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# Improved inhibitory activities against tumor-cell migration and invasion by 15-benzylidene substitution derivatives of andrographolide

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ABSTRACT

In the present study, andrographolide (Andro, 1) derivatives were screened to identify potent inhibitors against tumor-cell migration and invasion, and associated structure–activity relationships were studied. Compared to **1**, compounds **8a**–**8d** exhibited more potent activities against migration in SGC-7901, PC-3, A549, HT-29 and Ec109 cell lines. Improved activities against tumor-cell migration and invasion were proved to be associated with the down-regulation of MMPs.



Andrographolide (Andro, **1**) is a major active diterpenoid lactone of *Andrographis paniculata* that has been extensively used in traditional medicines of China, India and other Asian countries.[1a,b] This compound has been reported to have multiple pharmacological properties and has been widely used in clinical treatments for fever, cold, inflammation, diarrhea and other infectious diseases.[2a–e] Recent studies have suggested that Andro is an interesting pharmacophore with anti-cancer activity.[3a,b] The cytotoxicity of Andro has been mainly attributed to its ability to induce cell-cycle arrest and trigger apoptosis in human cancer cells.[4a–e] Therefore, it has the potential to be developed as a chemotherapeutic agent against cancer, and various semi-synthetic analogues are being developed and evaluated in order to increase this agent's cytotoxicity to tumor cells.[5a–f]

Some studies have indicated that Andro derivatives with ester side chain(s) at C-14 and/or C-3 and C-19 exhibit improved cytotoxicities compared to the parent molecule.[3b,5d,5e] Recent studies have reported the effectiveness of Andro against migration and invasion in human non-small-cell lung cancer A549 and colorectal carcinomal Lovo cells. This effect has been associated with down-regulation of matrix metalloproteinase (MMP)-7 and inhibition of MMP-2 activity.[6a,b] Hence, we are interested in developing Andro-based anti-invasive and anti-metastatic agents. To the best of our knowledge, to date, there have been no attempts to improve the inhibitory activities of Andro against tumor-cell migration and invasion.

Previous studies in this research laboratory designed and synthesized Andro analogues and investigated their α-glucosidase inhibitory activities and cytotoxicities against tumor cells.[7a–c] In the present study, the inhibitory effects of Andro and its derivatives (Scheme 1), which were first reported for compounds **3**, **8e** and **9a**, were evaluated with regard to tumor-cell migration, and associated structure–activity relationships were analyzed. Furthermore, the invasive abilities as well as mRNA and protein expression levels of MMP-9, -7 and -2 in human gastric carcinoma SGC-7901 cells treated with compound **8b** were explored and compared to Andro.

As shown in Table 1, bioactivity evaluation results indicated that, at its maximal noncytotoxic concentration 2.5 μM, Andro had no obvious inhibitory effects on migration in human bladder carcinoma 5637 cells, while at 5.0 μM, it inhibited the cell migration as well as the cell proliferation; whereas Andro at 10.0 μM,

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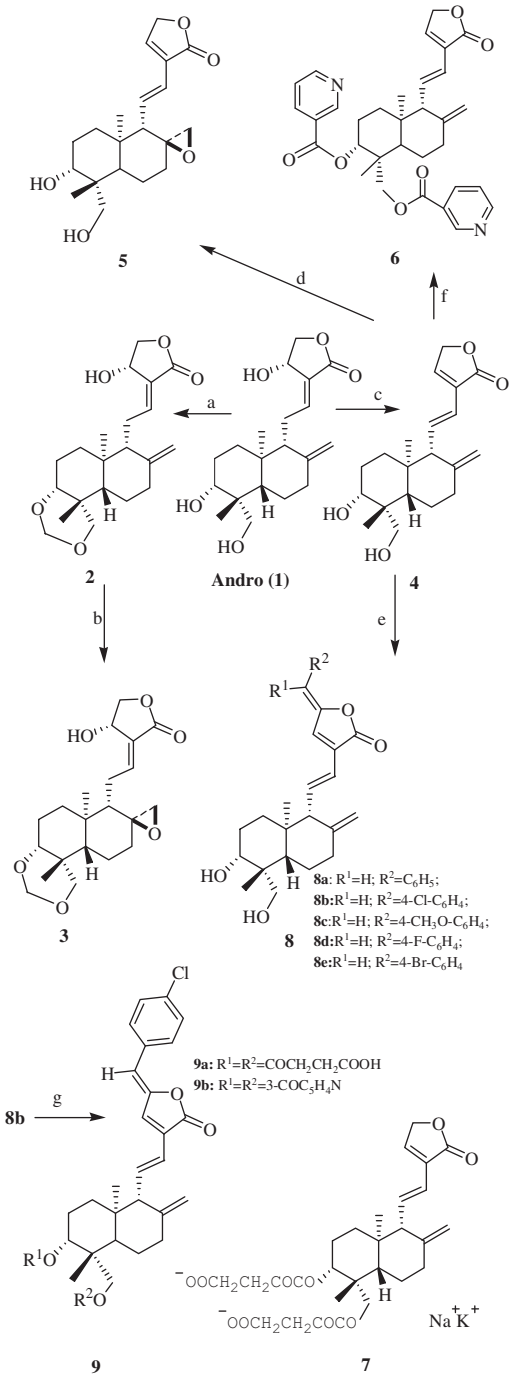


**Scheme 1.** Synthesis of compounds **2**–**6** and **8**–**9**. Reagents and conditions: (a) THF, $H_2SO_4$, paraform, refluxing, 1 h; (b) *m*-CPBA, $CHCl_3$, refluxing, 2 h; (c) pyridine, $Al_2O_3$, refluxing, 6 h; (d) (i) acetone, *p*-TsOH, 2, 2-dimethoxy propane, refluxing, 10 h, (ii) *m*-CPBA, $CHCl_3$, refluxing, 2 h; (e) aldehydes, $Na_2CO_3$, methanol, refluxing, 3–5 h; (f) $CHCl_3$, nicotinic chloride, $Et_3N$, refluxing, 3 h; (g) $CHCl_3$, succinic anhydride (**9a**) or nicotinic chloride (**9b**), $Et_3N$, refluxing, 3 h.

noncytotoxic concentration, demonstrated 20.8% inhibition against SGC-7901 cells.[8a,b] On the other hand, Andro had no remarkable effects on either migration or proliferation in human prostate carcinoma PC-3 cells. To determine if improvements in bioactivity could be achieved by modifying Andro at the C-3 and C-19 hydroxyls and by epoxidizing the exocyclic double bond ($\triangle^{(8,17)}$), compounds **2** and **3** were synthesized and evaluated. However, satisfactory results were not obtained, although the migration inhibition on 5637 cells treated with **2** was slightly stronger than the proliferation inhibition.

To evaluate the activity-related effects of the allylic hydroxyl at C-14 and the conjugated double bond, we appraised the anti-migratory effects of 14-deoxy-11,12-didehydroandrographolide (**4**), in which the exocyclic double bond ($\triangle^{(12,13)}$) was isomerized to the endocyclic double bond and the C-14 hydroxyl was simultaneously removed. The inhibition of migration in 5637 cells by compound **4** was obviously and remarkably stronger than that obtained with Andro, whereas the cytotoxicity of compound **4** was greatly decreased compared to Andro (Table 1).

Compound **5**, which was synthesized by epoxidizing the exocyclic double bond ($\triangle^{(8,17)}$) of compound **4**, did not show significantly enhanced bioactivity compared to compound **4**. Similar results were observed in the case of compound **6**, the 3,19-dinicotinate derivative of compound **4**.

Compound **7** (Yanhuning), the 3,19-disuccinate derivative of **4** (in which the molecular structure at C-3 and C-19 is similar to **6**) has been widely used in clinical studies. However, Yanhuning (**7**) observed no inhibitory effects on 5637 cell migration, implying that no considerable improvements in activity result from converting compound **4** to its epoxide at C-8 (**5**) or to its esters at C-3 and C-19 (**6**,**7**).

Interestingly, 15-benzylidene substitution analogues (**8a**–**8e**) of **4** demonstrated greater potency compared to compound **4** as well as their parent compound Andro. Compound **8a** was effective against all three cell lines (Table 1), whereas **8b**–**8e** potently inhibited the migration of SGC-7901 (as shown in Table 1 and Fig. 1B) and PC-3 cells but did not significantly inhibit 5637 cells at 5.0 μM, the minimum effective concentration for cell migration. Compound **8b** was the most potent against migration in SGC-7901 cells and was followed by **8d** and **8e**. Compounds **8b**–**8e** were more cytotoxic to 5637 than to other cell lines.

Moreover, compounds **9a** and **9b**, the 3,19-disuccinate and the 3,19-dinicotinate of compound **8b**, respectively, were synthesized and evaluated biologically. The results revealed inhibitory activity against migration of 5637, SGC-7901 and PC-3 cells that was lost when **8b** was converted to **9a**. Contrarily, when **8b** was converted to **9b**, the activity against 5637 improved significantly, while activities against SGC-7901 and PC-3 were lowered.

Furthermore, the anti-migration activities of compounds **8a**–**8d** against A549, human esophageal carcinoma Ec109 and human colon cancer HT-29 cells were investigated (Table 2). All of these demonstrated improved activities (compared to Andro) against all cell lines tested.

To better understand its value as an anti-metastasis agent or as a lead compound, the anti-invasive effect of **8b** on SGC-7901 cells was further appraised via matrigel invasion assay and compared with that of Andro, as shown in Figure 1(C and D).[9] Compared to control, Andro (5.0–10.0 μM; $P$ <0.05) and **8b** (2.5–10.0 μM; $P$ <0.01) significantly diminished the number of membrane-associated cells. The inhibitory activity of **8b** against the invasive ability of SGC-7901, for which an $IC_{50}$ value of 4.6 μM was obtained, was much more potent than that of Andro, which only caused 17.7% inhibition at 10.0 μM.

Considering the importance of matrix metalloproteinases (MMPs) to tumor-cell migration, invasion and metastasis, we studied the effects of Andro and **8b** on mRNA expression levels of MMP-7, MMP-2 and MMP-9 in SGC-7901 cells using a semi-quantitative RT-PCR method.[10] As shown in Figure 2, Andro decreased the MMP-7 mRNA expression levels in a dose-dependent manner ranging from 2.5 to 10.0 μM ($P$ <0.01); whereas even at concentrations as high as 10.0 μM, Andro did not alter MMP-2 mRNA expression levels but slightly lowered MMP-9 expression levels ($P$ <0.05). Compared with Andro, compound **8b**, at concentrations as low as 1.25 μM, could significantly down-regulate the mRNA expression