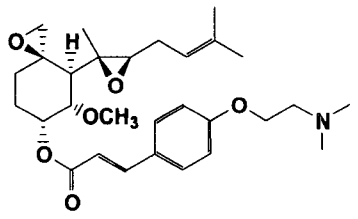
S4-5

Antiangiogenic Anticancer Agent, CKD-732 from *Aspergillus fumigatu*

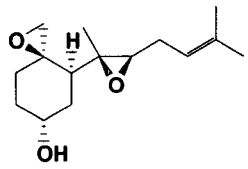
Soon Kil Ahn

Chong Kun Dang Research Institute 15-20, Osackdang-ri, Seonggeo-up, Cheon An, Chungcheongnamdo, 330-830, Kore.

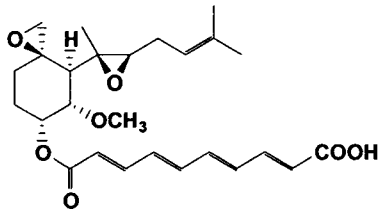
CKD-732, 6-(4-dimethylaminoethoxy)cinnamoyl fumagillol, is a new anticancer drug candidate, which is being developed by Chong Kun Dang Pharm in the stage of Ph I clinical trial in Korea. CKD-732, was semi-synthesized from natural product, fumagillin isolated from *Aspergillus fumigatus*. CKD-732 showed a highly selective inhibition of endothelial cell proliferation *in vitro* and a potent antiangiogenic anticancer activity *in vivo*. In addition, CKD-732 has better water solubility and less toxicity compared to TNP-470. We also found a new fumagillin derivative, 5-demehoxyfumagillol, CKD-733 during our fermentation for fumagillin from *Aspergillus fumigatus*. In this report our research results of preclinical studies on CKD-732 and the fermentation, isolation, structural assignment and anti-proliferation activity of CKD-733 will be described.



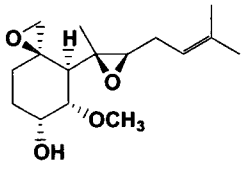
CKD-732



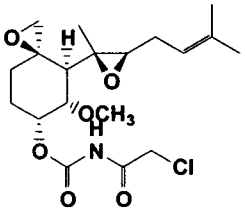
CKD-733



Fumagillin



Fumagillol



TNP-470

in vitro & *in vivo* activity of CKD-732

CKD-732 showed a potent inhibitory activity on *in vitro* proliferation of various cell lines at nano mole

concentrations. IC_{50}s of CKD-732 on CPAE , HUVEC and EL-4 were 0.048, 0.00436 and 0.379 ng/ml, respectively. CKD-732 demonstrated a greater selectivity than TNP-470 and fumagillin comparing their inhibitory activities on the proliferation of endothelial cells and non-endothelial cell. Against CPAE IC_{50}s of CKD-732, TNP-470 and fumagillin were 0.048, 0.599 and 3.15 ng/ml, respectively whereas IC_{50}s of them against non-endothelial cell, L5178Y were 77.32, 44.24 and 9.30 μg/ml, respectively. The selectivity index ($L5178Y_{IC50}/CPAE_{IC50}$) were $1610x10^3$, $931x10^3$ and $2.95x10^3$, respectively.

In the antiangiogenic activity study of CKD-732 in rat aorta organ culture assay, a tube formation was not observed at fifty times lower concentration than that of affecting cell growth. And *in vitro* angiogenesis models including cell migration and tube formation CKD-732 showed comparable activity to TNP-470. The inhibition effect of CKD-732 on *in vivo* angiogenesis in mice was determined by Matrigel™ implantation assay. CKD-732 inhibited *in vivo* angiogenesis dose-dependently and its inhibitory activity was comparable to that of TNP-470. At the dose of 500 mg/kg which showed a maximum inhibition (85%) body weight loss (17.4%) was observed.

CKD-732 showed a comparable antitumor activity to TNP-470 dose-dependently against 3LL and B16 cell lines in mice (IR% = 63.2 and 67.0%, respectively, at a total dose of 600 mg/kg s.c., q2dx5). In human tumor xenografts including PC-3, MDA-MB-231, CX-1, LX-1 and SKOV-3 CKD-732 also demonstrated comparable antitumor activity to TNP-470 (IR% = 63.7, 69.9, 73.5, 68.6 and 69.4%, respectively, at a total dose of 900 mg/kg s.c., q2dx5) and its R/Emax were 1.4 to 3.4. In an experimental metastatic model in mice CKD-732 also showed a comparable antimetastatic activity to TNP-470 dose-dependently against 3LL and B16 cell lines (IR% = 89.0 and 85.6%, respectively, at a total dose of 720 mg/kg s.c., q2dx6). The *in vivo*kg i.p., single dose) showed synergistic antimetastatic activity (IR% = 82.9, 70.3 and 75.9%, respectively).

Pharmacokinetics and metabolism of CKD-732

CKD-732 was rapidly metabolized *in vitro* and *in vivo* into the major N-oxide form (M 11) of CKD-732 and others by N-oxidation, hydroxylation, demethylation and epoxide hydrolysis. In the metabolism study of CKD-732 *in vitro* rat hepatic microsome, 14 metabolites were found and the major metabolite was identified as the N-oxide form of CKD-732. In rat plasma two metabolites were only found and the N-oxide form was the major metabolite. CKD-732 was metabolized into over 24 metabolites in rat urine and bile. Among them the metabolites covered >1% of total ion peak area were almost identical to those of the 14 metabolites *in vitro*.

Pharmacokinetic studies were performed in mice, rats and dogs after single and multiple *i.v.* or *i.p.* doses of CKD-732. CKD-732 and M 11 exhibited similar plasma kinetic profiles to the linear pharmacokinetics. After administration of a single *i.v.* bolus of CKD-732 in rats, AUCs increased in a dose-dependent manner and elimination half-lives, total clearances, volumes of distribution and mean residence times were not significantly changed with the increase in doses. The apparent plasma elimination half-life ($T_{1/2}$) values were 0.72 - 0.78 hrs for CKD-732 and 0.92 - 1.09 hrs for M 11 in rats. In dogs, $T_{1/2}$ values of CKD-732 and

M 11 were 1.54 and 1.79 hrs, respectively. After *i.v.* administration of CKD-732, 3.42 and 2.28% of the total administered dose in rats and dogs, respectively, were only excreted as an unchanged parent drug and M 11 within 72hrs into urine, bile and feces. The tissue concentration of CKD-732 after an *i.v.* boluse dose in mice were very high in lung followed by ovary, spleen, stomach and spleen and CKD-732 was not detected in all tissues in 6 hrs after administration.

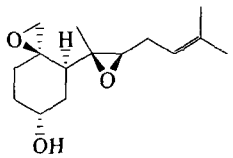
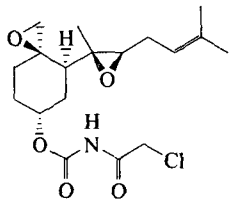
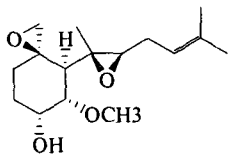
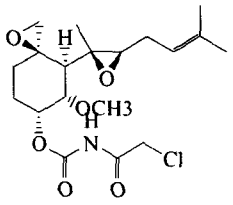
Toxicity of CKD-732

Ina single dose administration of CKD-732 in mice a capillary dilation of subcutis was not observed compared to control, whereas a significant capillary dilation with a local blood leaking was observed in TNP-470 treated group. The size of spleen was not also significantly changed compared to control, whereas a significant shrinkage of spleen was observed in TNP-470 treated group. In hematological toxicity study of CKD-732 (240 mg/kg i.v., 5-daily) in rats a significant change in WBC counts was only observed , however the effect was less severe than that of TNP-470 (40 and 73% reduction of WBC counts, respectively).

Fermentation, isolation and structural assignment of CKD-733

Several types of angiogenesis inhibitors, natural or synthetic, have been discovered and among them over fifty compounds are in clinical trials. In our extensive screening program for novel angiogenesis inhibitors from soil microorganisms, we found a novel 5-demethoxyfumagillol (CKD-733). 5-demethoxyfumagillol was produced by the saponification of 5-demethoxyfumagillin isolated from the cultured broth of *Aspergillus fumigatus* (IMI-069714). 5-Demethoxyfumagillol showed potent anti-proliferation activity against calf pulmonary artery endothelial (CPAE) cells. Spectral data for 5-demethoxyfumagillin were very similar to those of known fumagillin except for the

Table 1. Anti-proliferation activity of fumagillol and demethoxyfumgillol analogs

Compound	Structure	CPAE IC50 (μM)	L5178Y IC50 (μM)
CKD-733		7.06	>39.6
		1.147	>2.67
Fumagillol		8.09	>35.4
TNP-470		0.0011	>24.5

5- demethoxy moiety. Saponification of 5-demethoxyfumagillin to remove the polyene moiety gave the corresponding 5-demethoxyfumagillol in 48% yield. The chemical structure of 5-demethoxyfumagillol was established by an extensive spectroscopic analysis of NMR (^{1}H, ^{13}C), FT-IR, and HR-Mass (FAB).

In order to definitely confirm the structure of 5- demethoxyfumagillol, semi-synthesis of 5-demethoxyfumagillol from fumagillin was accomplished. Thus, the structure of 5-demethoxyfumagillol was elucidated to be (3*R*,4*R*,6*R*)-4-[(2*R*,3*R*)-2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]-octan-6-ol.

In order to examine the *in vitro* inhibitory activity of 5-demthoxyfumagillol and its derivative against endothelial cell proliferation, chemical modification of 5-demethoxy fumagillol was carried out. Chemical modification of 5-demethoxyfumagillol gave 6-*O*-(chloroacetylcarbamoyl)-5-demethoxyfumagillol using chloroacetyl isocyanate in the presence of DMAP as a representative compound. The anti-proliferation activity and cytotoxicity of a novel 5-demethoxyfumagillol and 6-*O*-(chloroacetylcarbamoyl)-5-demethoxyfumagillol were examined in CPAE cells and lymphoma (L5178Y, mice) cells, respectively. The anti-proliferation activity of 5-demethoxyfumagillol was comparable to that of fumagillol, whereas 6-*O*-(chloroacetylcarbamoyl)-5-demethoxyfumagillol showed at least eight times more potent anti-proliferation activity compared to 5-demethoxyfumagillol. However, both 5-demethoxyfumagillol and its carbamoyl derivative, 6-*O*-(chloroacetyl-car-bamoyl)-5-demethoxyfumagillol, exhibited less potent activity compared to TNP-470. Taking into consideration these results, the methoxy moiety on the 5-position of fumagillol compounds seems to be necessary and important for the anti-proliferation activity.

Conclusion

A new drug candidate, antiangiogenic fumagillin derivative, CKD-732 showed potent antitumor and antimetastatic activities comparable to TNP-470. Moreover CKD-732 was less toxic than TNP-470 against hematological system. Those results warrant further studies of CKD-732 as a new drug candidate.

Acknowledgements

The author thanks Dr. C. I. Hong, Dr. N. S. Choi, Dr. H. J. Son, Mr. R. K. Hong, Mr. D. J. Jeon, Dr. H. S. Chun, Mr. S. K. Moon, Dr. J. K. Kim, Mr. H. J. Park, Mr. J. H. Lee, Mr. H. S. Lee, Mr. Y. S. Son, Mr. B. T. Choi, Mr. C. H. Shin, Dr. H. J. Shin, Mr. J. H. Kim, Ms. S. K. Lee (CKD Research Institute), Dr. D. Kim (Seoul National University), Dr. C. K. Han, Dr. J. H. Yoon (IDR) and Dr. K. T. No (Soong Sil University) for their efforts on this work.

References

1. Kim, D.; Min, J.; Ahn, S. K.; Lee, H. W.; Choi, N.S.; Moon, S. K. *Chem. Pharm. Bull.* 2004, *52*, 447
2. Park, H. J.; Son, Y. S.; Son, H. J.; Moon. S. K.; Kim, J. G.; Kim, J. K.; Ahn, S. K.; Hong, C. I. *Proc.*

Amer. Assoc. Cancer Res., **2001**, *42*, Abst 471.

3. Lee, H. S.; Kim, H. Y.; Min, H. K.; Choi, W. K., Son, H. J.; Lee, S. S.; Myung, S. W.; Kim, J. K.; Ahn S. K.; Hong, C. I. *Proc. Amer. Assoc. Cancer Res.*, **2002**, *43*, Abst 907.
4. Han, C. K.; Ahn, S. K.; Choi, N. S.; Hong, R. K.; Moon, S. K.; Chun, H. S.; Lee, S. J.; Kim, J. W. Hong, C. I.; Kim, D.; Yoon, J. H.; No, K. T. *Bioorganic Med. Chem. Lett.*, **2000**, *10*, 39.
5. Lee, H. S.; Park, H. J.; Lee, J. H.; Lee. J. M.; Lee, J. S.; Shin, J. S.; Kim, J. K.; Kim, J. G.; Ahn, S. K. Shin, H. J.; Hong, C. I. *Proc. Amer. Assoc. Cancer Res.*, **2000**, *41*, Abst 3097.
6. Kim, D.; Ahn, S. K.; Bae, H.; Choi, W. J.; Kim, H. S. *Tetrahedron Lett.* **1997**, *38*, 4437.
7. Korean Patent Application 98-17636 and PCT/KR99/00229.
8. U.S. Patent 6,063,812.