Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 39–43

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Highly Potent Fumagillin Analogues from Homology Modeling for a Human MetAP-2

Cheol Kyu Han,[a,c] Soon Kil Ahn,[a,*] Nam Song Choi,[a] Ryung Kee Hong,[a] Seung Kee Moon,[a] Hyoung Sik Chun,[a] Sang Joon Lee,[a] Jung Woo Kim,[a] Chung Il Hong,[a] Deukjoon Kim,[b] Jeong Hyeok Yoon[c] and Kyoung Tai No[c,*]

[a]*Chong Kun Dang Research Institute 15-20, Osackdang-ri, Seonggeo-up, Cheon An, Chungcheongnamdo, 330-830, South Korea*
[b]*College of Pharmacy, Seoul National University, San 56-1, Shinrim-dong, Kwanak-gu, Seoul, 151-742, South Korea*
[c]*CAMD Research Center and Department of Chemistry, Soong Sil University, Seoul, 156-743, South Korea*

Received 2 August 1999; accepted 14 October 1999

Abstract—New fumagillin analogues were designed through structure-based molecular modeling with a human methionine aminopeptidase-2. Among the fumagillin analogues, cinnamic acid ester derivative **CKD-731** showed 1000-fold more potent proliferation inhibitory activity on endothelial cell than **TNP-470**.

Introduction

The concept of treating cancer by inhibition of angiogenesis (new blood vessel formation), which was proposed by Folkman,[1,2] is a promising strategy for cancer therapy.[3] Since Ingber et al. discovered that fumagillin (**1**) from *Aspergillus fumigatus* inhibits new blood vessel growth, many semisynthetic fumagillin analogues have been synthesized from fumagillol (**2**), the hydrolysis product of fumagillin.[4] Among these analogues, *O*-(chloroacetylcarbamoyl)fumagillol (TNP-470, AGM-1470) is currently in phase III clinical trials for the treatment of a variety of cancers.[5]

The underlying molecular mechanism of the inhibition of angiogenesis by these fumagillin derivatives remained unknown until Crews and Liu independently identified a fumagillin-binding protein, methionine aminopeptidase type 2 (MetAP-2).[6,7] Recently, Clardy and coworkers have reported the structure of a human MetAP-2 fumagillin complex, where the spiro-epoxide of fumagillin forms a covalent bond with the His231 in the active site of MetAP-2.[8] These reports have prompted us to disclose our study on the structure-based drug design of fumagillin analogues from homology modeling.[9]

In this communication, we wish to describe our effort to develop highly potent fumagillin analogues based on a 3D structure of human MetAP-2 by means of homology modeling.

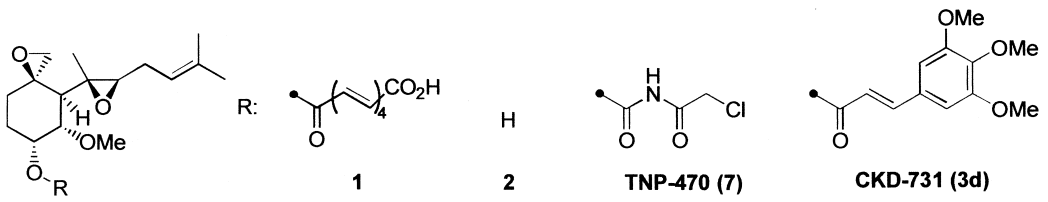
Results and Discussion

Homology models

MetAPs are metal-dependent enzymes, and the crystal structure of *E. coli* MetAP-1 reveals that, two cobalt ions are ligated by two aspartic acids, a histidine and two glutamic acids in an active site. Five amino acids (**D, D, H, E** and **E**; boxed letters) which bind to two cobalts ions are conserved in both MetAP-1[10,11] and MetAP-2 (Fig. 1).[12–14] Based on these findings, we performed the homology modeling of human MetAP-2. The sequence alignments of *Pyrococcus furiosus* MetAP-2 and *E. coli* MetAP-1 showed a sequence identity of 35 and 28% to human MetAP-2, respectively. The five amino acids in the R_1–R_5 domains are also conserved in *P. furiosus* MetAP-2 (Fig. 1). A three dimensional model of human MetAP-2 was built from known X-ray coordinates of *E. coli* MetAP-1 (PDB: 1MAT) and *P. furiosus* MetAP-2 (PDB: 1XGS) using MODELER.[15]

Keywords: Antitumor compounds; molecular modeling/mechanics.
*Corresponding author. E-mail: skahn@ckd.co.kr

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00577-6



Structure optimization of fumagillin derivatives using structure-based design

With the human MetAP-2 model, a series of fumagillin derivatives **1**–**7** were designed (Table 1). In designing the inhibitors, the distance between the methylene carbon of the spiro-epoxide moiety of the fumagillin and the NH group of His231 was constrained to 3 Å because they form a covalent bond in the complex. The docked structure of human MetAP-2 and **CKD-731**, the most potent inhibitor of the series is illustrated in Figure 2. In the complex, a water molecule is bound to two cobalt ions. The distances between the cobalt ions and the water oxygen are 1.95 Å and 1.97 Å. The same water forms a hydrogen bond with the oxygen of the spiro-epoxide of **CKD-731**, which might facilitate the opening of the epoxide ring. The structure also reveals another water molecule which forms hydrogen bonds with the oxygens of the methoxy group and the epoxide of the alkyl side chain of **CKD-731**. This water also plays a role in stabilizing His231. A pocket formed with His339, Ile338, Phe219 and Tyr444 is almost fully occupied by the terminal isopropylidene group. Since there are a well-defined hydrophobic valley formed with Leu328 and Leu447, and a large pocket which is connected to this valley and surrounded by Asn327, Val374, Asp376 and His375, we designed fumagillin analogues with functional groups which can bind efficiently to this valley and pocket. To optimize hydrophobic interactions with the valley and van der Waals contacts at the pocket, we introduced several functional groups as described in Table 1. We have designed fumagillin derivatives by introducing functional groups with an aromatic ring which can interact hydrophobically with the Leu447 of human MetAP-2. Cinnamic acid esters **3b**–**3e**, phenylalkanoic acid esters **3f**–**3g**, benzyl carbamates **4a**–**4d** and benzyl carbonates **5a**–**5c** have been designed. For comparison purposes, alkyl acid ester **3a** and xanthate **6** were also prepared.

Synthesis of fumagillin derivatives

Fumagillol (**2**) was acylated with NaH and an acid chloride to provide compound **3**.[5] Treatment of the known phenoxycabonyl fumagillol with amine produced the carbamate **4**. Preparation of carbonate **5** was achieved by coupling of fumagillol (**2**) with benzyl chloroformate. Xanthate derivative **6** was prepared from **2** using CS_2 and benzyl bromide (Scheme 1).

Biological assays

Antiproliferating activities of fumagillin derivatives were evaluated against calf pulmonary artery (SPAE, ATTIC HRL 209) endothelial cells, lymphoma EL-4 cells and murine leukemia P388D1. IC_{50} values were colorimetrically measured by SRB (CPAE cell) or MTT (EL-4, P388D) methods. The biological data for compounds **1**–**7** are shown in Table 1.

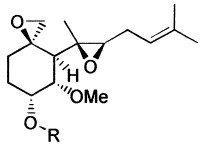
Structure–activity relationships

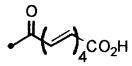
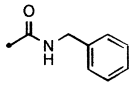
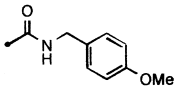
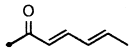
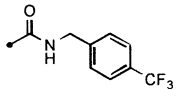
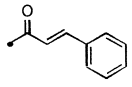
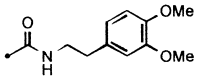
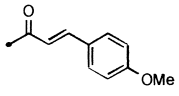
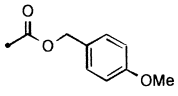
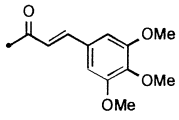
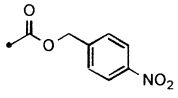
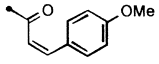
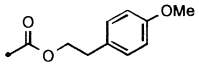
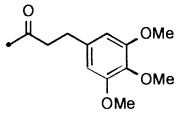
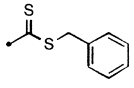
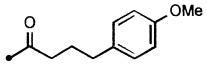
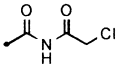
Among the designed compounds, the cinnamic acid esters **3b**–**3d** and benzyl carbamates **4a**–**4d** showed more potent activity than TNP-470, while the phenylalkanoic acid esters **3f**–**3g** and benzyl carbonates **5a**–**5c** were less active. It seems that the aromatic ring should be positioned to contact with Leu447 for maximizing hydrophobic interaction. The *trans*-cinnamic acid esters have an optimum fixed geometry for the hydrophobic interaction with Leu447. The activity tends to decrease as the bonds between the carbonyl group and the aromatic ring in the group R are more freely rotatable. In the docking model of the phenylalkanoic acid esters **3f**–**3g** and benzyl carbonate derivatives **5a**–**5c**, the aromatic rings point away from the Leu447. To support this assumption, we also prepared *cis*-cinnamic acid ester derivative **3e** which has an aromatic ring but cannot interact with Leu447.[16] The cell proliferation inhibitory

		R_1		R_2		R_3		R_4		R_5		
E.-coli MetAP-1	I VN I	**D**	VTV I KDGFHG	**D**	TSKM	EYCG	**H**	GIGR	FTI	**E**	PMV	AQY	**E**	HT I V	295
Human MetAP-1	I VNV	**D**	I TLYRNGYHG	**D**	LNDF	SYCG	**H**	GIHK	FTI	**E**	PMI	AQF	**E**	HTL L	394
Rat MetAP-2	I CK I	**D**	FGTH I SGR I I	**D**	CAFT	NLNG	**H**	SIGP	YAI	**E**	TFG	AQF	**E**	HT I L	480
Human MetAP-2	I CK I	**D**	FGTH I SGR I I	**D**	CAFT	NLNG	**H**	SIGQ	YAI	**E**	TFG	AQF	**E**	HT I L	478
P.f. MetAP-2	YLK I	**D**	VGVH I DGFTA	**D**	TAVT	NLSG	**H**	KIER	FAI	**E**	PEA	AQF	**E**	HT I V	295

Figure 1. Sequence alignment of the R_1–R_5 domains of MetAP-1 and MetAP-2.

Table 1. In vitro cell proliferation inhibitory activity of fumagillin derivatives against lymphoma EL-4 cell, calf pulmonary artery endothelial (CPAE) cell and murine leukemia P388D1[a]



	Subst. R	EL-4	CPAE	P388D1		Subst. R	EL-4	CPAE	P388D1
1		0.42	0.37	>1	**4a**		0.01	0.07	>1
2		5.16	15	>10	**4b**		0.042	0.044	>1
3a		5.05	12	>1	**4c**		0.014	0.0054	>1
3b		0.05	0.046	>1	**4d**		0.042	0.044	>1
3c		0.00019	0.00006	>1	**5a**		42	18	ND
3d		0.00015	0.00003	>1	**5b**		2.47	0.11	>1
3e		12	24	ND[b]	**5c**		153	2.3	ND
3f		36	44	ND	**6**		14	42	ND
3g		83	125	ND	**7**		0.03	0.04	>10

[a]IC_{50} values in ng/mL.
[b]ND: not determined.

activity of the *cis*-cinnamic acid ester derivative **3e** was 10^5 fold less than that of the *trans*-cinnamic acid ester derivatives **3b**–**3d**. Moreover the xanthate **6** was less active than the carbonyl ester derivatives **3b**–**3d**.

In summary, a human MetAP-2 model was built through homology modeling and several potent inhibitors were designed based on this model structure. Although the cell-level assay results do not always

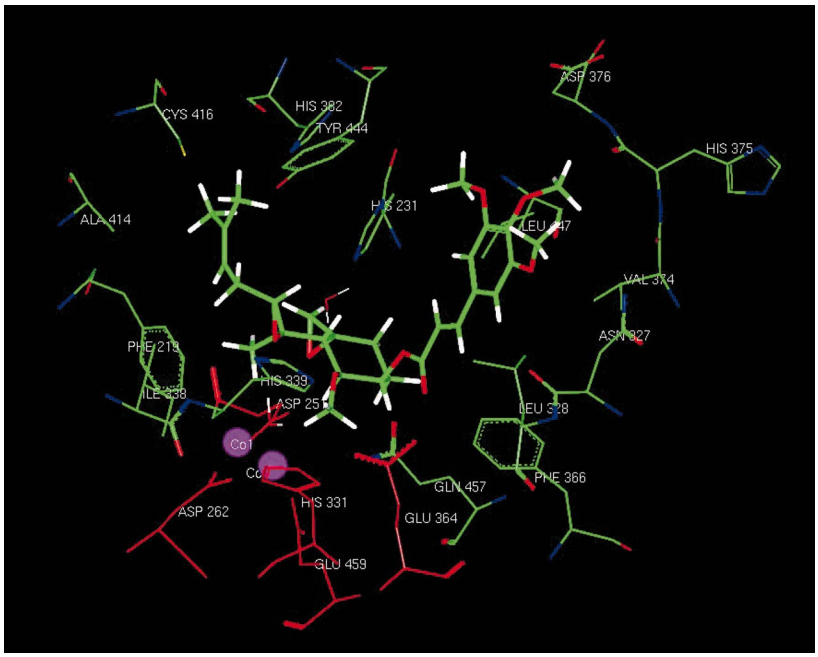
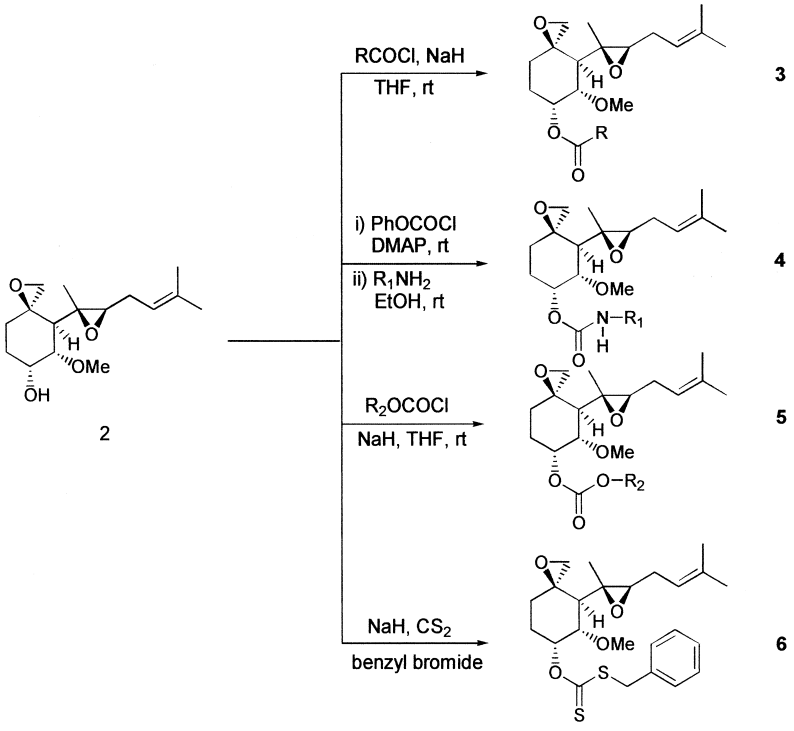


Figure 2. The docked structure of **CKD-731** complex with MetAP-2.



Scheme 1.

reflect the molecular-level interactions between enzyme and ligands, this study does provide some clues to designing fumagillin analogues as antiangiogenic anticancer agents.

Acknowledgements

We acknowledge financial support from the Ministry of Health and Welfare (HMP-98-D-4-0027). Dr. K. T. No thanks the Basic Science Research Institute Program of the Ministry of Education (BSRI-96-3448) for research grants.

References and Notes

1. Folkman, J.; Shing, Y. *J. Biol. Chem.* **1992**, *267*, 10931.
2. Folkman, J. *Adv. Cancer Res.* **1985**, *43*, 175.
3. Kohn, E. C.; Liotta, L. A. *Cancer Res.* **1995**, *55*, 1856.

4. Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* **1990**, *348*, 555.
5. Marui, S.; Itoh, F.; Kozai, Y.; Sudo, K.; Kishimoto, S. *Chem. Pharm. Bull.* **1992**, *40*, 96.
6. Sin, N.; Meng, L.; Wang, M. Q. W.; Wen, J. J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6099.
7. Griffith, E. C.; Su, Z.; Turk, B. E.; Chen, S.; Chang, Y.-H.; Wu, Z.; Biemann, K.; Liu, J. O. *Chem. Biol.* **1997**, *4*, 461.
8. Liu, S.; Widom, J.; Kemp, C. W.; Crews, C. M.; Clardy, J. *Science* **1998**, *282*, 1324.
9. Korean Patent Application 98-17636 and PCT/KR99/00229.
10. Roderick, S. L.; Matthews, B. W. *Biochemistry* **1993**, *32*, 3907.
11. Nagase, T.; Miyajima, N.; Tanaka, A.; Sazuka, T.; Seki, N.; Sato, S.; Tabata, S.; Ishikawa, K. I.; Kawarabayasi, Y.; Kotani, H.; Nomura, N. *DNA RES* **1995**, *2*, 37.
12. Wu, S.; Gupta, S.; Chatterjee, N.; Hileman, R. E.; Kinzy, T. G.; Denslow, N. D.; Merrick, W. C.; Chakrabarti, D.; Osterman, J. C.; Gupta, N. K. *J. Biol. Chem.* **1993**, *268*, 10796.
13. Arfin, S. M.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. E.; Matthews, B. W.; Bradshow, R. A. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7714.
14. Tsunasawa, S.; Izu, Y.; Miyagi, M.; Kato, I. *J. Biochem.* **1997**, *122*, 843.
15. Sali, A.; Blundell, T. L. *Mol. Biol.* **1993**, *234*, 779.
16. All the calculations were carried out with DISCOVER/INSIGHT(MSI) modeling software and for energy calculation CVFF force field was used.