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Discovery of Trp-His and His-Arg Analogues as New Structural Classes of Short Antimicrobial Peptides

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Naturally occurring antimicrobial peptides contain a large number of amino acid residues, which limits their clinical applicability. In search of short antimicrobial peptides, which represent a possible alternative for lead structures to fight antibiotic resistant microbial infections, a series of synthetic peptide analogues based on Trp-His and His-Arg structural frameworks have been prepared and found to be active against several Gram-negative and Gram-positive bacterial strains as well as against a fungal strain with MIC values of the most potent structures in the range of 5−20 μg/mL ((IC_{50} in the range of 1−5 μg/mL). The synthesized peptides showed no cytotoxic effect in an MTT assay up to the highest test concentration of 200 μg/mL. A combination of small size, presence of unnatural amino acids, high antimicrobial activity, and absence of cytotoxicity reveals the synthesized Trp-His and His-Arg analogues as promising candidates for novel antimicrobial therapeutics.

Introduction

Microbial infections continue to be a major cause of morbidity and mortality worldwide. Moreover, the problems have been continuously exacerbated by widespread antibiotic resistance, emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics. The scientific efforts of the last 50 years centered around about a dozen antimicrobial core chemotype scaffolds.[1,2] The major ones among these, which have become available on the market, are the oxazolidinone synthetic core (e.g., linezolid), the lipopeptides (e.g., daptomycin), and the ketolides (e.g., telithromycin), which are modified macrolides.[3,4] In the recent years, many reports in the literature have suggested that naturally occurring antimicrobial peptides (AMPs[a]), which constitute a major component of the innate self-defense system, have the potential to represent such a class of antibiotics.[5−7] These AMPs are not only lethal to a broad spectrum of pathogens but also have a unique low tendency for resistance development. Although the exact mode of action of AMPs is still not completely understood, it has been well established that AMPs interact with the cell membrane of susceptible microorganisms, where either their accumulation in the membrane causes increased permeability and loss of barrier function or they cross the membrane to access cytoplasmic targets.[8,9] The selective action of generally positively charged AMPs is demonstrated by preferential interaction with the anionic phospholipids of the bacterial cell membrane rather than with the neutral mammalian cell membrane, which is made of zwitterionic phospholipids and cholesterol.[10−12]

However, in spite of the rapid action of naturally occurring AMPs against a broad spectrum of microorganisms and the fact that the development of resistance by the microorganisms against them is slow because it requires substantial changes in the lipid composition of cell membranes of the microorganisms, there are some serious drawbacks that limit their practical use. One main disadvantage with naturally occurring AMPs is their large size, which poses several challenges regarding synthesis, metabolic stability, immunogenicity, bioavailability, route of administration, and production costs. One approach that could be adopted to resolve these problems is to design and develop smaller synthetic peptidomimetics having unnatural residues without compromising the minimum requirement for being antimicrobial in nature. Thus, the focus of the present work has been to discover small synthetic antimicrobial peptidomimetics that can ultimately led to promising candidates for novel antimicrobial therapeutics.

In our earlier research endeavor that has been recently reported,[13] we tried to ascertain the minimum pharmacophore by employing the HipHop module[14] of the software package CATALYST (Accelrys, San Diego, CA). For this purpose, the experimental data reported by Svendsen and coworkers was used wherein the synthesis and evaluation of smaller cationic AMPs composed of two to six natural and synthetic amino acids has been reported.[15,16] In absence of any substantial report regarding the presence of a particular receptor or protein target for cationic AMPs, the study was restricted to include only positively ionizable (PI) and hydrophobic (HYD) features for pharmacophore generation, as

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[a] Abbreviations: AMP, antimicrobial peptide; CDI, 1,1′-carbonyldiimidazole; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIC, *N*,*N*′-diisopropylcarbodiimide; HPLC, high-pressure liquid chromatography; HONB, *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide; HYD, hydrobhobic; IC_{50}, inhibitory concentration that affords 50% inhibition of microbial growth; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin resistant *S. epidermidis*; MTT, 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; PI, positively ionizable; YPD, yeast extract/peptone/dextrose.

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these are the only two important features for antimicrobial activity analysis. The generated hypotheses for *Escherichia coli* and *Staphylococcus aureus* showed that the minimum motif required for antibacterial activity is two PI features in both cases but four and three HYD features in the case of *E. coli* and *S. aureus*, respectively.

Out of all the compounds consisting from dipeptides to hexapeptides tested by Svendsen and co-workers, the most active compound reported is Tbt-Arg-NHBzl [where Tbt is β-(2,5,7-tri-*tert*-butylindol-3-yl)alanine] with MIC values of 10 μg/mL and 2.5 μg/mL against *E. coli* and *S. aureus,* respectively.[16] Interestingly, it is a dipeptidic molecule having a cationic feature in the form of the guanidine group of arginine and the other amino acid as tryptophan with bulky *tert*-butyl groups substituting on its indole ring. Therefore, it belongs to the Trp-Arg class of AMPs, which have been widely reported to be active.[17] The activity of this class of AMPs is attributed to the fact that Trp has a distinct preference for the interfacial region of lipid bilayers, while Arg residues endow the peptides with cationic charges and hydrogen bonding properties necessary for interaction with the abundant anionic components of bacterial membranes. These two residues are also capable of participating in cation$-\pi$ interactions, thereby facilitating enhanced peptide$-$membrane interactions. It is also well-known that the indole ring of Trp implicates itself in peptide and protein folding in aqueous solution, where it contributes by maintaining native and non-native hydrophobic contacts.

Although the proposed pharmacophore in the above-discussed dipeptide is small and interesting, there was no mention of the effect of varying the charge to bulk ratio on the activity of the compounds. Also, it has been reported that replacement of basic amino acids such as arginine and lysine increases the antimicrobial activity of peptides, particularly in acidic conditions.[18] The imidazole ring of histidine also implicates itself in peptide and protein folding in the same manner as done by the indole ring of Trp. Thus, histidine, an amphiphillic amino acid, offers as a logical replacement for arginine as well as tryptophan to examine the influence of charge to bulk ratio modification.

Results and Discussion

On the lines as discussed in the Introduction, we synthesized two series of dipeptides (Series 1 and 2) by replacing Arg and Trp in Tbt-Arg-NHBzl, respectively (Figure 1). In other words, the focus of these chemical manipulations on the Trp-Arg lead compound was to observe the influence of substitution of histidine on both amino acid positions, thereby introducing two new classes of AMPs (Trp-His and His-Arg). Moreover, to further understand the importance of magnitude and relative position of PI and HYD features, three more structural variations were performed. First, histidine was substituted with bulkier alkyl groups like *i*-propyl, *t*-butyl, cyclobutyl, cyclohexyl, and adamantan-1-yl at the second position of the imidazole ring to investigate the effect of change in hydrophobicity at the side chain of the involved amino acid. Second, apart from the benzyl amide group at the C-terminus, similar compounds with a methyl ester group at the same position were synthesized to note the effect of decreased bulk at the C-terminus. Third, to observe the effect of decreased cationicity and addition of bulk at the free amino group on the N-terminus, we decided to biologically evaluate the Boc protected final peptides too. The synthesized

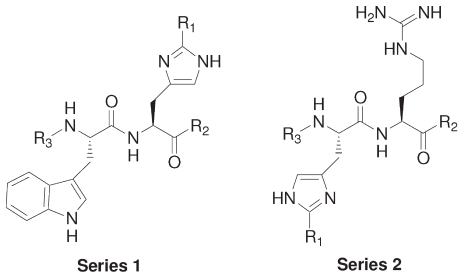


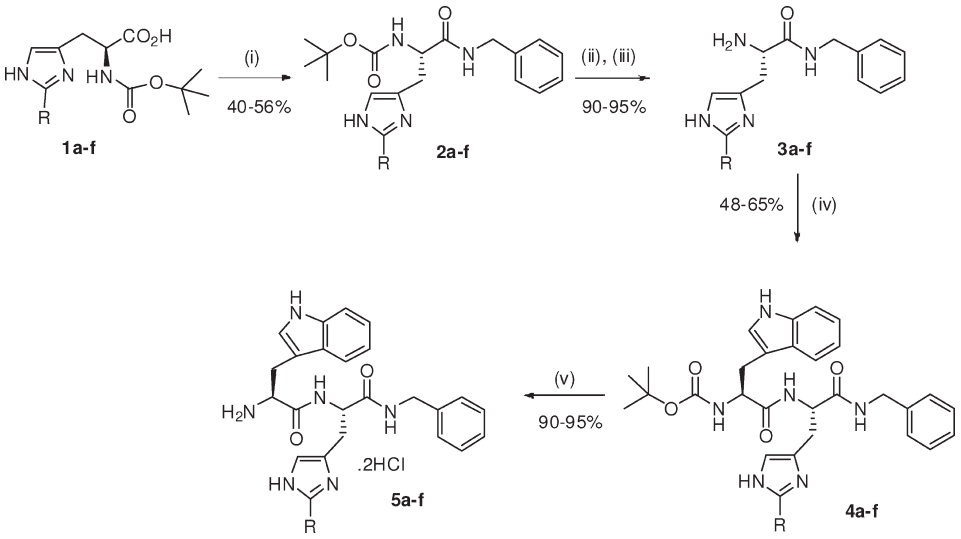
Figure 1. General structure of synthesized dipeptides.

dipeptides were evaluated for antimicrobial activities against two Gram-positive bacteria, three Gram-negative bacteria, and one fungal strain. To further assess their potential as novel antibiotics, cytotoxicity studies were also performed with the MTT test on mouse fibroblasts.

Chemistry. The synthetic strategy adopted to synthesize the peptides belonging to series 1 has been described in Schemes 1 and 2. First, *N*-α-Boc-2-alkyl-L-histidines (**1a**$-$**f**), precursors for final compounds, were synthesized using a reported procedure in four convenient steps.[19$-$21] The alkylation reaction at position C-2 of the imidazole ring proceeds through a homolytic mechanism and is highly selective.[22,23] The reaction involves nucleophilic addition of an alkyl radical (generated from silver catalyzed oxidative decarboxylation of alkylcarboxylic acid with ammonium persulfate) to a protonated imidazole ring followed by rearomatization, leading to direct C-2 alkylation. The method is highly useful as illustrated by the direct introduction of isopropyl, *tert*-butyl, cyclobutyl, cyclohexyl, adamantan-1-yl, and many other alkyl groups into the imidazole ring containing histidine system. Further, *N*-α-Boc-2-alkyl-L-histidines (**1a**$-$**f**) upon condensation reaction with benzylamine in the presence *N*,*N*′-dicyclohexylcarbodiimide (DCC) in DMF afforded Boc protected 2-alky-L-histidine benzylamides (**2a**$-$**f**). The latter compounds **2a**$-$**f** upon acidolysis afforded the unprotected 2-alkyl-L-histidine benzylamide dihydrochloride salts, which were neutralized to obtain 2-alkyl-L-histidine benzylamides (**3a**$-$**f**). These modified amino acid derivatives **3a**$-$**f** were then coupled with commercially available Boc-Trp-OH in the presence of *N*,*N*′-diisopropylcarbodiimide (DIC) and *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) in DMF to produce Boc protected dipeptides (**4a**$-$**f**). The Boc protection was finally removed by subjecting compounds **4a**$-$**f** to acidolysis to yield the desired dipeptides **5a**$-$**f** (Scheme 1). For synthesizing similar dipeptides with a methyl ester group at the C-terminus, 2-alkyl-L-histidine methyl ester dihydrochlorides (**7a**$-$**f**) were obtained by the reaction of 2-alkyl-L-histidine dihydrochloride salts (**6a**$-$**f**)[19$-$21] (Scheme 2). As discussed earlier, compounds **7a**$-$**f** were first neutralized and then coupled with Boc-Trp-OH to produce *N*-α-Boc-L-Trp-L-His(2-alkyl)-OMe (**8a**$-$**f**). Finally, removal of the Boc group afforded the desired dipeptides **9a**$-$**f**.

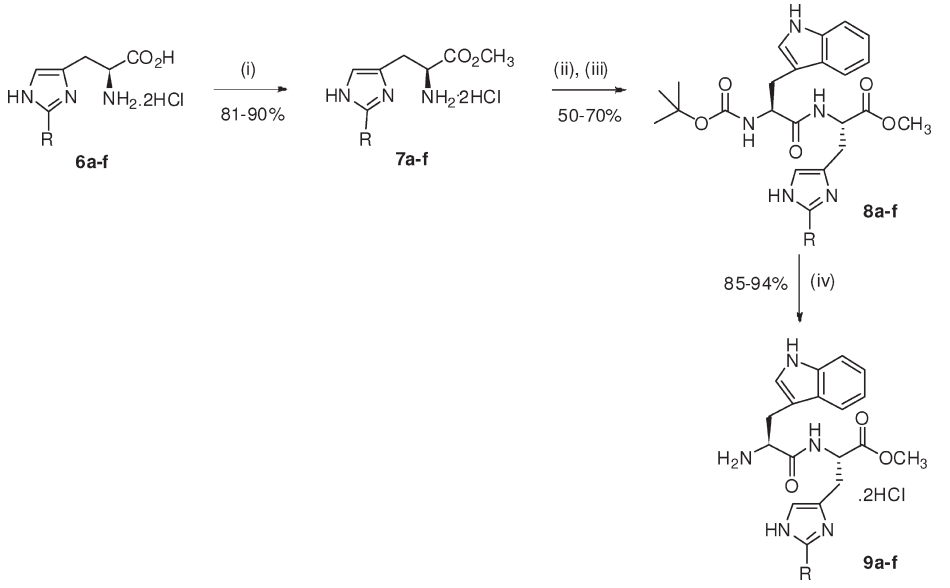
Similarly, the peptides belonging to series 2 were synthesized as described in Schemes 3 and 4. For the presence of a benzylamide group at the C-terminus, we required L-Arg-NHBzl as a component of the designed dipeptides. Under normal circumstances, the reaction would involve coupling of suitably protected Arg with benzylamine in the presence of a coupling reagent like DCC, followed by removal of

Scheme 1[a]



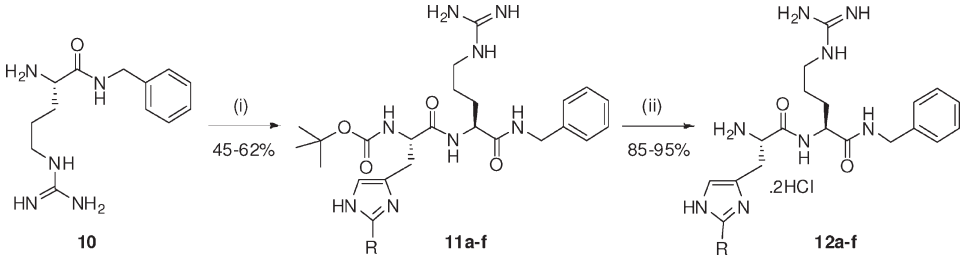
[a] Reagents and conditions: (i) $C_6H_5CH_2NH_2$, DCC, DMF, 4 °C, 12 h; (ii) 3 N HCl in 1,4-dioxane, rt, 15 min; (iii) 7 N NH_3 in MeOH, 0 °C, 10 min; (iv) Boc-Trp-OH, HONB, DIC, DMF, 4 °C, 48 h; (v) 3 N HCl in 1,4-dioxane, rt, 15 min.

Scheme 2[a]



[a] Reagents and conditions: (i) MeOH, HCl gas, 4 °C, 2 h; reflux, 8 h; (ii) 7 N NH_3 in MeOH, 0 °C, 10 min; (iii) Boc-Trp-OH, HONB, DIC, DMF, 4 °C, 48 h; (v) 3 N HCl in 1,4-dioxane, rt, 15 min.

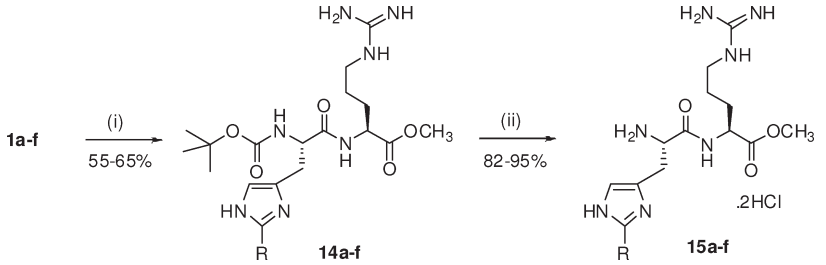
Scheme 3[a]



[a] Reagents and conditions: (i) Boc-L-His(2-alkyl)-OH (**1a**−**f**); HONB, DIC, DMF, 4 °C, 48 h; (ii) 3 N HCl in 1,4-dioxane, rt, 15 min.

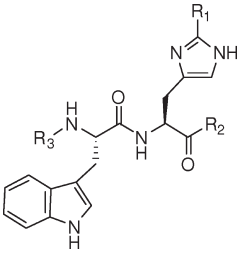
protective groups to obtain the desired amide in several steps. However, we required an amide in which the α-amino group of Arg remained unprotected and thus initiated a study to achieve the amidation reaction on fully unprotected Arg. A study that has been recently reported by us proved successful with identification of 1,1′-carbonyldiimidazole (CDI) as a useful reagent for the desired purpose.[24] The unprotected arginine upon reaction with benzylamine in

Scheme 4[a]



[a] Reagents and conditions: (i) H-Arg-OMe (**13**), HONB, DIC, DMF, 4 °C, 48 h; (ii) 3 N HCl in 1,4-dioxane, rt, 15 min.

Table 1. Antimicrobial Activities of Synthesized Dipeptides (Series 1)



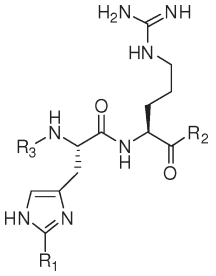
				MRSA		MRSE		*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*C. albicans*	
no.	R_1	R_2	R_3	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]
4a	$CH(CH_3)_2$	NHBzl	*t*-Boc	200	10.26	>200		>200		>200		>200		>200	
4b	$C(CH_3)_3$	NHBzl	*t*-Boc	>200		>200		>200		>200		>200		>200	
4c	*c*-C_4H_7	NHBzl	*t*-Boc	200	22.36	>200		>200		>200		>200		200	33.17
4d	*c*-C_6H_{11}	NHBzl	*t*-Boc	200	31.69	200	23.81	>200		>200		>200		>200	
4e	adamantan-1-yl	NHBzl	*t*-Boc	100	7.59	200	29.07	>200		200		>200		200	28.34
4f	H	NHBzl	*t*-Boc	>200		200	15.62	>200		200	21.24	200	40.70	200	9.70
5a	$CH(CH_3)_2$	NHBzl	H	>200		100	12.80	>200		>200		>200		>200	
5b	$C(CH_3)_3$	NHBzl	H	>200		100	9.24	>200		>200		>200		>200	
5c	*c*-C_4H_7	NHBzl	H	100	27.10	50	8.51	200	22.49	>200		>200		100	10.30
5d	*c*-C_6H_{11}	NHBzl	H	10	4.80	50	7.54	>200		50	17.37	>200		100	8.41
5e	adamantan-1-yl	NHBzl	H	100	15.96	50	4.70	200	21.34	200	31.99	>200		50	8.25
5f	H	NHBzl	H	200	12.26	200	28.98	>200		>200		>200		200	15.17
8a	$CH(CH_3)_2$	OMe	*t*-Boc	>200		>200		>200		>200		>200		>200	
8b	$C(CH_3)_3$	OMe	*t*-Boc	50	4.35	100	8.12	200	16.27	>200		>200		>200	
8c	*c*-C_4H_7	OMe	*t*-Boc	100	2.95	100	5.44	200	24.61	>200		>200		>200	
8d	*c*-C_6H_{11}	OMe	*t*-Boc	200	13.96	50	6.29	200	20.87	>200		>200		200	17.70
8e	adamantan-1-yl	OMe	*t*-Boc	20	1.69	50	4.03	100	6.59	>200		>200		200	26.61
8f	H	OMe	*t*-Boc	200	16.37	>200		>200		>200		>200		200	31.39
9a	$CH(CH_3)_2$	OMe	H	200	13.13	>200		200	12.19	>200		>200		200	15.62
9b	$C(CH_3)_3$	OMe	H	10	4.21	100	6.73	200	19.03	>200		>200		>200	
9c	*c*-C_4H_7	OMe	H	200	9.54	200	14.52	200	9.48	>200		>200		200	18.25
9d	*c*-C_6H_{11}	OMe	H	100	10.12	200	12.46	>200		200	29.38	>200		>200	
9e	adamantan-1-yl	OMe	H	20	2.30	20	4.59	>200		200	24.41	>200		>200	
9f	H	OMe	H	>200		>200		200	18.52	>200		>200		200	20.62

[a] In μg/mL. Standard used: *C. albicans* (amphotericin B, IC_{50} = 0.34 μg/mL), MRSA (vancomycin, IC_{50} = 0.14 μg/mL), MRSE (vancomycin, IC_{50} = 0.14 μg/mL), *E. coli* (streptomycin, IC_{50} = 0.73 μg/mL), *K. pneumoniae* (neomycin, IC_{50} = 0.6 μg/mL), *P. aeruginosa* (ciprofloxacin, IC_{50} = 1.18 μg/mL).

water in the presence of CDI conveniently produced the desired amide product. The reaction was found to be stereoconservative in nature, and the side chain functionality was found to be relatively unaffected by the reagents and reaction conditions. Further, L-Arg-NHBzl (**10**) upon coupling with *N*-α-Boc-2-alkyl-L-histidines (**1a**−**f**) in the presence of DIC and HONB in DMF produced the Boc protected dipeptides **11a**−**f** (Scheme 3). As discussed earlier, removal of the Boc group afforded the desired dipeptides **12a**−**f**. On the other hand, the synthesis of similar peptides having a methyl ester group at the C-terminus was carried out by coupling compounds **1a**−**f** with commercially available L-Arg-OMe (**13**) in the same way as earlier to produce protected dipeptides **14a**−**f**, which finally afforded the desired dipeptides **15a**−**f** upon cleavage of the Boc group (Scheme 4).

Antimicrobial Activity. The results of antibacterial and antifungal activities of the Trp-His peptides (series 1) are provided in Table 1. Analogues **8e**, **9e**, **8c**, **8b**, and **5d** were most potent against methicillin-resistant *Staphylococcus aureus* (MRSA) with IC_{50} values of 1.69, 2.30, 2.95, 4.35, and 4.80 μg/mL, respectively. Thus, it can be interpreted that the presence of hydrophobicity (bulk) at the C-2 position of

Table 2. Antimicrobial Activities of Synthesized Dipeptides (Series 2)



				MRSA		MRSE		*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*C. albicans*	
no.	R_1	R_2	R_3	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]	MIC[a]	IC_{50}[a]
11a	$CH(CH_3)_2$	NHBzl	*t*-Boc	100	17.95	100	12.66	200	23.69	>200		200	45.15	>200	
11b	$C(CH_3)_3$	NHBzl	*t*-Boc	200	17.52	>200		100	12.03	>200		>200		200	33.15
11c	*c*-C_4H_7	NHBzl	*t*-Boc	>200		200	19.30	>200		200	20.81	>200		>200	
11d	*c*-C_6H_{11}	NHBzl	*t*-Boc	100	12.07	100	18.42	200		>200		>200		>200	
11e	adamantan-1-yl	NHBzl	*t*-Boc	20	7.47	100	11.50	>200		100	15.08	100	27.21	>200	
11f	H	NHBzl	*t*-Boc	>200		100	8.79	>200		>200		>200		>200	
12a	$CH(CH_3)_2$	NHBzl	H	100	4.85	200	12.76	>200		>200		200	27.76	>200	
12b	$C(CH_3)_3$	NHBzl	H	20	4.18	10	3.13	>200		>200		>200		>200	
12c	*c*-C_4H_7	NHBzl	H	50	6.99	50	3.94	>200		>200		>200		100	9.95
12d	*c*-C_6H_{11}	NHBzl	H	20	2.06	5	1.00	200	17.51	200	23.52	>200		200	18.00
12e	adamantan-1-yl	NHBzl	H	5	2.15	10	1.19	200	9.29	100	16.15	>200		50	7.46
12f	H	NHBzl	H	100	7.04	100	8.07	>200		>200		>200		200	16.64
14a	$CH(CH_3)_2$	OMe	*t*-Boc	200	12.45	200	13.10	>200		>200		>200		200	20.86
14b	$C(CH_3)_3$	OMe	*t*-Boc	100	4.15	200	20.14	>200		>200		>200		>200	
14c	*c*-C_4H_7	OMe	*t*-Boc	>200		>200		200	18.12	200	14.97	>200		>200	
14d	*c*-C_6H_{11}	OMe	*t*-Boc	200	10.08	100	10.00	100	4.93	>200		200	27.53	100	9.44
14e	adamantan-1-yl	OMe	*t*-Boc	50	9.77	50	6.87	100	8.33	200	31.47	100	25.71	200	16.11
14f	H	OMe	*t*-Boc	>200		100	5.07	>200		>200		>200		>200	
15a	$CH(CH_3)_2$	OMe	H	50	4.82	50	5.42	200	9.98	>200		>200		>200	
15b	$C(CH_3)_3$	OMe	H	20	5.39	20	2.53	200	18.48	200	10.52	>200		100	9.65
15c	*c*-C_4H_7	OMe	H	200	9.28	100	6.66	200	15.31	>200		>200		200	13.96
15d	*c*-C_6H_{11}	OMe	H	50	20.95	10	1.92	>200		>200		>200		200	27.55
15e	adamantan-1-yl	OMe	H	10	3.67	20	4.28	>200		200	19.38	200	35.48	>200	
15f	H	OMe	H	100	4.88	50	2.72	>200		>200		>200		>200	

[a] In μg/mL. Standard used: *C. albicans* (amphotericin B, IC_{50} = 0.34 μg/mL), MRSA (vancomycin, IC_{50} = 0.14 μg/mL), MRSE (vancomycin, IC_{50} = 0.14 μg/mL), *E. coli* (streptomycin, IC_{50} = 0.73 μg/mL), *K. pneumoniae* (neomycin, IC_{50} = 0.6 μg/mL), *P. aeruginosa* (ciprofloxacin, IC_{50} = 1.18 μg/mL).

the imidazole ring affects the antimicrobial activity against MRSA in a positive way. As all but one of the above stated active peptides have a methyl ester group at the C-terminus, it can also be stated that OMe is more effective as compared to the NHBzl group for increasing the activity against MRSA for such Trp-His peptides. Also, it was observed that analogues **4a**, **4c**, **4d**, **4e**, **5c**, **5e**, **5f**, **8d**, **8f**, **9a**, and **9d** were found to be moderately effective, with IC_{50} values ranged between 7 and 32 μg/mL against MRSA. Whereas against methicillin resistant *S. epidermidis* (MRSE), analogues **8e**, **9e**, and **5e** were found to be most potent with IC_{50} values of 4.03, 4.59, and 4.70 μg/mL, respectively. The importance of bulk at position R_1 has been underlined with the fact that all these active peptides contain adamantan-1-yl at this position. Some other analogues like **5b**, **5c**, **5d**, **8b**, **8c**, **8d**, **9b**, **9c**, and **9d** also displayed encouraging activities with IC_{50} values in the range between 5 and 15 μg/mL. Coming to Gram-negative bacterial strains, some compounds were found to be active against *E. coli*. Analogue **8e** was found to be most potent against *E. coli* with an IC_{50} value of 6.59 μg/mL. Some other analogues like **9c**, **9a**, and **8a** also displayed encouraging activities with IC_{50} values of 9.48, 12.19, and 16.27 μg/mL, respectively. Interestingly, only peptides having a Boc group at the N-terminus were found most active against *E. coli*. This can be interpreted in a way that the presence of hydrophobicity and/or the deficiency of positively ionizable features at the N-terminus of His-Trp peptides is essential for activity against *E. coli*. However, the importance of a bulkier residue at position R_1 seems to be equally important as discussed earlier. In the case of *Klebsiella pneumoniae*, very few peptides exhibited moderate activities such as analogues **5d** (IC_{50} = 17.37 μg/mL), **5e** (IC_{50} = 31.99 μg/mL), and **4f** (IC_{50} = 21.24 μg/mL). However, except **4f** (IC_{50} = 40.70 μg/mL), no analogue of this series was found to be active against *Pseudomonas aeruginosa*. Analogues **5e** and **5d** with adamantan-1-yl and cyclohexyl groups at the imidazole ring and an NHBzl group at the C-terminus were most potent against the fungus *Candida albicans* with IC_{50} values of 8.25 and 8.41 μg/mL, respectively. This leads to the conclusion that the presence of a bulky group at the C-terminus as well as at the C-2 position of the imidazole ring increases the activity of Trp-His peptides against *C. albicans*. Also, the presence of a free amino group at the N-terminus, which increases the overall cationicity of the peptide, seems to be important for a good activity against the same strain. Apart from these peptides, many other analogues such as **4c**, **4e**, **5c**, **5f, 8d**, **8e**, **8f**, **9a**, **9c**, and **9f** produced modest activities with IC_{50} values in the range of

10−35 μg/mL for the same strain. Thus, broadly, it is observed that out of all synthesized Trp-His peptides, **5e**, which is substituted with a bulky adamantan-1-yl group at the C-2-position in the imidazole ring and has a NHBzl group at the C-terminus, was active against five strains exhibiting a broad spectrum of antimicrobial activities. Analogues **4f**, **5c**, **5d**, **8e**, and **9c** displayed promising antimicrobial activity against four tested strains.

The antimicrobial results for the His-Arg peptides (series 2) are shown in Table 2. These peptides showed high activities against MRSA. Analogue **12d** exhibited the most potent activity with an IC_{50} of 2.06 μg/mL. In total, the eight compounds **12a**, **12b**, **12d**, **12e**, **14b**, **15a**, **15e**, and **15f** exhibited IC_{50} values ≤5 μg/mL. In the case of the His-Arg peptides, the peptides with a Boc group at the N-terminus are more active as compared to the unprotected ones. A possible explanation for this observation is that the reduction in hydrophobicity that is caused by the substitution of tryptophan with histidine is compensated by the bulkier Boc group, whereas the cationicity imparted by the guanidinium group of the arginine side chain plays an important role in the activity. Some of the tested peptides also showed encouraging activities against MRSE. Analogues **12c**, **15d**, **15b**, and **15f** were highly potent with IC_{50} values of 1.00, 1.92, 2.53, and 2.72 μg/mL, respectively. Apart from these analogues, at least 10 compounds from series 2 exhibited IC_{50} values in the range of 2−10 μg/mL. Contrary to the observation made in case of MRSA, peptides with a free amino group at the N-terminus are most active against MRSE. Very few compounds were active against *E. coli*. Analogue **14d** was found to be the most potent peptide against *E. coli* (IC_{50} = 4.93 μg/mL). Analogues **14e**, **12e**, and **15a** exhibited modest activity with IC_{50} values of 8.33, 9.29, and 9.98 and μg/mL, respectively. Similarly, analogues **15b** (IC_{50} = 10.52 μg/mL), **14c** (IC_{50} = 14.97 μg/mL), **11e** (IC_{50} = 15.08 μg/mL), **12e** (IC_{50} = 16.15 μg/mL), and **11c** (IC_{50} = 20.81 μg/mL) were the only compounds exhibiting moderate activities against *K. pneumoniae*. However, except of a few peptides that were moderately active, most peptides belonging to series 2 were inactive against *P. aeruginosa*. Analogues **12e**, **14d**, **15b**, and **12c** were found to be most potent against the fungus *C. albicans* with IC_{50} values of 7.46, 9.44, 9.65, and 9.95 μg/mL, respectively. Many other analogues produced modest activities with IC_{50} values in the range of 16−34 μg/mL for the same pathogen. It seems that the presence of a bulky group at position R_1 is much more important than the variations tried at positions R_2 and R_3 for the His-Arg peptides concerning activity against *C. albicans*.

As a conclusion, it can be interpreted that out of all synthesized His-Arg analogues, compound **12e** (substituted with a bulky and hydrophobic adamantan-1-yl group at the C-2-position of the imidazole ring with NHBzl at the C-terminus) and **14d** and **14e** substituted with bulky cyclohexyl and adamantan-1-yl groups, respectively, were active against five strains exhibiting a broad spectrum of antimicrobial activities. Analogue **15d** displayed promising antimicrobial activity against four tested strains.

Cytotoxic Experiments. Upon the basis of the results from the antimicrobial testing, the most active peptides were screened for cytotoxicity using a MTT assay with mouse fibroblasts. All of the tested dipeptides, belonging to both series 1 and 2, showed no cytotoxic effect up to the highest test concentration of 200 μg/mL.

Conclusions

Our experiments have resulted in the identification of novel dipeptide analogues composed of the modified natural amino acids His, Trp, and Arg, which will help in contributing to increase the enzymatic stability of the peptides. Apart from containing synthetic residues, these peptides have an extremely small size, allowing the possibility of designing active peptides far smaller than previously described. Also, with the help of this study, we have established that His and its derivatives can replace both Trp and Arg to different extents in the Trp-Arg class of AMPs, thereby introducing His-Arg and Trp-His classes of AMPs. It has also been noted that variation of hydrophobicity, particularly as a substituent of the imidazole ring of histidine, plays an important role in effecting antimicrobial activity of compounds of both classes. In general, the His-Arg peptides were more active against the tested strains. This is mainly attributed to enhanced cationic character as compared to the other class.

Experimental Section

The synthesized amino acids and dipeptides were checked for their purity on precoated silica gel G_{254} TLC plates (Merck), and the spots were visualized under UV light and by exposing them to iodine vapors. Column chromatographic purification was carried out on Merck silica gel (230−400 mesh) or neutral alumina. Melting points were recorded on a capillary melting point apparatus and are uncorrected. All solvents used for synthesis were of analytical grade and used without any further purification unless otherwise stated. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker FT-NMR (Avance DPX 300) spectrometer using tetramethylsilane as internal standard, and the chemical shifts are reported in δ units. Mass spectra were recorded on a Finnigan Mat LCQ spectrometer (APCI/ESI). Optical rotations were recorded on a Perkin-Elmer 241MC polarimeter. Elemental analyses were recorded on Elementar Vario EL spectrometer. The elemental analyses of all final peptides were within ±0.4% of the expected values unless otherwise stated. All final peptides were checked for their homogeneity on a Shimadzu SPD-M20A HPLC system using a Supelcosil LC-8, 5 μm (25 cm × 4.6 mm ID) column. The peptides were analyzed by using a solvent system of CH_3CN-H_2O (0.1% TFA) in the form of a 20 min gradient: 5−65% CH_3CN in 12 min, 65−95% CH_3CN in 3 min, and 100% CH_3CN in 5 min. The peptides were ≥95% pure on HPLC analysis. Amino acids, and coupling reagents, DMF, DCC, DIC, and TFA, were purchased from either Chem-Impex International or NovaBiochem (Merck Ltd.).

General Method for the Synthesis of *N*-α-Boc-L-Trp-L-His(2-alkyl)-NHBzl (4a−f, Scheme 1). HONB (1.2 mmol) was added to *N*-α-Boc-L-Trp (1 mmol) in water-free DMF (10 mL). At −10 °C, DIC (1.2 mmol) was added, and after 5 min stirring, L-His(2-alkyl)-NHBzl (**3a**−**f**, 1 mmol) was added. After stirring for 36 h at 4 °C, the solvent was removed and the crude product chromatographed on neutral alumina using CH_3OH:$CHCl_3$ (7:93) to afford *N*-α-Boc-L-Trp-L-His(2-alkyl)-NHBzl (**4a**−**f**).

***N*-α-Boc-L-Trp-L-His(2-isopropyl)-NHBzl (4a).** Yield: 52%, light-yellowish solid; mp 209−210 °C. ^1H NMR (CD_3OD): δ 1.17 (d, 6H, J = 7.1 Hz), 1.28 (s, 9H), 2.88 (m, 2H), 3.07 (m, 1H), 3.09 (m, 2H), 3.88 (t, 1H, J = 7.3 Hz), 4.37 (t, 1H, J = 6.8 Hz), 4.69 (m, 1H), 4.87 (m, 1H), 6.66 (s, 1H), 7.08−7.11 (m, 3H), 7.30−7.38 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.53, 27.94, 28.29, 28.60, 29.83, 42.71, 52.45, 56.95, 80.32, 107.29, 111.28, 117.40, 118.59, 120.32, 122.46, 123.85, 126.15, 128.59, 128.91, 129.69, 134.81, 138.34, 155.37, 170.79, 171.40. MS (APCI): m/z 573.3 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-*tert*-butyl)-NHBzl (4b).** Yield: 62%, colorless solid; mp 214−216 °C. ^1H NMR (CD_3OD): δ 1.24 (s,

9H), 1.31 (s, 9H), 2.71 (m, 2H), 3.14 (m, 2H), 3.68 (t, 1H, J = 7.0 Hz), 4.26 (t, 1H, J = 6.9 Hz), 4.70 (m, 1H), 4.92 (m, 1H), 6.89 (s, 1H), 7.10−7.13 (m, 3H), 7.20−7.22 (m, 2H), 7.35−7.40 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 28.27, 28.91, 29.37, 30.17, 31.82, 37.29, 42.28, 52.37, 55.93, 79.80, 109.71, 111.48, 116.82, 118.38, 119.38, 123.60, 124.31, 127.74, 128.09, 129.51, 137.11, 138.91, 156.27, 172.52, 174.51. MS (APCI): m/z 587.2 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-cyclobutyl)-NHBzl (4c).** Yield: 48%, colorless solid; mp 224−227 °C (dec).; ^{1}H NMR (CD_3OD): δ 1.27 (s, 9H), 1.86 (m, 2H), 2.29 (m, 4H), 2.89 (m, 2H), 3.06 (m, 1H), 3.17 (m, 2H, CH_2), 3.57 (t, 1H, J = 6.9 Hz), 4.27 (t, 1H, J = 6.8 Hz), 4.48 (m, 1H), 4.71 (m, 1H), 6.58 (s, 1H), 7.05−7.09 (m, 3H), 7.21−7.32 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 19.37, 25.73, 27.91, 28.39, 28.90, 29.81, 31.81, 43.82, 52.17, 54.71, 78.96, 110.15, 111.63, 116.87, 118.71, 119.35, 123.04, 124.10, 126.19, 127.89, 129.00, 130.46, 136.51, 139.90, 158.27, 173.80, 181.61. MS (APCI): m/z 585.1 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-cyclohexyl)-NHBzl (4d).** Yield: 60%, light- yellowish solid; mp 241−242 °C. ^{1}H NMR (CD_3OD): δ 1.23 (m, 6H), 1.35 (s, 9H), 1.71 (m, 4H), 1.96 (m, 1H), 2.97 (m, 2H), 3.24 (m, 2H), 4.04 (t, 1H, J = 7.0 Hz), 4.35 (t, 1H, J = 6.6 Hz), 4.63 (m, 1H), 4.88 (m, 1H), 6.83 (s, 1H), 7.02−7.10 (m, 3H), 7.16−7.21 (m, 2H), 7.25−7.30 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.90, 26.41, 26.84, 28.05, 29.17, 30.37, 32.77, 38.27, 44.10, 52.78, 55.38, 77.71, 111.25, 111.34, 115.72, 119.10, 120.73, 123.38, 124.29, 126.16, 127.90, 128.15, 129.70, 137.33, 142.51, 156.21, 171.92, 177.52. MS (APCI): m/z 613.2 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His[2-(adamantan-1-yl)]-NHBzl (4e).** Yield: 63%, colorless solid; mp 254−256 °C.; ^{1}H NMR (CD_3OD): δ 1.24 (s, 9H), 1.51−1.54 (m, 12H), 1.97 (m, 3H), 3.00 (m, 2H), 3.22 (m, 2H), 4.09 (t, 1H, J = 7.2 Hz), 4.28 (t, 1H, J = 6.8 Hz), 4.61 (m, 1H,, 4.85 (m, 1H), 6.89 (s, 1H), 7.01−7.09 (m, 3H), 7.17−7.30 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 26.34, 27.05, 27.70, 28.17, 34.69, 38.46, 41.23, 44.57, 53.82, 55.38, 80.02, 110.14, 111.61, 115.41, 118.60, 120.51, 122.28, 124.55, 126.81, 127.68, 128.73, 129.12, 136.07, 144.28, 159.61, 172.28, 175.04. MS (APCI): m/z 665.2 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His-NHBzl (4f).** Yield: 65%, colorless solid; mp 194−196 °C. ^{1}H NMR (CD_3OD): δ 1.37 (s, 9H), 2.77 (m, 2H), 3.25 (m, 2H), 3.71 (t, 1H, J = 6.9 Hz), 4.29 (t, 1H, J = 6.8 Hz), 4.51 (m, 1H), 4.72 (m, 1H), 6.79 (s, 1H), 7.03−7.09 (m, 3H), 7.23−7.34 (m, 7H, Ar-H), 7.41 (s, 1H, CH). ^{13}C NMR (75 MHz, CD_3OD): δ 26.61, 28.56, 29.18, 42.26, 53.75, 59.02, 73.29, 109.18, 111.39, 117.56, 118.12, 119.19, 121.89, 123.48, 128.76, 129.19, 129.82, 135.80, 137.92, 153.21, 170.74, 173.96. MS (APCI): m/z 531.0 $[MH]^+$.

General Method for the Synthesis of L-Trp-L-His(2-alkyl)-NHBzl·2HCl (5a−f, Scheme 1). A solution of *N*-α-Boc-L-Trp-L-His(2-alkyl)-NHBzl (**4a**−**f**, 1 mmol) in 3 N HCl in 1,4-dioxane (15 mL) was stirred at ambient temperature for 15 min. The solvent was evaporated under reduced pressure to produce L-Trp-L-His(2-alkyl)-NHBzl·2HCl (**5a**−**f**).

L-Trp-L-His(2-isopropyl)-NHBzl·2HCl (5a). Yield: 90%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.27 (d, 6H), 2.96 (m, 2H), 3.15 (m, 1H), 3.20 (m, 2H), 3.82 (t, 1H, J = 7.0 Hz), 4.42 (t, 1H, J = 6.9 Hz), 4.68 (m, 1H), 4.89 (m, 1H), 6.91 (s, 1H), 7.11−7.18 (m, 3H), 7.34−7.41 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 20.59, 26.28, 28.02, 29.61, 41.38, 52.70, 58.93, 109.22, 112.65, 117.42, 118.97, 121.64, 122.86, 125.32, 127.78, 128.81, 129.10, 129.93, 138.13, 145.34, 168.58, 173.46. MS (APCI): m/z 473.0 $[MH]^+$. $[\alpha]_D^{25} = -14.9°$ (c = 1.0, CH_3OH).

L-Trp-L-His(2-*tert*-butyl)-NHBzl·2HCl (5b). Yield: 94%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.26 (s, 9H), 2.61 (m, 2H), 2.99 (m, 2H), 3.16 (t, 1H, J = 7.2 Hz,), 4.32 (t, 1H, J = 6.9 Hz,), 4.57 (m, 1H), 4.79 (m, 1H), 6.75 (s, 1H), 7.03 (m, 3H), 7.26−7.35 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 28.70, 28.66, 29.58, 30.48, 32.51, 36.51, 43.02, 53.16, 57.81, 111.19, 113.34, 117.41, 118.30, 119.83, 122.65, 124.00, 127.67, 128.89, 129.26, 135.31, 138.35, 170.57, 178.13. MS (APCI): m/z 487.1 $[MH]^+$. $[\alpha]_D^{25} = -19.2°$ (c = 1.8, CH_3OH).

L-Trp-L-His(2-cyclobutyl)-NHBzl·2HCl (5c). Yield: 93%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.73 (m, 2H), 2.18 (m, 4H), 2.83 (m, 2H), 3.08 (m, 1H), 3.21 (m, 2H), 3.71 (t, 1H, J = 6.8 Hz), 4.37 (t, 1H, J = 6.7 Hz), 4.56 (m, 1H), 4.87 (m, 1H), 6.80 (s, 1H), 7.09−714 (m, 3H), 7.29−7.37 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.32, 25.81, 27.45, 28.79, 30.37, 31.94, 44.18, 53.78, 54.61, 109.19, 111.61, 118.04, 118.96, 120.24, 122.40, 124.37, 127.02, 128.83, 129.55, 130.68, 133.83, 138.28, 171.83, 176.91. MS (APCI): m/z 485.2 $[MH]^+$. $[\alpha]_D^{25} = -15.0°$ (c =1.5, CH_3OH).

L-Trp-L-His(2-cyclohexyl)-NHBzl·2HCl (5d). Yield: 90%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.19−1.23 (m, 6H), 1.66 (m, 4H), 2.07 (m, 1H), 2.89 (m, 2H), 3.18 (m), 3.94 (t, 1H, J = 7.1 Hz), 4.27 (t, 1H, J = 6.7 Hz), 4.60 (m, 1H), 4.98 (m, 1H), 6.68 (s, 1H), 6.99−7.06 (m, 3H), 7.21 (m, 2H), 7.29−7.35 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.19, 26.79, 27.44, 28.10, 30.07, 32.77, 36.12, 43.72, 52.76, 56.31, 109.25, 111.08, 116.51, 119.89, 121.76, 124.35, 125.29, 127.21, 128.54, 129.80, 136.67, 145.14, 173.27, 175.55. MS (APCI): m/z 513.3 $[MH]^+$. $[\alpha]_D^{25} = -22.6°$ (c = 1.6, CH_3OH).

L-Trp-L-His[2-(adamantan-1-yl)]-NHBzl·2HCl (5e). Yield: 95%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.49 (m, 12H), 2.07 (m, 3H), 2.95 (m, 2H), 3.13 (m, 2H), 3.95 (t, 1H, J = 6.8 Hz), 4.26 (t, 1H, J = 6.9 Hz), 4.72 (m, 1H), 4.88 (m, 1H), 6.95 (s, 1H), 7.07−7.14 (m, 3H), 7.26−7.33 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.94, 26.89, 27.41, 28.07, 35.16, 39.30, 42.27, 44.36, 52.63, 57.17, 110.19, 111.99, 116.80, 118.68, 122.00, 122.93, 124.51, 126.58, 127.37, 128.56, 129.33, 135.88, 143.28, 170.98, 174.49. MS (APCI): m/z 565.3 $[MH]^+$. $[\alpha]_D^{25} = -17.0°$ (c = 1.5, CH_3OH).

L-Trp-L-His-NHBzl·2HCl (5f). Yield: 95%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 2.69 (m, 2H), 3.16 (m, 2H), 3.83 (t, 1H, J = 6.7 Hz), 4.37 (t, 1H, J = 6.8 Hz), 4.69 (m, 1H), 4.95 (m, 1H), 6.86 (s, 1H), 7.06−7.10 (m, 3H), 7.28−7.37 (m, 7H), 7.55 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 27.51, 29.66, 43.76, 52.42, 57.81, 107.61, 112.25, 118.28, 119.69, 119.99, 120.56, 123.14, 127.43, 128.71, 129.35, 137.90, 139.28, 172.40, 177.22. MS (APCI): m/z 431.2 $[MH]^+$. $[\alpha]_D^{25} = -11.6°$ (c = 1.2, CH_3OH).

General Method for the Synthesis of *N*-α-Boc-L-Trp-L-His(2-alkyl)-OMe (8a−f, Scheme 2). To a solution of L-His(2-alkyl)-OMe·2HCl (**7a**−**f**, 1 mmol) was added ammonia (7 N solution in CH_3OH) at 0 °C. The reaction mixture was stirred for 10 min, and the solvent was removed under reduced pressure to produce L-His(2-alkyl)-OMe. HONB (1.2 mmol) was added to *N*-α-Boc-L-Trp (1 mmol) in water-free DMF (10 mL). At −10 °C, DIC (1.2 mmol) was added, and after 5 min stirring, L-His(2-alkyl)-OMe (1 mmol) was added. After stirring for 36 h at 4 °C, the solvent was removed and the crude product chromatographed on neutral alumina using CH_3OH:$CHCl_3$ (5:95) to afford *N*-α-Boc-L-Trp-L-His(2-alkyl)-OMe (**8a**−**f**).

***N*-α-Boc-L-Trp-L-His(2-isopropyl)-OMe (8a).** Yield: 70%, colorless solid; mp 188−190 °C. ^{1}H NMR (CD_3OD): δ 1.26 (d, 6H, J = 7.1 Hz), 1.35 (s, 9H), 2.96 (m, 2H), 3.17 (m, 1H), 3.34 (m, 2H), 3.63 (s, 3H), 3.66 (t, 1H, J = 7.2 Hz), 4.35 (t, 1H, J = 6.9 Hz), 6.71 (s, 1H), 7.02−7.05 (m, 3H), 7.31−7.33 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.26, 28.10, 28.54, 30.17, 43.21, 51.68, 56.43, 80.12, 110.32, 111.74, 117.46, 118.77, 119.21, 121.81, 124.13, 128.28, 129.32, 134.85, 154.18, 157. 01, 172.15, 175.12. MS (APCI): m/z 498.8 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-*tert*-butyl)-OMe (8b).** Yield: 67%, colorless solid; mp 198 °C. ^{1}H NMR (CD_3OD): δ 1.30 (s, 9H), 1.34 (s, 9H), 2.96 (m, 2H), 3.17 (m, 2H), 3.66 (s, 3H), 3.78 (t, 1H, J = 7.1 Hz), 4.34 (t, 1H, J = 6.9 Hz), 6.68 (s, 1H), 7.01−7.08 (m, 3H), 7.30−7.33 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 28.07, 28.66, 29.19, 29.86, 30.16, 33.19, 43.20, 51.66, 52.19, 56.40, 80.08, 110.31, 111.69, 117.65, 118.76, 119.18, 121.78, 124.08, 128.28, 131.73, 134.85, 137.43, 156.60, 172.28, 175.64.; MS (APCI): m/z 512.2 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-cyclobutyl)-OMe (8c).** Yield: 50%, light-yellowish solid; mp 194−196 °C.; ^{1}H NMR (CD_3OD): δ

1.35 (s, 9H), 1.50 (m, 2H), 2.31 (m, 4H), 2.95 (m, 2H), 3.04 (m, 1H), 3.19 (m, 2H), 3.61 (s, 3H), 3.81 (t, 1H, J = 6.8 Hz), 4.35 (t, 1H, J = 6.9 Hz), 6.73 (s, 1H), 7.04−7.08 (m, 3H), 7.31−7.33 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 18.91, 25.23, 27.61, 28.02, 28.47, 29.67, 31.98, 42.18, 51.92, 52.31, 54.89, 79.83, 107.67, 111.72, 112.56, 117.34, 118.70, 119.89, 120.56, 125.23, 128.13, 134.39, 141.90, 157.36, 171.67, 176.28. MS (APCI): m/z 510.9 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His(2-cyclohexyl)-OMe (8d).** Yield: 61%, colorless solid; mp 211−214 °C. ^{1}H NMR (CD_3OD): δ 1.25 (m, 6H), 1.39 (s, 9H), 1.65 (m, 4H), 1.90 (m, 1H), 2.93 (m, 2H), 3.20 (m, 2H), 3.59 (s, 3H), 3.69 (t, 1H, J = 7.1 Hz), 4.35 (t, 1H, J = 6.8 Hz), 6.49 (s, 1H), 7.05−7.10 (m, 3H), 7.33−7.35 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.94, 26.14, 26.45, 28.33, 28.79, 30.21, 31.98, 37.10, 43.18, 51.91, 53.01, 56.51, 80.83, 109.97, 112.09, 118.90, 119.81, 122.36, 124.15, 128.02, 128.86, 134.77, 153.48, 156.47, 171.60, 172.75. MS (APCI): m/z 538.2 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His[2-(adamantan-1-yl)]-OMe (8e).** Yield: 63%, colorless solid; mp 233−235 °C. ^{1}H NMR (CD_3OD): δ 1.36 (s, 9H), 1.52−1.55 (m, 12H), 1.99 (m, 3H), 3.01 (m, 2H), 3.22 (m, 2H), 3.65 (s, 3H), 3.73 (t, 1H, J = 7.0 Hz), 4.32 (t, 1H, J = 6.8 Hz), 6.83 (s, 1H), 7.01−7.08 (m, 3H), 7.31−7.33 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 26.53, 26.62, 27.35, 28.91, 35.15, 39.63, 41.66, 43.47, 51.23, 54.89, 78.52, 108.71, 110.15, 115.95, 117.16, 119.89, 120.23, 122.51, 126.68, 129.14, 133.30, 135.85, 155.38, 158.19, 170.34, 173.63. MS (APCI): m/z 591.0 $[MH]^+$.

***N*-α-Boc-L-Trp-L-His-OMe (8f).** Yield: 67%, white solid; mp 171−173 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.36 (s, 9H), 3.05 (m, 2H), 3.23 (m, 2H), 3.64 (s, 3H), 3.77 (t, 1H, J = 7.1 Hz), 4.35 (t, 1H, J = 6.9 Hz), 6.85 (s, 1H), 7.07 (m, 3H), 7.31 (m, 2H), 7.59 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 22.97, 27.69, 29.09, 51.68, 53.20, 56.36, 110.31, 111.69, 118.03, 119.19, 121.54, 124.11, 128.25, 134.88, 155.62, 172.14, 175.37. MS (APCI): m/z 455.9 $[MH]^+$.

General Method for the Synthesis of L-Trp-2-alkyl-L-His-OMe·2HCl (9a−f, Scheme 2). A solution of *N*-α-Boc-L-Trp-L-His(2-alkyl)-OMe (**8a−f**, 1 mmol) in 3 N HCl in 1,4-dioxane (15 mL) was stirred at ambient temperature for 15 min. The solvent was evaporated under reduced pressure to afford L-Trp-2-alkyl-L-His-OMe·2HCl (**9a−f**).

L-Trp-L-His(2-isopropyl)-OMe·2HCl (9a). Yield: 90%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.32 (d, 6H), 2.93 (m, 2H), 3.24 (m, 1H), 3.34 (m, 2H), 3.57 (s, 3H), 3.76 (t, 1H, J = 6.9 Hz), 4.07 (t, 1H, J = 6.6 Hz), 6.88 (s, 1H), 7.02−7.04 (m, 3H), 7.22 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 20.11, 27.43, 28.56, 30.13, 51.67, 54.40, 63.69, 114.58, 117.20, 118.57, 120.41, 122.23, 124.53, 127.52, 134.92, 170.50, 174.76. MS (APCI): m/z 398.2 $[MH]^+$. $[\alpha]_D^{25} = -13.0°$ (c = 1.0, CH_3OH).

L-Trp-L-His(2-*tert*-butyl)-OMe·2HCl (9b). Yield: 92%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.44 (s, 9H), 2.76 (m, 2H), 3.16 (m, 2H), 3.67 (s, 3H), 3.73 (t, 1H, J = 7.0 Hz,), 4.43 (t, 1H, J = 6.7 Hz), 6.91 (s, 1H), 7.09−7.12 (m, 3H), 7.21−7.23 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 27.16, 28.17, 29.53, 30.13, 43.26, 51.66, 52.69, 54.36, 111.80, 117.24, 118.58, 119.67, 120.04, 122.24, 125.22, 129.48, 134.92, 170.89, 174.75. MS (APCI): m/z 412.2 $[MH]^+$. $[\alpha]_D^{25} = -22.9°$ (c = 1.7, CH_3OH).

L-Trp-L-His(2-cyclobutyl)-OMe·2HCl (9c). Yield: 86%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.49 (m, 2H), 2.33 (m, 4H), 2.87 (m, 2H), 3.21 (m, 1H), 3.29 (m, 2H), 3.54 (s, 3H), 3.73 (t, 1H, J = 6.7 Hz), 4.32 (t, 1H, J = 6.8 Hz), 6.94 (s, 1H), 7.10−7.13 (m, 3H,), 7.28 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 18.74, 27.17, 28.18, 29.46, 31.43, 52.55, 52.78, 54.44, 107.19, 112.014, 117.25, 118.64, 119.65, 119.99, 122.22, 125.15, 128.56, 134.93, 169.67, 170.82. MS (APCI): m/z 410.3 $[MH]^+$. $[\alpha]_D^{25} = -16.7°$ (c = 1.0, CH_3OH).

L-Trp-L-His(2-cyclohexyl)-OMe·2HCl (9d). Yield: 95%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.28 (m, 6H), 1.74 (m, 4H), 2.02 (m, 1H), 2.98 (m, 2H), 3.30 (m, 2H), 3.64 (s, 3H), 3.73 (t, 1H, J = 6.9 Hz), 4.24 (t, 1H, J = 6.8 Hz), 6.88 (s, 1H), 7.03−7.08 (m, 3H), 7.25 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.71, 25.98, 27.19, 28.86, 30.12, 31.24, 36.44, 43.29, 51.70, 52.78, 63.70, 107.18, 111.80, 117.16, 118.10, 119.66, 120.04, 122.24, 125.26, 129.03, 134.94, 170.82, 170.75. MS (APCI): m/z 438.4 $[MH]^+$. $[\alpha]_D^{25} = -13.9°$ (c = 1.5, CH_3OH).

L-Trp-L-His[2-(adamantan-1-yl)]-OMe·2HCl (9e). Yield: 85%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.58−1.65 (m, 12H), 2.07 (m, 3H), 2.89 (m, 2H), 3.31 (m, 2H), 3.65 (s, 3H), 3.74 (t, 1H, J = 7.1 Hz), 4.17 (t, 1H, J = 6.8 Hz), 6.78 (s, 1H), 7.07−7.13 (m, 3H), 7.28−7.30 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.39, 28.58, 28.91, 30.12, 31.34, 36.29, 43.27, 51.67, 52.02, 67.54, 108.43, 111.88, 116.86, 118.30, 119.82, 121.29, 122.61, 125.92, 128.42, 134.92, 171.02, 174.75. MS (APCI): m/z 490.3 $[MH]^+$. $[\alpha]_D^{25} = -25.4°$ (c = 2.0, CH_3OH).

L-Trp-L-His-OMe·2HCl (9f). Yield: 88%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 2.90 (m, 2H), 3.21 (m, 2H), 3.55 (s, 3H), 3.64 (t, 1H, J = 7.0 Hz), 3.74 (t, 1H, J = 6.8 Hz), 6.93 (s, 1H), 7.12−7.15 (m, 3H), 7.27 (m, 2H), 7.42 (s, 1H).; ^{13}C NMR (75 MHz, CD_3OD): δ 27.12, 30.12, 52.58, 63.67, 67.55, 111.81, 118.19, 118.60, 119.72, 120.04, 124.53, 128.69, 134.94, 171.75, 174.97. MS (APCI): m/z 356.1 $[MH]^+$. $[\alpha]_D^{25} = -18.4°$ (c = 1.3, CH_3OH).

General Method for the Synthesis of *N*-α-Boc-L-His(2-alkyl)-L-Arg-NHBzl (11a−f, Scheme 3). HONB (1.2 mmol) was added to *N*-α-Boc-L-His(2-alkyl)-OH (**1a−f**, 1 mmol) in water-free DMF (10 mL). At −10 °C, DIC (1.2 mmol) was added, and after 5 min stirring, L-Arg-NHBzl (**10**, 1 mmol) was added. After stirring for 36 h at 4 °C, the solvent was removed and the crude product chromatographed on neutral alumina using CH_3OH:$CHCl_3$ (1:9) to afford *N*-α-Boc-L-His(2-alkyl)-L-Arg-NHBzl (**11a−f**).

***N*-α-Boc-L-His(2-isopropyl)-L-Arg-NHBzl (11a).** Yield: 58%, colorless solid; mp 182−183 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (d, 6H, J = 7.0 Hz), 1.37 (s, 9H), 1.63 (m, 2H), 1.84 (m, 2H), 2.67 (t, 2H, J = 7.1 Hz), 3.03 (m, 2H), 3.29 (m, 1H), 4.46 (s, 2H), 4.68 (t, 1H, J = 6.7 Hz), 4.93 (t, 1H, J = 6.9 Hz), 6.90 (s, 1H), 7.26−7.31 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 23.11, 28.08, 29.5, 29.84, 30.12, 41.89, 44.81, 54.27, 58.1, 75.7, 116.06, 124.88, 126.12, 127.8, 128.95, 129.26, 131.83, 139.53, 151.37, 157.99, 172.92.; MS (APCI): m/z 543.3 $[MH]^+$.

***N*-α-Boc-L-His(2-*tert*-butyl)-L-Arg-NHBzl (11b).** Yield: 53%, colorless solid; mp 197−200 °C. ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 1.46 (s, 9H), 1.70 (m, 2H), 1.84 (m, 2H), 2.71 (t, 2H, J = 6.9 Hz), 3.04 (m, 2H), 4.34 (s, 2H), 4.51 (t, 1H, J = 6.7 Hz), 4.85 (t, 1H, J = 6.8 Hz), 6.78 (s, 1H), 7.24−7.27 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.78, 28.72, 29.36, 29.79, 40.12, 43.92, 55.7, 58.86, 73.2, 119.61, 125.11, 126.6, 127.44, 128.72, 137.85, 148.17, 154.31, 158.63, 174.8. MS (APCI): m/z 557.3 $[MH]^+$.

***N*-α-Boc-L-His(2-cyclobutyl)-L-Arg-NHBzl (11c).** Yield: 50%, colorless solid; mp 213-215 °C. ^{1}H NMR ($CDCl_3$): δ 1.34 (s, 9H), 1.57 (m, 2H), 1.87 (m, 2H), 1.93 (m, 2H), 2.14 (m, 4H), 2.56 (t, 2H, J = 7.1 Hz), 3.12 (m, 2H), 3.27 (m, 1H), 4.37 (s, 2H), 4.58 (t, 1H, J = 6.9 Hz), 4.80 (t, 1H, J = 6.8 Hz), 6.94 (s, 1H), 7.27−7.32 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.57, 24.73, 26.2, 27.84, 28.3, 29.45, 31.92, 35.56, 40.9, 44.6, 56.76, 59.44, 78.3, 119.52, 125.4, 126.55, 127.78, 129.6, 136.48, 146.2, 155.0, 158.28, 178.4. MS (APCI): m/z 555.3 $[MH]^+$.

***N*-α-Boc-L-His(2-cyclohexyl)-L-Arg-NHBzl (11d).** Yield: 52%, colorless solid; mp 220 °C. ^{1}H NMR ($CDCl_3$): δ 1.33−1.36 (m, 6H), 1.40 (s, 9H), 1.73 (m, 2H), 1.80 (m, 4H), 1.92 (m, 2H), 2.67 (t, 2H, J = 7.2 Hz), 3.03 (m, 2H), 4.39 (s, 2H), 4.55 (t, 1H, J = 6.9 Hz), 4.81 (t, 1H, J = 6.8 Hz), 6.68 (s, 1H), 7.28−7.31 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.57, 26.42, 26.66, 28.10, 29.86, 30.14, 32.47, 38.60, 41.29, 43.47, 53.76, 56.72, 72.13, 119.80, 126.18, 127.61, 128.93, 130.16, 137.71, 147.74, 154.62, 158.63, 177.52. MS (APCI): m/z 583.3 $[MH]^+$.

***N*-α-Boc-L-His[2-(adamantan-1-yl)]-L-Arg-NHBzl (11e).** Yield: 45%, colorless solid; mp 268−270 °C. ^{1}H NMR ($CDCl_3$): δ 1.31 (s, 9H), 1.58−1.62 (m, 12H), 1.72 (m, 2H), 1.85 (m, 2H), 2.01 (m, 3H), 2.59 (t, 2H, J = 7.0 Hz), 3.08 (m, 2H), 4.30 (s, 2H), 4.54 (t, 1H, J = 6.8 Hz), 4.86 (t, 1H, J = 6.7 Hz), 6.67 (s, 1H), 7.24−7.28

(m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.12, 27.81, 28.3, 29.66, 30.82, 32.67, 35.97, 40.94, 43.45, 44.68, 53.56, 59.45, 73.1, 119.38, 125.85, 126.34, 128.04, 137.91, 138.73, 146.51, 152.15, 157.8, 176.17.; MS (APCI): *m*/*z* 635.3 $[MH]^+$.

***N*-α-Boc-L-His-L-Arg-NHBzl (11f).** Yield: 62%, colorless solid; mp 161−163 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H), 1.61 (m, 2H), 1.89 (m, 2H), 2.71 (t, 2H, *J* = 7.0 Hz), 3.12 (m, 2H), 4.29 (s, 2H), 4.57 (t, 1H, *J* = 6.7 Hz), 4.82 (t, 1H, *J* = 6.7 Hz), 6.86 (s, 1H), 7.27−7.30 (m, 5H), 7.65 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.17, 28.08, 29.57, 30.62, 39.96, 43.52, 54.04, 59.83, 77.63, 119.42, 124.21, 124.82, 126.18, 126.92, 127.91, 131.7, 136.0, 139.8, 153.15, 157.82, 177.1. MS (APCI): *m*/*z* 501.2 $[MH]^+$.

General Method for the Synthesis of L-His(2-alkyl)-L-Arg-NHBzl·2HCl (12a−f, Scheme 3). A solution of *N*-α-Boc-L-His(2-alkyl)-L-Arg-NHBzl (**11a−f**, 1 mmol) in 3 N HCl in 1,4-dioxane (15 mL) was stirred at ambient temperature for 15 min. The solvent was evaporated under reduced pressure to produce L-His(2-alkyl)-L-Arg-NHBzl·2HCl (**12a−f**).

L-His(2-isopropyl)-L-Arg-NHBzl·2HCl (12a). Yield: 87%, light-yellowish semisolid. ^{1}H NMR (CD_3OD): δ 1.12 (d, 6H, *J* = 7.1 Hz), 1.67 (m, 2H), 1.93 (m, 2H), 2.87 (t, 2H, *J* = 7.2 Hz), 3.09 (m, 2H), 3.22 (m, 1H), 3.74 (t, 1H, *J* = 6.8 Hz), 4.45 (m, 2H), 4.62 (t, 1H, *J* = 6.7 Hz), 6.97 (s, 1H), 7.28−7.33 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 22.39, 24.54, 28.7, 29.91, 30.67, 39.96, 45.41, 55.7, 59.71, 119.52, 125.4, 126.88, 127.2, 127.92, 128.19, 132.07, 136.5, 138.74, 153.82, 176.89. MS (APCI): *m*/*z* 443.9 $[MH]^+$. $[\alpha]_D^{25} = -18.6°$ (*c* = 1.5, H_2O).

L-His(2-*tert*-butyl)-L-Arg-NHBzl·2HCl (12b). Yield: 89%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.43 (s, 9H), 1.79 (m, 2H), 1.86 (m, 2H), 2.77 (t, 2H, *J* = 7.1 Hz), 3.14 (m, 2H), 3.85 (t, 1H, *J* = 6.8 Hz), 4.45 (s, 2H), 4.57 (t, 1H, *J* = 6.7 Hz), 6.91 (s, 1H), 7.30−7.35 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.08, 25.74, 29.17, 30.25, 33.93, 41.22, 42.72, 53.79, 58.24, 120.61, 126.21, 126.6, 127.32, 128.47, 133.35, 146.7, 159.46, 173.58. MS (APCI): *m*/*z* 457.9 $[MH]^+$. $[\alpha]_D^{25} = -16.5°$ (*c* = 1, H_2O).

L-His(2-cyclobutyl)-L-Arg-NHBzl·2HCl (12c). Yield: 93%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.64 (m, 2H), 1.82 (m, 2H), 1.98 (m, 2H), 2.23 (m, 4H), 2.68 (t, 2H, *J* = 7.0 Hz), 3.05 (m, 2H), 3.31 (m, 1H), 3.72 (t, 1H, *J* = 6.7 Hz), 4.33 (s, 2H), 4.51 (t, 1H, *J* = 6.8 Hz), 6.78 (s, 1H), 7.25−7.28 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 20.31, 24.51, 25.32, 28.2, 31.92, 36.73, 41.82, 44.31, 54.47, 59.04, 122.24, 126.4, 126.9, 127.62, 128.71, 134.67, 147.22, 157.63, 174.72. MS (APCI): *m*/*z* 455.7 $[MH]^+$. $[\alpha]_D^{25} = -11.7°$ (*c* = 2.0, H_2O).

L-His(2-cyclohexyl)-L-Arg-NHBzl·2HCl (12d). Yield: 95%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.39−1.41 (m, 6H), 1.78 (m, 2H), 1.85 (m, 4H), 1.95 (m, 2H), 2.56 (t, 2H, *J* = 7.1 Hz), 3.10 (m, 2H), 3.88 (t, 1H, *J* = 6.9 Hz), 4.30 (s, 2H), 4.47 (t, 1H, *J* = 6.7 Hz), 6.87 (s, 1H), 7.29−7.34 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.1, 25.2, 26.42, 28.32, 31.78, 33.5, 38.95, 41.67, 44.0, 55.27, 58.84, 121.52, 126.1, 126.78, 128.62, 128.9, 130.16, 138.3, 145.42, 159.36, 175.5. MS (APCI): *m*/*z* 483.8 $[MH]^+$. $[\alpha]_D^{25} = -12.2°$ (*c* = 1.8, H_2O).

L-His[2-(adamantan-1-yl)]-L-Arg-NHBzl·2HCl (12e). Yield: 90%, colorless semisolid. ^{1}H NMR (CD_3OD): δ 1.63−1.68 (m, 12H), 1.76 (m, 2H), 1.90 (m, 2H), 2.06 (m, 3H), 2.66 (t, 2H, *J* = 7.2 Hz), 3.18 (m, 2H), 3.98 (t, 1H, *J* = 6.8 Hz), 4.44 (s, 2H), 4.61 (t, 1H, *J* = 6.7 Hz), 6.79 (s, 1H), 7.28−7.31 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.42, 28.47, 28.93, 30.5, 32.82, 36.32, 41.24, 43.32, 44.06, 52.77, 58.61, 120.43, 126.19, 126.8, 128.41, 138.16, 138.3, 147.66, 156.13, 173.4. MS (APCI): *m*/*z* 535.9 $[MH]^+$. $[\alpha]_D^{25} = -15.8°$ (*c* = 1.4, H_2O).

L-His-L-Arg-NHBzl·2HCl (12f). Yield: 95%, light-yellowish semisolid. ^{1}H NMR (CD_3OD): δ 1.59 (m, 2H), 1.86 (m, 2H), 2.79 (t, 2H, *J* = 7.2 Hz), 3.05 (m, 2H), 3.81 (t, 1H, *J* = 6.8 Hz), 4.36 (s, 2H), 4.51 (t, 1H, *J* = 6.7 Hz), 6.87 (s, 1H), 7.29−7.34 (m, 5H), 7.55 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.78, 29.23, 30.12, 40.56, 44.18, 53.67, 61.20, 116.28, 123.85, 124.34, 124.78, 125.82, 126.52, 128.11, 130.72, 135.25, 138.13, 155.27, 178.91. MS (APCI): *m*/*z* 401.5 $[MH]^+$. $[\alpha]_D^{25} = -8.8°$ (*c* = 1, H_2O). H

General Method for the Synthesis of *N*-α-Boc-L-His(2-alkyl)-L-Arg-OMe (14a−f, Scheme 4). HONB (1.2 mmol) was added to *N*-α-Boc-L-His(2-alkyl)-OH (**1a−e**, 1 mmol) in water-free DMF (10 mL). At −10 °C, DIC (1.2 mmol) was added, and after 5 min stirring, L-Arg-OMe (**13**, 1 mmol) was added. After stirring for 36 h at 4 °C, the solvent was removed and the crude product chromatographed on neutral alumina using CH_3OH:$CHCl_3$ (1:9) to afford *N*-α-Boc-L-His(2-alkyl)-L-Arg-OMe (**14a−f**).

***N*-α-Boc-L-His(2-isopropyl)-L-Arg-OMe (14a).** Yield: 61%, white solid; mp 167−168 °C. ^{1}H NMR (CD_3OD): δ 1.23 (d, 6H, *J* = 7.0 Hz), 1.37 (s, 9H), 1.55 (m, 2H), 1.81 (m, 2H), 2.45 (t, 2H, *J* = 6.7 Hz), 3.12 (m, 2H), 3.25 (m, 1H), 3.68 (s, 3H), 3.91 (t, 1H, *J* = 6.8 Hz), 4.36 (t, 1H, *J* = 6.5 Hz), 6.99 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 22.80, 25.15, 26.15, 29.27, 31.82, 32.55, 45.29, 51.86, 52.71, 57.81, 83.76, 118.45, 128.19, 144.23, 157.96, 160.73, 176.64, 180.02. MS (APCI): *m*/*z* 468.2 $[MH]^+$.

***N*-α-Boc-L-His(2-*tert*-butyl)-L-Arg-OMe (14b).** Yield: 58%, colorless solid; mp 174 °C. ^{1}H NMR (CD_3OD): δ 1.38 (s, 9H), 1.41 (s, 9H), 1.68 (m, 2H), 1.91 (m, 2H), 3.00 (t, 2H, *J* = 6.8 Hz), 3.18 (m, 2H), 3.69 (s, 3H), 3.83 (t, 1H, *J* = 6.9 Hz), 4.40 (t, 1H, *J* = 6.7 Hz), 6.72 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.62, 28.29, 29.17, 29.51, 31.90, 33.24, 41.66, 52.52, 52.67, 54.16, 80.15, 118.02, 132.49, 156.69, 157.06, 158.08, 172.99, 176.37. MS (APCI): *m*/*z* 482.3 $[MH]^+$.

***N*-α-Boc-L-His(2-cyclobutyl)-L-Arg-OMe (14c).** Yield: 55%, colorless solid; mp 180−182 °C. ^{1}H NMR (CD_3OD): δ 1.28 (m, 2H), 1.40 (s, 9H), 1.68 (m, 2H), 1.92 (m, 2H), 2.34 (m, 4H), 2.51 (t, 2H, *J* = 6.8 Hz), 3.22 (m, 2H), 3.29 (m, 1H), 3.36 (s, 3H), 3.93 (t, 1H, *J* = 6.9 Hz), 4.44 (t, 1H, *J* = 6.6 Hz), 6.75 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 18.80, 25.62, 28.31, 28.88, 29.14, 30.25, 34.33, 41.68, 52.55, 54.56, 62.97, 78.39, 117.89, 132.82, 139.43, 155.47, 158.09, 173.03, 176.33. MS (APCI): *m*/*z* 480.2 $[MH]^+$.

***N*-α-Boc-L-His(2-cyclohexyl)-L-Arg-OMe (14d).** Yield: 55%, colorless solid; mp 187 °C. ^{1}H NMR (CD_3OD): δ 1.30−1.32 (m, 6H),1.39 (s, 9H), 1.72 (m, 2H), 1.82 (m, 4H), 1.93 (m, 2H), 2.51 (t, 2H, *J* = 7.0 Hz), 2.83 (m, 1H), 3.20 (m, 2H), 3.75 (s, 3H), 3.85 (t, 1H, *J* = 6.8 Hz), 4.41 (t, 1H, *J* = 6.6 Hz), 7.04 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 18.94, 25.97, 26.18, 26.40, 28.64, 33.83, 36.87, 39.45, 41.38, 52.64, 53.13, 58.70, 119.53, 128.29, 140.48, 156.43, 158.09, 173.14, 174.99. MS (APCI): *m*/*z* 508.4 $[MH]^+$.

***N*-α-Boc-L-His[2-(adamantan-1-yl)]-L-Arg-OMe (14e).** Yield: 56%, colorless solid; mp 202−204 °C. ^{1}H NMR (CD_3OD): δ 1.39 (s, 9H), 1.49−1.53 (m, 12H), 1.56 (m, 2H), 1.82 (m, 2H), 2.04 (m, 3H), 2.43 (t, 2H, *J* = 7.2 Hz), 3.21 (m, 2H), 3.72 (s, 3H), 3.97 (t, 1H, *J* = 6.9 Hz), 4.53 (t, 1H, *J* = 6.7 Hz), 6.84 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.22, 24.30, 27.49, 28.40, 29.02, 30.15, 33.90, 38.28, 41.41, 46.30, 52.23, 56.95, 60.26, 120.81, 125.10, 128.11, 129.28, 141.62, 155.17, 158.08, 172.70, 177.52. MS (APCI): *m*/*z* 560.3 $[MH]^+$.

***N*-α-Boc-L-His-L-Arg-OMe (14f).** Yield: 65%, colorless solid; mp 153−155 °C. ^{1}H NMR (CD_3OD): δ 1.40 (s, 9H) 1.48 (m, 2H), 1.67 (m, 2H), 2.51 (t, 2H, *J* = 6.9 Hz), 3.11 (m, 2H), 3.72 (s, 3H), 3.84 (t, 1H, *J* = 6.9 Hz), 4.41 (t, 1H, *J* = 6.5 Hz), 6.78 (s, 1H), 7.08 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.71, 28.18, 28.97, 29.49, 41.51, 52.56, 53.26, 55.32, 80.34, 118.23, 132.83, 135.41, 156.99, 158.07, 171.08, 173.72. MS (APCI): *m*/*z* 426.1 $[MH]^+$.

General Method for the Synthesis of L-His(2-alkyl)-L-Arg-OMe·2HCl (15a−f, Scheme 4). A solution of *N*-α-Boc-L-His(2-alkyl)-L-Arg-OMe (**14a−f**, 1 mmol) in 3 N HCl in 1,4-dioxane (15 mL) was stirred at ambient temperature for 15 min. The solvent was evaporated under reduced pressure to produce L-His(2-alkyl)-L-Arg-OMe·2HCl (**15a−f**).

L-His(2-isopropyl)-L-Arg-OMe·2HCl (15a). Yield: 90%, light-yellowish semisolid. ^{1}H NMR (CD_3OD): δ 1.28 (d, 6H), 1.57 (m, 2H), 1.87 (m, 2H), 2.65 (t, 2H, *J* = 6.9 Hz), 3.07 (m, 2H), 3.31 (m, 1H), 3.75 (s, 3H), 3.86 (t, 1H, *J* = 6.9 Hz), 4.43 (t, 1H, *J* = 6.6 Hz), 6.88 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 20.22, 25.15, 27.63, 28.09, 28.63, 41.41, 52.74, 53.36, 58.34, 118.67,

126.39, 148.2, 159.74, 170.48, 177.51. MS (APCI): m/z 368.3 $[MH]^+$. $[\alpha]_D^{25} = -21.0°$ ($c = 1.8$, H_2O).

L-His(2-*tert*-butyl)-L-Arg-OMe·2HCl (15b). Yield: 95%, light-yellowish semisolid. ^1H NMR (CD_3OD): δ 1.51 (s, 9H), 1.75 (m, 2H), 1.98 (m, 2H), 2.72 (t, 2H, $J = 6.9$ Hz), 3.12 (m, 2H), 3.40 (s, 3H), 3.73 (t, 1H, $J = 6.9$ Hz), 4.47 (t, 1H, $J = 6.6$ Hz), 7.41 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.29, 25.78, 28.21, 28.57, 33.55, 41.4, 53.07, 56.73, 118.79, 126.57, 141.23, 158.09, 174.01, 176.12. MS (APCI): m/z 382.3 $[MH]^+$. $[\alpha]_D^{25} = -10.7°$ ($c = 2.0$, H_2O).

L-His(2-cyclobutyl)-L-Arg-OMe·2HCl (15c). Yield: 86%, light-yellowish semisolid. ^1H NMR (CD_3OD): δ 1.46 (m, 2H), 1.75 (m, 2H), 1.99 (m, 2H), 2.46 (m, 4H), 2.73 (t, 2H, $J = 6.8$ Hz), 3.34 (m, 2H), 3.43 (m, 1H), 3.74 (s, 3H), 3.97 (t, 1H, $J = 6.7$ Hz), 4.40 (t, 1H, $J = 6.5$ Hz), 7.55 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 18.78, 25.87, 27.1, 28.06, 30.07, 35.17, 41.4, 52.47, 53.66, 58.32, 119.28, 127.75, 135.32, 158.07, 168.77, 173.14. MS (APCI): m/z 380.3 $[MH]^+$. $[\alpha]_D^{25} = -9.0°$ ($c = 1.0$, H_2O).

L-His(2-cyclohexyl)-L-Arg-OMe·2HCl (15d). Yield: 90%, light-yellowish semisolid. ^1H NMR (CD_3OD): δ 1.46−1.49 (m, 6H), 1.77 (m, 2H), 1.83 (m, 4H), 1.92 (m, 2H), 2.59 (t, 2H, $J = 7.0$ Hz), 2.98 (m, 1H), 3.11 (m, 2H), 3.63 (s, 3H), 3.89 (t, 1H, $J = 6.8$ Hz), 4.44 (t, 1H, $J = 6.6$ Hz), 6.98 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.86, 25.72, 26.56, 28.96, 30.63, 34.13, 37.84, 41.49, 53.44, 54.71, 58.81, 118.12, 128.93, 137.63, 157.88, 170.71, 176.7. MS (APCI): m/z 408.3 $[MH]^+$. $[\alpha]_D^{25} = -15.6°$ ($c = 1.1$, H_2O).

L-His[2-(adamantan-1-yl)]-L-Arg-OMe·2HCl (15e). Yield: 82%, light-yellowish semisolid. ^1H NMR (CD_3OD): δ 1.57 (m, 2H), 1.66−1.69 (m, 12H), 1.82 (m, 2H), 2.08 (m, 3H), 2.54 (t, 2H, $J = 7.0$ Hz), 3.09 (m, 2H), 3.62 (s, 3H), 3.88 (t, 1H, $J = 6.7$ Hz), 4.48 (t, 1H, $J = 6.9$ Hz), 6.93 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 24.34, 26.25, 27.71, 28.56, 30.89, 32.14, 37.67, 41.72, 43.61, 52.74, 55.31, 59.49, 119.81, 128.75, 139.67, 159.08, 171.17, 174.67. MS (APCI): m/z 460.4 $[MH]^+$. $[\alpha]_D^{25} = -21.3°$ ($c = 1.0$, H_2O).

L-His-L-Arg-OMe·2HCl (15f). Yield: 92%, light-yellowish semisolid. ^1H NMR (CD_3OD): δ 1.66 (m, 2H), 1.93 (m, 2H), 2.71 (t, 2H, $J = 7.1$ Hz), 3.11 (m, 2H), 3.66 (s, 3H), 3.75 (t, 1H, $J = 6.8$ Hz), 4.38 (t, 1H, $J = 6.6$ Hz), 6.78 (s, 1H), 7.58 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 28.84, 29.42, 30.73, 45.12, 51.61, 52.75, 56.43, 123.24, 130.94, 138.92, 161.76, 171.88, 176.53. MS (APCI): m/z 326.2 $[MH]^+$. $[\alpha]_D^{25} = -12.7°$ ($c = 1.2$, H_2O).

Antimicrobial Activity Determination. The synthesized dipeptides as well as their Boc protected derivatives were evaluated for antibacterial and antifungal activities against three Gram-negative bacteria [*E. coli* (ATCC 35218), *P. aeruginosa* (ATCC 9027), and *K. pneumoniae* (ATCC 700603)], two Gram-positive bacteria [MRSA (DSM 50128509) and MRSE (DSM 50160384)], and one fungal strain [*C. albicans* (ATCC 10231)], which were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Minimum inhibition concentration (MIC), defined as the lowest test concentration that completely suppresses growth of microorganism, was measured using a modified broth microdilution method[25] whereby the assay was performed in sterile 96-well, flat-bottom polypropylene microtiter plates (Nunc, Denmark) with inocula of 5×10^5 bacteria or fungi per mL. The yeast extract/peptone/dextrose (YPD) medium was used in the case of the fungal strain, whereas a modification of a reported medium, with addition of a buffer substance (MOPS) to stabilize the pH for the addition of acidic peptides, was used for the bacterial strains.[26] After aerobic incubation for 15−18 h at 37 °C, inhibition of bacterial and fungal growth was determined by measuring absorbance at 600 nm with a Fusion universal microplate analyzer (Perkin-Elmer, MA). Half-maximal inhibitory concentration (IC_{50}) is expressed as the concentration that affords 50% inhibition of microbial growth. IC_{50} values were obtained, in triplicate per assay, from nonlinear regression analysis of plots of percentage inhibition versus log [concentration of peptide].

Cytotoxicity Experiments. Murine L-929 fibroblasts were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and cultivated in the media recommended by the supplier at 37 °C and 10% CO_2. MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] was used to measure growth and viability of cells, which are capable of reducing it by dehydrogenases of the mitochondria to a violet formazan product. Serial dilutions of the test compounds (60 μL) were added to 120 μL aliquots of a cell suspension (5×10^4/mL) in a 96-well microplate. Blank and solvent controls were incubated under identical conditions. MTT in phosphate buffered saline (PBS) (20 μL) was added to a final concentration of 0.5 mg/mL after 5 days. The precipitate of formazan crystals was centrifuged after 2 h and the supernatant discarded. The precipitate was washed with 100 μL of PBS and dissolved in 100 μL of isopropyl alcohol containing 0.4% hydrochloric acid. The microplates were gently shaken for 20 min to ensure a complete dissolution of the formazan and finally measured at 595 nm using an ELISA plate reader. All studies were carried out in two parallel experiments; the percentage of viable cells was calculated as the mean with respect to the controls set to 100%.

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Supporting Information Available: Detailed experimental procedures and spectral data for the intermediates **2a**−**f**, **3a**−**f**, and **7a**−**e**. The HPLC analysis results of all final peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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