

Multifunctional anti-angiogenic activity of the novel compounds with antitumor activity

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Angiogenesis is often the primary physiologic response that leads to the development of chronic, sometimes fatal diseases. Indeed, angiogenesis is an essential step that allows tumors to survive initially and to grow at the primary site. Moreover, tumor cells that break away from the primary tumor form metastatic tumors by causing new blood vessels to grow from existing ones. In this way, angiogenesis plays a significant role in both initial tumor development and tumor metastasis. Consequently, compounds that inhibit angiogenesis would be useful in treating not only cancer but also rheumatoid arthritis, diabetic retinopathy and other chronic diseases that depend upon angiogenesis for initial development.

The system that comprises urokinase-type plasminogen activator (u-PA) and its specific receptor (u-PAR) has been a target for anti-angiogenic agent research, since many reports have shown that either the inhibition of u-PA enzymatic activity or the disruption of the u-PA/u-PAR system by small molecules results in decreased metastasis and angiogenesis *in vivo*. However, these u-PA-targeting compounds have not yet been used clinically. Therefore, we aimed to develop a novel anti-angiogenic agent that inhibits u-PA production and also other protease cascades in both endothelial and tumor cells.

During preliminary screening, the effects of 13 ozonides on the inhibition of u-PA production in human fibrosarcoma HT-1080 cells and on the inhibition of angiogenesis on chicken embryonic chorioallantoic membranes were determined. Of the ozonides tested, 9 inhibited *in vitro* u-PA production of HT-1080 cells and 7 of these 9 exhibited strong anti-angiogenic activity. Interestingly, 6 of the 13 ozonides also inhibited cathepsin B activity. 1-Phenyl-1, 4-epoxy-1*H*, 4*H*-naphtho[1, 8-*de*][1, 2] dioxepin (ANO-2) potently inhibited cathepsin B ($IC_{50} = 0.47 \mu M$) as well as u-PA production. Consequently, ANO-2 was selected for further study. ANO-2 inhibited tube formation by human umbilical vein endothelial cells cultured on Matrigel while exhibiting no cytotoxicity. Additionally, *in vivo* administration of ANO-2 inhibited angiogenesis induced by Sarcoma-180 cells tested using the mouse dorsal air sac assay. Moreover, ANO-2 also suppressed primary tumor growth and reduced the number of pulmonary metastases caused by Lewis lung carcinoma cells in mice. These *in vitro* and *in vivo* activities indicate that ANO-2 has considerable potential as a new and potent anti-angiogenic drug that inhibits both u-PA production and enzymatic activity of cathepsins, indicating that ANO-2 may be a multifunctional inhibitor of angiogenesis.

Moreover, the therapeutic potential of a diaminotriazine, 2, 4-diamino-6-(pyridine-4-yl)-1, 3, 5-triazine (4PyDAT), was investigated in a metastatic model using the mouse colon 26 carcinoma variant. The antimetastatic and antitumor activities of 4PyDAT are due in part to inhibition of angiogenesis, rather than direct antiproliferative action on the tumor cells. 4PyDAT may become a lead compound to develop antitumor triazine derivatives based on antiangiogenic action.

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