 μM) the guanidines **65** and **66** proved to be the most potent inotropic substances in this series. Comparing equieffective concentrations **65** and **66** were about 2 and 10 times, respectively, more potent than enoximone in the guinea pig papillary muscle (Fig. 2) (**65**: EC_{50} 16 μM, **66**: EC_{50} 3 μM). The intrinsic activity of both compounds was the same as that found for the reference substance isoprenaline (Fig. 2), whereas enoximone as well as most of the other compounds tested did not produce more than 40 – 60 % of isoprenaline's response (intrinsic activities (i.a.) cf. Table 1 and Fig. 2). The concentration response curves of compounds **65** and **66** were shifted to the right in the presence of the H_2-antagonist famotidine (10 μM), and the maximal response was reduced by more than 50 %, resulting in a concentration response curve similar to that of PDE inhibitors such as enoximone or **47b**, the cyanoguanidine analogue of **65** (Fig. 2). The effect of **47b** was not influenced by famotidine (10 μM) (data not shown).

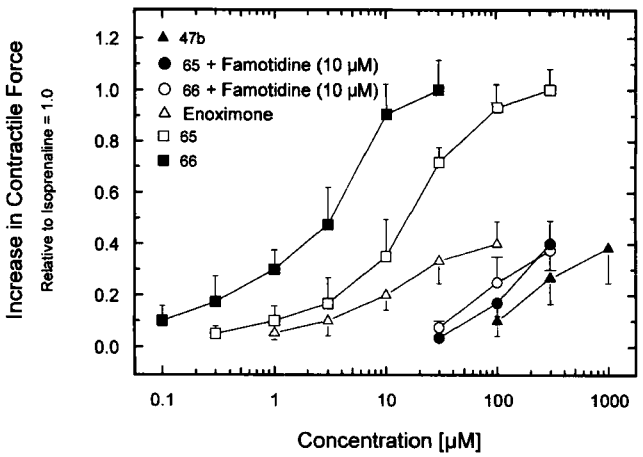
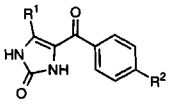


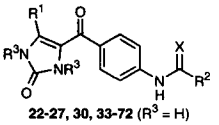
Fig. 2. Increase in contractile force on the electrically stimulated guinea pig papillary muscle relative to isoprenaline = 1.0. Mean values ± SEM; **65**, **66**: $n = 10$, **47b**, enoximone: $n = 5$.

Quantitative evaluation of the contribution of H_2 agonism to the overall inotropic response induced by the pharmacological hybrids is complicated due to the fact that cAMP is involved in both mechanisms of action. Some additional investigations should be useful to give further evidence that H_2 receptor agonism is involved. As shown above for the

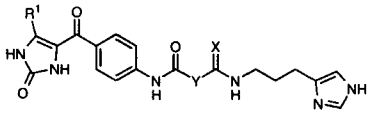
Table 1. Structures, formulas, and results of the pharmacological screening for PDE III inhibition (guinea pig cardiac PDE III) and positive inotropic activity (isolated electrically stimulated guinea pig papillary muscle)



9-15, 29, 78-81



22-27, 30, 33-72 (R^3 = H)
28 (R^3 = COOEt)



74-77

No.	R^1	R^2 or Y	X	Yield %	Mp, °C (solvent)[a]	Analysis C, H, N[b]	PDE III inhibition IC_{50} [μM][c]	Papillary muscle EC_{50} [μM][d]	i. a[d]
9	Me	H		85	253–255[e] (iPrOH/H_2O)	$C_{11}H_{10}N_2O_2$	n.d.	inactive	
10	Me	F		63	290[f] (iPrOH/H_2O)	$C_{11}H_9FN_2O_2$	n.d.	100	0.2
11[g]	Me	NO_2		85	>320 (DMSO/H_2O)	$C_{11}H_9N_3O_4$	n.d.	inactive	
12	Me	NPht		88	>320 (EGME/H_2O)	$C_{19}H_{13}N_3O_4$	n.d.	inactive	
13	Et	NPht		95	>320 (EGME/H_2O)	$C_{20}H_{15}N_3O_4$	n.d.	inactive	
14[g]	Me	NH_2		85	>320 (iPrOH/H_2O)	$C_{11}H_{11}N_3O_4$	16.5 ± 1.8	100	0.3
15	Et	NH_2		94	265 (iPrOH/H_2O)	$C_{12}H_{13}N_3O_4$	11.3 ± 1.5	100	0.3
22	Me	Me	O	93	286–208 (EtOH/H_2O)	$C_{13}H_{13}N_3O_3$	42.0 ± 2.3	100	0.1
23	Me	OEt	O	95	279 (EtOH/H_2O)	$C_{14}H_{15}N_3O_4$	38.0 ± 2.0	100	0.2
24	Me	CHClMe	O	89	259 (MeOH)	$C_{14}H_{14}ClN_3O_3 \cdot CH_3OH$	70.4 ± 4.7	inactive	
25	Me	$(CH_2)_3Cl$	O	94	202–203 (EtOH)	$C_{15}H_{16}ClN_3O_3$	n.d.	inactive	
26	Me	$(CH_2)_3$NPht	O	80	258 (EtOH/H_2O)	$C_{23}H_{20}N_4O_5 \cdot H_2O$	n.d.	inactive	
27	Me	$(CH_2)_2$COOH	O	95	250 (EtOH/H_2O)	$C_{15}H_{15}N_3O_5$	n.d.	inactive	
28	Me	OEt	O	96	171–173 (EtOH/H_2O)	$C_{20}H_{23}N_3O_8$	n.d.	inactive	
29	Me	2-oxo-1-pyrrolidinyl		91	>300 (EtOH/H_2O)	$C_{15}H_{15}N_3O_3$	62.3 ± 4.1	inactive	
30	Me	$(CH_2)_3NH_2$	O	40	123 (MeOH)	$C_{15}H_{18}N_4O_3 \cdot H_2O$	n.d.	inactive	
33a	Me	OPh	O	98	227 (EtOH)	$C_{18}H_{15}N_3O_4$	n.d.	n.d.	
33b	Me	OPh	NCN	95	260 (EtOH)	$C_{19}H_{15}N_5O_3$	n.d.	n.d.	
33c	Me	OPh	NBoc	93	146 (EtOH)	$C_{23}H_{24}N_4O_5 \cdot 0.5H_2O$	n.d.	n.d.	
33d	Me	OPh	NCOOEt	53	192 (EtOH)	$C_{21}H_{20}N_4O_5 \cdot H_2O$	n.d.	n.d.	
34c	Et	OPh	NBoc	85	185–186 (EtOH)	$C_{24}H_{26}N_4O_5 \cdot 0.5H_2O$	n.d.	n.d.	
35a	Me	NHMe	O	93	284 (EtOH/H_2O)	$C_{13}H_{14}N_4O_3 \cdot H_2O$	49.3 ± 2.1	inactive	
36a	Me	$NH(CH_2)_2$(4-Im)	O	87	105 (EtOH)	$C_{17}H_{18}N_6O_3 \cdot C_2H_5OH$	57.6 ± 2.8	630	0.5
37a	Me	$NH(CH_2)_3$(4-Im)	O	92	153 (MeOH)	$C_{18}H_{20}N_6O_3 \cdot H_2O$	46.1 ± 3.0	100	0.3
38a	Me	$NH(CH_2)_3Ph$	O	93	253 (EtOH)	$C_{21}H_{22}N_4O_3$	9.3 ± 0.9	1000	0.2
39a	Me	$NH(CH_2)_3$(1-Im)	O	98	247 (MeOH/H_2O)	$C_{18}H_{20}N_6O_3$	n.d.	inactive	
40a	Me	$NH(CH_2)_2$(2-Py)	O	91	224 (EtOH)	$C_{19}H_{19}N_5O_3 \cdot 0.5H_2O$	n.d.	inactive	
41a	Me	$NH(CH_2)_2SCH_2$ (5-Me-4-Im)	O	97	187 (iPrOH/H_2O)	$C_{19}H_{22}N_6O_3S \cdot 0.5\ H_2O$	n.d.	inactive	
42a	Me	$NH(CH_2)_2NH_2$	O	97	170 (EtOH/H_2O)	$C_{14}H_{17}N_5O_3 \cdot \cdot C_6H_3N_3O_7 \cdot H_2O$	n.d.	inactive	
43a	Me	4-Ph-1-piperazinyl	O	90	257 (MeOH)	$C_{22}H_{23}N_6O_3$	n.d.	630	0.3
44b	Me	NH_2	NCN	96	>300 (EtOH/H_2O)	$C_{13}H_{12}N_6O_2$	27.0 ± 2.0	100	0.4
45b	Me	NHMe	NCN	97	226–229 (EtOH/H_2O)	$C_{14}H_{14}N_6O_2$	59.0 ± 3.3	630	0.3
46b	Me	$NH(CH_2)_2$(4-Im)	NCN	96	173 (EtOH)	$C_{18}H_{18}N_8O_2 \cdot H_2O$	6.4 ± 0.8	63	0.9
47b	Me	$NH(CH_2)_3$(4-Im)	NCN	95	232–234 (MeOH)	$C_{19}H_{20}N_8O_2$	13.1 ± 1.1	300	0.4
48b	Me	$NH(CH_2)_3Ph$	NCN	80	226–228 (MeOH)	$C_{22}H_{22}N_6O_2$	1.3 ± 0.5	300	0.3
49b	Me	1-piperidinyl	NCN	91	250 (EtOH)	$C_{18}H_{20}N_6O_2$	n.d.	inactive	
50b	Me	4-morpholinyl	NCN	88	260–262 (EtOH/H_2O)	$C_{17}H_{18}N_6O_3 \cdot 0.5H_2O$	n.d.	inactive	
51b	Me	4-Ph-1-piperazinyl	NCN	80	283 (EtOH/H_2O)	$C_{23}H_{23}N_7O_2$	n.d.	100	0.4
52b	Me	4-Bzl-1-piperazinyl	NCN	82	172–174 (EGME/H_2O)	$C_{24}H_{25}N_7O_2 \cdot H_2O$	n.d.	100	0.3

Table 1. *Continued.*

No.	R^1	R^2 or Y	X	Yield %	Mp, °C (solvent)[a]	Analysis C, H, N[b]	PDE III inhibition IC_{50} [μM][c]	Papillary muscle EC_{50} [μM][d]	i. a[d]
53c	Me	NHMe	NBoc	75	198 (EtOH/Et_2O)	$C_{18}H_{23}N_5O_4 \cdot H_2O$	n.d.	inactive	
54c	Me	$NH(CH_2)_2$(4-Im)	NBoc	75	256 (EtOH/Et_2O)	$C_{22}H_{27}N_7O_4$	49.2 ± 3.2	125	0.5
55c	Me	$NH(CH_2)_3$(4-Im)	NBoc	91	211 (MeOH/Et_2O)	$C_{23}H_{29}N_7O_4 \cdot H_2O$	29.3 ± 2.9	100	0.6
56c	Et	$NH(CH_2)_3$(4-Im)	NBoc	87	140 (EtOH)	$C_{24}H_{31}N_7O_4 \cdot H_2O$[h]	34.3 ± 3.3	63	0.7
57c	Me	$NH(CH_2)_3$(1-Im)	NBoc	85	94–95 (EtOH/Et_2O)	$C_{23}H_{29}N_7O_4 \cdot H_2O$	54.3 ± 3.9	inactive	
58c	Me	$NH(CH_2)_2$ (1-pyrrolidinyl)	NBoc	61	219 (EtOH)	$C_{23}H_{32}N_6O_4 \cdot H_2O$	n.d.	inactive	
59c	Me	$NH(CH_2)_2Ph$	NBoc	50	195 (MeCN)	$C_{25}H_{29}N_5O_4 \cdot H_2O$	3.8 ± 0.7	794	0.2
60c	Me	$NH(CH_2)_3Ph$	NBoc	81	175 (MeCN)	$C_{26}H_{31}N_5O_4$	5.8 ± 1.0	630	0.3
61c	Me	$NH(CH_2)_4Ph$	NBoc	77	197 (EtOH)	$C_{27}H_{33}N_5O_4 \cdot 0.5H_2O$	3.4 ± 0.7	398	0.2
62d	Me	$NH(CH_2)_3$(4-Im)	NCOOEt	80	234–236 (EtOH/Et_2O)	$C_{21}H_{25}N_7O_4 \cdot H_2O$	n.d.	100	0.5
63	Me	NHMe	NH	49	281 (EtOH/H_2O)	$C_{13}H_{15}N_5O_2 \cdot C_6H_3N_3O_7$	n.d.	inactive	
64	Me	$NH(CH_2)_2$(4-Im)	NH	45	219–222 (EtOH/H_2O)	$C_{17}H_{19}N_7O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$	>100	inactive	
65	Me	$NH(CH_2)_3$(4-Im)	NH	65	176–179 (EtOH/H_2O)	$C_{18}H_{21}N_7O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$	51.6 ± 3.9	16	1.0
66	Et	$NH(CH_2)_3$(4-Im)	NH	30	129 (EtOH/H_2O)	$C_{19}H_{23}N_7O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$	50.1 ± 3.8	3	1.0
67	Me	$NH(CH_2)_3$(1-Im)	NH	40	131 (EtOH/H_2O)	$C_{18}H_{21}N_7O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$	>100	inactive	
68	Me	$NH(CH_2)_2$ (1-pyrrolidinyl)	NH	42	177–179 (EtOH/H_2O)	$C_{18}H_{24}N_6O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$[i]	n.d.	inactive	
69	Me	$NH(CH_2)_2Ph$	NH	65	257 (EtOH)	$C_{20}H_{21}N_5O_2 \cdot H_2O$	28.5 ± 1.3	63	0.5
70	Me	$NH(CH_2)_3Ph$	NH	72	267 (EtOH)	$C_{21}H_{23}N_5O_2 \cdot 2H_2O$	42.4 ± 3.2	30	0.6
71	Me	$NH(CH_2)_4Ph$	NH	75	269 (EtOH)	$C_{22}H_{25}N_5O_2 \cdot 2H_2O$	3.8 ± 0.9	100	0.5
72	Me	$NH(CH_2)_2Ph$	$NCONH(CH_2)_2Ph$	95	214 (EtOH)	$C_{29}H_{30}N_6O_3 \cdot HCl$	n.d.	inactive	
74	Me	$(CH_2)_2$	O	75	196 (EtOH)	$C_{21}H_{24}N_6O_4 \cdot H_2O$	55.5 ± 3.6	inactive	
75	Me	$(CH_2)_3NH$	NBoc	42	159 (EtOH)	$C_{27}H_{36}N_8O_5 \cdot 2H_2O$[j]	n.d.	125	0.7
76	Me	$NH(CH_2)_2NH$	NBoc	51	208 (EtOH/Et_2O)	$C_{26}H_{35}N_9O_5 \cdot H_2O$	n.d.	63	0.6
77	Me	$NH(CH_2)_2NH$	NH	92	204–206 (EtOH/H_2O)	$C_{21}H_{27}N_9O_3 \cdot 2C_6H_3N_3O_7 \cdot H_2O$	67.3 ± 5.0	16	0.8
78	Me	3-NH_2-1,2,4-triazol-5-yl		99	>320 (EGME/H_2O)	$C_{13}H_{13}N_7O_2 \cdot 0.5H_2O$	27.0 ± 1.8	100	0.3
79a,b[k]	Me	3(5)-NH_2-1-Me-1,2,4-triaz.-5(3)-yl		98	277 (EGME/H_2O)	$C_{14}H_{15}N_7O_2 \cdot 0.5H_2O$	54.0 ± 4.2	inactive	
80	Me	$NHNC(CN)_2$		98	267 (EtOH/H_2O)	$C_{14}H_{10}N_6O_2 \cdot 0.5H_2O$	0.6 ± 0.3	30	0.3
81	Me	NHN(3,5-$(NH_2)_2$-4-pyraz.)		80	>320 (EtOH/H_2O)	$C_{14}H_{14}N_8O_2 \cdot H_2O$	52.6 ± 3.7	inactive	
Enoximone							2.6 ± 0.8	10	0.4
Histamine							–	0.56[l]	0.95

a: Solvents used for recrystallization; EGME = ethylene glycol monomethylether. b: Analysis C, H, N within ± 0.4 % unless otherwise indicated. c: Mean of 3–5 independent experiments; n. d. = not determined. d: Mean of at least 3–5 independent experiments (compounds **65**, **66**: n = 10); i. a. = intrinsic activity (SEM within ± 0.1) relative to the maximal increase in contractile force induced by isoprenaline (i. a. = 1.0); 'inactive' compounds were not tested in concentrations >100 μM. e: Ref.[19]: mp 329–330 °C. f: Ref.[19] mp 289–291 °C. g: Ref[30] no data. h: Analysis C, H; N: calcd. 19.6; found 19.1. $^+$FAB-MS: *m/z* (%) = 482 (11) $[M+H]^+$, 109 (100). i: Analysis H, N; C: calcd. 43.4, found 42.8; $^+$FAB-MS: *m/z* (%) = 357 (25) $[M+H]^+$. j: Analysis C, N; H: calcd. 6.85; found 6.34. $^+$FAB-MS: *m/z* (%) = 553 (1) $[M+H]^+$, 109 (100). k: 92 : 8 mixture of isomers **a** and **b**. l: n > 30.

papillary muscle in perfused guinea pig hearts the dose response curve for **65** (EC_{50} 2 μM) was also shifted to the right in the presence of famotidine (10 μM) (complete dose response curves for **65** including concentrations ≥ 100 μM in the presence of famotidine could not be constructed due to the limited amount of compound available). In the rat heart, which is reported to be devoid of H_2 receptors[27], very high concentrations (≥ 100 μM) of **65** were required to produce an increase in contractile force, presumably as a consequence of PDE inhibition being the only relevant mechanism of action in this species. In contrast to the increase in contractile force (papillary muscle) the positive chronotropic response induced by **65** or **66** *in vitro* (guinea pig atrium) was considerably lower than that found on stimulation with either isoprenaline or histamine. In the isolated spontaneously beating guinea pig right atrium **65** (EC_{50} 11 μM) and **66** (EC_{50} 4 μM) were found to act as partial histamine H_2 receptor agonists. The maximal increase in heart rate reached only about 20 % of that of histamine. The positive chronotropic response was reduced by about 60–70 % but was not completely blocked by famotidine (10 μM) again indicating both mechanisms of action, *viz.* H_2 agonism and PDE inhibition, to be involved. Moderate H_2 receptor affinity was exemplarily confirmed for **65** (K_i 7.14 μM, Hill coefficient n_H 0.9966) in a binding study using [^{3}H]tiotidine as the radioligand[28]. The H_2 agonist activity of **65** was also demonstrated in the adenylyl cyclase assay where **65** produced about 25 % of the maximal increase in cAMP formation induced by histamine. This effect could be completely blocked by famotidine (10 μM). The H_2 agonist impromidine produced about 85 % of histamine's maximal response, whereas cyanoguanidine **47b**, which is devoid of H_2 agonistic properties, was inactive in this assay. Nevertheless, despite weak potency at H_2 receptors this mechanism of action considerably contributes to the overall action of **65** and **66** at the papillary muscle, as shown by blocking the H_2 agonist effect with famotidine (Fig. 2).

Compounds **65** and **66** are superior to guanidine **77**, although the latter is nearly as active as **65** and **66** with respect to PDE inhibition. Additionally, **77** should be a more potent histamine H_2 agonist owing to a more basic aliphatic guanidine system. However, the stronger basicity may be a major disadvantage with respect to the diffusion across the cell membrane. Thus, the reduced basicity of the aryl substituted guanidino group in **65**, **66** appears to allow both moderate stimulation of H_2 receptors and inhibition of PDE III in the intact organ.

Although the PDE inhibitor activity of compounds **48b**, **59–61c**, and **71** was in the range of enoximone or about 8–40 times that of **65** and **66**, there are obviously considerable discrepancies concerning inotropic potency at the papillary muscle. Similar results were found for the hydrazonopropanedinitrile **80** which was the most potent PDE inhibitor tested in this series (IC_{50} 0.6 μM) achieving an approximately 80-fold higher activity than **65**, **66** in this assay. However, the inotropic activity at the papillary muscle was about one order of magnitude lower than that of compound **66**, producing only 30 % of the maximal response. Reduced inotropic response in the isolated organ may reflect inadequate penetration into the cell. This could, for instance, explain the inactivity of a series of compounds (e.g. **79**, **81**, **74**) at the papillary muscle in spite of PDE III inhibitor activities in the range of the guanidines **65**, **66**.

The partial effects of symbiotic drugs should be quantitatively well balanced in order to potentiate each other. The results in Fig. 2 and Table 1 indicate a favourable ratio of the two effect, H_2 agonism and PDE inhibition, for compounds **65** and **66**. This initial finding must be further investigated by additional pharmacological tests to provide a basis for the development of new compounds with increased inotropic and vasodilator activity. However, the general availability of drugs combining H_2 agonism and PDE inhibition becomes obvious.

Conclusion

In summary, both moderate PDE III inhibition and weak H_2 agonism account for the positive inotropic effect of the hybrid compounds. The optimal structural features were found in an imidazolylpropylguanidine moiety directly attached in *p*-position of a benzoylimidazolone partial structure. It remains to be investigated if the combination of both qualities of action in a symbiotic drug offers advantages (e.g. concerning dosage, route of administration, pharmacokinetics, or minor H_2 receptor down-regulation during long-term treatment) over the single or combined application of a PDE III inhibitor such as enoximone and an H_2 agonist such as arpromidine.

Acknowledgement

The authors thank Mrs. I. Walter and Mrs. H. Lucka (Institut für Pharmazie, FU Berlin) for technical assistance in the investigations on the guinea pig papillary muscle and Dr. L. Unger (Knoll AG, Ludwigshafen, Germany) for performing the H_2 receptor binding study with compound **65**. Generous support of this work by Prof. Dr. Dr. W. Schunack (FU Berlin) and a grant of the Fonds der Chemischen Industrie to A.B. are gratefully acknowledged.

Experimental Part

Chemistry

M.p. (uncorrected): melting point apparatus Büchi 512.– Elemental analyses: Perkin-Elmer 240B and 240C.– IR: Perkin-Elmer 297 and 1420.– ^{1}H NMR: Bruker WM 250 (250 MHz) and Bruker AC 300 (300 MHz), TMS as internal reference. All N*H* and O*H* were exchangeable with D_2O. Im = imidazole.– EI-MS: Finnigan MAT CH7A (170 °C, 70 eV), Finnigan MAT 711 (200 °C, 80 eV), and Kratos MS 25 RF (250 °C, 70 eV).– $^+$FAB-MS (xenon; DMSO/glycerol): Finnigan MAT CH5DF.– Prep. chromatography: Chromatotron 7924T (Harison Research); glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck).– Short path distillation: Kugelrohr apparatus (Büchi GKR-50).

1,3-Dihydro-4-methyl-3H-imidazol-2-one (**3**) *and 4-ethyl-1,3-dihydro-3H-imidazol-2-one* (**4**)

The imidazolones **3** and **4** were prepared according to standard procedures[19,21,22] from **1** (obtained from phthalimidoacetone, mp 112 °C (ref.[29] 112 °C), by hydrolysis in 20 % HCl) or **2**[22], respectively, by reaction with HCl (1 equivalent) and KOCN (10 % excess) in aqueous solution.

3: Yield 56 %, mp 202 °C (H_2O) (ref.[21] 202–204 °C), Anal. ($C_4H_6N_2O$) C, H, N. 5-Ethyl-1,3-dihydro-2-oxo-3*H*-imidazole-4-carboxylate: Yield 65 %, mp 180–182 °C (H_2O) (ref.[22] 182–184 °C). Anal. ($C_8H_{12}N_2O_3$) C, H, N. **4**: Yield 60 %, mp 191 °C (ref.[22] 192–194 °C), Anal. ($C_5H_8N_2O$) C, H, N.

Preparation of the benzoylimidazolones **9–13**

1,3-Dihydro-5-methyl-4-(4-phthalimidobenzoyl)-3*H*-imidazol-2-one (**12**). The imidazolone **3** (0.25 g, 2.5 mmol) and anhydrous $AlCl_3$ (1.3 g, 10 mmol) are dissolved in nitrobenzene (10 ml). Phthalimidobenzoylchloride **8** (0.71 g, 2.5 mmol) is slowly added, the mixture is stirred for 6 h at 60 °C and subsequently poured onto 150 g of ice. Precipitated **12** is collected and recrystallized from ethylene glycol monomethylether/water. Yield 0.76 g (88 %), mp > 320 °C. Anal. ($C_{19}H_{13}N_3O_4$) C, H, N.– IR (KBr): ν = 1716 cm^{-1} vs, 1703 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.92 (s, 3H, CH_3), 7.60 (d, *J* = 8.5 Hz, 2H, aromatic), 7.78 (d, *J* = 8.5 Hz, 2H, aromatic), 7.92 – 7.99 (m, 4H, Pht), 10.39 (s, 1H, NH), 10.95 (s, 1H, NH).– EI-MS (70 eV): *m/z* (%) = 347 (100) [M^+], 200 (52)

The benzoylimidazolones **9–11** and **13** are analogously synthesized starting from either **3** or **4** and the pertinent acyl chlorides **5–8**. Solvents for recrystallization cf. Table 1.

Preparation of the p-aminobenzoylimidazolones **14** *and* **15**

4-(4-Aminobenzoyl)-1,3-dihydro-5-methyl-3*H*-imidazol-2-one (**14**). Phthalimide **12** (3.5 g, 10 mmol) is stirred at room temp. for 10 h in a mixture of 15 ml 1M Na_2CO_3 solution and 1 ml hydrazine hydrate. The product is collected and washed with water. An analytical sample is recrystallized from *i*PrOH/H_2O. Yield 1.85 g (85 %), mp. > 320 °C (ref.[30]: no data). Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.– IR (KBr): ν = 1708 cm^{-1} vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.95 (s, 3H, CH_3), 5.95 (s, 2H, NH_2), 6.57 (d, *J* = 8.5 Hz, 2H, aromatic), 7.42 (d, *J* = 8.5 Hz, 2H, aromatic), 10.09 (s, 1H, NH), 10.60 (s, 1H, NH).– EI-MS (70 eV): *m/z* (%) = 217 (100) [M^+].

Compound **15** is analogously prepared from **13** (cf. Table 1).

Preparation of the carboxamides **22–28**

N-{4-[1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl} -4-phthalimidobutanamide (**26**). A solution of **14** (0.43 g, 2 mmol), **20** (0.5 g, 2 mmol) and a catalytic amount of DMAP in pyridine (5 ml) is stirred for 2 h at room temp. The solvent is removed *in vacuo* and the residue is recrystallized from EtOH/H_2O. Yield 0.72 g (80 %), mp 258 °C. Anal. ($C_{23}H_{20}N_4O_5 \cdot H_2O$) C, H, N.– IR (KBr): ν = 1766 cm^{-1} m, 1707 vs, 1595 s.– ^1H NMR ([D$_6$]DMSO): δ = 1.88 (s, 3H, CH_3), 1.92 (m, 2H, $CH_2CH_2CH_2$), 2.41 (t, *J* = 7.3 Hz, 2H, CH_2CH_2CO), 3.66 (t, *J* = 6.5 Hz, 2H, CH_2CH_2N), 7.54 (d, *J* = 8.7 Hz, 2H, aromatic), 7.62 (d, *J* = 8.7 Hz, 2H, aromatic), 7.83 (m, 4H, Pht), 10.16 (s, 1H, NH), 10.24 (s, 1H, NH), 10.81 (s, 1H, NH).– EI-MS (80 eV): *m/z* (%) = 432 (20) [M^+], 217 (100).

Compounds **22–25, 27, 28** (cf. Table 1) are synthesized analogously using the acyl chlorides **16–19** or succinic anhydride (**21**). For the preparation of **28** ethyl chloroformate **17** is used in 10-fold excess.

1,3-Dihydro-5-methyl-4-[4-(2-oxopyrrolidin-1-yl)benzoyl]-3H-imidazol-2-one (**29**)

The 4-chlorobutanamide **25** (0.64 g, 2 mmol) is stirred for 0.5 h at room temp. in 5 ml 1N NaOH. The solution is neutralized with 1N hydrochloric acid and the precipitated lactam **29** is recrystallized from EtOH/H_2O. Yield 0.52 g (91 %), mp > 300 °C. Anal. ($C_{15}H_{15}N_3O_3$) C, H, N.– IR (KBr): ν = 1705 cm^{-1} vs, 1685 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.87 (s, 3H, CH_3), 2.08 (m, 2H, $CH_2CH_2CH_2$), 2.55 (t, *J* = 8, 2H, CH_2CH_2CO), 3.89 (t, *J* = 7, 2 Hz, CH_2CH_2N), 7.64 (d, 2H, *J* = 8.7 Hz, aromatic), 7.79 (d, 2H, *J* = 8.7 Hz, aromatic), 10.28 (s, 1H, NH), 10.84 (s, 1H, NH).– EI-MS (70 eV): *m/z* (%) = 285 (85) [M^+], 284 (100) [M–1]$^+$, 200 (26), 188 (58).

4-Amino-N-[4-[1,3-dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)carbonyl]-phenyl]butanamide (**30**)

Phthalimide **26** (2.2 g, 5 mmol) is treated for 24 h at room temp. with 28 ml of 1M Na_2CO_3 solution and 2 ml of hydrazine hydrate. The precipitate is filtered off, dried *in vacuo* over P_2O_5 and recrystallized from MeOH, yielding 0.64 g (40 %) of **30**, mp 123 °C. Anal. ($C_{15}H_{18}N_4O_3 \cdot H_2O$) C, H, N.– IR (KBr): ν = 1701 cm^{-1} vs, 1593 vs.– ^1H NMR ([D$_6$]DMSO) **30**·HCl: δ = 1.90 (m, 5H, CH_3, $CH_2CH_2CH_2$), 2.5 (m, 2H, CH_2CH_2CO), 2.85 (m, 2H, $CH_2CH_2NH_3^+$), 7.59 (d, *J* = 8.5 Hz, 2H, aromatic), 7.74 (d, *J* = 8.5 Hz, 2H, aromatic), 8.0 (br, 3H, $CH_2NH_3^+$), 10.26 (s, 1H, NH), 10.58 (s, 1H, NH), 10.86 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%) = 303 (100) [M+H]$^+$.

N-[4-[1,3-Dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)carbonyl]phenyl]-carbamic acid phenyl ester (**33a**)

Compound **14** (4.34 g, 20 mmol) is stirred with phenyl chloroformate **31** (2.64 ml, 21 mmol) in 100 ml of anhydrous pyridine at room temp. The solution is evaporated *in vacuo* and the residue is crystallized from EtOH yielding 6.61 g (98 %) **33a**, mp. 227 °C. Anal. ($C_{18}H_{15}N_3O_4$) C, H, N.– IR (KBr): ν = 3225 cm^{-1} m, 1750 s, 1705 vs, 1598 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.89 (s, 3H, CH_3), 7.28 (m, 3H, aromatic), 7.44 (m, 2H, aromatic), 7.63 (m, 4H, aromatic), 10.28 (s, 1H, NH), 10.58 (s, 1H, NH), 10.83 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%) = 338 (57) [M+H]$^+$, 125 (100).

N^1-Cyano-N^2-[4-[(1,3-dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)-carbonyl]phenyl]-O-phenyl isourea (**33b**)

Diphenyl *N*-cyanocarbonimidate (**32b**) (2.38 g, 10 mmol) and **14** (2.17 g, 10 mmol) are stirred for 5 h in 10 ml anhydrous pyridine at room temp. The solution is evaporated *in vacuo*, the residue is triturated with Et_2O and filtered off yielding 3.43 g (95 %) of **33b** which is used for further reactions without purification. An analytical sample is recrystallized from EtOH, mp 260 °C. Anal. ($C_{19}H_{15}N_5O_3$) C, H, N.– IR (KBr): ν = 3219 cm^{-1} m, 2195 vs, 1769 s, 1702 vs, 1598 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.89 (s, 3H, CH_3), 7.32 (m, 3H, aromatic), 7.47 (m, 2H, aromatic), 7.63 (m, 4H, aromatic), 10.32 (s, 1H, NH), 10.89 (s, 1H, NH), 11.11 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%) = 362 (18) [M+H]$^+$, 93 (100).

Preparation of the phenoxymethylene carbamic acid esters **33c,d** *and* **34c**

{[4-[(1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl-amino]phenoxymethylene}carbamic acid *tert*-butylester (**33c**). Carbonimidate **32c** (3.13 g, 10 mmol) and compound **14** (2.17 g, 10 mmol) are stirred for 5 h in 10 ml anhydrous pyridine at room temp. The solvent is removed *in vacuo* and the residue is crystallized from EtOH yielding 4.14 g (93 %) of **33c**, mp 146 °C. Anal. ($C_{23}H_{24}N_4O_5 \cdot 0.5\ H_2O$) C, H, N.– IR (KBr): ν = 1706 cm^{-1} vs, 1634 vs, 1599 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.27 (s, 9H, tBu), 1.90 (s, 3H, CH_3), 7.27 (m, 3H, aromatic), 7.45 (m, 2H, aromatic), 7.64 (m, 4H, aromatic), 10.29 (s, 1H, NH), 10.69 (s, 1H, NH), 10.85 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%) = 437 (6) [M+H]$^+$, 337 (12).

Compounds **33d** and **34c** are analogously prepared from equimolar amounts of **14** and **32d** or **15** and **32c**, respectively (cf. Table 1)

Preparation of the ureas **35–43a**

N^1-{4-[(1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl}-N^2-(2-aminoethyl)urea (**42a**). Urethane **33a** (0,34 g, 1 mmol) and ethylenediamine (0.67 ml, 10 mmol) in 10 ml of MeCN are stirred at room temp. for 0.5 h. The product is filtered off and washed repeatedly with Et_2O. Yield 3.31 g (98 %). An analytical sample is converted into the picrate with aqueous picric acid solution and recrystallized from EtOH/H_2O, mp 170 °C. Anal. ($C_{14}H_{17}N_5O_3 \cdot C_6H_3N_3O_7 \cdot H_2O$). C, H, N.– IR (KBr): ν = 1706 cm^{-1} vs, 1596 s.– ^1H NMR ([D$_6$]DMSO): δ = 1.90 (m, 3H, CH_3), 2.92 (m, 2H, $CH_2CH_2NH_3^+$), 3.35 (m, 2H, CH_2CH_2NH), 6.42 (t, *J* = 5.6 Hz, 1H, $NHCH_2$), 7.54 (m, 4H, aromatic), 7.70 (m, 3H, $CH_2NH_3^+$), 8.59 (s, 2H, picric acid), 9.10 (s, 1H, NH), 10.22 (s, 1H, NH), 10.78 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%)= 304 (60) [M+H]$^+$, 149 (100).

For the preparation of the ureas **35–41a** and **43a** (cf. Table 1) urethane **33a** and the corresponding amines are used in equimolar amounts and the reaction mixture is heated under reflux for 0.5 h (**35–41a**) or 2 h (**43a**), respectively.

Preparation of the cyanoguanidines **44–52b**

N^1-Cyano-N^2-{4-[(1,3-dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl}-N^3-[2-(1*H*-imidazol-4-yl)ethyl]guanidine (**46b**). A solution of **33b** (0.36 g, 1 mmol) and 2-(4-imidazolyl)ethanamine (0.11 g, 1 mmol) in 15 ml of anhydrous MeCN is refluxed for 2 h. Precipitated chromatographically pure **46b** is filtered off and washed repeatedly with Et_2O. Yield 0.38 g (96 %). A sample is recrystallized from EtOH, mp 173 °C. Anal. ($C_{18}H_{18}N_8O_2 \cdot H_2O$) C, H, N.– IR (KBr): ν = 3205 cm^{-1} br. w, 2193 vs, 1705 vs, 1598 vs.– ^1H NMR ([D$_6$]DMSO): δ = 1.90 (s, 3H, CH_3), 2.78 (t, *J* = 6.6 Hz, 2H, $ImCH_2CH_2$), 3.54 (m, 2H, CH_2CH_2NH), 6.93 (s, 1H, Im-5-*H*), 7.31 (d, *J* = 8.5 Hz, 2H, aromatic), 7.57 (d, *J* = 8.5 Hz, 2H, aromatic), 7.65 (m, 1H, $NHCH_2$), 7.71 (s, 1H, Im-2-*H*), 9.52 (s, 1H, NH), 10.28 (s, 1H, NH),

10.85 (s, 1H, NH), 11.8 (br, 1H, Im-N*H*).– $^+$FAB-MS: *m/z* (%) = 379 (100) $[M+H]^+$.

Compounds **44, 45b** and **47–52b** are analogously prepared from **33b** and the pertinent amines, however, for the synthesis of **49–52b** the isourea **33b** (3.61 g, 10 mmol) was heated under reflux for 12 h in 10 ml of ethylene glycol monomethylether with 20 mmol of the corresponding amine.

Preparation of the diaminomethylene carbamic acid esters **53–61c, 62d, 75, 76** *and of the urea* **72**

{N^1-[4-[(1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]-phenyl]-N^2-[3-(1*H*-imidazol-4-yl)propyl]diaminomethylene}carbamic acid *tert*-butyl ester **(55c).** Compound **33c** (0.44 g, 1 mmol) and amine **73** (0.13 g, 1 mmol) are stirred for 24 h in 10 ml of anhydrous MeCN at room temp. The solvent is removed *in vacuo* and the residue is crystallized from MeOH/Et_2O, yielding 0.44 g (91 %) **55c**, mp 211 °C. Anal. ($C_{23}H_{29}N_7O_4 \cdot H_2O$) C, H, N.– ^{1}H NMR ([D$_6$]DMSO): (300 MHz) δ = 1.38 (s, 9H, tBu), 1.83 (m, 2H, $CH_2CH_2CH_2$), 1.91 (s, 3H, CH_3), 2.56 (m, 2H, ImCH_2), 3.30 (m, 2H, CH_2CH_2NH), 6.81 (s, 1H, Im-5-H), 7.49 – 7.59 (m, 5H, 4Ph-H, Im-2-H), 8.52 (br, 1H, N*H*CH_2), 9.67 (br, 1H, NH), 10.27 (s, 1H, NH), 10.82 (s, 1H, NH), 11.87 (br, 1H, Im-N*H*).– $^+$FAB-MS: *m/z* (%) = 468 (8) $[M+H]^+$, 368 (35), 109 (100).

Compounds **53c, 54c, 56–61c** are obtained under the same conditions starting either from **33c** or **34c** and the corresponding amines, whereas compound **62d** is prepared from **33d** and the equimolar amount of **73** by stirring for 4 h at 50 °C in anhydrous MeCN. Compounds **75** and **76** are synthesized in a one-pot procedure: The amines **30, 42** (1 mmol) are first allowed to react with the equimolar amount of **32c** at room temp. in 5 ml of anhydrous MeCN. Subsequently, **73** (1 mmol) is added and the mixture is heated for 20 h under reflux. After evaporation *in vacuo* **75** and **76** are isolated and purified chromatographically (Chromatotron, silica gel 60 PF_{254} containing gypsum; $CHCl_3$/MeOH, 95 + 5, NH_3 atmosphere) (cf. Table 1). The urea **72** is obtained in a similar way from **33c** (0.44 g, 1 mmol) and an excess of phenylethylamine 0.61 g (5 mmol) by heating under reflux in anhydrous MeCN for 2 h.

Preparation of the guanidines **63–71** *and* **77**

N^1-{4-[(1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl}-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (**65**).To the Boc-protected guanidine **55c** (0.49 g, 1 mmol) 1M hydrochloric acid (5 ml) is added and directly removed under reduced pressure at a maximum bath temp. of 60 °C. Guanidine **65** is isolated chromatographically (Chromatotron, silica gel 60 PF_{254}, containing gypsum; $CHCl_3$/MeOH, 90 + 10, NH_3 atmosphere) and converted into the amorphous dihydrochloride, yield 0.29 g (65 %). For characterization of **65** the dipicrate is formed using aqueous picric acid solution, mp 176–179 °C (EtOH/H_2O). Anal. ($C_{18}H_{21}N_7O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$) C,H,N.– ^{1}H NMR ([D$_6$]DMSO): (**65**·2HCl) δ = 1.91 (m, 5H, $CH_2CH_2CH_2$, CH_3), 2.77 (t, *J* = 7.4 Hz, 2H, ImCH_2CH_2), 3.33 (m, 2H, CH_2CH_2NH), 7.32 (d, *J* = 8.3 Hz, 2H, aromatic), 7.50 (s, 1H, Im-5-H), 7.66 (d, *J* = 8.3 Hz, 2H, aromatic), 8.20 (br, 2H, C=NH_2^+), 8.45 (br, 1H, CH_2N*H*), 9.05 (s, 1H, Im-2-H), 10.28 (br, 1H, NH), 10.36 (s, 1H, NH), 10.95 (s, 1H, NH), 14.64 (br, 2H, imidazolium-NH).– $^+$FAB-MS: *m/z* (%) = 368 (60) $[M+H]^+$, 109 (100).

Analogously are prepared the guanidines **63, 64, 66–68**. The phenylalkyl-guanidines **69–71** are synthesized by hydrolysis of the corresponding Boc-protected guanidines in 5 ml of 1M hydrochloric acid for 20 min at a temp. of 60 °C in maximum. Subsequently, the solutions are neutralized with $NaHCO_3$, the guanidines are filtered off and recrystallized (cf. Table 1). For the preparation of guanidine **77** the acid solution is evaporated *in vacuo*. Traces of water and HCl are removed by repeated evaporation of an ethanolic solution. The dihydrochloride of **77** is obtained as chromatographically pure amorphous solid. An analytical sample is converted into the dipicrate.

*N-{4-[1,3-Dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)carbonyl]phenyl-N'-[3-(1H-imidazol-4-yl)propyl]}butanediamide (***74***)*

Carboxylic acid **27** (0.64 g, 2 mmol) and CDI (0.32 g, 2 mmol) are stirred at room temp. in 10 ml of DMF. Subsequently, amine **73** (0.25 g, 2 mmol) is added and the mixture is stirred for 24 h. The solution is evaporated *in vacuo* and the residue is crystallized from EtOH yielding 0.33 g (75 %) of diamide **74**, mp 196 °C. Anal. ($C_{21}H_{24}N_6O_4 \cdot H_2O$) C, H, N.– IR (KBr): ν = 1709 cm^{-1} vs, 1688 vs, 1646 vs, 1586 vs.– ^{1}H NMR ([D$_6$]DMSO): δ = 1.67 (m, 2H, $CH_2CH_2CH_2$), 1.88 (s, 3H, CH_3), 2.43 (t, *J* = 6.7 Hz, 2H, CH_2CH_2Im), 2.62 (m, 4H, $CO(CH_2)_2CO$), 3.08 (m, 2H, CH_2CH_2NH), 6.72 (s, 1H, Im-2-H), 7.50 (s, 1H, Im-5-H), 7.60 (d, *J* = 8.6 Hz , 2H, aromatic), 7.71 (d, *J* = 8.6 Hz, 2H, aromatic), 7.95 (t, *J* = 5.4 Hz, 1H, CON*H*CH_2), 10.26 (m, 2H, 2NH), 10.82 (s, 1H, NH).– $^+$FAB-MS : *m/z* (%) = 425 (100) $[M+H]^+$, 223 (40), 197 (31).

Preparation of triazolediamines **78, 79a,b**

N^5-{4-[(1,3-Dihydro-5-methyl-2-oxo-3*H*-imidazol-4-yl)carbonyl]phenyl}-1*H*-1,2,4-triazole-3,5-diamine (**78**). A mixture of *N*-cyano-*O*-phenyl isourea **33b** (0,36 g, 1 mmol) and 2 mmol of hydrazine hydrate (0.1 g) in 15 ml of MeCN is stirred at room temp. for about 2 h (control by TLC, silica gel, CH_2Cl_2/MeOH, 9 + 1). Precipitated chromatographically pure **78** is filtered off and recrystallized from ethylene glycol monomethyl ether/H_2O, yield 0.31 g (99 %), mp > 320 °C. Anal. ($C_{13}H_{13}N_7O_2 \cdot 0.5H_2O$) C, H, N.– IR (KBr): ν = 3188 cm^{-1} m, 1691 vs, 1595 vs.– ^{1}H NMR ([D$_6$]DMSO): (300 MHz) δ = 1.93 (s, 3H, CH_3), 5.98 (s, 2H, NH_2), 7.54 (m, 4H, aromatic), 9.24 (s, 1H, NH), 10.19 (s, 1H, NH), 10.70 (br, 1H, NH), 11.33 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%)= 300 (100) $[M+H]^+$.

The triazoles **79a,b** are analogously obtained from **33b** and methylhydrazine as a mixture of both isomers (a:b = 92:8, determined by ^{1}H NMR spectroscopy; cf. ref.[31]). A separation was not carried out but the mixture was used for analytical and pharmacological investigations. Yield (**79a,b**) 0.32 g (98 %), mp of the mixture 277 °C.– Anal. ($C_{14}H_{15}N_7O_2 \cdot 0.5H_2O$) C, H, N.– **79a**: ^{1}H NMR ([D$_6$]DMSO): δ = 1.9 (s, 3H, CH_3), 3.53 (s, 3H, NCH_3), 5.2 (s, 2H, NH_2), 7.55–7.65 (m, 4H, aromatic), 9.1 (s, 1H, NH), 10.25 (s, 1H, NH), 10.77 (s, 1H, NH).– **79b**: ^{1}H NMR ([D$_6$]DMSO): δ = 1.9 (s, 3H, CH_3), 3.47 (s, 3H, CH_3), 6.2 (s, 2H, NH_2), 7.55–7.65 (m, 4H, aromatic), 9.26 (s, 1H, NH), 10.2 (s, 1H, NH), 10.7 (s, 1H, NH).– $^+$FAB-MS: *m/z* (%)= 314 (100) $[M+H]^+$.

*2-{4-[(1,3-Dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)carbonyl]phenyl}-hydrazonopropanedinitrile (***80***)*

$NaNO_2$ (0.18 g, 2.5 mmol) in 1.5 ml of water is slowly added with cooling (0–5 °C) to a solution of **14** (0.5 g, 2.3 mmol) in 12.5 ml of 1M hydrochloric acid. Then malonitrile (0.17 g, 2.5 mmol) in 1.5 ml of water is added. After stirring for 1 h at room temp. pH is adjusted to 6 with NaOAc. Precipitated product is filtered off, washed with water and recrystallized from EtOH/H_2O yielding 0.68 g (98 %) **80**, mp 267 °C. Anal. ($C_{14}H_{10}N_6O_2 \cdot 0.5H_2O$) C, H, N.– IR (KBr): ν = 3212 cm^{-1} s, 2227 vs, 1700 vs.– ^{1}H NMR ([D$_6$]DMSO): δ = 1.89 (s, 3H, CH_3), 7.54 (2H, aromatic), 7.68 (2H, aromatic), 10.31 (s, 1H, NH), 10.89 (s, 1H, NH), 13.10 (br, 1H, NH).– $^+$FAB–MS: *m/z* (%) = 295 (100) $[M+H]^+$, 217 (17).

*4-{4-[(1,3-Dihydro-5-methyl-2-oxo-3H-imidazol-4-yl)carbonyl]phenyl}-hydrazono-4H-pyrazole-3,5-diamine (***81***)*

The dinitrile **80** (0.3 g, 1 mmol) is heated for 1 h under reflux with one drop of hydrazine hydrate (about 1–1.5 mmol) in 5 ml of water. The product is collected, washed with water and recrystallized from EtOH/H_2O, yield 0.28 g (80 %) **81**, mp 320 °C.– Anal. ($C_{14}H_{14}N_8O_2 \cdot H_2O$) C, H, N.– IR (KBr): ν = 3290 cm^{-1} s, 1707 vs, 1595 vs.– ^{1}H NMR ([D$_6$]DMSO): δ = 1.90 (s, 3H, CH_3), 6.0 – 6.4 (br, 4H, $2NH_2$), 7.63 (2H, aromatic), 7.74 (2H, aromatic), 10.31 (s, 1H, NH), 10.84 (s, 2H, 2NH).– EI-MS (70 eV): *m/z* (%) = 326 (88) $[M^+]$, 216 (78), 201 (78), 125 (100).

Pharmacology

The compounds were tested as bases (e.g., the guanidine carboxylates) or hydrochlorides in all pharmacological experiments. Stock solutions (1 mM) were prepared using water as the solvent or if necessary water containing a maximum of 10 % (*v/v*) DMSO. Dilutions were exlusively prepared with water.

Positive inotropic activity on the guinea pig papillary muscle

The evaluation of the positive inotropic activity on the isolated electrically stimulated (1 Hz, duration 1 ms) guinea pig papillary muscle was performed analogously to a method reported[32] using papillary muscles of the right ventricle and Krebs-Henseleit solution, containing [mM] NaCl 118, $NaHCO_3$ 25 KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5. $MgSO_4$ 1.6, glucose 6.2, gassed with 95 % O_2/5 % CO_2, bath temp. 32.5 °C. The EC_{50} values were calculated from increase in contractile force in cumulative concentration-response curves as mean of at least 3–5 independent experiments.

For better comparison of the maximal increase in contractile force the intrinsic activities were determined using (*RS*)-isoprenaline as the internal reference. As the doses of isoprenaline required to produce a maximal response may induce pronounced disrhythmias in many cases, the subsequent construction of concentration response curves for the test compounds may be impaired or even impossible. Therefore, in accordance with other authors[33] the maximum effect of the β-adrenergic agonist was always measured after the test compound. After washing out of the test compounds from the tissue and return to basal values a high dose (1 μM) of isoprenaline was used in order to produce a maximal response[34]. The maximal increase in contractile force produced by the test compounds was calculated as intrinsic activity relative to isoprenaline = 1.0.

The participation of histamine H_2 receptors in the positive inotropic effect was checked by pretreatment with the H_2 receptor antagonist famotidine (10 μM). Additionally, compounds with intrinsic activities > 0.5 (**46b**, **55c**, **56c**, **65**, **66**, **70**, **75–77**, cf. Table 1) were investigated at the papillary muscle in presence of the β-adrenoceptor blocker metoprolol (1 μM) or the muscarinic agonist carbachol (1 μM). The inotropic potency was not affected by metoprolol but was reduced by carbachol indicating that significant participation of β-adrenoceptors may be ruled out but the cAMP pathway is involved.

Positive chronotropic activity (histamine H_2 receptor agonism) on the guinea pig right atrium[35]

The investigations on the isolated spontaneously beating guinea pig right atrium were performed according to the procedure previously described[36] with minor modifications. In brief, male guinea pigs (350–400 g) were killed by a blow on the head and exsanguinated. Right atria were rapidly removed, attached to a tissue holder in an organ bath (32.5 °C) containing 20 ml of Krebs-Henseleit solution (see above) gassed with 95 % O_2/5 % CO_2. The EC_{50} values and intrinsic activities were determined from isometrically recorded cumulative concentration response curves[37] using histamine dihydrochloride (0.1–10 μM) as the reference agonist.

Contractility studies on isolated perfused rat and guinea pig hearts

Male guinea pigs (350–500 g) were killed by a blow on the head and exsanguinated. The heart was rapidly excised and perfusion was started within one minute. For the investigations on rat hearts male Wistar rats (250–280 g) pretreated with heparin (8 000 IU/kg) and anaesthetized with pentobarbital (30 mg/kg i.p.) were used. The hearts were perfused according to the Langendorff technique[38] with a solution containing [mM] NaCl 118, KCl 4.7, EDTA 0.06, $NaHCO_3$ 24.7, KH_2PO_4 0.23, $CaCl_2$ 1.5, $MgSO_4$ 2.1, glucose 11.1 (flow rate 10 ml/min), gassed with 95 % O_2/5 % CO_2 at 37 °C. After equilibration (15 min) cumulative concentration response curves were constructed by stimulation of the organs with the test compounds dissolved in the perfusion medium.

Inhibition of Phosphodiesterases

The PDE activity assay was performed according to Thompson et al.[39] in a two step procedure as described in ref.[40]. The purification followed a known procedure[41] with minor modifications. Briefly, guinea-pig heart tissue was cut into small pieces, frozen and thawed, and homogenized in 10 volumes of extraction buffer with a Polytron (2 times at setting 6 for 10 s) after addition of phenylmethansulfonyl fluoride (final concentration: 50 μM) and aprotinin (50 KIU/ml). After centrifugation for 20 min at 600 g the supernatant was sonicated (12 mA/30 s/ml) and recentrifugated for 30 min at 20000 g. The supernatant was applied to a DEAE-Sephacel column (17 × 2.5 cm) pre-equilibrated with extraction buffer (20 mM Tris, 2 mM EDTA, 50 mM sodium acetate, 5 mM 2-mercaptoethanol, pH 6.5). A flow rate of 30 ml/h was used throughout the ion exchange chromatography. After washing with 120 ml of buffer solution the PDE activities were eluted with a 0.05–1 M sodium acetate linear gradient. Fractions of 6 ml were collected and assayed for cAMP and cGMP PDE activity (substrate concentration 1μM [^{3}H]cAMP or [^{3}H]cGMP). PDE isoenzyme activity was identified in presence and absence of calmodulin (20 nM) and 10 μM $CaCl_2$ (PDE I), in presence of 1 μM cGMP (PDE II and III), and by addition of the selective PDE IV inhibitor, rolipram (PDE IV). Peak III and IV activities were rechromatographed as described, only the sodium acetate gradient was changed to 0.2–0.7 M. PDE fractions were pooled, dialyzed against extraction buffer and stored in 30 % (*v/v*) ethylene glycol at –20 °C. IC_{50} values (mean of 3 experiments) were determined from 9-point concentration response curves.

Adenylyl cyclase activity

The investigations for stimulation of adenylyl cyclase activity were performed according to ref.[42] using membrane preparations of the guinea pig papillary muscle[43].

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☆ Dedicated to Prof. Dr. Dr. W. Schunack, Berlin, on the occasion of his 60th birthday.

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Received: June 20, 1995 [FP032]