RESEARCH ARTICLE

[View Article Online](#)
[View Journal](#) | [View Issue](#) Cite this: *Med. Chem. Commun.*,
2017, 8, 1845

One-pot synthesis, anti-tumor evaluation and structure–activity relationships of novel 25-OCH₃-PPD derivatives†

Fan-Zhi Qu,^a Chen Zhao,^a Jia-Qing Cao,^a Yan Zhang^a and Yu-Qing Zhao^{*ab}

Based on the fact that 25-OCH₃-PPD, a natural ginsengenin isolated from the leaves of *Panax ginseng*, is a promising lead compound, novel 25-OCH₃-PPD derivatives were synthesized to find more potent anti-tumor agents by a simple and facile synthetic method. These derivatives were classified into three types and screened for their cytotoxic activities against seven human cancer cell lines. Compared with 25-OCH₃-PPD, compounds **a5**, **a7**, **b5** and **b7** exhibited higher anti-tumor activities on all tested cell lines with almost 5-fold to 15-fold increases. In particular, compound **a7** showed the greatest cytotoxic activity against α -2 cells (IC₅₀ = 2.4 $\pm$ 0.4 μ M). The preliminary study on the mechanisms indicated that compound **a7** could induce α -2 cell apoptosis. Structure–activity relationships demonstrated that the carbon–carbon double bond at the C-20 position could enhance the antiproliferative activity. In conclusion, the novel derivatives **a5**, **a7**, **b5** and **b7** could be further studied as potential candidates for the treatment of cancer. This research provides a theoretical reference for the exploration of new antiproliferative agents.

Received 15th July 2017,
Accepted 21st July 2017

DOI: 10.1039/c7md00358g

rsc.li/medchemcomm

1. Introduction

25-OCH₃-PPD [AD-1, Fig. 1], a dammarane-type ginsengenin, has been obtained from the leaves of *Panax ginseng*.¹ Previous studies demonstrated that it could suppress the proliferation of tumor cells and induce apoptosis.^{2,3} Western blot analysis revealed that it decreased MDM2, E2F1, Cyclin D1, Cyclin E and cdk4 expression, whereas it increased p21, p27 and Bcl-2 expression.^{4–6} In addition, it was found to exhibit strong cytotoxicity against a wide range of tumor cells (IC₅₀ = 20–40 μ M), about 10–40 times more potent than that of the clinically applied anti-cancer agent ginsenoside-Rg₃.⁷ What's more, it also has no significant toxicity to normal cells (IMR90, IMR90-E1A and IOSE144) and animal models (rats and dogs) compared with the severe side effects of chemotherapy.^{8,9} Nevertheless, it's impractical to develop it directly as a clinical drug due to its weak anti-tumor effects.

To enhance the bioactivity of AD-1, chemical modifications are mainly focused on the alcoholic hydroxyl groups in

the dammarane backbone. According to the reported pharmacokinetic studies^{10–14} and structure–activity relationships (SARs)^{15,16} of ginsenosides, previously we carried out the structural modification of AD-1 by introducing saturated fatty-acid groups, and found that some derivatives possessed highly promising anti-tumor activities. SARs showed that introduction of short-chain groups could contribute to a significant improvement of the anti-proliferative activity.¹⁷

On the other hand, the importance of halogens in medicinal chemistry is well known. Halogens exist extensively in commonly used clinical anti-tumor agents, such as carmustine, lapatinib and clofarabine. As electronegative elements, halogens could affect the properties of neighboring functional groups. Furthermore, they could contribute to improving the metabolic stability of compounds.^{18,19} As described in the reported work, halogen atoms or halogen-containing substituents could obviously increase the anti-tumor potency of compounds.^{20,21} Combination of the parent compound with halogen moieties in seeking novel anti-tumor agents has attracted extensive attention.

Based on these observations, additional halogen groups were introduced to AD-1, which had the biologically active polycyclic dammarane skeleton. Herein, we reported the synthesis of AD-1 derivatives with chloroacetyl and bromoacetyl groups. These analogues were tested for their inhibitory activities on seven human tumour cells (HCT-116, A549, α -2, BGC-823, C4-2B, MCF-7 and SGC-7901).²² Meanwhile, the potential

^a Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China. E-mail: zyq4885@126.com; Fax: +86 24 23986521; Tel: +86 24 23986521

^b Key Laboratory of Structure-based Drug Design and Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7md00358g

Fig. 1 Chemical structures of AD-1, **A** (acetic acid derivative of AD-1, 20*r*-25-methoxyl-3 β -*o*-acetyl-dammarane-12 β ,20-diol) and **B** (acetic acid derivative of AD-1, 20*r*-25-methoxyl-3 β ,12 β -*o*-diacetyl-dammarane-20-ol).

mechanisms of the most active compound were also investigated by microscope observation and flow cytometric analysis. Finally, the SARs of these derivatives were briefly discussed.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, AD-1 was esterified with chloroacetyl chloride or bromoacetyl bromide in dry dichloromethane. Their structures were determined accurately by comparing the spectroscopic data with those of AD-1 and its reported analogues. These data are provided in the ESI.† According to their structural characteristics, the derivatives could be classified into three types. The first class was composed of compounds **a1**–**a4** and **b1**–**b4**. The second class constituted com-

pounds with a common carbon–carbon double bond (**a5**, **a6** and **b5**, **b6** with special carbon signals at δ 137 and 124). And the other compounds with a terminal double bond (**a7**, **a8** and **b7** with special carbon signals at δ 152 and 107) belonged to the last class.

2.2. Biological activity

The derivatives were evaluated in terms of their anti-proliferative activities on colorectal cancer cells (HCT-116), lung cancer cells (A549 and α -2), gastric cancer cells (BGC-823 and SGC-7901), prostate cancer cells (C4-2B), and breast cancer cells (MCF-7). 5-Fluorouracil (5-Fu) and AD-1 were used as positive controls. The IC₅₀ values of the tested analogues are listed in Table 1.

Scheme 1 Synthesis of AD-1 with chloroacetyl chloride (compounds **a1**–**a8**) and bromoacetyl bromide (compounds **b1**–**b7**).

Table 1 Antiproliferative activities (IC₅₀, μM, 48 h) of the compounds against seven human cancer cell lines

Compounds	HCT-116	A549	α-2	BGC-823	C4-2B	MCF-7	SGC-7901
a1	43.8 ± 1.5	85.7 ± 3.1	33.1 ± 2.2	43.2 ± 1.6	36.4 ± 2.1	29.4 ± 2.4	40.8 ± 3.3
a2	53.5 ± 2.2	73.9 ± 4.6	39.7 ± 1.4	45.8 ± 2.8	30.8 ± 2.4	32.4 ± 2.2	61.5 ± 2.4
a3	48.7 ± 3.1	46.4 ± 2.8	30.2 ± 1.5	26.2 ± 2.1*	23.1 ± 1.2**	36.6 ± 2.7	34.5 ± 1.0
a4	36.6 ± 1.7	48.4 ± 2.5	37.2 ± 2.1	35.0 ± 2.6	25.0 ± 1.3	29.5 ± 1.1	30.8 ± 1.4
a5	4.9 ± 0.4***	10.5 ± 1.0**	5.5 ± 0.8***	9.2 ± 1.1***	7.1 ± 0.8***	3.4 ± 1.1***	9.4 ± 0.8***
a6	22.9 ± 0.8***	30.7 ± 1.2	25.9 ± 0.9	18.2 ± 0.9***	25.4 ± 1.2**	18.6 ± 0.7*	28.4 ± 1.5
a7	8.0 ± 0.7***	7.8 ± 0.6***	2.4 ± 0.4***	8.7 ± 0.9***	6.3 ± 0.4***	6.5 ± 0.3***	10.3 ± 0.5***
a8	22.3 ± 1.3**	17.2 ± 0.8*	15.1 ± 0.9**	21.8 ± 1.2**	16.7 ± 1.0***	18.5 ± 1.8*	26.2 ± 3.1
b1	29.8 ± 1.1*	54.5 ± 2.3	44.2 ± 2.0	39.3 ± 1.4	29.8 ± 0.9	27.8 ± 1.3	31.5 ± 1.4
b2	31.3 ± 1.2	41.5 ± 1.6	25.3 ± 1.2	40.7 ± 2.5	25.4 ± 1.8*	26.5 ± 1.2	37.9 ± 2.2
b3	31.4 ± 1.4	36.2 ± 2.8	25.7 ± 1.0	32.5 ± 1.8	20.6 ± 1.3**	33.8 ± 2.1	34.8 ± 1.7
b4	32.1 ± 1.4	43.5 ± 1.2	22.4 ± 0.8	26.7 ± 1.5*	22.8 ± 0.9	19.6 ± 1.0*	31.1 ± 1.8
b5	7.5 ± 0.5***	9.8 ± 1.0***	5.4 ± 0.7***	6.4 ± 0.4***	3.2 ± 0.3***	6.3 ± 0.4***	8.1 ± 0.6***
b6	22.4 ± 1.7***	14.5 ± 0.6**	13.1 ± 0.9**	15.7 ± 0.8***	13.2 ± 0.9***	19.7 ± 0.8**	24.8 ± 1.3*
b7	7.1 ± 0.4***	10.4 ± 0.8**	7.3 ± 0.6***	6.5 ± 0.6***	5.7 ± 0.3***	4.5 ± 0.4***	6.7 ± 0.3***
A	27.8 ± 0.8**	26.7 ± 1.3	19.3 ± 0.9*	23.6 ± 2.3**	30.1 ± 2.2*	32.9 ± 1.7	37.2 ± 1.6
B	30.9 ± 1.5*	36.9 ± 1.2	22.7 ± 1.3	17.8 ± 1.1*	26.4 ± 0.8**	38.5 ± 1.8	31.2 ± 2.3
AD-1	44.2 ± 2.0	38.5 ± 1.9	33.6 ± 3.1	39.6 ± 2.5	37.5 ± 1.4	36.9 ± 1.0	44.7 ± 2.4
5-Fu ^a	37.6 ± 2.9	23.5 ± 1.8	27.5 ± 1.7	34.8 ± 1.0	38.2 ± 2.9	29.4 ± 0.7	36.4 ± 1.1

^a Positive control. The results are shown as mean value ± S.D. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, when compared to positive control values by Student's *t*-test.

For HCT-116 cells (Table 1), the most active compound was a5 (IC₅₀ = 4.9 ± 0.4 μM). In addition, compounds a6, a7, a8, b5, b6 and b7 displayed better anti-tumour activities than AD-1 (44.2 ± 2.0 μM), with IC₅₀ values of 22.9 ± 0.8, 8.0 ± 0.7, 22.3 ± 1.3, 7.5 ± 0.5, 22.4 ± 1.7 and 7.1 ± 0.4 μM, respectively. Some compounds of type 1 like a1, a2 and b1 showed weaker activities compared to the positive controls (AD-1 and 5-Fu). For α-2 cells, among the analogues, compound a7 (2.4 ± 0.4 μM) had the strongest inhibitory activity. Compared with AD-1 (33.6 ± 3.1 μM) and 5-Fu (27.5 ± 1.7 μM), compounds a5, a8, b5, b6 and b7 showed better bioactivities with IC₅₀ values within the range of 5.4 to 15.1 μM. For C4-2B cells, the IC₅₀ values for compounds a5, a7, b5 and b7 (IC₅₀ = 3.2–7.1 μM) were much lower than those of A (30.1 ± 2.2 μM) and B (26.4 ± 0.8 μM), with almost 4-fold to 10-fold increases. Compounds a8 and b6 also had strong antiproliferative activity (IC₅₀ = 16.7 ± 1.0 and 13.2 ± 0.9 μM, respectively). Interestingly, compound a6 (25.4 ± 1.2 μM) was more potent than AD-1 (37.5 ± 1.4 μM).

At the same time, compounds a5 and b7 showed better anti-tumour activities against the MCF-7 cells than AD-1 (36.9 ± 1.0 μM) and 5-Fu (29.4 ± 0.7 μM) with IC₅₀ values of 3.4 ± 1.1 and 4.5 ± 0.4 μM, respectively. Compound a7 (7.8 ± 0.6 μM) displayed the most potent inhibitory effect on A549 cells. And compound b5 had the most powerful potency against BGC-823 (6.4 ± 0.4 μM). The IC₅₀ value of compound b7 (6.7 ± 0.3 μM) against the SGC-7901 cells was the lowest. Overall, amongst the tested compounds, compounds a5, a7, b5 and b7 exhibited the strongest bioactivities *in vitro*.

2.3. SARs of the 25-OCH₃-PPD derivatives

Based on the above results, compounds of types 2 and 3 had stronger activities than those of type 1, as shown by comparisons between compounds a1 and a5, a3 and a8, b1 and b5, and b2 and b6. So the carbon-carbon double bond at the C-20 position resulted in a significant increase in the cytotoxicity of the molecule. As shown in Fig. 2, the additional

Fig. 2 IC₅₀ values of compounds a2, a6, a7, b6 and AD-1.

Fig. 3 Effects of compound a7 on α -2 cells. The arrows indicate apoptotic bodies. (A) Cells were incubated with 20 μ M a7 for 12 h, and cell appearances were observed using an inverted microscope ($\times 40$). (B) Nuclear morphology of treated and control cells as observed using a fluorescence microscope ($\times 40$) following staining with DAPI. (C and D) Cells were cultured in the presence of a7 for 6, 12 and 24 h, and cell cycle analysis was conducted by flow cytometry.

double bond, similar to that in compound a6, also improved the activity and indicated the above conclusion when compared with AD-1. For compounds of types 2 and 3, the number of substituent groups affected the anti-proliferation activities in the following order: a5 or a7 > a8 > a6. The same results could be obtained by comparing the potencies of b5 and b6. Briefly, more substituent groups yielded stronger activity. This observation suggested that the side-chain substituents of ginsengenin could influence the anti-tumour potency. However, the first type of compounds didn't show the same result. And a marked decrease in bioactivity was observed when just the halogen group was introduced to the C-3 and C-12 position (except for the MCF-7 cell line), which meant that the halogen group wasn't the main reason for the increased activity. We need to emphasise that the carbon-carbon double bond played a more important role in enhancing the anti-proliferative effect compared with the number of substituent groups. We also noted that the bromoacetyl derivatives showed a more potent cytotoxicity than the corresponding chloroacetyl derivatives, but the increase was not evident.

2.4. Analyses of apoptosis

To clarify how compound a7 affects the cell growth of α -2 cells, we observed the morphological changes of the treated cells.²³ Relative to the control group, cells treated with compound a7 (20 μ M) were found to undergo a series of obvious morphological changes, including the appearance of membrane blebbing and granular apoptotic bodies (Fig. 3A). Cells stained with DAPI displayed a condensed chromatin nucleus (Fig. 3B), which was a peculiar phenomenon of cell apoptosis. We also evaluated the effects of compound a7 on cell cycle progression by flow cytometry. As shown in Fig. 3C and D, the percentage of cells in the SubG1 phase after treatment with compound a7 for 6, 12 and 24 h increased to 7.41%, 13.28% and 38.15%, respectively, compared with 2.72% for the non-treated group. These results indicated that compound a7 could significantly induce α -2 cell apoptosis.

3. Conclusions

In summary, we synthesised a series of new ginsengenin derivatives with halogen groups by a one-pot reaction. Results

showed that derivatives with a carbon–carbon double bond at the C-20 position and two substituents displayed higher anti-proliferative activities than AD-1 and 5-Fu, with almost 5-fold to 15-fold increases. In addition, the pharmacological experiments preliminarily showed that compound **a7** significantly induced α -2 cell apoptosis. Given the current data, we concluded that the double bond and multi-substituted groups increased the anti-proliferative effects. The carbon–carbon double bond in compounds of types 2 and 3 may be of great importance for the bioactivities and enrichment of a variety of ginseng skeleton. It may be able to decrease the steric hindrance of the side chain. More halogen-containing substituents may help compounds achieve an appropriate lipophilicity. Hence, the present study provided new ideas and directions for the chemical modification and development of dammarane-type saponins. The results indicated that the representative compounds **a5**, **a7**, **b5** and **b7** were chosen as lead compounds for further research.

Live subject statement

All experiments were performed in compliance with the relevant laws and institutional guidelines for the Care and Use of Laboratory Animals of Shenyang Pharmaceutical University. The experiments were approved by the Ethics Committee of Shenyang Pharmaceutical University.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

The research was supported by the National Science Foundation of China (No. 81273389). We thank the Analytical Center of Shenyang Pharmaceutical University for NMR measurement.

Notes and references

- 1 Y. Zhao, W. Wang, L. Han, E. R. Rayburn, D. L. Hill, H. Wang and R. Zhang, *Med. Chem.*, 2007, 3, 51–60.
- 2 W. Wang, H. Wang, E. R. Rayburn, Y. Zhao, D. L. Hill and R. Zhang, *Br. J. Cancer*, 2008, 98, 792–802.
- 3 X. Bi, Y. Zhao, W. Fang and W. Yang, *Clin. Exp. Pharmacol. Physiol.*, 2009, 36, 1074–1078.
- 4 W. Wang, E. R. Rayburn, J. Hang, Y. Zhao, H. Wang and R. Zhang, *Lung Cancer*, 2009, 65, 306–311.
- 5 W. Wang, E. R. Rayburn, Y. Zhao, H. Wang and R. Zhang, *Cancer Lett.*, 2009, 278, 241–248.
- 6 L. H. Zhang, Y. L. Jia, X. X. Lin, H. Q. Zhang, X. W. Dong, J. M. Zhao, J. Shen, H. J. Shen, F. F. Li, X. F. Yan, W. Li, Y. Q. Zhao and Q. M. Xie, *Biochim. Biophys. Acta*, 2013, 1830, 4148–4159.
- 7 S. Y. Lee, G. T. Kim, S. H. Roh, J. S. Song, H. J. Kim, S. S. Hong, S. W. Kwon and J. H. Park, *Biosci., Biotechnol., Biochem.*, 2009, 73, 811–816.
- 8 X. Zhang, J. Xu, D. Zhang, J. Gu and Y. Zhao, *Xenobiotica*, 2009, 39, 457–464.
- 9 X. Zhang, J. Zhang, W. Li, L. Liu, B. Sun, Z. Guo, C. Shi and Y. Zhao, *PLoS One*, 2014, 9, e94962.
- 10 E. A. Bae, M. J. Han, E. J. Kim and D. H. Kim, *Arch. Pharmacol. Res.*, 2004, 27, 61–67.
- 11 H. Hasegawa, K. S. Lee, T. Nagaoka, Y. Tezuka, M. Uchiyama, S. Kadota and I. Saiki, *Biol. Pharm. Bull.*, 2000, 23, 298–304.
- 12 C. Wakabayashi, H. Hasegawa, J. Murata and I. Saiki, *Oncol. Res.*, 1997, 9, 411–417.
- 13 H. Hasegawa, R. Suzuki, T. Nagaoka, Y. Tezuka, S. Kadota and I. Saiki, *Biol. Pharm. Bull.*, 2002, 25, 861–866.
- 14 H. Hasegawa, *J. Pharmacol. Sci.*, 2004, 95, 153–157.
- 15 J. M. Zhao, N. Li, H. Zhang, C. F. Wu, H. R. Piao and Y. Q. Zhao, *Bioorg. Med. Chem. Lett.*, 2011, 21, 1027–1031.
- 16 T. Akao, H. Kida, M. Kanaoka, M. Hattori and K. Kobashi, *J. Pharm. Pharmacol.*, 1998, 50, 1155–1160.
- 17 Y. F. Liu, H. N. Yuan, X. L. Bi, H. R. Piao, J. Q. Cao, W. Li, P. Wang and Y. Q. Zhao, *Steroids*, 2013, 78, 1305–1311.
- 18 E. L. Luzina and A. V. Popov, *Eur. J. Med. Chem.*, 2009, 44, 4944–4953.
- 19 Y. J. Da, W. D. Yuan, T. Xin, Y. Y. Nie, Y. Ye, Y. J. Yan, L. S. Liang and Z. L. Chen, *Bioorg. Med. Chem.*, 2012, 20, 7101–7111.
- 20 J. Francuz, B. Srećo, M. Popsavin, G. Benedeković, V. Divjaković, V. Kojić, G. Bogdanović, A. Kapor and V. Popsavin, *Tetrahedron Lett.*, 2012, 53, 1819–1822.
- 21 J. Francuz, I. Kovačević, M. Popsavin, G. Benedeković, B. S. Zelenović, V. Kojić, D. Jakimov, L. Aleksić, G. Bogdanović, T. Srdić-Rajić, E. Lončar, M. V. Rodić, V. Divjaković and V. Popsavin, *Eur. J. Med. Chem.*, 2017, 128, 13–24.
- 22 T. Mosmann and J. Immunol, *Methods*, 1983, 65, 55–63.
- 23 J. Shao, Y. Dai, W. Zhao, J. Xie, J. Xue, J. Ye and L. Jia, *Cancer Lett.*, 2013, 330, 49–56.