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Quantitative Structure–Intestinal Permeability Relationship of Benzamidine Analogue Thrombin Inhibitor

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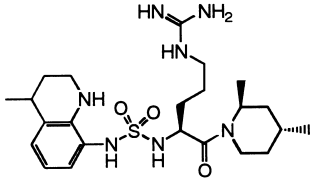
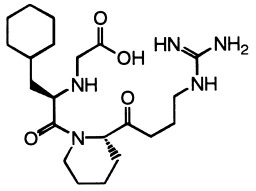
Abstract—The intestinal permeability of benzamidine analogue thrombin inhibitor is correlated with molecular volume, lipophilicity (calculated log *P* and IAM column capacity factor), hydrogen bond acidity/basicity and dipolarity.

Introduction

Thrombin plays a key role in hemostasis by mediating conversion of fibrinogen to fibrin and activating platelets. Thrombin inhibitors prevent intravascular clot formations which cause many cardiovascular diseases such as myocardial infarction, deep vein thrombosis, and ischemic stroke. Some thrombin inhibitors (argatroban, heparin, etc.) are available but these agents are not orally active. Therefore, an orally active thrombin inhibitor would be useful in clinical practice. Many pharmaceutical companies have made concentrated efforts to develop an orally active synthetic thrombin inhibitor, but in many cases, only limited oral activity was achieved. The low oral activity is due to the low intestinal permeability, rather than low solubility and metabolic instability. Most of the synthetic thrombin inhibitors reported before have a highly basic functional group like guanidine or benzamidine which interacts with Asp 189 (P_1 pocket) of thrombin,[1] and these highly basic functional groups cause low membrane permeability. Recently, some compounds have been reported to have oral activity despite their high basicity, for example inogatran.[2] To develop an orally active thrombin inhibitor, we performed the intestinal permeability screening of many benzamidine derivatives, which have thrombin inhibition activity. In this report, we describe the structure–permeability relationship of benzamidine analogue thrombin inhibitors.



Argatroban



Inogatran

Results and Discussion

We measured the everted sac permeability of benzamidine analogues[3] with an IC_{50} of thrombin inhibition less than 100 μM (ESA, Table 1).[4,5] All of our compounds have a benzamidine group whose pK_a is more than 10.[6] According to the pH partition theory,[7] benzamidine analogues can not penetrate the intestinal membrane via the transcellular pathway, because they are completely positively charged at the neutral pH of the small intestine. Therefore, benzamidine analogues are thought to penetrate mainly via paracellular aqueous pore diffusion, otherwise it would be the substrate of transporters. By Caco-2 cell experiment, we determined that opening the tight junction dramatically increased the permeability of some of our compounds (data not shown). This phenomenon indicates that the paracellular pathway is the dominant pathway in this class of compound.

Usually, permeation via paracellular aqueous pore diffusion is a function of molecular size reflected by the sieving coefficient on aqueous pores and the diffusion coefficient in water.[8] The larger the molecular size of the compound the lower its permeability. So we first studied

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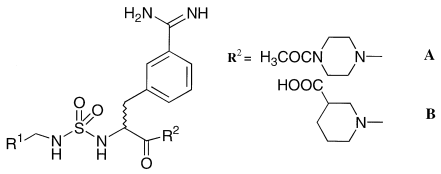
Table 1. ESA value, log k and calculated solute properties of benzamidine analogues



No.	R^1	R^2	log ESA[a]	prolog P[b]	log k[c]	R_2[d]	π_2[d]	$\Sigma\alpha_2^{H,d}$	$\Sigma\beta_2^{H,d}$	Vx[d]
1	Phenyl	A	−0.810	−0.75	−0.049	1.452	3.16	0	1.70	2.226
2	1-Naphthyl	A	−0.842	0.47	0.736	2.182	3.51	0	1.76	2.595
3	2-Naphthyl	A	−0.932	0.47	0.707	2.222	3.52	0	1.76	2.595
4	1-Phenylethyl	A	−0.889	0.21	0.367	1.452	3.16	0	1.70	2.508
5	2-Phenylphenyl	A	−1.071	1.19	0.919	2.211	3.63	0	1.78	2.834
6	3-Phenoxyphenyl	A	−1.149	1.04	1.047	2.067	3.72	0	1.76	2.893
7	4-Phenylphenyl	A	−1.174	1.20	1.176	2.211	3.63	0	1.78	2.834
8	4-Phenethylphenyl	A	−1.252	1.83	1.323	2.053	3.68	0	1.84	3.116
9	1-Phenylethyl	B	−0.762	1.82	0.268	1.412	2.54	0.60	1.37	2.467
10	1-Naphthyl	B	−0.752	2.07	0.600	2.182	2.90	0.60	1.43	2.554
11	1-Phenylethyl	B	−0.759	2.32	—	1.412	2.54	0.60	1.37	2.467
12	1-Naphthyl	B	−0.893	2.57	0.924	2.182	2.90	0.60	1.43	2.554
13	1,1-Diphenylethyl	A	−1.456	1.68	1.165	2.053	3.68	0	1.84	3.116
14	5-Quinolylmethyl	A	−0.686	−0.60	0.132	2.119	3.61	0	2.10	2.554
15	1,1-Diphenylmethyl	A	−1.092	1.17	0.956	2.053	3.68	0	1.84	2.975
16	2-Methoxyphenyl	A	−0.815	−0.79	0.069	1.576	3.38	0	1.86	2.426
17	2-Ethoxyphenyl	A	−0.876	−0.33	0.269	1.532	3.34	0	1.88	2.567
18	3-Methoxyphenyl	A	−0.804	−0.69	0.066	1.560	3.42	0	1.86	2.426
19	2,4-Dimethoxyphenyl	A	−0.602	−0.83	−0.057	1.657	3.64	0	2.06	2.626
20	2-Pheylphenyl	A	−0.987	1.37	1.087	2.053	3.68	0	1.84	2.975
21	1-Isoquinolylmethyl	A	−0.635	−0.63	0.249	2.119	3.61	0	2.10	2.554
22	3,4,5-Trimethylphenyl	A	−0.818	−1.95	−0.142	1.755	3.87	0	2.21	2.825
23	*N*-Phenylmethyl-2-pyrrolidinyl	A	−0.963	−0.78	−0.305	1.816	3.41	0	2.49	2.988
24	3-Indolyl	A	−0.914	0.43	0.863	2.051	3.70	0.44	1.91	2.738
25	1-Naphthylmethyl	A	−1.114	0.92	0.918	2.222	3.52	0	1.76	2.736
26	2-Naphthylmethyl	A	−1.032	0.92	0.918	2.182	3.51	0	1.76	2.736
27	2-Phenyl-1-(1-pyrrolidinylcarbonyl)-ethyl	A	−1.027	−0.59	0.129	1.988	4.53	0	2.48	3.220
28	3-Nitrophenylmethyl	A	−0.688	−0.32	0.314	1.725	3.74	0	1.81	2.401
29	3-Bromo-1-naphthyl	A	−1.367	1.41	1.303	2.463	3.77	0	1.71	2.770
30	3-Aminophenylmethyl	A	−0.750	−1.21	−0.167	1.797	3.59	0.23	2.01	2.467
31	1,2,3,4-Tetrahydroquinol-3-yl	A	−0.674	−0.85	0.068	2.101	3.59	0.17	2.07	2.640
32	3,4-Methylenedioxyphenylmethyl	A	−0.636	−0.92	0.209	1.988	3.81	0	1.91	2.376
33	1,4-Benzodioxan-6-yl-methyl	A	−0.654	−1.00	0.235	2.030	3.81	0	1.91	2.517
34	2-Nitro-4,5-methylenedioxyphenylmethyl	A	−0.564	−0.75	0.350	2.249	4.40	0	2.05	2.550
35	2-Tetrahydropyranyl	A	−0.708	−1.44	−0.543	1.197	2.99	0	2.01	2.273
36	4-Chloro-2-nitrophenyl	A	−0.670	−0.54	0.578	1.831	3.81	0	1.81	2.523
37	1,4-Benzodioxan-2-yl	A	−0.726	−2.28	0.237	2.030	3.81	0	1.91	2.517
38	3-Ethoxy-benzothiophen-2-yl	A	−0.983	−0.68	0.844	2.245	3.70	0	1.94	2.861
39	Cyclohexyloxymethyl	A	−0.708	−0.47	0.024	1.197	2.99	0	2.01	2.273
40	2-Hydroxyphenyl	A	−0.514	−1.29	0.284	1.682	3.48	0.52	1.93	2.285
41	3,4-Dimethoxyphenyl	A	−0.633	−0.85	−0.135	1.657	3.64	0	2.06	2.626
42	Pentafluorophenyl	A	−0.650	−0.09	0.191	0.851	3.41	0	1.56	2.315
43	3-Methoxy-4-nitrophenyl	A	−0.559	−0.69	0.275	1.820	3.98	0	1.99	2.600
44	6-Methoxy-1-naphthyl	A	−0.860	0.55	0.820	2.289	3.79	0	1.91	2.795
45	6-Hydroxy-1-naphthyl	A	−0.590	0.05	0.853	2.371	3.72	0.61	1.96	2.654
46	2-Hydroxy-3-methoxyphenyl	A	−0.833	−1.33	—	1.688	3.55	0.22	2.08	2.426
47	4-Hydroxymehtylphenyl	A	−0.836	−1.65	—	1.688	3.58	0.37	2.18	2.426
48	2,3-Dimethoxyphenyl	4-(2-Hydroxyethyl)-1-piperazinyl	−0.796	−0.79	0.045	1.721	2.94	0.37	2.55	2.669
49	2,3-Dimethoxyphenyl	1-Carboxy-2-isoindolinyl	−0.879	1.42	0.486	2.176	3.54	0.60	1.87	2.770
50	2,3-Dimethoxyphenyl	4-Aminomethyl-1-piperidinyl	−0.686	0.46	0.141	1.609	2.72	0.16	1.89	2.610
51	2,3-Dimethoxyphenyl	4-Methyl-2-carboxy-1-piperidinyl	−1.000	1.57	0.245	1.617	3.02	0.60	1.73	2.725

[a]Everted sac permeability assay.[5]
[b]Calculated with Pallas 2.0.[10]
[c]IAM column capacity factor.[13]
[d]Abraham's solute descriptors. R_2: molar refraction, π_2^H: dipolarity/polarizability parameter, $\Sigma\alpha_2^H$: hydrogen bond acidity, $\Sigma\beta_2^H$: hydrogen bond basicity, Vx: McGouen characteristic volume.[9,10,14–17]

the correlation between molecular size and permeability. We selected the McGouen characteristic volume (Vx) as a descriptor of molecular size[9,10] because Vx is a more quantitative molecular size descriptor than molecular weight and it can be easily calculated from molecular structure, using a table of atomic constants. Vx was originally used in Abraham's LFER equation as the descriptor of molecular size (described below). Actually, there was a negative correlation ($r = 0.704$) between log ESA and Vx (Fig. 1).

$$\log \mathrm{ESA} = 0.844 - 0.642\mathrm{Vx}$$
$$(n = 51,\ \ r = 0.704,\ \ \mathrm{S.D.} = 0.150,\ \ F = 48) \quad (1)$$

In this equation, and following equations, n is the number of data points, r is the overall correlation coefficient, S.D. is the overall standard deviation and F is the Fisher F statistic.

Even though molecular size is generally known to be the most influential factor in permeation via the paracellular pathway, Kristl et al. reported that permeation via the paracellular pathway was correlated with lipophilicity in the case of acyclovir derivatives.[11] So we next studied the correlation between lipophilicity and permeability. We calculated log P (prolog P) as a lipophilicity parameter. We also found negative correlation ($r = 0.557$) between lipophilicity and log ESA (Fig. 2).

$$\log\ \mathrm{ESA} = -0.840 - 0.0977\mathrm{prolog}\ P$$
$$(n = 51,\ \ r = 0.557,\ \ \mathrm{S.D.} = 0.175,\ \ F = 22) \quad (2)$$

We thought that the somewhat weak correlation coefficient of eq (2) is due to the calculation error of prolog P.

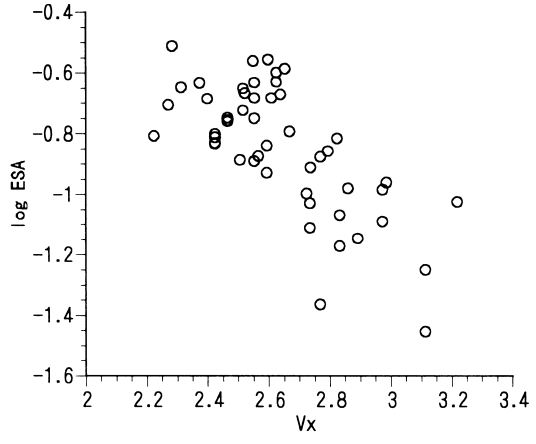


Figure 1. Negative correlation between everted sac permeability (log ESA) and molecular volume (Vx).

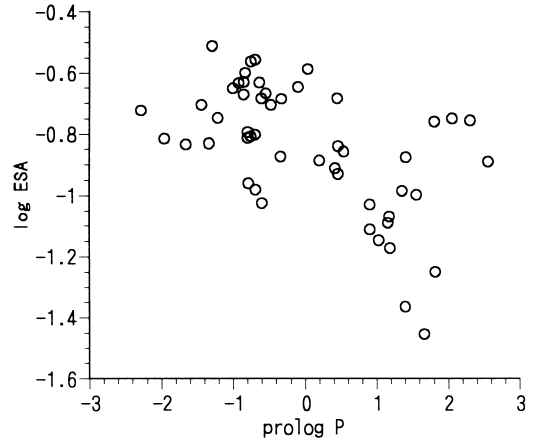


Figure 2. Negative correlation between everted sac permeability (log ESA) and calculated log P (prolog P).

Therefore, we experimentally determined the lipophilicity parameter. The IAM column capacity factor, log k, was selected as an experimental lipophilicity parameter because log k is known to have a better correlation with intestinal permeability.[12,13] We measured log k at pH 5.4, because the effective pH at the surface of the intestinal epithelium is about 5.4. We found a correlation ($r = 0.672$) between log k and log ESA.

$$\log\ \mathrm{ESA} = 0.709 - 0.315\log\ k$$
$$(n = 48,\ \ r = 0.672,\ \ \mathrm{S.D.} = 0.161,\ \ F = 38) \quad (3)$$

Furthermore, combination of lipophilicity parameters and molecular volume parameter showed a better correlation to log ESA without lowering the F value.

$$\log\ \mathrm{ESA} = 0.470 - 0.0661\ \mathrm{prolog}\ P - 0.500\mathrm{Vx}$$
$$(n = 51,\ \ r = 0.766,\ \ \mathrm{S.D.} = 0.137,\ \ F = 34) \quad (4)$$

$$\log\ \mathrm{ESA} = 0.456 - 0.191\log\ k - 0.461\mathrm{Vx}$$
$$(n = 48,\ \ r = 0.792,\ \ \mathrm{S.D.} = 0.134,\ \ F = 38) \quad (5)$$

However, there was a weak correlation between lipophilicity parameters (prolog P and log k) and molecular volume (Vx) ($r = 0.397$ and 0.534, respectively), because molecular size is one factor of lipophilicity. Therefore, we did the multiple linear regression (MLR) analysis with Abraham's solute descriptors to separate the molecular volume factor from the electrostatic interaction. Abraham's solute descriptors are excess molar refraction (R_2), solute dipolarity/polarizability parameter (π_2^H), hydrogen bond acidity and basicity ($\Sigma\alpha_2^H$, $\Sigma\beta_2^H$) and McGouen characteristic volume (Vx). These parameters are previously used to analyze skin and blood brain barrier permeability.[14–17]

We calculated four of these parameters (R_2, π_2^H, $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$) by the fragment addition method. The benzamidine and sulfonurea groups were excluded from the calculation because these functional groups are common to all the compounds. The log ESA was expressed as eq (6).

$$\log \mathrm{ESA} = 0.092 - 0.065R_2 + 0.158\pi_2^H + 0.216\Sigma\alpha_2^H + 0.263\Sigma\beta_2^H - 0.721\mathrm{Vx}$$
$$(n = 51,\ r = 0.807,\ \mathrm{S.D.} = 0.130,\ F = 17) \quad (6)$$

In eq (6), the coefficient of R_2 is not significant ($p = 0.399$) and could be omitted to give eq (7).

$$\log \mathrm{ESA} = 0.150 + 0.126\pi_2^H + 0.188\Sigma\alpha_2^H + 0.287\Sigma\beta_2^H - 0.764\mathrm{Vx}$$
$$(n = 51,\ r = 0.805,\ \mathrm{S.D.} = 0.129,\ F = 21) \quad (7)$$

In this equation, the coefficient of π_2^H and $\Sigma\alpha_2^H$ is weakly significant ($p = 0.052$ and $p = 0.051$, respectively) and log ESA can be described by $\Sigma\beta_2^H$ and Vx as eq (8) (Fig. 3).

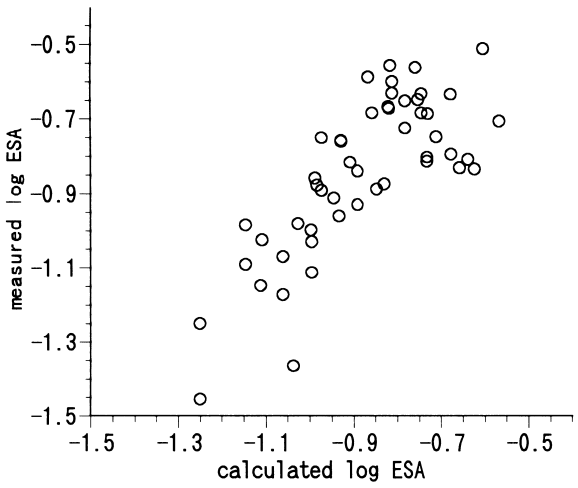


Figure 3. Calculated and measured log ESA using eq (8).

$$\log \mathrm{ESA} = 0.433 + 0.314\Sigma\beta_2^{\mathrm{H}} - 0.714\mathrm{Vx} \quad (n = 51,\ r = 0.772,\ \mathrm{S.D.} = 0.136,\ F = 35) \tag{8}$$

This result indicates that both molecular volume and electrostatic parameters are the dominant factors of intestinal permeation of benzamidine analogue. As the substituents (R^1, R^2) become smaller and more hydrogen bond basic, the intestinal permeability becomes larger. Hydrogen bond basicity mainly comes from the lone pair of electrons of oxygen and nitrogen atom. Therefore, functional groups with these atoms will improve permeability. Even though a clear explanation can not be given for the effect of the electrostatic parameter on the intestinal permeability at this time, this information will facilitate investigation of orally active benzamidine analogue drugs.

Conclusion

Negative correlation between intestinal permeability and both molecular volume and lipophilicity was found for the thrombin inhibitor composed of benzamidine analogues. MLR analysis with Abraham's solute descriptors revealed that not only molecular volume but also hydrogen bond acidity/basicity and dipolarity are factors that determine the intestinal permeability of benzamidine analogue thrombin inhibitor.

References and Notes

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