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Synthesis and structure–activity relationships of sinenxan A derivatives as multidrug resistance reversal agents

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ABSTRACT

Two types of sinenxan A derivatives with different side chains at C-5 were synthesized and evaluated for their in vitro multidrug resistant reversal activities. Several derivatives exhibited better activities than the positive control verapamil. The structure–activity relationships of these derivatives suggested that a carbonyl group at C-13 and the length of side chain at C-5 are important for the activity.

Multidrug resistance (MDR) refers to a phenomenon whereby cancer cells undergoing chemotherapy simultaneously develop cross resistance to a number of unrelated anticancer drugs with diverse structures and mechanisms of action.[1] MDR attributes to a variety of mechanisms of action which are not fully understood yet so far. One of the most important mechanisms is due to the overexpression of a transmembrane protein called P-glycoprotein (P-gp), which can actively transport anticancer drugs out of the cancer cells and thus result in a decreased intracellular accumulation of the anticancer drugs.[2] Therefore, P-gp has emerged as a promising target for cancer therapy and great efforts have been focused on the development of effective reversal agents to overcome P-gp-mediated MDR.

Some chemically diverse compounds such as verapamil, quinine, and cyclosporin A have been previously reported to directly bind to P-gp with subsequent inhibition of pump activity and thus resensitize MDR cells to anticancer drugs.[3] It was found recently that some natural taxoids isolated from the Japanese yew *Taxus cuspidata* also enhanced the cytotoxicity of vincristine (VCR) in MDR human ovarian cancer 2780AD cells and efficiently inhibited [^{3}H]-azidopine photolabeling of P-gp.[4] In addition, surprisingly, these natural taxoids exhibited weak or no cytotoxicity. The strongest increase of VCR cellular accumulation by taxezopidine G (Fig. 1) corresponded to be 323% of that by verapamil.[5] This has paved the way to the synthesis and study of potent MDR reversal agents of the taxoid family. Taxinine (Fig. 1), a major natural taxoid, has been chemically modified to yield many compounds showing an increase in activity to reverse MDR and weak cytotoxicity.[6] However, taxinine and taxezopidine G can be only isolated from the seeds of the Chinese yew *Taxus chinensis* and Japanese yew *T. cuspidata* in very low yields of 0.014% and 0.0056% (w/w), respectively.[7]

Sinenxan A (Fig. 1), a biosynthetic taxoid consisting of a 6/8/6-membered ring system, can be obtained in a higher yield of 5.0% (w/w).[8] Nine sinenxan A derivatives with different side chains at C-5 have been semi-synthesized in our group and showed MDR reversal activity against VCR-resistant human oral epidermoid carcinoma KB/V cells.[9] Among these sinenxan A derivatives, three compounds were selected for further investigation on their in vitro MDR reversal activities and one compound was evaluated regarding its in vivo sensitizing activity with VCR-resistant KB/V tumor xenografts.[10] Our preliminary structure–activity relationships (SAR) results[9] showed that change of 5-*O* acyl substituents has a remarkable impact on the MDR reversal activity.

However, 5-*O* acyl substituents in our previous study were limited to side chains containing a phenyl ring. Therefore, side chains with different lengths containing another similar ring, such as a heterocycle, a cyclohexane or a naphthalin, could be introduced at C-5 to investigate the electronic effect and space effect on the activity. Especially, in view of the poor aqueous solubility of taxoids, introduction of side chains containing a heterocycle is expected to improve physicochemical property and subsequent drug metabolism and pharmacokinetics (DMPK) profile of the whole

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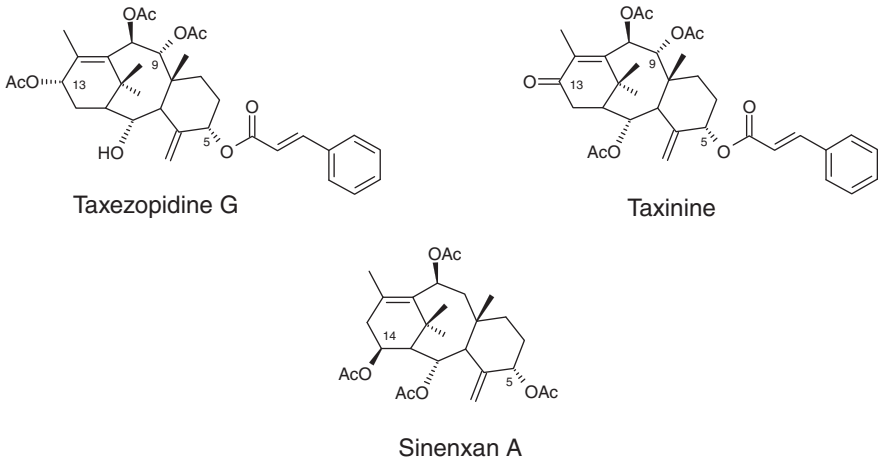
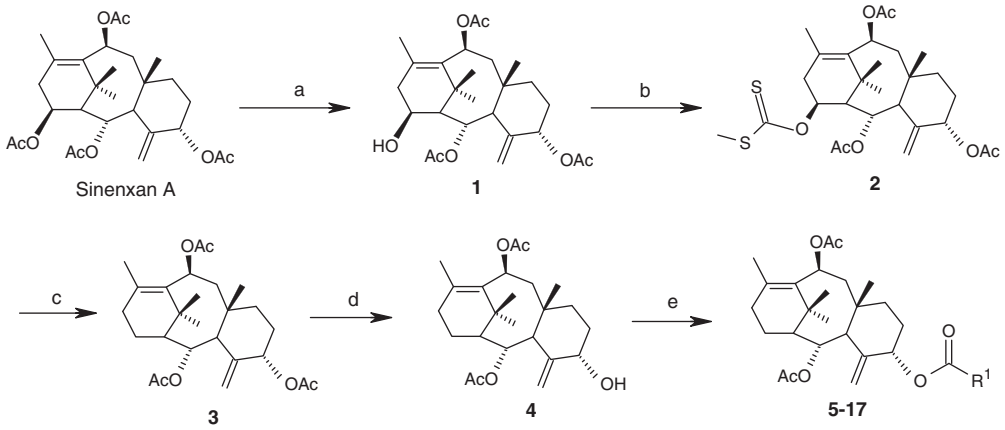


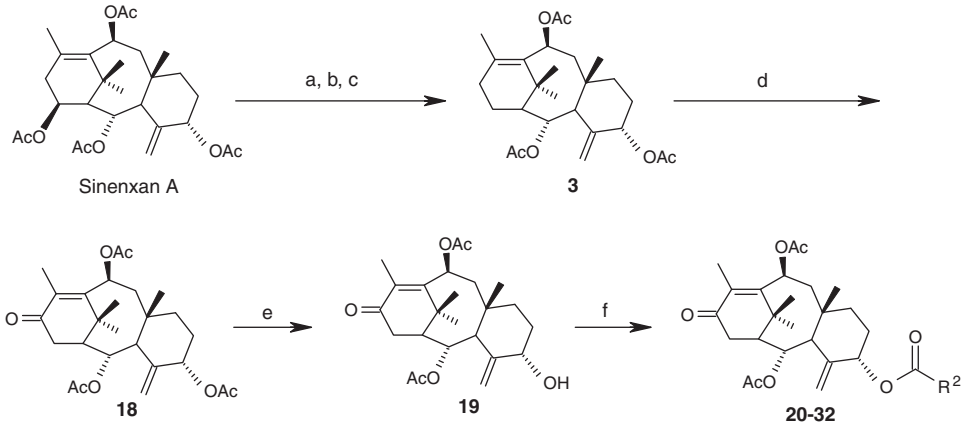
Figure 1. Chemical structures of taxezopidine G, taxinine, and sinenxan A.



Scheme 1. Reagents and conditions: (a) 10 N KOH, CH_3OH, 0 °C, 36%; (b) (i) NaH, CS_2, THF, reflux, 90%; (ii) CH_3I, THF, 45 °C, 90%; (c) Bu_3SnH, AIBN, toluene, 80 °C, 90%; (d) *t*BuOK, THF, −78 °C, 80%; (e) RCOOH, DCC, DMAP.

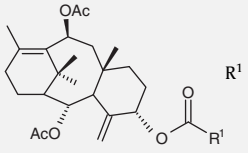
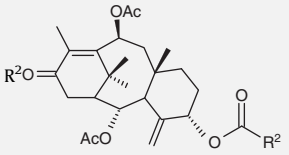
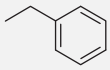
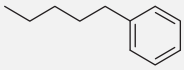
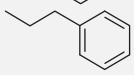
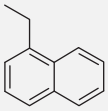
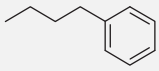
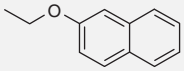
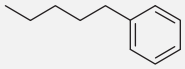
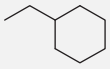
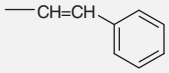
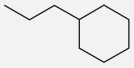
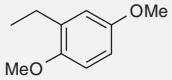
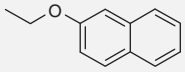
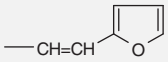
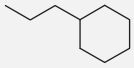
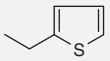
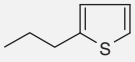
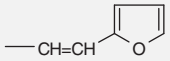
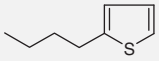
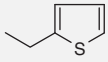
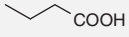
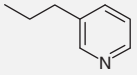
taxoid molecule. On the other hand, to our best knowledge, the information about the effect of 13-oxo on the activity is limited. It would be necessary to investigate the role of 13-oxo related to MDR reversal activities.

Herein, we report the most recent progress in this research. Two series of compounds with different 5-O acylations were synthesized to investigate the structure–activity relationships (SAR) of 5-*O* acyl substituents as well as that of 13-oxo. The key intermediates **4** and **19** were synthesized starting from sinenxan A (Schemes 1 and 2)[11] followed by 5-O acylation to give targets **5**–**17** and **20**–**32,** respectively. The structures of these target compounds were validated by ^1H NMR, FAB-MS or ESI-MS.



Scheme 2. Reagents and conditions: (a) 10 N KOH, CH_3OH, 0 °C, 36%; (b) (i) NaH, CS_2, THF, reflux, 90%; (ii) CH_3I, THF, 45 °C, 90%; (c) Bu_3SnH, AIBN, toluene, 80 °C, 90%; (d) PCC, NaOAc, celite, benzene, reflux, 60%; (e) *t*BuOK, THF, −78 °C, 90%; (f) RCOOH, DCC, DMAP or RCOCl.

Table 1
Effects of sinenxan A derivatives on resensitizing to VCR in KB/V cells

Compds		IC_{50} (nM)	Compds		IC_{50} (nM)
5		706.14	**20**		1795.99
6		403.58	**21**		1203.69
7		1616.67	**22**		191.77
8		48.23	**23**		2.80
9		509.83	**24**		55.98
10		370.47	**25**		71.21
11		45.36	**26**		11.05
12		537.24	**27**		1184.72
13		763.99	**28**		356.51
14		347.62	**29**		24.53
15		346.09	**30**		0.87
16		52.68	**31**		63.32
17		902.74	**32**		12.97
			Verapamil		18.47

The IC_{50} values of VCR in KB/V cells were determined in the presence of 10 μM of sinenxan A derivatives. Verapamil was used as a positive control.

MDR reversal activity of the derivatives was tested in vitro on the cellular accumulation of VCR in KB/V cells, which is 100-fold more resistent to VCR and cross resistance to doxorubicin, paclitaxel, and colchicine. The results were shown in Table 1. Among these sinenxan A derivatives, compounds **30** (IC_{50} = 0.87 nM), **23** (IC_{50} = 2.80 nM), **26** (IC_{50} = 11.05 nM), and **32** (IC_{50} = 12.97 nM), that were 21.23, 6.60, 1.67, and 1.42 times more potent than verapamil, completely reversed the resistance to VCR in KB/V cells overexpressing P-gp. Compounds **8**, **11**, **16**, **24**, **25**, **29**, and **31** partially reversed the MDR in KB/V cells. The others had no MDR reversal activities.

Most of these sinenxan A derivatives were also evaluated in vitro on the cytotoxicity against KB cells and exhibited weak or no cytotoxicity (data not shown).

Most of the derivatives with a heterocyclic acyl at O-5 and a 13-oxo displayed increased MDR reversal activity in KB/V cells. In particular, all of the compounds bearing nitrogen-containing heterocycles (**16**, **30**, **31**, and **32**) showed better potency. Compounds **23** possessing a cyclohexyl also showed improved MDR reversal activity.

In summary, the in vitro SAR suggested that the presence of a carbonyl group at C-13 and two or three atoms length of side chain at C-5 might be important for the activity of reversing MDR. Some derivatives bearing a cyclohexyl or a heterocycle at C-5 side chain are favorable for this type of activity.

Acknowledgments

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References and notes

1. Litman, T.; Druley, T. E.; Stein, W. D.; Bates, S. E. *Cell Mol. Life Sci.* **2001**, *58*, 931.
2. Sikic, B. I.; Fisher, G. A.; Lum, B. L.; Halsey, J.; Beketic-Oreskovic, L.; Chen, G. *Cancer Chemother. Pharmacol.* **1997**, *40*.
3. Tan, B.; Piwnica-Worms, D.; Ratner, L. *Curr. Opin. Oncol.* **2000**, *12*, 450.

4. (a) Kobayashi, J.; Hosoyama, H.; Wang, X.-X.; Shigemori, H.; Koiso, Y.; Iwasaki, S.; Sasaki, T.; Naito, M.; Tsuruo, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 393; (b) Kosugi, K.; Sakai, J.; Zhang, S.; Watanabe, Y.; Sasaki, H.; Suzuki, T.; Hagiwara, H.; Hirata, N.; Hirose, K.; Ando, M.; Tomida, A.; Tsuruo, T. *Phytochemistry* **2000**, *54*, 839; (c) Sakai, J.; Sasaki, H.; Kosugi, K.; Zhang, S.; Hirata, N.; Hirose, K.; Tomida, A.; Tsuruo, T.; Ando, M. *Heterocycles* **2001**, *54*, 999.
5. Zhang, S.; Wang, J.; Hirose, K.; Ando, M. *J. Nat. Prod.* **2002**, *65*, 1786.
6. (a) Sako, M.; Suzuki, H.; Yamamoto, N.; Hirota, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3403; (b) Hosoyama, H.; Shigemori, H.; Tomida, A.; Tsuruo, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 389.
7. Yin-Ju, C.; Chin-Yeu, C.; Ya-Ching, S. *J. Nat. Prod.* **1999**, *62*, 149.
8. Cheng, K.-K.; Chen, W.-M.; Zhu, W.-H.; Fang, Q.-C.; Liang, X.-T.; Guo, J.-Y.; Meng, C.; Kong, M.; He, W.-Y.; Lu, J.; Tong, X.-J.; Obuchi, T. WO Patent, 9,406,740, A1, 1994.
9. Zhang, M.; Yin, D.-L.; Liu, H.-Y.; Guo, J.-Y.; Liang, X.-T. *Acta Pharm. Sinica* **2003**, *38*, 424.
10. Zhao, X.; Gu, J.; Yin, D.-L.; Chen, X.-G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4767.
11. (a) Yin, D.-L.; Liu, R.-W.; Wang, D.-H.; Guo, J.-Y.; Liang, X.-T.; Yoshinori, S.; Kazuya, K. *J. Chin. Pharm. Sci.* **1999**, *8*, 191; (b) Zhang, M.; Yin, D.-L.; Guo, J.-Y.; Liang, X.-T. *Chin. Chem. Lett.* **2002**, *13*, 135; (c) Zhang, M.; Yin, D.-L.; Guo, J.-Y.; Liang, X.-T. *Chin. Chem. Lett.* **2002**, *13*, 501.