Renin Inhibitors. Free-Wilson and Correlation Analysis of the Inhibitory Potency of a Series of Pepstatin Analogues on Plasma Renin

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Free-Wilson and correlation analysis were combined to study a series of 34 pepstatin analogues in which mainly position 2 was varied. A statistically highly significant correlation was found between the inhibitory activity of the analogues on an enriched plasma renin preparation and structural parameters of the amino acid side chain in position 2. The crucial parameters were found to be the NMR chemical shift of the α-carbon, the localized electrical (inductive) effect, and the van der Waals radius related steric parameter, which demonstrated the dominating influence of electronic inductive effects compared to steric bulk. The model gives insight into the structural requirements for effective inhibition and suggests the histidine-2 derivative, a positive outlier in this series, as a lead compound for further structure–activity studies.

Inhibitors of renin, as aspartyl protease that catalyzes the first, rate-limiting step in the conversion of angiotensinogen to angiotensin II, are of potential value in the treatment of hypertension.[1] For this reason, great effort has been expended in order to design potent and stable renin inhibitors.[2] In particular, modification of the aspartyl proteinase pepstatin, Iva-Val-Val-Sta-Ala-Sta-OH (Iva, isovaleryl; Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine)), has been shown to be a valuable approach.[3,4] A series of analogues with the general formula A-X-Y-Sta-Ala-Sta-R (A and R, protecting groups; X and Y, amino acid residues) has been prepared recently.[5] This has allowed us to investigate the interaction of the variable substituents in the peptide with the subsites S_2 and S_3 in the catalytic center of renin. The compounds displayed inhibitory potencies over 3 orders of magnitude, most of them being more potent than the natural inhibitor, pepstatin. Structure–activity relationships were evaluated both qualitatively,[5] with the known three-dimensional features of renin and its species differences taken into account, and quantitatively,[6] with consideration of the group contributions to the inhibitory activity and the variations of the steric, hydrophobic, and electronic properties of the variable side chains in the peptide.

In this paper, we extend our quantitative structure–activity relationship (QSAR) studies to pepstatin analogues of the same general formula in which mainly the position Y was varied. Using the methodology described first by Pliska,[7] we combined a Free–Wilson with a classical correlation analysis in order (i) to estimate the value of the side-chain contributions (SCC) to the IC_{50} value and to prove their additivity, (ii) to identify the structural factors influencing the binding of the inhibitor to the active site of renin, (iii) to detect outliers that could be used as lead compounds for the development of more potent derivatives, and (iv) to further evaluate the usefulness of QSAR analyses of peptides.

Materials and Methods

Free–Wilson analysis[8] was performed on 34 compounds (Table I) with variations in each of the positions A, X, Y, and R. In order to meet the requirements of the analysis, only those analogues were included in which each A, X, Y, and R group or residue was contained at least twice in the series. Thus 16 compounds described in ref 5 and 18 new compounds were available. Position Y was subjected to an extensive correlation analysis; here, exceptionally, a few side chains were represented only once.

Synthesis followed classical methods of peptide synthesis in solution, and inhibition studies were conducted as described earlier.[5] Nonnatural amino acids that were not commercially available were prepared in their L form, appropriately protected in the N^{α}-position, and introduced in the peptide by usual coupling methods. O-Alkylation of *N*-tritylserine was performed according to Barlos et al.,[9] and L-cyclopentylglycine was obtained by the method of Hill and Dunn.[10] Optical purity of the nonnatural amino acids was checked by proton NMR spectroscopy (250 MHz) of their diastereoisomeric derivatives. Purity of the final compounds was assessed by elemental, amino acid, and HPLC analysis. The ratio of the main peak in HPLC analysis to the sum of all observed peaks (λ 210 nm) was higher than 95%, except for compounds **22** and **25** (Table I), for which it was 92% and 91%, respectively.

The potency of the inhibitors was estimated on an enriched human plasma renin preparation, with endogenous angiotensinogen (about 2×10^{-6} M). Angiotensin I liberated in the presence of increasing concentrations of the inhibitor to be tested was measured by RIA. The results were expressed as IC_{50}, the molar concentration of the analogue causing 50% inhibition of plasma renin activity. As shown by Pliska and Marbach[11] and by Pliska,[12] when *S* is constant for a series of inhibitors, as in the case treated here, IC_{50}'s are linearly proportional to K_i's, the dissociation constants of the enzyme–inhibitor complex. Side-chain contributions (SCC) to IC_{50} and to K_i are, therefore, likewise proportional within the investigated series.

Amino acid side chain parameters were taken mainly

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Table I. Structure and Biological Activity of the Analogues Subjected to QSAR Analysis[a,b]

	A-X-Y	-R	log IC_{50}		A-X-Y	-R	log IC_{50}
1	Z-Phe-Phe	-OH	6.00	18	Boc-Phe-Phg	-OMe	7.03
2	Z-Phe-Phe	-OMe	5.46	19	Boc-Phe-Ser(Et)	-OMe	6.95
3	Ac-Phe-Phe	-OMe	5.85	20	Boc-Phe-Nle	-OMe	6.92
4	Iva-Phe-Phe	-OMe	6.50	21	Boc-Phe-Chg	-OMe	6.82
5	Z-Phe-Trp	-OMe	5.85	22	Boc-Phe-Ser(Bzl)	-OMe	6.76
6	Boc-Phe-His	-OMe	7.57	23	Boc-Phe-Phe	-OMe	6.72
7	Z-Phe-Val	-OMe	6.75	24	Boc-Phe-Thg	-OMe	6.60
8	Ac-Phe-Nva	-OMe	6.59	25	Boc-Phe-Ser(Pym)	-OMe	6.50
9	Iva-Phe-Nva	-OMe	7.38	26	Boc-Phe-Gln	-OMe	6.50
10	Boc-Phe-Nle	-OH	7.52	27	Boc-Phe-Met	-OMe	6.50
11	Iva-Phe-Nle	-OH	7.55	28	Boc-Phe-Ser	-OMe	6.36
12	Boc-Trp-Trp	-OMe	6.82	29	Boc-Phe-Cha	-OMe	6.31
13	Z-Trp-Val	-OMe	6.82	30	Boc-Phe-Asn	-OMe	5.92
14	Boc-Trp-His	-OMe	6.85	31	Boc-Phe-Tyr(Bzl)	-OMe	5.85
15	Boc-Phe-Cpg	-OMe	7.43	32	Boc-Phe-Asn(Ph)	-OMe	5.85
16	Boc-Phe-Nva	-OMe	7.29	33	Boc-Phe-Met(O_2)	-OMe	5.00
17	Boc-Phe-Val	-OMe	7.19	34	Boc-Phe-Trp	-OMe	6.92

[a] The full structure of the analogues is A-X-Y-Sta-Ala-Sta-R; A, acyl group; X and Y, amino acid residues; Sta, statine; R, C-terminal hydroxyl or methyl ester. [b] Other abbreviations: Iva, isovaleryl; Cpg, cyclopentyl glycine; Phg, phenylglycine; Chg, cyclohexylglycine; Thg, 2-thienylglycine; Ser(Pym), *O*-(3-pyridinylmethyl)serine; Asn(Ph), N_γ-phenylasparagine; Met(O_2), methionine sulfone.

Table II. Free–Wilson Contribution and Side-Chain Parameters of the Residue Y in Position 2

no.	residue	abbrev	SCC[a]	π[b]	$-E_s$[c]	ν[d]	α[e]	ν_V[f]	δH_C[g]	σ_I[h]	SCC predicted[i]
1	histidine	His	0.505	0.13	0.08	0.64	0.230	4.66	10.2	0.08	(−0.37),[j] (−0.34)[k]
2	cyclopentylglycine	Cpg	0.680	1.99	0.85	0.74	0.214	4.64	18.2	−0.01	0.982
3	norvaline	Nva	0.563	1.35	0.13	0.73	0.139	3.0	10.3	−0.01	0.003
4	valine	Val	0.790	1.22	0.88	0.89	0.140	3.0	17.2	0.01	0.467
5	phenylglycine	Phg	0.280	1.60	0.51	0.81	0.244	4.89	18.5	0.12	0.081
6	*O*-ethylserine	Ser(Et)	0.200	0.79	0.29	0.60	0.155	3.60	13.1	0.11	−0.168
7	norleucine	Nle	0.252	1.70	0.55	0.71	0.186	4.0	10.5	−0.01	0.064
8	cyclohexylglycine	Chg	0.070	2.40	0.98	1.08	0.257	5.43	18.5	0.00	0.360
9	*O*-benzylserine	Ser(Bzl)	0.010	2.34	0.56	0.60	0.352	7.49	13.1	0.11	−0.168
10	phenylalanine	Phe	−0.256	1.79	0.56	0.71	0.290	5.89	13.9	0.03	0.242
11	2-thienylglycine	Thg	−0.149	1.25	0.26	0.74	0.230	5.05	18.2	0.19	−0.273
12	*O*-(3-pyridinylmethyl)serine	Ser(Pym)	−0.249	0.86	0.05	0.60	0.328	7.30	13.1	0.11	−0.168
13	glutamine	Gln	−0.249	−0.22	0.16	0.71	0.180	3.95	10.6	0.05	−0.301
14	methionine	Met	−0.249	1.23	0.47	0.77	0.221	4.43	10.4	0.04	−0.369
15	serine	Ser	−0.390	−0.04	−0.01	0.55	0.062	1.60	13.1	0.11	−0.080
16	cyclohexylalanine	Cha	−0.440	2.72	0.94	0.98	0.303	6.43	9.8	−0.01	−0.499
17	asparagine	Asn	−0.830	−0.60	0.14	0.58	0.134	2.95	8.0	0.06	−0.463
18	*O*-benzyltyrosine	Tyr(Bzl)	−0.900								(−0.73),[j] (0.17)[k]
19	N_γ-phenylasparagine	Asn(Ph)	−0.900	1.40	0.14	0.58	0.377	7.84	8.0	0.10	−0.714
20	methioninesulfone	Met(O_2)	−1.750	0.20	0.66	1.23	0.217	5.63	10.4	0.11	−1.617
21	tryptophan	Trp	0.108	2.25	0.31	0.84	0.409	6.08	13.2	0.00	0.113

[a] Side-chain contribution to the overall inhibitory activity. [b] Hydrophobic constant. [c] Negative Taft steric index. [d] Van der Waals radius related steric parameter according to Charton[17] and Fauchère.[18] [e] Polarizability. [f] Normalized van der Waals volume.[18] [g] NMR chemical shift ofthe α-carbon in ppm from glycine.[19] [h] Localized electrical effect. [i] Side-chain contribution predicted by the correlation model comprising three descriptors (δH_C, σ_I, ν). [j,k] Estimates for the outliers His and Tyr(Bzl) from the seven- and three-parameter model, respectively (all data).

from ref 13. So far unknown descriptors for several uncommon residues had to be determined either by experiment or by calculation. The hydrophobic constants π of the side chains of phenylglycine, cyclopentylglycine, *O*-(3-pyridinylmethyl)serine, and N^γ-phenylasparagine were calculated by the fragmental constant method of Leo.[14] The π values for Ser(Et), Ser(Bzl), Thg, Tyr(Bzl), and Met(O_2) were obtained experimentally by thin-layer chromatography, with the same calibration as in ref 15 at neutral pH. The set of Taft steric indices E_s was obtained from the graph shape index by using a correlation equation as described by Kier.[16] The second steric parameter, ν, was derived for new groups, from the van der Waals radius based index of Charton[17] according to a published method and regression equation.[18] The normalized van der Waals volume[18] and the NMR chemical shift of the α-carbon[19] were measured or calculated in our laboratory.

Free–Wilson, correlation, and principal components analyses were obtained by using computer programs of the BMDP library.[20]

Results

Free–Wilson Analysis. From the biological activity of the analogues displayed in Table I, we have evaluated contributions of the individual side chains in each of the four A-, X-, Y-, and R-positions (results not shown) to the

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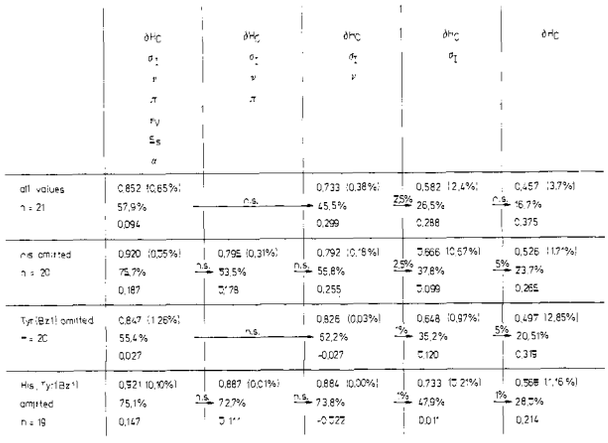

Figure 1. Correlation of the complete and reduced set of analogues with selected linear combinations of the structural parameters. For each set of data characterized in the left column, the first line shows the multiple correlation coefficient with level of significance (in parentheses), the second line the explained variance (ev), and the third line the coefficient of serial correlation of residuals (r_{res}). Significance of parameter reduction is indicated as probability level in % at arrows connecting the corresponding columns; n.s., nonsignificant.

overall inhibitory activity. These side-chain contributions (SCC) are reported in Table II for side chains in position Y. They vary over more than 2 orders of magnitude, the more favorable side chains being those of valine, cyclopentylglycine, norvaline, and histidine, while methionine sulfone appears very detrimental to activity. Statistical analysis of the Free–Wilson regression confirms the expected additivity of the contributions ψ from different positions, the constant term being the arithmetic mean of log $1/IC_{50}$ values used in computation:

$$\log 1/IC_{50} = 6.62 + \psi_A + \psi_X + \psi_Y + \psi_R \qquad (1)$$

$$n = 34,\ r = 0.979,\ F = 7.57 \text{ (DF 28 and 5)},\ \text{ev} = 0.83$$

The correlation is highly significant and explains 83% of the overall variance of IC_{50} (explained variance, ev, cf. ref 21).

Correlation Analysis. Side-chain descriptors for a number of uncommon amino acids are given in Table II.

1. Stepwise Reduction of the Number of Independent Variables. The primary task of the analysis was to specify a regression model that would enable a description of SCCs in terms of a minimal number of physicochemical descriptors. To identify the significant descriptors, multiple regression analysis was first performed with all seven parameters listed in Table II. The degree of correspondence between found SCCs and their values predicted by regression was expressed by means of the multiple correlation coefficient r, together with its appropriate significance test, and by the explained variance ev.[21] The latter criterion stands for the fraction of the total variance of the independent variable that can be accounted for by changes precisely described by the employed regression model. Our arbitrary conditions were that r must be significant at the 5% probability level and that ev must have a minimum value of 50%.

The resulting regression coefficients were tested on the significance of their difference from 0, by using a common t test. Descriptors with regression coefficients not having reached the 5% level were excluded, and a new computation was carried out with a reduced model. Evaluation of the effect of such a reduction was based upon comparison of residual variances associated with the two models under investigation.[7] The computation itself employs correlation coefficients and the corresponding degrees of freedom.

Reduction of the model was continued until all nonsignificant independent variables were eliminated; this was achieved in the second or the third step (Figure 1). The resulting regression model comprises three descriptors significant at least on a 1.5% probability level: δH_C, σ_I, and ν. To complete the reduction, further variables were omitted in the sequence of their decreasing significance. However, the additional omissions visibly deteriorated the regression model: the significance of the omission was high, and the explained variance dropped considerably. This suggested that the three-parameter model optimizes the requirements postulated above. Results of the computational procedure are summarized on the diagram in Figure 1.

2. Investigation of Outlying IC_{50} Values. The low value of explained variance (around 45%) apparently reveals some outliers in the data set. Two side chains, His and Tyr(Bzl), indeed display extreme values of residuals, i.e., difference found minus predicted SCC: His a positive and Tyr(Bzl) a negative residual. Moreover, the coefficient of the serial correlation of residuals[22] (r_{res}), although insignificant in statistical terms, indicates that the residuals are not entirely random and independent of the SCC values. Correlation coefficients found in individual runs are indicated in Figure 1. We have assumed that the outliers may account for this irregular distribution of re-

Table III. Correlation between SCC and Descriptors of Physicochemical Features of the Side Chain in Position 2 of Renin Inhibitors: Three-Parameter Regression Model (n = 19)

term	regression coeff	standard deviation	significance,[a] %
constant	−0.076		
δH_C	0.126	0.021	0.00
σ^I	−6.278	1.270	0.02
ν	−1.758	0.429	0.09

[a] Probability that the regression coefficient is not different from 0.

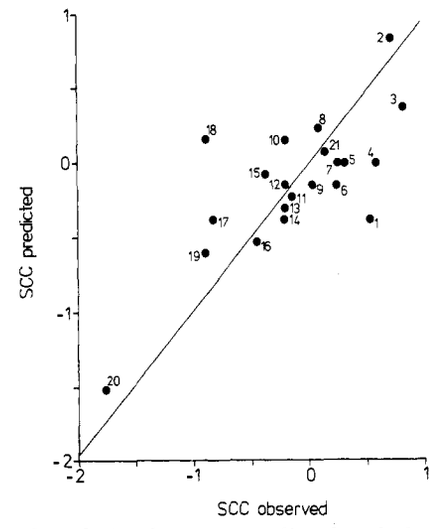

Figure 2. Correlation between predicted and observed SCC values: regression model with three parameters (n = 21, numbering as in Table II).

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Table IV. Correlations between Structural Descriptors: Matrix of Correlation Coefficients[a]

	α	π	σ_I	ν	ν_V	δH_C
π	0.649**					
σ^I	−0.057	−0.414				
ν	0.044	0.266	−0.279			
ν_V	0.957**	0.586**	0.025	0.054		
δH_C	0.025	0.386	0.084	0.229	0.015	
E_s	−0.210	−0.654**	0.486*	−0.682**	−0.246	−0.447*

[a] Significance of the correlation coefficient (19 degrees of freedom): (*) <0.05, (**) <0.01.

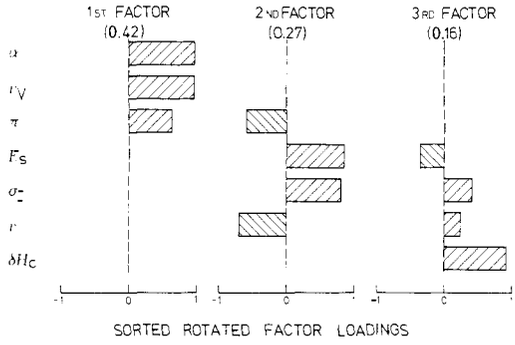

Figure 3. Composition of the first three principal components: factor loading matrix. The factors explain 84.1% of the total variance (fraction of explained variance by individual factors in parentheses). Loadings less than 0.25 have been omitted.

siduals, which, on itself, questions the suitability of the employed model. The elimination of outliers as a method of regrouping analogues has been discussed recently.[23] We have omitted subsequently each of them and observed the change in the individual criteria. In both cases, the explained variance and r were elevated, but r_{res} was still rather high when only His was eliminated. Finally, after omission of both His and Tyr(Bzl), all criteria were fully satisfactory (cf. Figure 1). Regression coefficients and their standard deviations are indicated in Table III. Predicted values of SCC were added into Table II (last column). Figure 2 shows the correlation between predicted and found SCCs.

3. Properties of the Structural Descriptors. Correlation coefficients comprised in Table IV indicate rather tight intercorrelation between certain pairs of descriptors. Moreover, descriptors α, ν_V, and π are joint in a cluster, as shown by the cluster analysis of variables. Obviously, they all relate to two physical properties which are also closely related: hydrophobicity and bulkiness. The analysis suggests further potential clusters, for instance, E_s, ν, and π, although some correlations within them are weak and do not support their existence. The parameters δH_C and to a large extent σ_I appear to be well-separated from all other descriptors; the corresponding correlation coefficients are low (Table IV).

The principal component analysis performed on the seven descriptors displays a similar trend (Figure 3). The first three components explain 84% of the variance. The first factor, after sorting of rotated loadings, comprises the descriptors α, ν_V, and π (all positive). In the second factor, the descriptors E_s and σ_I show positive loadings and π and ν show negative loadings. Whereas the first factor can be again ascribed to bulkiness and the associated hydrophobicity, the interpretation of the second is more ambiguous. The dominating influence of δH_C can be seen in the third factor. However, this analysis is still insufficient for a representation of individual molecular features by a factor, since the number of cases used for their computation was too low (21 side chains).

Discussion

An undeniable advantage of a QSAR study rests with its simplicity and the power of condensing information obtained from the response of a complex system. Provided that the biological activity and the physicochemical properties of the side chains can be expressed as numeric descriptors, the analysis generally yields precise and lucid results. In the present system, a qualitative inspection of the SCC values would have revealed that valine, cyclopentylglycine, and norvaline are favorable substitutions in position 2, while histidine, although favorable too and present in this position in the natural substrate, would not have been considered as the better possible choice. Among the residues most detrimental to bioactivity, asparagine, *O*-benzyltyrosine, and N^{γ}-phenylasparagine would have come to attention and methionine sulfone been considered as a strikingly activity lowering outlier. Here, chemical intuition might lead only to fragmentary results. In particular, it would have been difficult to explain the high activity of both the histidine and cyclopentylglycine derivatives, since they strongly differ in their polarity. Explanations for the extremely low activity of the methionine sulfone analogue or for the stronger activity-lowering effect of Tyr(Bzl) compared to Ser(Bzl) could be also difficult to find. Due to the complexity of the effects involved in the interaction of the pepstatin-like inhibitors with the active site of renin, a quantitative investigation seemed to be appropriate.

The present study revealed that three structural parameters (δH_C, σ_I and ν) were able to explain 45% of the variance when all 21 side chains were considered and 73% when two outliers, His and Tyr(Bzl), were discarded. Interestingly, no improvement has been achieved by adding hydrophobicity π as the fourth parameter. These results provide an explanation of the structure–activity relationships in this system and suggest that the three parameters are representative for the variation of the inhibitory activity over 2.6 logarithmic units (400-fold variation). The best correlation with one single parameter was obtained with the normalized NMR chemical shift δH_C of the α-carbon. The chemical shift is related to the shielding of the ^{13}C nucleus against the external magnetic field, which in turn depends on the inductive effect of other groups attached to the α-carbon. Obviously, δH_C is not free of steric and hydrophobic effects, as also seen from a certain level of correlation between δH_C and π (0.38) and ν (0.21) in this series, and cannot be considered as a purely electronic parameter. However, when it is taken into account that the next significantly correlating parameter is the localized electrical effect σ_I, it can be assumed that polar effects in this position play a major role in the inhibitory potency. Steric bulk expressed by the normalized Charton/Taft parameter[17,18] is also of importance, as shown by the great improvement of the statistical significance when this parameter was included (Figure 1). The normalized van der Waals radius, on the other hand, is irrelevant as

(23) Pliska, V.; Heiniger, J. In *QSAR in Drug Design and Toxicology*; Hadzi, D., Jerman-Blazic, B., Eds.; Elsevier: Amsterdam, 1987; p 263.

a parameter of the correlation. This result implies that a side chain with a large projection surface perpendicular to the C_β–C_γ bond[18] is not favorable to activity. In other words, the catalytic site of renin seems to accommodate linear chains (norvaline) and β-branched chains (valine, cyclopentylglycine) but not γ- nor δ-branched side chains (cyclohexylalanine, phenylalanine). From this point of view, the side chain of histidine should not be favorable.

The model that resulted from multiple regression (Table III) predicts higher inhibitory activity for relatively high δH_C values and lower activity for relatively high σ_1 and ν values. Screening of a number of amino acid side chains for which the three constants are available[13] and which have not yet been introduced in position 2 of the analogues suggests that threonine (calculated SCC = 0.7) and to a lesser extent aminobutyric acid (0.6) and *tert*-butylglycine (0.45) should lead to very active compounds while aspartic acid (−0.9) or adamantylalanine (−1.3) should not. Although it cannot be guaranteed that these precise inhibitory activities will be observed, the model provides at least a rational basis for the choice of the next analogues to be synthesized.

Two clear-cut outliers were discovered in the series. For the first one, Tyr(Bzl), the model predicts a higher activity than observed. The chosen structural descriptors do not apparently reflect the properties of this side chain properly. One reason might be the fact that this substituent is the only one in the series that contains two aromatic rings: it is therefore likely that the values of δH_C, σ_1, and ν are insensitive to the C_α-remote benzyl aromatic ring. As a matter of fact, these three constants are identical for Tyr(Bzl) and Phe, and further descriptors are required such as ν_V for a proper distinction of the two side chains; in contrast, a very different behavior may be expected for Ser(Bzl). Of greater significance is the case of the second outlier, histidine, since here the observed activity is much higher than predicted. The result implies that some property of the histidine side chain potentiates the inhibitory effect of its pepstatin derivative significantly more than in other derivatives. This property is likely to be less related to the steric properties of the imidazole ring; rather, it may be accounted for by charge, hydrogen bond donor and acceptor, aromaticity, or tautomerism. Although the structural reason for the outstanding behavior of histidine is unknown, the histidine-2 derivative may obviously serve as a lead compound for the synthesis of more potent inhibitors. Moreover, the presence of a histidine residue in the corresponding site of the natural N-terminal angiotensinogen substrate and also in several highly potent renin inhibitors[24] suggests that the availability of pepstatin derivatives containing analogues of histidine[25] in this position should contribute to the refinement of our quantitative model and serve as a guide for future design purposes.

Although this QSAR study of a bioactive peptide did not focus on selectivity toward aspartyl proteinases, but only on potency toward renin, it is one more example wherein several useful conclusions as to the factors effective in binding and proteolysis could be drawn, which could not have been deduced from simple inspection of the chemical structure.

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Potent Vasopressin Antagonists Modified at the Carboxy-Terminal Tripeptide Tail

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In a continuing effort to design more potent renal vasopressin (V_2 receptor) antagonists, we have focused our attention on the carboxy-terminal tripeptide tail (Pro-Arg-Gly-NH_2), a fragment common to both agonists and antagonists. Vasopressin antagonist analogues having a dibasic dipeptide tail, e.g., Arg-Arg-NH_2 or Arg-Lys-NH_2, attached directly to the cyclic hexapeptide ring are potent V_2-receptor antagonists. Similar modification of a representative agonist drastically reduces its potency. We report the synthesis and pharmacological properties of a series of potent V_2-receptor antagonists **3–9** where a combination of D or L dibasic dipeptide has been utilized to replace the common tripeptide fragment. Our results suggest a difference in the way agonists and antagonists bind to vasopressin receptor and further support the difference in the structure–activity relationships of agonists and antagonists. These results provide potentially useful insights for the design of novel V_2-receptor antagonists.

Antagonists of the antidiuretic response to vasopressin can be powerful pharmacological tools and physiological probes and may have clinical potential for the treatment of vasopressin-induced water-retention states of diverse etiologies.[1-4] The design and synthesis of effective antagonists of the vasopressor (V_1 receptor) and antidiuretic (V_2 receptor) responses to vasopressin have been major goals of structure–activity studies on this peptide.[5-10] As part of a continuing effort to design and synthesize potent antagonists of the vasopressin V_2 receptor and to delineate in greater detail the structural requirements for such ac-

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