Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 9, pp. 1157-1160, 1994

0960-894X/94 $6.00+0 00

0960-894X(94)E0119-Y

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS STUDY OF ENDOTHELIN-1 ANALOGS

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Abstract: Endothelin-1 analogs replaced by various amino acids at position 20 ([X^{20}]-ET-1) were synthesized and evaluated for the ET_A and ET_B receptor binding activities. In order to obtain the structural requirements for the ET_A and ET_B receptor binding activities, the quantitative structure-activity relationships(QSAR) of sixteen analogs was investigated by the PLS method.

Endothelin-1(ET-1) is a 21-amino acid peptide with two intramolecular disulfide linkages and provides a potent constriction of various smooth muscles. Since the isolation of ET-1 from the cultured porcine endothelial aortic cells[1], it has attracted a great interest of many groups for clinical purposes. Several ET-1 analogs and linear or monocyclic fragment analogs have been synthesized and tested for their biological activities[2,3]. The importance of C-terminal amino acid(Trp) as well as the loop structure linked by the disulfide bonds in ET-1 molecule has been reported[2]. In addition, Aramori et al.[4] have found that a tripeptide (FR 139317) ia an ET_A selective antagonist.

Recently two endothelin receptor subtypes(ET_A and ET_B) have been identified and characterized[5]. These distributions and physiological roles in many species are different from each other. ET_A receptor is mainly distributed in peripheral tissues and its action is believed to mediate vasoconstriction. ET_B receptor is found predominantly in the central nervous system and endothelial cells. It is suggested that ET_B receptor controls vasodilation via a relaxing factor.

We have aimed at developing the specific antagonists for both ET_A and ET_B receptors. ET-1 analogs replaced by various amino acids at position 21([X^{21}]-ET-1) or 20([X^{20}]-ET-1) were synthesized and their receptor binding activities were tested[6,7]. In order to obtain the structural requirements for the ET_A and ET_B receptor binding activities, we have performed a quantitative structure-activity relationships(QSAR) analysis of [X^{20}]-ET-1 analogs using the PLS(partial least squares) method.

Sixteen ET-1 analogs([X^{20}]-ET-1) with the binding activities for ET_A and ET_B receptors were used for this QSAR study(Due to the global conformation change of proline and D-proline analogs they were deleted from a data set[7]). The receptor binding activity was expressed as a logarithm of the reciprocal of IC_{50} value. IC_{50} is a millimolar concentration of an analog required to produce 50% inhibition. The chemical structures and receptor binding activities are listed in Table 1. In this study thoracic aortic and rat

Table 1. Physico-chemical parameters and receptor binding activities of ET-1 analogs ([X^{20}]-ET-1).

No.	X	π	MR	L	Wl	Wu	Wd	σ*	log(1/IC50) ET_A	log(1/IC50) ET_B
1	Ile(ET-1)	2.04	19.59	3.75	3.27	5.13	1.42	-0.21	9.93	10 07
2	Phg[a]	1.96	25.36	4.06	4.24	4.95	1.48	0.60	8.89	9.76
3	Chg[b]	2.51	26.69	5.73	3.20	5.18	1.46	-0.26	9.88	10.01
4	D-Val	1.53	14.96	4.46	3.28	1.42	2.64	-0 19	9.00	9.05
5	Ala	0 56	5.65	3.59	2.29	2.64	1.42	0.00	7.53	9.40
6	D-NVal	1.55	14 96	5.52	3.51	1.46	3.83	-0.12	7.60	10.00
7	D-Leu	2.00	19.59	5 57	3.24	1.43	3.93	-0.13	7.97	9.94
8	D-Phg	1.96	25.36	4.06	4.24	1.48	4.95	0.60	6.85	8.60
9	Trp	2.68	41.09	8.19	3.06	3.94	1.42	0.23	6.71	9.77
10	D-His	0.29	24.50	6.86	3.13	1.42	2 64	0.23	6.13	7.55
11	D-Phe	2.01	30 01	7.86	3.31	1.42	3.17	0.23	6.07	8.60
12	D-Ala	0 56	5 65	3.59	2.29	1.42	2 64	0 00	5 77	7.96
13	D-Gln	-1.22	19.58	4.92	3.48	1.42	4 83	0.03	5 27	5.59
14	D-Dea[c]	2 61	24.24	4.00	4 55	1.89	4.60	-0.23	8.06	9.78
15	D-Ile	2.04	19.59	3.75	3.27	1.42	5 13	-0.21	7 59	9 40
16	D-Chg	2 51	26.69	5 73	3.20	1.46	5.18	-0.26	7.04	9.69

[a] Phenyl glycine [b] Cyclohexyl glycine [c] Diethyl alanine

brain membrane fraction were used as ET_A and ET_B receptors, respectively.

The structural descriptors are seven physico-chemical parameters; hydrophobic substituent constant π, molar refractivity MR, Iwamura parameters(L,Wl,Wu,Wd) and Taft inductive constant σ* of an amino acid residue at position 20. The π and MR values were estimated by the empirical model with an additive rule[8]. The σ* value was referred from the Hansch-Leo table[8]. The Iwamura parameters[9] were calculated by setting the amino acids in three dimensional space shown in Figure 1. This coordinate system can easily differentiate the L configuration from D configuration of the amino acid residue. The three dimensional coordinate of the amino acid was derived from the amino acid library in Chem-X[10].

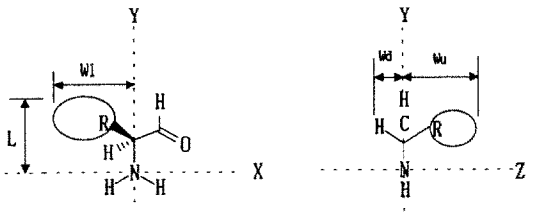

Figure 1. Definition of Iwamura parameters(L,Wl,Wu,Wd)

The PLS method was employed to obtain the structural requirements for the receptor binding activities. PLS is a novel multivariate statistical method based on latent variables[11,12]. A dependent variable y is expressed by the linear combination of latent variables t and independent variables X is decomposed into principal component-like model.

$$\mathbf{y} = \Sigma \mathbf{t}q + \mathbf{f} \qquad \mathbf{X} = \Sigma \mathbf{t}\mathbf{p} + \mathbf{E}$$

where q is a coefficient and **p** is a loadings for **y** and **X**, respectively.

The PLS model equation $\hat{y}$ is converted into a multiple linear regression-like model(MLR-like model) in terms of independent variables X.

$$\hat{y} = \mathbf{xW(PW)^{-1}q}$$

where **W**, **P** and **q** are a PLS weight matrix, loading matrix and coefficient vector, respectively. The number of PLS components A is determined by the leave-one-out cross-validation procedure[14] to derive the predictive model.

In this QSAR study the resulting PLS model equations were compared with each other to make clear the selectivity of analogs for two types of receptor. PLS analysis was carried out using the Unscrambler software package on IBM PS/2 microcomputer[11].

The binding activity for ET_A receptor was first studied. The activity and seven physico-chemical parameters were autoscaled to unit variance. A two-component(A=2) PLS model was obtained. Converting the PLS model to the MLR-like model, the following equation was obtained

$$\log(1/IC50) = 0.506\pi - 0.017MR - 0.291L + 0.460Wl + 0.384Wu - 0.229Wd - 1.596\sigma^* + 6.865 \quad (1)$$

$$n = 16,\ A = 2,\ r = 0.913,\ r_{pred} = 0.787,\ s = 0.615$$

where r and r_{pred} are conventional correlation coefficient and predictive one, respectively. r_{pred}^2 is an important measure of predictive ability of PLS model and is defined by the following equation.

$$r_{pred}^2 = 1 - \Sigma(y_{obs} - y_{pred})^2 / \Sigma(y_{obs} - y_{mean})^2$$

where y_{obs}, y_{pred} and y_{mean} are observed, leave-one predicted and mean activity values, respectively. The binding activity for ET_B receptor were next studied. Similaly a four-component(A=4) PLS model was obtained and transformed into the MLR-like equation.

$$\log(1/IC50) = 1.138\pi - 0.039MR - 0.033L - 0.068Wl + 0.023Wu - 0.169Wd - 0.288\sigma^* + 8.972 \quad (2)$$

$$n = 16,\ A = 4,\ r = 0.946,\ r_{pred} = 0.853,\ s = 0.454$$

The loadings for ETA and TEB activities are shown in Table 2.

Table 2. The PLS loadings of independent variables for ET_A and ET_B activities

Descriptor	ET_A		ET_B			
	p_1	p_2	p_1	p_2	p_3	p_4
π	0.524	-0.106	0.691	0.109	0.588	-0.160
MR	0.145	-0.566	0.400	-0.601	0.286	-0.373
L	-0.168	-0.609	0.112	-0.557	0.403	-0.086
Wl	0.134	0.010	0.193	-0.213	0.213	-0.527
Wu	0.705	-0.252	0.580	-0.215	-0.687	0.284
Wd	-0.468	0.323	-0.335	0.202	0.451	-0.710
σ^*	-0.160	-0.467	-0.120	-0.605	0.478	0.268

The established MLR-like equations(eq.1 and eq.2) are fair with better predictive capability and it is meaningful to interpret these equations for the design of the other potent ET-1 antagonists. Examining the regression coefficients of eq.(1) indicates the strong importance of Taft inductive constant σ^* of the amino acid residue at position 20 for the ET_A receptor binding activity. The coefficient for MR is low indicating that steric/bulk is not an important descriptor for activity. However, molecular shape (L,Wl,Wu,Wd) is important for ET_A activity. Increasing the ability for electron donating and hydrophobicity of the amino acid residue taking into consideration of its molecular shape is expected to

produce the potent ET-1 antagonists for ET_A receptor.

On the other hand, the regression coefficients of eq.(2) suggests that the hydrophobicity π of the amino acid residue is very important for the ET_B receptor binding activity but a specific molecular shape(L,Wl,Wu,Wd) of the amino acid residue at position 20 is not so important for the ET_B receptor binding activity. Increasing the ability for electron donating and the hydrophobicity of the amino acid residue is expected to produce the potent ET-1 analogs for inhibiting ET_B receptor.

Hibert et al.[14] have proposed a three dimensional model of ET_A receptor taking bacteriorhodopsin as a template using a molecular modeling software. Their model of ET_A receptor corresponds to the result of the first QSAR model(eq.1) and this correspondence implicitly validates the QSAR analysis in this study: The amino acid residue(Ile) at position 20 of ET-1 is near Trp613 of ET_A receptor and increasing hydrophobicity of the amino acid residue at position 20 of ET-1 makes the residue interact with Trp613 favorably and produces high receptor binding activity.

Ackowledgments: We gratefully acknowlege the financial support of the Ishida Foundation and wish to thank Chiyoka Suzuki at Tokyo Research Laboratories, Kowa for technical assistance.

REFERENCES

1. Yanagisawa, M.;Kurihara, H;Kimura, S.;Tomobe, Y.;Kobayashi, M.;Mitusi, Y.; Yazaki, Y.; Goto, K.;Masaki, T. *Nature* **1988**, *332*, 411.
2. Kimura, S.;Kasuya, Y.;Sawamura, T.;Shinmi, O.;Sugita, Y.;Yanagisawa, M.;Goto, K.;Masaki, T. *Biochem.Biophys.Res.Commun.* **1988**, *156*, 1182.
3. (a) Ihara, M.;Fukuroda, T.;Saeki, T.;Nishikibe, M.;Kojiri, K.;Suda, H.;Yano, M. *Biochem. Biophys. Res. Commun.* **1991**, *178*, 132.(b) Cody, W.L;Doherty, A.M.;He, J.X.;DePue, P.L.;Rapundalo, S.T.;Hingorani, G.A.;Major, T.C.; Panek, R.L.;Dudley, D.T.;Haleen, SS.J.;LaDouceur, D.;Hill, K.E.;Flynn, M.A.;Reynolds, E.E. *J.Med.Chem.* **1992**, *35*, 3301.
4. Aramori, I.;Nirei, H.;Shoubo, M.;Sogabe, K.;Nakamura, K.;Kojo, H.;Notsu, Y.;Ono, T.; Nakanishi, S. *Mol. Parmacol.* **1993**,43,127-131.
5. Sakurai, T.;Yanagisawa, M.;Masaki, T. *Trends Pharmacol.Sci.* **1992**, *13*, 103.
6. Koshi, T.;Suzuki, C.;Arai, K.;Mizoguchi, T.;Torii, T.;Hirata, T.;Ohkuchi, M.;Okabe, T. *Chem.Pharm.Bull.* **1991**, *39*, 3061.
7. Hirata, T. et al. to be published.
8. Hansch, C.;Leo, A. *Substituent Constants for Correlation Analysis in Chemistry*; John Wiley & Sons: New York, **1979**.
9. Iwamura, H. *J.Med.Chem.* **1980**, *23*, 308.
10. Chem-X is a product of Chemical Design Ltd., 7 West Way, Oxford, OX2 0JB, England.
11. Martens, H.;Næs, T. *Multivariate Calibration*,John Wiely & Sons:Chichester, **1989**.
12. Miyashita, Y.;Li, Z.;Sasaki, S. *Trends Anal. Chem.***1993**, *12*,50.
13. Wold, S. *Technometrics* **1978**, *20*, 397.
14. Huggins, J.P.;Trumpp-Kallmeyer, S.;Hibert, M.F.;Hoflack, J.M.;Fanger, B.O.;Jones, C.R. *EMBO J.* **1993**, *245*, 203.

(*Received in USA* 2 *February* 1994; *accepted* 30 *March* 1994)