are about as effective as pravastatin and lovastatin in inhibiting cholesterol biosynthesis on iv administration in this assay. Indole (**3r**) and indene (**3v**) analogues also show oral activity equivalent to that of pravastatin and lovastatin in this assay and are also effective as hypocholesterolemic agents on chronic oral dosing in rabbits, dogs, and cynomologus monkeys.[21] On the basis of its overall pharmacological profile, the disodium salt of **3r** (SQ 33,600) has been chosen for clinical study in humans.

In summary, a new class of hydroxyphosphinyl-containing inhibitors of HMG-CoA reductase has been designed on the basis of mechanistic considerations of the enzymatic reaction. These compounds are as effective in inhibiting cholesterol biosynthesis in vitro and in vivo as compounds currently in clinical study. Several analogues appear to be at least 1 order of magnitude more hepatocyte selective than pravastatin, as estimated from their ability to inhibit cholesterol biosynthesis in fibroblasts and hepatocytes. The extension of this inhibitor design concept to other aromatic and heteroaromatic systems will be the subject of future disclosures.

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(20) In this assay male Sprague-Dawley rats adapted to a reverse light cycle were dosed with drug po 2.5 h or iv 2 h, 5 min before the middark cycle of maximum cholesterol biosynthesis. [^{14}C]Acetate was administered ip 2 h before middark and blood was drawn 2 h after middark. Plasma was separated and saponified and the nonsaponifiable lipids were counted. Percent inhibition of cholesterol biosynthesis was calculated from the percent decrease in the number of counts in the treated animals relative to controls. Plots of percent inhibition vs log dose were used to determine ED_{50}'s for the test compounds.

(21) Tanaka, R.; Arbeeny, C., unpublished results.

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Selective Inhibition of Urokinase by Substituted Phenylguanidines: Quantitative Structure-Activity Relationship Analyses

Altered proteinase regulation has been related to various pathological manifestations of altered cell function such as inflammatory disease, emphysema, and the development of invasive malignant behavior in tumors. Both synthetic and natural inhibitors of proteolytic enzymes have been widely studied.[1] High levels of expression by tumor cells of plasminogen activators, particularly urokinase (UK), have been implicated in cancer of digestive tracts, breast cancer, endometrial and cervical cancer, the metastasis of colon cancer, and mouse melanoma.[2] Autoimmune disease of the skin has also been reported to involve the excessive activity of UK.[3] Inhibitors of UK might be therapeutic agents for the above conditions or at least be used experimentally to assess the role of UK. Nevertheless, reports on reversible synthetic UK inhibitors have been few.

Geratz and co-workers[4] have investigated the inhibition of UK by a series of amidine compounds, among which the tightest binding to UK was displayed by bis(5-amidino-2-benzimidazolyl)methane with K_i of 2.3 μM. However, it also inhibited plasmin, trypsin, and thrombin with high potency, giving K_i values of 2.6 μM, 17 nM, and 4.15 μM, respectively.[4] In 1987 Vassalli and Belin[5] reported that amiloride, a potassium sparing diuretic drug, was an inhibitor of UK with K_i of 7 μM with selectivity over other serine proteinases including plasmin, tissue-type plasminogen activator (tPA), thrombin, and kallikrein. However, we found that amiloride is also a potent inhibitor of bovine trypsin with an apparent K_i of 17.2 μM at pH 8.3 when pyroGlu-Gly-Arg-*p*-nitroanilide (S-2444, Abbott Chemical and Agricultural Division, North Chicago, IL) was used as the chromogenic substrate. Therefore it was considered desirable to explore the possibility of developing small non-peptide compounds with selective inhibitory activity for UK over other serine proteinases.

UK hydrolyzes its biological substrate, plasminogen, at the arginyl-valyl bond 560 amino acids from the N-terminus. Although the structure of the active site of UK has not been experimentally elucidated, from the sequence homology and from comparative modeling studies[6,7] it is expected to have a binding pocket for a positively charged group as do the trypsin-like members of the serine proteinase family. After an initial screening of various ammonium, amidinium, or guanidinium compounds having aromatic or aliphatic side chains, we found that a series of simple phenylguanidine compounds are competitive inhibitors of UK with K_i values in the micromolar range (Table I).[8,11] The compounds were further tested on other

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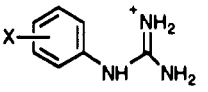
Table I. Inhibition of Serine Proteinases by Substituted Phenylguanidines



X	K_i, mM					
	UK	trypsin	plasmin	thrombin	tPA	kallikrein
H	0.0206	0.163	2.61	*b*	0.733	*d*
2-OCH_3	0.136	0.881	*a*	*b*	*c*	*d*
2-CH_3	0.259	3.55	*a*	*b*	*c*	*d*
2-NO_2	0.0543	0.133	*a*	*b*	*c*	*d*
2-Cl	0.103	0.898	*a*	*b*	*c*	*d*
2-NH_2	0.388	1.18	*a*	*b*	*c*	*d*
3-CF_3	0.419	10.7	*a*	*b*	*c*	*d*
3-Cl	0.0618	1.10	*a*	*b*	*c*	*d*
4-COO^-	0.745	10.0	*a*	*b*	*c*	*d*
4-NO_2	0.0206	0.352	*a*	*b*	0.592	*d*
4-CH_3	0.0156	0.318	*a*	*b*	*c*	*d*
4-F	0.0724	0.808	*a*	*b*	*c*	*d*
4-$COOCH_3$	1.45	0.652	*a*	*b*	*c*	*d*
4-Cl	0.00607	0.120	*a*	*b*	*c*	*d*
4-OCH_3	0.676	5.89	*a*	*b*	*c*	*d*
4-Br	0.0307	2.78	*a*	*b*	*c*	*d*
4-CH_2CH_3	0.0907	5.77	*a*	*b*	*c*	*d*
4-CH=CHCOOH	1.33	4.92	*a*	0.766	*c*	0.205
4-CF_3	0.00649	0.0631	*a*	*b*	*c*	*d*

[a] Less than 10% inhibition at 0.5 mM when assayed against 0.3 mM S-2251 (K_m = 0.664 mM). [b] Less than 10% inhibition at 1.0 mM when assayed against 7.0 μM S-2288 (K_m = 7.10 μM). [c] Less than 45% inhibition at 1.0 mM when assayed against 0.3 mM S-2288 (K_m = 0.673 mM). [d] Less than 30% inhibition at 2.0 mM when assayed against 0.1 mM S-2302 (K_m = 0.604 mM).

serine proteinases including human plasmin, human plasma thrombin, human plasma kallikrein, tPA, and bovine trypsin,[13] and the results are shown in Table I. Among these other serine proteinases, only trypsin showed any significant binding to the phenylguanidine compounds, albeit much weaker than was observed with UK.

The inhibition constants of these compounds were then analyzed by QSAR (quantitative structure–activity relationship) as pioneered by C. Hansch.[15] The log ($1/K_i$) values for UK and trypsin inhibition are listed in Table II. Parameters investigated include π, hydrophobic parameter, F and R, electronic parameters, MR, molar refractivity, and STERIMOL parameters (L, B1, B2, B3, and B4) for the steric effects due to the substituents. All the parameter values were taken from the literature[16,17] unless otherwise noted. Since the negative logarithm of K_i is used, a large log ($1/K_i$) pertains to a tight binding compound. The errors in the experimentally determined K_i values are between 8 and 11% CV.

QSAR of Para-Substituted Phenylguanidines[18] (Compounds 1, 9–19 in Table II). Equations 1 and 2 are the most important three-variable equations developed from the 12 compounds for the UK and the trypsin inhibition, respectively. In eqs 1 and 2, n is the number of

$$\log 1/K_i\ (\text{UK}) = 0.41\ (\pm 0.06)\pi_4 - 0.55\ (\pm 0.26)\text{MR}_4 + 2.07\ (\pm 0.53)R_4 + 4.89\ (\pm 0.21) \quad (1)$$

$$n = 12 \quad s = 0.344 \quad r = 0.944 \quad F = 21.8 \quad p < 0.0003 \quad press\ s = 0.383$$

$$\log 1/K_i\ (\text{trypsin}) = 0.26\ (\pm 0.09)\pi_4 - 0.82\ (\pm 0.35)\text{MR}_4 + 2.15\ (\pm 0.72)R_4 + 3.84\ (\pm 0.30) \quad (2)$$

$$n = 12 \quad s = 0.480 \quad r = 0.839 \quad F = 6.40 \quad p < 0.02 \quad press\ s = 0.663$$

compounds used in the correlation, s is the residual standard deviation, r is the correlation coefficient, F and p are the F statistics and significance probability, and *press s* is the average residual standard deviation of the predicted potency from the leave-one-out Jackknife analyses. The standard error of estimation of each coefficient is given in parentheses.

In both UK and trypsin inhibition, the positive coefficient of π_4 and the negative coefficient of MR_4 indicate that hydrophobic para substituents increase the inhibitory potency while bulky substituents at para position decrease the potency. The positive R_4 coefficient suggests that the

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(11) Assay of UK was done by measuring the initial rates of amidolysis of S-2444 by low molecular weight UK (Abbokinase from Abbott Laboratories, Abbott Park, IL) in 50 mM Tris/HCl, 100 mM NaCl, pH 8.44 in the presence of various concentrations of the inhibitor. The data obtained in a Hewlett-Packard 8452A diode array spectrophotometer were fit into a competitive inhibition equation with a personal computer program as described by Perrella.[12]
(12) Perrella, F. W. *Anal. Biochem.* **1988**, *174*, 437.
(13) Human plasmin was prepared by activation of human Lys-plasminogen.[14] For K_i measurements, 0.1–0.9 mM H-D-Val-Leu-Lys-*p*-nitroanilide (S-2251, Sigma Chemical Co., St. Louis, MO) was used in 50 mM Tris/HCl, 200 mM arginine/HCl, pH 8.00. Highly purified human plasma thrombin was obtained from Sigma Chemical Co., and the K_i values were measured with 5–10 μM H-D-Ile-Pro-Arg-*p*-nitroanilide (S-2288, Helena Laboratories, Beaumont, TX) in 0.2 M Tris/AcOH, pH 8.00. Human plasma kallikrein (Sigma Chemical Co.) was assayed in 50 mM Tris/HCl, 100 mM NaCl, pH 8.44 using 0.1–0.3 mM H-D-Pro-Phe-Arg-*p*-nitroanilide (S-2302, Helena Laboratories) for K_i measurements. Tissue-type plasminogen activator was from Codon, Inc., South San Francisco, CA. K_i values were measured with 0.1–0.9 mM S-2288 in 0.2 mM Tris/AcOH, pH 8.00. Bovine trypsin freed of chymotrypsin was a gift from Dr. R. B. Credo of Abbott Laboratories and was assayed in 0.2 M Tris/HCl, pH 8.00 with 0.1–0.3 mM S-2444 for K_i measurements.
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(18) SAS program from SAS Institute, Inc., Raleigh, NC.

Table II. Observed and Calculated log $1/K_i$ Values from Eqs 5 and 6

			log $1/K_i$, (mol/L)						physicochemical parameters used in QSAR analyses[a]								
			urokinase			trypsin						F_2	MR_4				
no.	substituent	sym	obs	cal[b]	dev[b]	obs	cal[c]	dev[c]	MR_2	$B1_2$	$B2_2$	σ_{p2}	σ_{p2}	MR_4	π_4	R_4	MR_3
1	H	a	4.686	4.511	0.175	3.788	3.734	0.054	0.103	1.00	1.00	0.00	0.00	0.103	0.00	0.00	0.103
2	2-OCH_3	b	3.866	3.887	−0.021	3.055	2.883	0.172	0.787	1.35	1.90	0.26	−0.27	0.103	0.00	0.00	0.103
3	2-CH_3	c	3.587	3.776	−0.189	2.450	2.411	0.039	0.565	1.52	1.90	−0.04	−0.17	0.103	0.00	0.00	0.103
4	2-NO_2	d	4.265	4.357	−0.092	3.876	3.807	0.069	0.736	1.70	1.70	0.67	0.78	0.103	0.00	0.00	0.103
5	2-Cl	e	3.987	3.787	0.200	3.047	3.259	−0.212	0.603	1.80	1.80	0.41	0.23	0.103	0.00	0.00	0.103
6	2-NH_2	f	3.411	3.392	0.019	2.928	3.065	−0.137	0.542	1.50	1.50	0.02	−0.66	0.103	0.00	0.00	0.103
7	3-CF_3	g	3.378	3.921[d]	−0.543	1.971	2.662[d]	−0.691	0.103	1.00	1.00	0.00	0.00	0.103	0.00	0.00	0.502
8	3-Cl	h	4.209	3.775[d]	0.434	2.959	2.407[d]	0.552	0.103	1.00	1.00	0.00	0.00	0.103	0.00	0.00	0.603
9	4-CO_2H	i	3.128	2.852	0.276	2.000	2.461	−0.461	0.103	1.00	1.00	0.00	0.00	0.605	−4.36[e]	0.13[e]	0.103
10	4-NO_2	j	4.686	4.691	−0.005	3.453	3.499	−0.046	0.103	1.00	1.00	0.00	0.00	0.736	−0.28	0.16	0.103
11	4-CH_3	k	4.807	4.510	0.297	3.498	3.239	0.259	0.103	1.00	1.00	0.00	0.00	0.565	0.56	−0.13	0.103
12	4-F	l	4.140	3.929	0.211	3.093	3.055	0.038	0.103	1.00	1.00	0.00	0.00	0.092	0.14	−0.34	0.103
13	4-CO_2CH_3	m	2.839	2.852	−0.013	3.186	3.113	0.073	0.103	1.00	1.00	0.00	0.00	1.287	−0.01[f]	0.15[f]	0.103
14	4-Cl	n	5.217	4.538	0.679	3.921	3.206	0.715	0.103	1.00	1.00	0.00	0.00	0.603	0.71	−0.15	0.103
15	4-OCH_3	o	3.170	3.538	−0.368	2.230	2.100	0.130	0.103	1.00	1.00	0.00	0.00	0.787	−0.02	−0.51	0.103
16	4-Br	p	4.513	4.566	−0.053	2.556	2.977	−0.421	0.103	1.00	1.00	0.00	0.00	0.888	0.86	−0.17	0.103
17	4-C_2H_5	q	4.042	4.768	−0.726	2.239	3.056	−0.817	0.103	1.00	1.00	0.00	0.00	1.030	1.02	−0.10	0.103
18	4-$CH{=}CHCO_2H$	r	2.876	3.199	−0.323	2.308	1.910	0.398	0.103	1.00	1.00	0.00	0.00	1.703	−4.04[e]	0.24[g]	0.103
19	4-CF_3	s	5.188	5.255	−0.068	4.200	4.055	0.145	0.103	1.00	1.00	0.00	0.00	0.502	0.88	0.19	0.103

[a] Values were taken from Hansch et al.[16] and Verloop et al.[17] [b] Calculated with use of eq 5. [c] Calculated with use of eq 6. [d] Calculated with use of eqs 9 and 10. [e] Values are for CO_2^- or $CH{=}CHCO_2^-$ group. [f] A better correlation is obtained from UK inhibition by this compound if the parameter values π_4 (−4.36) and R_4 (0.13) for 4-CO_2^- are used. This may indicate that the ester is considerably hydrolyzed at pH 8.44. [g] Estimated value as suggesetd by reviewers.

para position prefers electron-withdrawing groups.[19]

Among the three para substituent parameters, the hydrophobic parameter (π_4) has the most influence on the UK inhibitory potency, accounting for more than 65% of the variance of the data. Electronic effects (R_4) contribute 18% and the effects of bulky substituent (MR_4) improve the correlation by additional 6%.

Contribution of the individual parameters for the trypsin inhibition is different from that for the UK inhibition, i.e., π_4, R_4, and MR_4 account for the variance of the data by 53%, 16%, and 13%, respectively. Here, the bulky substituent effect (MR_4) becomes more significant.

QSAR of Ortho-Substituted Phenylguanidines (Compounds 1–6 in Table II). Equations 3 and 4 are the most significant two-variable equations obtained from the six compounds for the UK and the trypsin inhibition, respectively. Despite the small number of ortho-substituted compounds studied, it is clear from eqs 3 and 4 that, in both UK and trypsin inhibition, electron-donating ortho-substituents decrease the inhibitory potency. Similar to the para position, a bulky group is not preferred at the ortho position judged by the negative coefficient of $B1_2$ or $B2_2$.

$$\log 1/K_i\ (\text{UK}) = -1.27\ (\pm 0.32)B1_2 + 0.86\ (\pm 0.19)\sigma_{p2} + 5.86\ (\pm 0.49) \quad (3)$$

$$n = 6 \quad s = 0.190 \quad r = 0.948 \quad F = 13.31 \quad p < 0.03 \quad press\ s = 0.343$$

$$\log 1/K_i\ (\text{trypsin}) = -1.38\ (\pm 0.26)B2_2 + 1.57\ (\pm 0.31)F_2 + 5.10\ (\pm 0.41) \quad (4)$$

$$n = 6 \quad s = 0.185 \quad r = 0.965 \quad F = 20.24 \quad p < 0.02 \quad press\ s = 0.269$$

Table III. Pearson Correlation Coefficients (n = 17)

	MR_2	$B1_2$	$B2_2$	σ_{p2}	F_2	MR_4	π_4	R_4
MR_2	1.00	0.91	0.97	0.06	0.73	−0.61	0.11	0.11
$B1_2$		1.00	0.91	0.19	0.75	−0.59	0.11	0.10
$B2_2$			1.00	0.02	0.63	−0.61	0.11	0.10
σ_{p2}				1.00	0.65	0.02	0.00	0.00
F_2					1.00	−0.40	0.08	0.07
MR_4						1.00	−0.36	0.23
π_4							1.00	−0.43
R_4								1.00

QSAR of the Combined Set of Ortho and Para Substituents (Compounds 1–6, 9–19 of Table II). Equations 5 and 6 were derived from all 17 compounds used in eqs 1–4 for the UK and the trypsin inhibition, respectively.[20,21] The coefficients of the variables in eqs

$$\log 1/K_i\ (\text{UK}) = 0.44\ (\pm 0.06)\pi_4 + 1.89\ (\pm 0.55)R_4 - 1.14\ (\pm 0.34)B1_2 + 0.83\ (\pm 0.33)\sigma_{p2} + 5.65\ (\pm 0.42) \quad (5)$$

$$n = 17 \quad s = 0.360 \quad r = 0.914 \quad F = 15.1 \quad p < 0.0001 \quad press\ s = 0.406$$

$$\log 1/K_i\ (\text{trypsin}) = 0.26\ (\pm 0.08)\pi_4 - 0.79\ (\pm 0.30)MR_4 + 2.13\ (\pm 0.63)R_4 - 1.40\ (\pm 0.44)B2_2 + 1.57\ (\pm 0.71)F_2 + 5.21\ (\pm 0.63) \quad (6)$$

$$n = 17 \quad s = 0.420 \quad r = 0.856 \quad F = 6.10 \quad p < 0.006 \quad press\ s = 0.558$$

(19) Since no literature values of substituent constants are available for the ionized form of compound 18, eqs 1 and 2 were derived from values estimated as noted in the footnotes *e* and *g* of Table II. If compound 18 is excluded in the analysis, the following equations are obtained. In each case, the alternative equation supports the conclusions based on eqs 1 and 2.

$$\log 1/K_i\ (\text{UK}) = 0.39\ (\pm 0.07)\pi_4 - 0.61\ (\pm 0.28)MR_4 + 2.07\ (\pm 0.55)R_4 + 4.93\ (\pm 0.22) \quad (1a)$$

$$n = 11 \quad s = 0.357 \quad r = 0.934 \quad F = 15.8 \quad p < 0.002 \quad press\ s = 0.146$$

$$\log 1/K_i\ (\text{trypsin}) = 0.26\ (\pm 0.10)\pi_4 - 0.84\ (\pm 0.41)MR_4 + 2.12\ (\pm 0.79)R_4 + 3.85\ (\pm 0.32) \quad (2a)$$

$$n = 11 \quad s = 0.512 \quad r = 0.839 \quad F = 5.60 \quad p < 0.03 \quad press\ s = 0.783$$

(20) Equation 5 was developed as follows.

$$\log 1/K_i\ (\text{UK}) = 0.31\ (\pm 0.07)\pi_4 + 4.18\ (\pm 0.14)$$

$$n = 17 \quad s = 0.639 \quad r = 0.729 \quad F = 17.0 \quad p < 0.0009 \quad press\ s = 0.556$$

$$\log 1/K_i\ (\text{UK}) = 0.38\ (\pm 0.08)\pi_4 + 1.45\ (\pm 0.74)\sigma_p + 4.27\ (\pm 0.13)$$

$$n = 17 \quad s = 0.499 \quad r = 0.794 \quad F = 11.9 \quad p < 0.0009 \quad press\ s = 0.533$$

$$\log 1/K_i\ (\text{UK}) = 0.43\ (\pm 0.07)\pi_4 + 1.82\ (\pm 0.65)R_4 - 0.98\ (\pm 0.39)B1_2 + 5.45\ (\pm 0.48)$$

$$n = 17 \quad s = 0.425 \quad r = 0.866 \quad F = 13.0 \quad p < 0.0003 \quad press\ s = 0.482$$

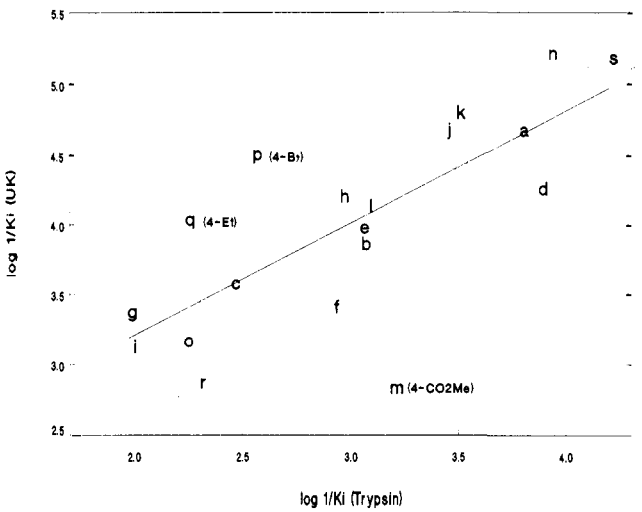

Figure 1. Plot of log $1/K_i$ (UK) versus log $1/K_i$ (trypsin).

5 and 6 are essentially identical with those in eqs 1–4 within the confidence limits except that MR_4, the least significant parameter in eq 1, is missing in eq 5. This may provide a further clue to the selectivity of phenylguanidine analogues for UK over trypsin. The negative effect of a bulky para substituent appears to be more tolerable for UK inhibition than for trypsin inhibition. Table II lists the calculated log $(1/K_i)$ values using eqs 5 and 6 for UK and trypsin, respectively, and the Pearson correlation coefficients of the variables used in eqs 5 and 6 are shown in Table III.

When the steric parameter $B1_2$ or $B2_2$ was replaced by MR_2, eqs 7 and 8 were obtained. Although eqs 7 and 8

$$\log 1/K_i\ (\text{UK}) = 0.43\ (\pm 0.06)\pi_4 + 1.87\ (\pm 0.58)R_4 - 1.17\ (\pm 0.38)\text{MR}_2 + 0.67\ (\pm 0.94)\sigma_{p2} + 4.62\ (\pm 0.15) \quad (7)$$

$$n = 17 \quad s = 0.375 \quad r = 0.906 \quad F = 13.7 \quad p < 0.0002 \quad press\ s = 0.424$$

$$\log 1/K_i\ (\text{trypsin}) = 0.26\ (\pm 0.08)\pi_4 - 0.79\ (\pm 0.30)\text{MR}_4 + 2.13\ (\pm 0.63)R_4 - 2.10\ (\pm 0.75)\text{MR}_2 + 2.00\ (\pm 0.86)F_2 + 4.01\ (\pm 0.33) \quad (8)$$

$$n = 17 \quad s = 0.445 \quad r = 0.837 \quad F = 5.10 \quad p < 0.01 \quad press\ s = 0.588$$

are poorer in quality than eqs 5 and 6, eqs 7 and 8 provide information about the size of available space around the ortho and para substituents in the same scale. The magnitude of the coefficients of MR_2 and MR_4 parameters indicates that bulky substituents are more tolerable in the para position than in the ortho position for the inhibition of both enzymes. The electronic effects of ortho and para substituents appear to be both field-inductive and resonance.

There are only two meta substituents (3-CF_3 and 3-Cl) included in this study. Thus no reliable QSAR can be derived from the set of meta-substituted compounds.[22]

The QSAR analyses so far indicate that electron-withdrawing ortho substituents and hydrophobic para substituents with electron-withdrawing property are preferred for tighter binding to both UK and trypsin. Bulky substituents with a large MR value at meta position are predicted to decrease the binding affinity to both enzymes. Bulky substituents at para position appear to interfere with tighter binding to trypsin more than to UK. However, the QSAR eqs 1–10 should be viewed with caution due to the relatively small number of samples analyzed in this study.

The difference in the intercept in the correlations for the inhibition of UK and trypsin is more than 1 log unit, indicating the binding affinity of phenylguanidines to UK is at least 10 times greater than that of trypsin. This is shown in Figure 1 and eq 11. In Figure 1, the 4-$COOCH_3$ analogue appears to be an outlier. This may be due to hydrolysis of the ester during the UK inhibition constant measurement as noted in the footnote f of Table II.

$$\log 1/K_i\ (\text{UK}) = 0.80\ (\pm 0.18)\ \log 1/K_i\ (\text{trypsin}) + 1.60\ (\pm 0.54) \quad (11)$$

$$n = 19 \quad s = 0.511 \quad r = 0.742 \quad F = 20.81 \quad p < 0.0003 \quad press\ s = 0.529$$

It is useful to examine the three-dimensional model structure of UK in order to help understand the reasons behind the QSAR results. The partial structure of UK shown in Figure 2 was constructed from the known experimental structures of serine proteinases by using comparative modeling methods.[6,7] Binding of the phenyl-

(21) Equation 6 was developed as follows.

$$\log 1/K_i\ (\text{trypsin}) = 0.22\ (\pm 0.10)\pi_4 + 3.11\ (\pm 0.15)$$

$$n = 17 \quad s = 0.608 \quad r = 0.490 \quad F = 4.73 \quad p < 0.05 \quad press\ s = 0.625$$

$$\log 1/K_i\ (\text{trypsin}) = 0.34\ (\pm 0.12)\pi_4 + 0.90\ (\pm 0.60)R_4 + 3.12\ (\pm 0.60)$$

$$n = 17 \quad s = 0.584 \quad r = 0.589 \quad F = 3.71 \quad p < 0.05 \quad press\ s = 0.560$$

$$\log 1/K_i\ (\text{trypsin}) = 0.33\ (\pm 0.12)\pi_4 - 0.58\ (\pm 0.33)\text{MR}_4 + 1.32\ (\pm 0.60)R_4 + 3.44\ (\pm 0.22)$$

$$n = 17 \quad s = 0.543 \quad r = 0.688 \quad F = 3.90 \quad p < 0.03 \quad press\ s = 0.581$$

$$\log 1/K_i\ (\text{trypsin}) = 0.36\ (\pm 0.10)\pi_4 - 1.26\ (\pm 0.37)\text{MR}_4 + 1.90\ (\pm 0.54)R_4 - 1.12\ (\pm 0.42)\text{B2}_2 + 5.19\ (\pm 0.68)$$

$$n = 17 \quad s = 0.449 \quad r = 0.817 \quad F = 6.04 \quad p < 0.007 \quad press\ s = 0.626$$

(22) However, since both the ortho and the para substrates demonstrated steric effects as well as electronic effects, it is likely that the meta substituents also show similar effects. In order to investigate whether meta substituents show negative steric effects, the two meta-substituted compounds were added to eqs 5 and 6 with MR_3 parameter. Eqs 9 and 10 show the results. All the coefficients in eqs 9 and 10 remained essentially identical with those in eqs 5 and 6. The negative coefficients of MR_3 parameter in eqs 9 and 10 clearly indicate that large substituents are not preferred at meta position. Since any electronic parameter for meta position cannot be included with the steric parameter due to the small number of data points, the standard errors of estimation of both MR_3 coefficients are relatively large.

$$\log 1/K_i\ (\text{UK}) = 0.43\ (\pm 0.06)\pi_4 + 1.87\ (\pm 0.61)R_4 - 1.12\ (\pm 0.37)\text{MR}_2 + 0.82\ (\pm 0.37)\sigma_{p2} - 1.45\ (\pm 0.68)\text{MR}_3 + 5.77\ (\pm 0.49) \quad (9)$$

$$n = 19 \quad s = 0.396 \quad r = 0.890 \quad F = 9.90 \quad p < 0.0004 \quad press\ s = 0.505$$

$$\log 1/K_i\ (\text{trypsin}) = 0.26\ (\pm 0.09)\pi_4 - 0.72\ (\pm 0.36)\text{MR}_4 + 2.06\ (\pm 0.75)R_4 - 2.01\ (\pm 0.84)\text{MR}_2 + 1.99\ (\pm 0.96)F_2 - 2.52\ (\pm 0.98)\text{MR}_3 + 4.21\ (\pm 0.43) \quad (10)$$

$$n = 19 \quad s = 0.497 \quad r = 0.804 \quad F = 3.60 \quad p < 0.03 \quad press\ s = 0.687$$

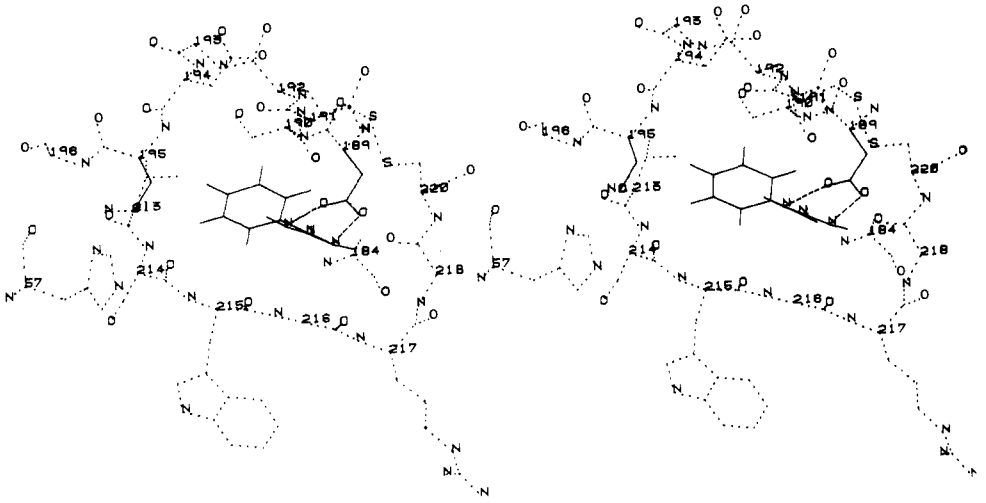

Figure 2. Stereo presentation of the active site of the UK model structure (···). The inhibitor, phenylguanidine (–) is shown bound to the active site. The guanidino group forms a salt bridge interaction (- - -) with Asp 189. The para position is near the Cβ of Ser 195. Both Asp 189 and Ser 195 are highlighted in solid lines. The main chain imino nitrogens of residues 193 and 195 mark the sites where the hydrogens emerge that favor the presence of electron-withdrawing groups in the para position.

guanidines to the active site of UK was modeled after the crystal structure of *p*-guanidinobenzoate bound to bovine trypsin.[23] Model studies show that the UK active site is very similar to that of trypsin. This is consistent with the similarity of QSAR equations for UK and trypsin for the interaction of the substituted phenylguanidines with UK and trypsin. In the crystal structure of ref 23, a covalent bond is formed between the carboxyl group of the benzoate and Ser 195[24] of the trypsin enzyme. Although a similar covalent bond can not form between UK and the compounds studied here, the model structure of phenylguanidine bound to the UK active site (Figure 2) shows that the para position lies very close to the Ser 195 in UK as well. It is probably this proximity which is limiting the size of the substituents at the para position in the phenylguanidine as seen from the negative coefficients for MR_4 in the QSAR eqs 1 and 2.

The positive R_4 coefficients of eqs 1 and 2 indicate that the para position prefers electron-withdrawing groups. This effect may be explained by the model structure which shows the main chain imino hydrogens of residues 193 and 195 as pointing toward the para position of the phenyl group (Figure 2) creating a positive environment that would favor electron-withdrawing groups on the para position of the phenylguanidine. These same imino hydrogens form hydrogen bonds to the carbonyl oxygen of the *p*-guanidinobenzoate–Ser 195 adduct in the reported trypsin crystal structure.[23] This site, which is close to the phenyl para substituent, is called the oxyanion binding site and stabilizes the anion formed in the tetrahedral transition state of the enzyme–substrate complex.[25]

The closest atom on the enzyme to the para carbon of the phenyl ring is Cβ of residue Ser 195 and its hydrogens. This hydrophobic interaction might explain why the para position has a tendency to prefer hydrophobic substituents as shown by the positive coefficient of π_4. The coefficients of π_4 for both UK and trypsin are less than 0.5, which supports the proposal by Hansch and Klein[26] that the hydrophobic interaction site of the ligand may be on a surface or shallow trough of the enzyme rather than in a cave.

The structural basis for the QSAR analyses of the ortho compounds is more complex since there are two possible positions for each ortho substitution (Figure 2). The relatively strong tendency against bulky substituents is the result of the nonplanar conformation of the phenylguanidine analogues. If the phenyl ring would lay in the plane of the guanidinium group, one of the ortho positions would point directly into the enzyme and the other would point out in the solution. However, because the phenyl group lies at about 45° from the plane of the guanidinium, both ortho positions are pointing toward the peptide strands that line the guanidinium binding pocket (Figure 2). In one case, the ortho substituent would lay near the carbonyl oxygen of residue 215 and a water molecule hydrogen bonding to it (the water molecule is not shown in the figure). The other ortho site points somewhat toward the outside but lies near the disulfide bridge between cystine 191 and 220. In both cases, there is only limited room for substituents. Larger modifications would require the molecule either to become more planar or disrupt the interaction between the guanidinium group and Asp 189 at the base of the pocket. Both of these effects would lower the binding energy of the compound to the enzyme.

In summary, the group of phenylguanidine compounds shows selectivity for UK over other serine proteinases (Table I). Trypsin has less binding affinity to the series of phenylguanidine compounds by at least 1 order of magnitude compared with UK. For plasmin, underivatized phenylguanidine is the tightest binding inhibitor in the series with a still rather poor K_i of 2.61 ± 0.39 mM. For kallikrein, 4-guanidinocinnamic acid is the tightest binding among the series of compounds with a K_i of 0.205 ± 0.033 mM. The same compound is also the best inhibitor among the group for thrombin with an apparent K_i of 0.766 ± 0.179 mM. The phenylguanidine compounds are generally poor inhibitors of tPA. Among them 4-nitrophenylguanidine and phenylguanidine are better inhibitors than others with the K_i values of 0.592 ± 0.060 and 0.733 ± 0.089 mM, respectively.

It is surprising to find that compounds as simple as phenylguanidines show such high selectivity for UK over the other trypsin-like serine proteinases tested. We are currently investigating ways to further improve the se-

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lectivity and the binding affinity by employing computer-assisted modeling as well as by expanding the series of compounds.

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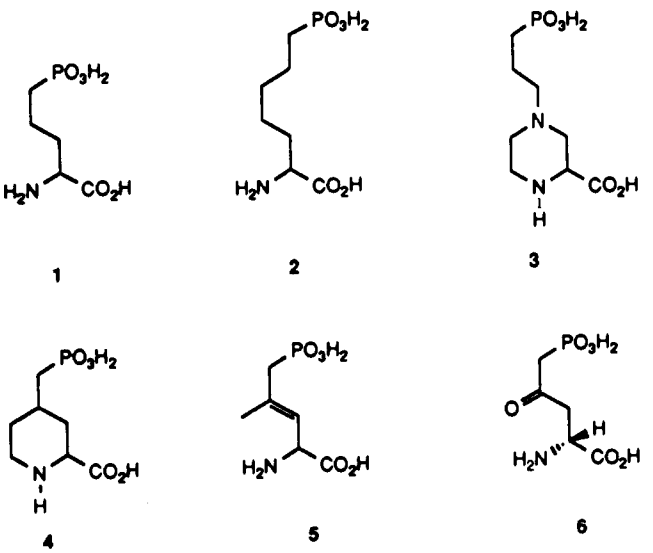
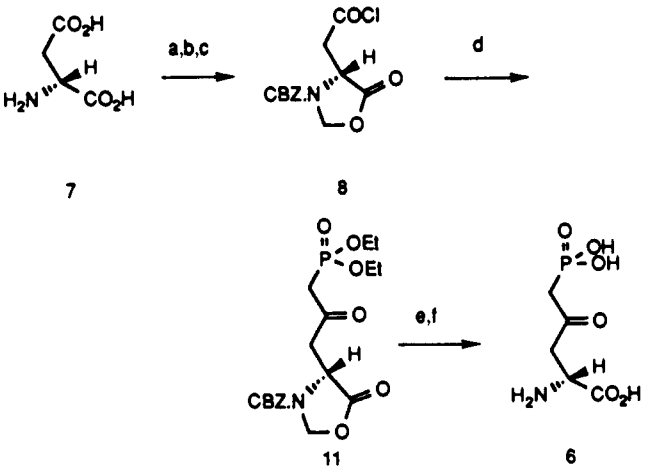
(*R*)-4-Oxo-5-phosphononorvaline: A New Competitive Glutamate Antagonist at the NMDA Receptor Complex

The neurochemical functions of the excitatory amino acids glutamic acid and aspartic acid have been extensively studied in recent years. These neurotransmitters can activate at least three receptor complexes, which are designated according to their sensitivity to the ligands *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid.[1] Abnormal physiological conditions, such as epilepsy, Huntington's chorea, memory disorders, and neuronal damage, which occur following an ischemic episode, have been associated with hyperactivity of one or more of these receptor complexes.[2] The NMDA receptor complex, which is the best understood to date, contains individual binding sites for glutamic acid,[3] glycine,[3] Mg^{2+},[4] Zn^{2+},[5] and polyamines,[6] all of which play a key role in modulating the flow of Ca^{2+} through the receptor-associated ion channel. In this paper we wish to communicate the discovery of a new, systematically active antagonist of the glutamate binding site of the NMDA receptor complex.

A number of antagonists have been reported in the literature. The first of these, 2-amino-5-phosphonopentanoic acid (AP5, 1) and 2-amino-7-phosphonoheptanoic acid (AP7, 2), while reasonably potent in binding assays, have difficulty in penetrating the blood–brain barrier, resulting in weak systemic activity in various animal models.[7] More recently, second-generation phosphonic acid derivatives, such as 4-(3-phosphonomethyl)-2-piperazinecarboxylic acid (CPP, 3),[8] *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19,755, 4),[9] and (*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37,849, 5)[10] have been shown to combine good binding affinities with the ability to cross the blood–brain barrier. In this context, on the basis of extensive molecular modeling studies, we designed and synthesized (*R*)-4-oxo-5-phosphononorvaline (6), a structural analogue of AP5 (1).[20]



Scheme I[a]



[a] Reagents: (a) CBZCl, NaOH; 93%; (b) CH_2O, PTSA refluxing benzene; 96%; (c) $SOCl_2$; 100%; (d) $((EtO)_2POCH_2)_2CuI$, Et_2O/THF, −30 °C; 37%; (f) TMSI; (g) Dowex 50W-X8; 68%.

Table I. Relative in Vitro Potency of Phosphono Amino Acid Antagonists[a]

compound	K_i, nM, vs [^{3}H]CPP	IC_{50}, μM, vs cGMP
1 (AP5)	176 ± 16	5.5
2 (AP7)	730 ± 64	14.1
3 (CPP)	131 ± 18	0.8
4 (CGS 19,755)	99 ± 32	1.1 ± 0.4
6 (MDL 100,453)	109 ± 12	5.9 ± 1.4

[a] Methods are described by Baron *et al.*[14] Results are means of at least three determinations except the functional assays with no range, which are the results of a single determination.

A convergent synthesis of 6 utilizing a key copper-catalyzed coupling step,[11] with D-aspartic acid as the chiral educt, is outlined in Scheme I. D-Aspartic acid (7), protected as the *N*-(carbobenzyloxy)-4-oxazolidone[12] by

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