



## Synthesis and anti-migrative evaluation of moverastin derivatives

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### ABSTRACT

Cell migration of tumor cells is essential for invasion of the extracellular matrix and for cell dissemination. Inhibition of the cell migration involved in the invasion process represents a potential therapeutic approach to the treatment of tumor metastasis; therefore, a novel series of derivatives of moverastins (moverastins A and B), an inhibitor of tumor cell migration, was designed and chemically synthesized. Among these moverastin derivatives, several compounds showed stronger cell migration inhibitory activity than parental moverastins, and UTKO1 was found to have the most potent inhibitory activity against the migration of human esophageal tumor EC17 cells in a chemotaxis cell chamber assay. Interestingly, although moverastins are considered to inhibit tumor cell migration by inhibiting farnesyltransferase (FTase), UTKO1 did not inhibit FTase, indicating that UTKO1 inhibited tumor cell migration by a mechanism other than the inhibition of FTase.

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Despite significant advances in understanding the fundamental aspects of cancer, the development of metastatic lesions remains the predominant cause of death for most cancer patients.<sup>1,2</sup> Cell migration is a crucial event in the spread of cancer and, consequently, the metastatic process.<sup>3,4</sup> This prompted us to develop inhibitors of tumor cell migration as novel anti-metastatic drugs.

Previously, we screened for inhibitors of cancer cell migration derived from microbial origin, and obtained moverastin A and B (**1** and **2**, respectively), new members of the cylindrol family, from *Aspergillus* sp. F7720.<sup>5</sup> Their structures including the absolute stereochemistries were confirmed unambiguously by the synthesis as outlined in Scheme 1. Furthermore, moverastin A and B were found to inhibit FTase; therefore, moverastins were considered to inhibit the migration of tumor cells by inhibiting the farnesylation of H-Ras, and subsequent H-Ras-dependent activation of the PI3K/Akt pathway. However, because the inhibitory activity of moverastins for tumor cell migration was rather modest (IC<sub>50</sub> value of 7 μM), we considered it an attractive lead compound in the search for other, more potent agents.

In this study, we chemically synthesized a series of moverastin derivatives and assessed their potential as tumor cell migration inhibitors in several in vitro assays.

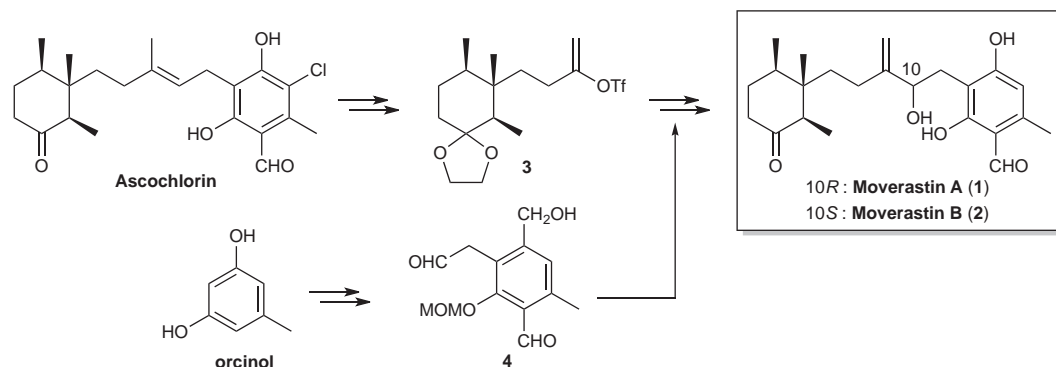
Structures of moverastin derivatives (UTKO1–12) synthesized in this study are shown in Figure 1. UTKO1–6 were synthesized by employing the same approach as that for our previous synthesis of moverastins.<sup>5</sup> The enol triflates (**7**, **10**, **12**, **15**, **18**) were prepared starting from readily available ketones or aldehydes (**5**, **9**, **11**, **13**, **16**, respectively) as shown in Scheme 2. Coupling reactions between the enol triflates (or 2-iodopropene for UTKO4) and aldehyde **4** were carried out successfully using the Nozaki–Hiyama–Kishi procedure<sup>6,7</sup> and UTKO1–6 were obtained after acid hydrolysis of MOM ether. The dihydro analog (UTKO7) and etherified analogs (UTKO9 and 10) of UTKO1 were also synthesized by its hydrogenation, Mitsunobu reaction or methylation (Scheme 3). The unsaturated ketone analog of UTKO1 was also obtained by the PDC oxidation-deprotection of **19** which is the intermediate from **7** to UTKO1. UTKO11 and 12, deformylated analogs of moverastin and UTKO1, respectively, were also synthesized from aldehyde **21** instead of **4** (Scheme 4). Detailed synthetic procedure for UTKO compounds will be published elsewhere.

Next, the cell migration inhibitory activity of these moverastin derivatives was examined by the chemotaxis cell chamber (BD Biosciences) assay using conditioned medium of human esophageal tumor EC17 cells as a source of chemoattractants as previously reported with some modifications.<sup>8</sup> In this assay, EC17-conditioned medium was initially placed in the lower compartment. EC17 cells were incubated in the upper chamber, where they were allowed to migrate and penetrate the filter separating the chambers in order to enter the lower chamber. After 24 h of incubation, the number of cells attached to the lower side of the filter was counted. The IC<sub>50</sub> values obtained in this study are listed in Table 1. Among

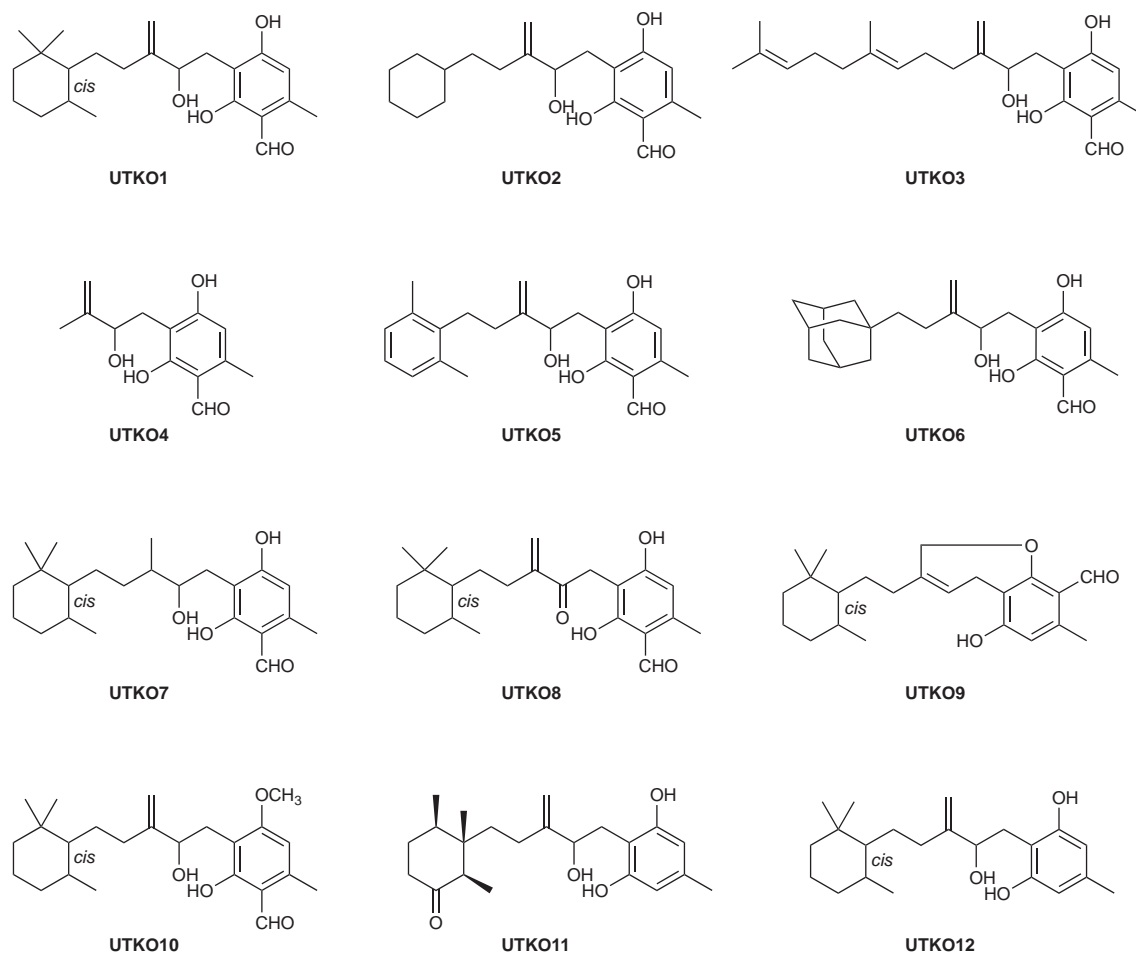
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**Scheme 1.** Synthesis and structures of moverastin A and B.

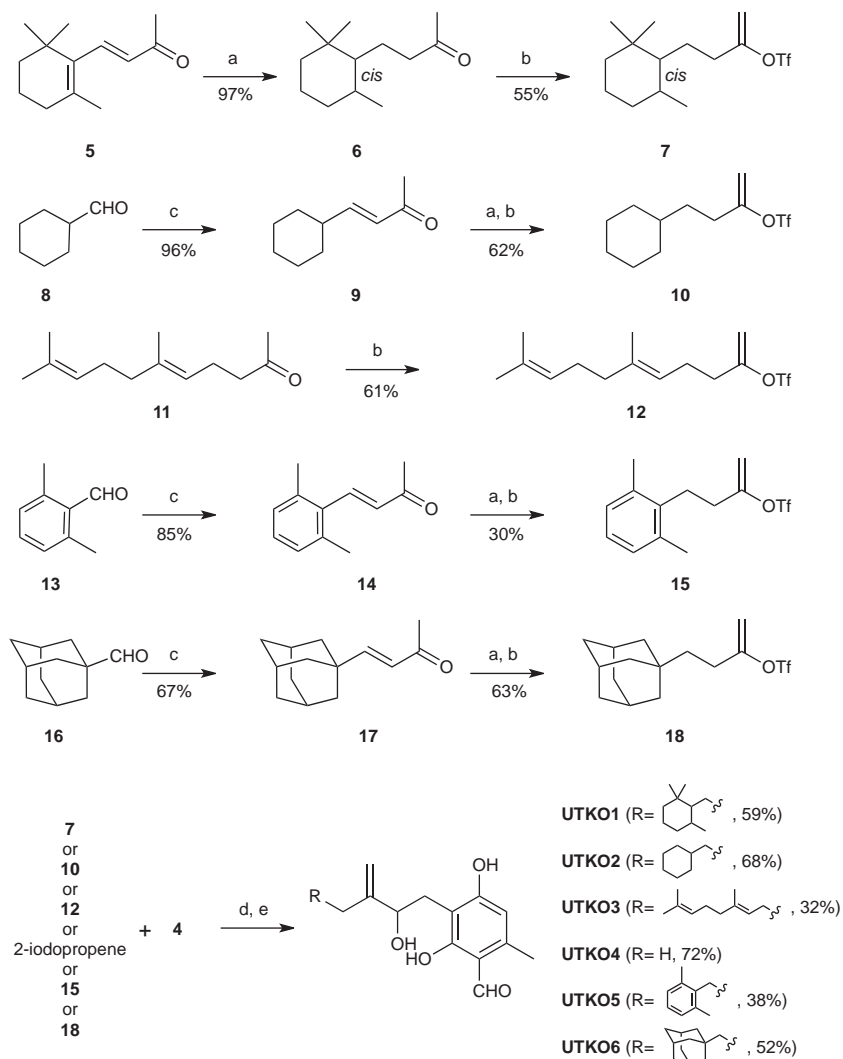


**Figure 1.** Structures of moverastin derivatives.

moverastin derivatives, UTKO1 showed the most potent inhibitory activity of EC17 cell migration with an  $IC_{50}$  of 1.98  $\mu\text{M}$  (Fig. 2). UTKO7, UTKO9 and UTKO12 are also significantly more active inhibitors of EC17 cell migration than the parental natural product, moverastin A, with  $IC_{50}$  values of 2.12, 2.00 and 2.17  $\mu\text{M}$ , respectively (Table 1). These inhibitory effects are not due to the toxic effect of the drug because their 50% inhibitory concentration for EC17 cell viability, as estimated by trypan blue dye exclusion assay, was at least five-fold higher than that for cell migration.

Previously, we found that moverastin A showed inhibitory activity against FTase, and demonstrated that moverastin A inhibited

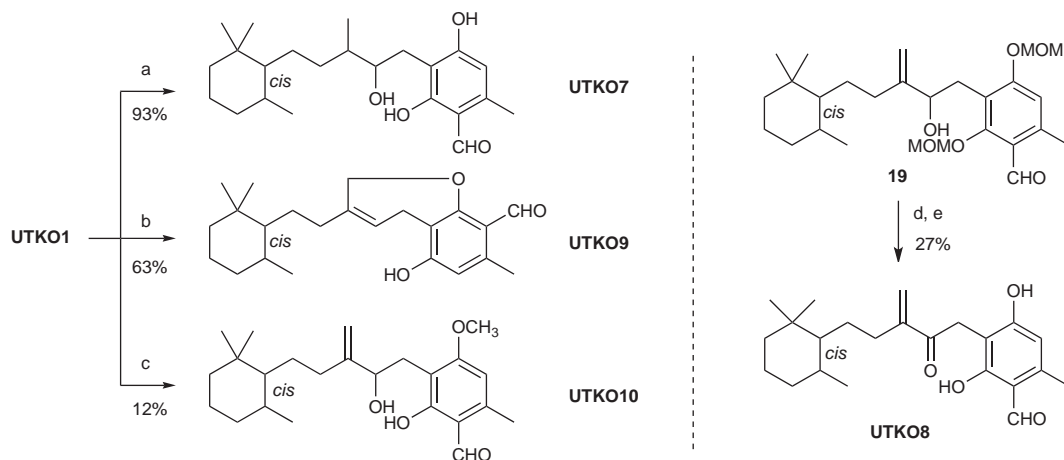
the migration of tumor cells by inhibiting the farnesylation of H-Ras;<sup>5</sup> therefore, next we examined the effect of moverastin derivatives on FTase in vitro. For this assay, FTase was partially purified from EC17 cells and recombinant GST-H-Ras and [<sup>3</sup>H]-farnesylpyrophosphate were used as the substrates as described before.<sup>5</sup> As shown in Table 1, all moverastin derivatives tested, including UTKO1, UTKO7, UTKO9, and UTKO12, which showed strong inhibition of cell migration, did not inhibit FTase in vitro up to 100  $\mu\text{M}$ . These results indicated that a mechanism other than the inhibition of FTase is responsible for UTKO-induced inhibition of EC17 cell migration. To examine this possibility, several cancer



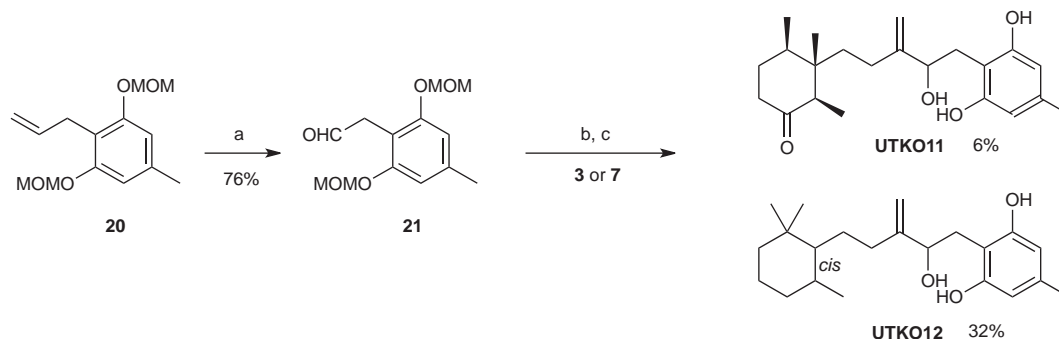
**Scheme 2.** Synthesis of UTKO1–6. Reagents and conditions: (a) H<sub>2</sub>, Pd-C, EtOAc (for **6**) or EtOH (for **10**, **15** and **18**); (b) KHMDS, Comin's reagent, THF, –78 °C; (c) Ph<sub>3</sub>P=CHCOMe, CH<sub>2</sub>Cl<sub>2</sub> (for **9** and **14**) or xylene (for **17**), reflux; (d) CrCl<sub>2</sub>, NiCl<sub>2</sub>, DMF, rt; (e) concd HCl, THF, rt.

cell lines were investigated with respect to the inhibitory potential of moverastin A and the most potent moverastin derivative, UTKO1. The inhibitory effect of moverastin A depends on cell type,

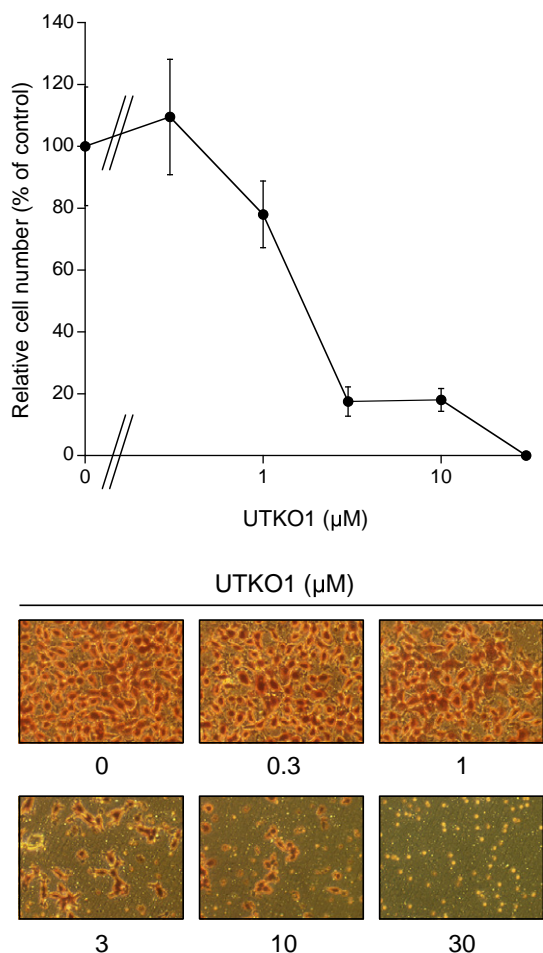
and there is a significant negative correlation between the sensitivity of each cell to moverastin A and the expression level of H-Ras, a substrate of FTase ( $r = -0.86$ ,  $p = 0.0013$ ) (Fig. 3). This result



**Scheme 3.** Synthesis of UTKO7–10. Reagents and conditions: (a) Rh-Al<sub>2</sub>O<sub>3</sub>, EtOH; (b) DEAD, PPh<sub>3</sub>, THF; (c) MeI, K<sub>2</sub>CO<sub>3</sub>, (n-Bu)<sub>4</sub>N-HSO<sub>4</sub>, EtOAc–toluene, reflux; (d) PDC, CH<sub>2</sub>Cl<sub>2</sub>; (e) concd HCl, THF, rt.

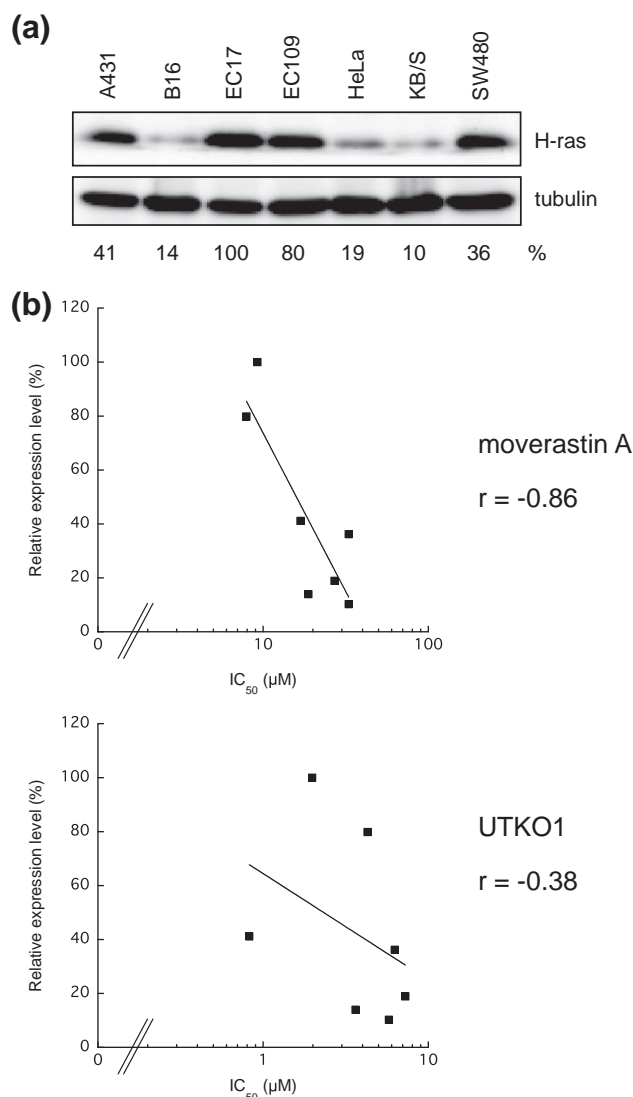


**Scheme 4.** Synthesis of UTKO11 and UTKO12. (a)  $O_3$ ,  $CH_2Cl_2$ ,  $-78^\circ C$ , then  $PPh_3$ ; (b)  $CrCl_2$ ,  $NiCl_2$ ,  $DMF$ ,  $rt$ ; (c)  $concd\ HCl$ ,  $THF$ ,  $rt$ .



**Figure 2.** Effect of UTKO1 on migration of EC17 cells. EC17 cells were incubated with various concentrations of UTKO1 in the top chamber; the lower chamber contained EC17-conditioned medium, obtained from 24-hour cultures of EC17 cells maintained in RPMI1640 supplemented with 1% FBS. After 24 h, the number of cells that migrated through the filter to the lower surface was counted. The results are the mean  $\pm$  SD of five different fields.

supported our previous conclusion that moverastins inhibited tumor cell migration due to the inhibition of FTase. On the other hand, the cell migration inhibitory activity of UTKO1 also depends on cell type, but there is no correlation with the expression levels of H-Ras ( $r = -0.38$ ,  $p = 0.40$ ) (Fig. 3). These results suggested that the inhibitory mechanism of cell migration by UTKO1 is different from that of moverastin A. To understand the molecular basis by which UTKO1 inhibits tumor cell migration, biochemical identification of the protein target for UTKO1 is now under investigation.



**Figure 3.** Correlation between expression levels of H-Ras and inhibition of migration by moverastin A and UTKO1. (a) Western blot analysis of H-Ras expression levels in seven tumor cell lines. Cell lysates were separated by SDS-PAGE and then subjected to immunoblotting using anti-H-Ras antibody. Quantitation of expression levels of H-Ras was analyzed by Image gauge and normalized with the level of tubulin. Percentages represent the relative expression level of H-Ras in tumor cells compared to the level in EC17 cells. (b) Simple linear correlations between two parameters were calculated. Correlation coefficients ( $r$ ) are shown in this figure.

**Table 1**  
Effects of UTKO compounds on cell migration, cell viability and in vitro FTase activity in EC17 cells

	IC <sub>50</sub> (μM)		
	Cell migration	Cell viability <sup>a</sup>	FTase <sup>b</sup>
Moverastin A	7.22	>77	14.7
UTKO1	1.98	46	>100
UTKO2	8.43	87	>100
UTKO3	7.53	>80	>100
UTKO4	30.0	>130	>100
UTKO5	10.6	45	>100
UTKO6	6.52	55	>100
UTKO7	2.12	16	>100
UTKO8	4.41	18	>100
UTKO9	2.00	18	>100
UTKO10	4.57	18	>100
UTKO11	21.4	>28	>100
UTKO12	2.17	17	>100

<sup>a</sup> For cell viability assay, a trypan blue dye (15250-061, Gibco, Invitrogen) exclusion assay were used to examine cell viability and performed according to previously reported protocols.<sup>9</sup>

<sup>b</sup> For in vitro FTase assay, partially purified enzymes from EC17 cells were incubated with [<sup>3</sup>H]-FPP plus recombinant GST-H-Ras in the presence or absence of test compound. The reaction was terminated by the addition of TCA. The radioactivity of the TCA insoluble fraction was measured.

Our preliminary structure–activity relationship study revealed that UTKO12 retained the same level of inhibitory activity toward EC17 cell migration as that of UTKO1, indicating that formyl group on benzene ring is not required for the inhibitory activity toward EC17 cell migration. On the other hand, the formyl groups of moverastins are essential for the FTase inhibition, because the inhibitory activity of UTKO11 toward FTase has been lost.

Although UTKO1 was initially synthesized as an analogous compound of moverastins, it possesses a different biological function from cylindrol family, and therefore, UTKO1 is expected to be a new lead compound in the search for more potent anti-metastatic and anti-cancer agents.

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.028. These data include MOL files and InChIKeys of the most important compounds described in this article.

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