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Modified Synthesis and Antiangiogenic Activity of Linomide

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Abstract—A modified procedure for the synthesis of Linomide is described. The synthesized drug was characterized and assessed for its in vivo antiangiogenic activity. In a murine angiogenesis assay Linomide treatment inhibited new blood vessel formation as documented by reduced microvessel area and blood volume.

Metastatic prostate cancer is the leading cause of cancer related deaths in American males accounting for approximately 40,000 deaths annually.[1] Androgen ablation therapy often initially elicits a positive response to prostate cancer patients. However, cancer cells ultimately progress to an androgen-independent state, greatly diminishing the efficacy of hormonal therapy. Several chemotherapy agents have shown some activity in hormone-refractory prostate cancer patients.[2,3] However, chemotherapy agents are dependent on the progression of cells through the cell cycle to exert antitumor effect (i.e., apoptotic cell death only occurs if the cell cycle is perturbed).[4] Unfortunately, less than 5% of androgen-independent cells are proliferating per day, while the remaining cells are in a quiescent state. Thus, the majority of tumor cells are poorly sensitive to anti-proliferative chemotherapeutic agents.[5] Based on this realization, alternative therapeutic approaches are urgently needed for prostate cancer treatment.

Linomide (*N*-phenylmethyl-2-dihydro-4-hydroxyl-1-methyl-2-oxoquinoline-3-carboxamide), a low molecular weight, water soluble quinoline-3-carboxamide, has been shown to inhibit the process of angiogenesis.[6] Angiogenesis or new blood vessel formation is an extremely vital step in tumor development. Sprouting of new blood vessels from endothelial cells lining preexisting blood vessels is a critical multistep process for tumor metastases, as it serves to provide oxygen and nutrients to the developing tumor. Inhibition of angiogenesis has been shown to induce tumor 'dormancy' and to reduce metastases. In particular, Linomide has been reported to decrease human prostate carcinoma growth in mice. This antitumor activity correlated with a decrease in microvessel density, an increase in necrotic areas, and an increase in the apoptotic index.[6] Moreover, Linomide has recently been shown to specifically inhibit vascular endothelial growth factor (VEGF)-elicited migration and growth of vascular endothelial cells.[7]

Edgar et al.[8] reported preparation of heterocyclic carboxamides, however in this communication, we report a simple, facile, high yield and less cumbersome synthesis of linomide.

Compound **1** was prepared as shown in Scheme 1. Briefly, a cold solution of methyl-3-chloro-3-oxopropionate in dichloromethane was reacted with *N*-methylaniline and triethylamine. The reaction mixture was stirred for 16 h at 0 °C to give **2** (80% yield).

Compound **2** was dissolved in DMF and then 95% dry NaH was added at 0°C and the reaction mixture was heated to 80 °C for 1 h. *N*-Methylisotoic anhydride in DMF was added through addition funnel and the reaction was heated to 115 °C for 1 h. Thereafter, work up and purification by silica column chromatography (9:1, CH_2Cl_2/MeOH) afforded **1** as a white solid powder (50% yield) (note: carbanion of **2** was also generated by LDA at 0 °C).[9,10]

The effect of newly synthesized Linomide on new blood vessel formation was assessed in a modified Matrigel angiogenesis assay.[11] Briefly, 4–6 week old male athymic nude mice (Taconic) (five animals for control and five animals for Linomide) were injected subcutaneously with 0.75 mL Matrigel (Collaborative Research), bilaterally in the abdomen. Matrigel was supplemented with 150

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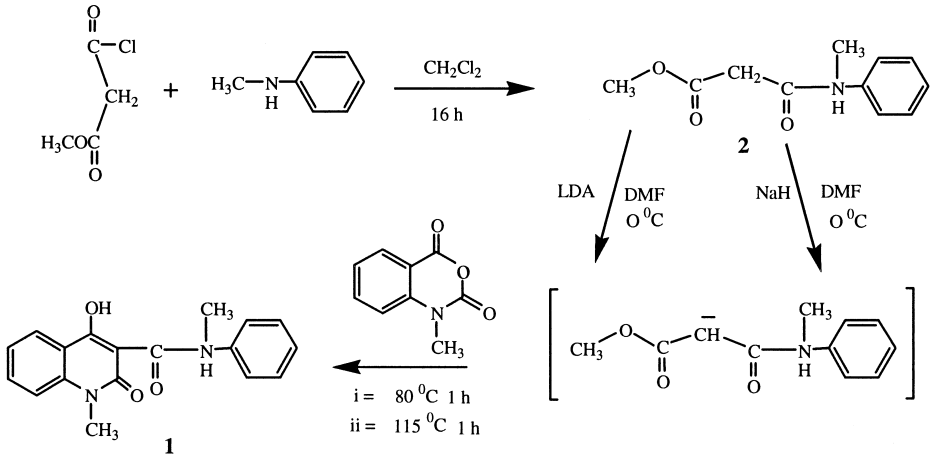

Scheme 1.

Table 1. Effect of Linomide on VEGF-induced angiogenesis in vivo

	Microvessel aea (%)	Blood volume (HB g/mL)
Control	5.38 ± 0.34[a]	1.06 ± 0.24
Linomide	3.48 ± 0.27[b]	0.33 ± 0.03[c]

[a]Mean ± standard error.
[b]$p < 0.00003$.
[c]$p < 0.015$.

ng/mL vascular endothelial growth factor (VEGF) (R&D Systems) and bovine aorta endothelial cells (10^6). Linomide was administered in the drinking water (100 mg/kg or 1 mg/mL) starting five days prior to the Matrigel injection and until the end of the experiment. Mice were sacrificed seven days after the Matrigel injection. Then the gels were recovered by dissection. Five plugs/group were fixed in PBS-buffered 10% formalin containing 0.25% glutaraldehyde prior to staining of the paraffin-embedded sections with Masson's Trichrome. The Image-Pro analysis software was used to quantify the area occupied by blood vessels (Microvessel area) in the histological sections. The mean area/field from 10 to 20 fields × section/plug (×200 = 20× objective lens and 10× ocular lens: Zeiss Axioskop) was calculated and expressed as mean ± standard error of the mean. The remaining five plugs/group were processed for hemoglobin content as a surrogate for blood volume. After overnight extraction in water, hemoglobin was measured using the Drabkin method and Drabkin reagent (Sigma). The hemoglobin was calculated and expressed as mean g/mL/plug ± standard error of the mean.

As shown in Table 1, Linomide treatment inhibited VEGF-induced angiogenesis, as documented by a 36% and 69% reduction in microvessel area and blood volume, respectively. The experiment was repeated once with similar results.

In conclusion, we have shown that Linomide can be synthesized in a two-step synthesis with reasonable yield. The resulting drug was biologically active and exerted antiangiogenesis activity in vivo.

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9. All compounds gave satisfactory NMR and MS results in accord with the assigned structures.
10. **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.21 (s, 3H, *N*-CH_3), 3.56 (s, 3H, CH_3O-), 5.78 (s, 2H, -$COCH_2CO$-), 7.36–7.52 (m, 5H, ArH). Linomide: ^{1}H NMR of (MeOH-*d*) δ 3.23 (s, 3H, *N*-CH_3), 3.28 (s, 3H, *N*-CH_3), 6.66–7.85 (m, 9H, ArH). MS (Maldi) 310 (M + 1).
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