Accepted Manuscript

Synthesis and evaluation of panaxtriol derivatives as Na⁺, K⁺-ATPase inhibitors

Qiong Wu, Peng Chen, Guangzhong Tu, Meng Li, Bowen Pan, Yan Guo, Jinbi Zhai, Hongzheng Fu

PII:	S0960-894X(18)30590-0
DOI:	https://doi.org/10.1016/j.bmcl.2018.07.027
Reference:	BMCL 25958
To appear in:	Bioorganic & Medicinal Chemistry Letters
Received Date:	23 March 2018
Revised Date:	12 June 2018
Accepted Date:	16 July 2018



Please cite this article as: Wu, Q., Chen, P., Tu, G., Li, M., Pan, B., Guo, Y., Zhai, J., Fu, H., Synthesis and evaluation of panaxtriol derivatives as Na⁺, K⁺-ATPase inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.07.027

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Synthesis and evaluation of panaxtriol derivatives as Na⁺, K⁺-ATPase

inhibitors

Qiong Wu^a, Peng Chen^a, Guangzhong Tu^b, Meng Li^c, Bowen Pan^a, Yan Guo^d, Jinbi Zhai^d and Hongzheng Fu^{a,*}

- ^a State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Science, Peking University, Beijing 100191, China
- ^b Beijing Institute of Microchemistry, Beijing 100091, China
- ^c University of Warwick, Coventry CV4 7AL, United Kingdom
- ^d College of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang 110016, China
- *Corresponding author. Hongzheng Fu, E-mail: drhzfu@sina.com

Keywords: Na⁺, K⁺-ATPase, panaxtriol, *seco*-A ring derivatives, molecular docking.

Abstract: Panaxatriol, a triterpene bearing a steroid-like structure similar to cardiac glycosides, was presumed to share the same bioactivity with cardiac glycosides, and may be a potential Na⁺, K⁺-ATPase inhibitor. In this paper, a series of panaxtriol derivatives were synthesized and evaluated for Na⁺, K⁺-ATPase inhibitory activities. The results of biological tests showed that more than half of the synthesized derivatives presented increased inhibitory activities compared with panaxatriol. Of these compounds, **13a** with a 3, 4-*seco* skeleton showed the most potent inhibitory activity, which was equal to that of the standard drug digoxin. To understand the binding mode of the most active compound, molecular docking study of **13a** with Na⁺, K⁺-ATPase was conducted. Therefore, **13a** may serve as a new lead compound for the development of novel Na⁺, K⁺-ATPase inhibitors.

Heart failure (HF) is a serious condition characterized by the incapability of the heart to supply sufficient blood flow to meet the body's needs.¹ Across the globe, about 26 million adults worldwide are living with HF.² However, survival rates remain poor and the five-year mortality rate for HF is nearly 50%.³ Digitalis cardiac glycosides, such as digoxin, have been used as positive inotrope for the treatment of HF for more than 200 years. Their mechanism of action is digitalis reversibly inhibits the membrane bound alpha subunits of the Na⁺, K⁺-ATPase in cardiomyocytes.⁴ Cardiac glycosides' aglycones, the steroidal structures, are considered to be responsible for their inhibitory activities. ⁵ Despite cardiac glycosides are extensively used in clinical therapy, its safety

is still a big problem due to the life-threatening cardiac arrhythmias toxicity and the narrow therapeutic index.⁶

Panax ginsing has been widely used in Chinese medicine for the promotion of physical strength and resistance to diseases for thousands of years.⁷ In addition, ginseng has been used for the treatment of HF, ⁸ and generally has a good safety profile and low adverse effects.^{9, 10} Ginsenosides are the main components of ginseng, ¹¹ and have been reported to inhibit Na⁺, K⁺-ATPase.¹² However, ginsenosides were poorly absorbed in the gastrointestinal tract, ¹³ and were tend to be metabolized to their final aglycones by intestinal bacterial deglycosylation after oral administration.¹⁴ The main aglycones of ginsenosides, such as protopanaxatriol (PPT) and protopanaxadiol, are dammarane-type tetracyclic triterpenes.

20(R)-panaxatriol (PT) is a pseudo-aglycone of PPT-type ginsenosides, and can be obtained by conversion of PPT during the acid hydrolysis. ¹⁵ PT was similar structurally to uzarigenin, which was a potent NKA inhibitor and 5α H-cardiotonic steroid ^{16, 17}. Thus, we presumed that PT may share the same inhibitory activity towards Na⁺, K⁺-ATPase. To our best knowledge, the inhibitory activities of PT and its derivatives on Na⁺, K⁺-ATPase have not been reported yet. With the aim to search for novel Na⁺, K⁺-ATPase inhibitors with low toxicity and high affinity from natural resources, a series of PT derivatives were synthesized for Na⁺, K⁺-ATPase inhibitory activities evaluation.



Figure 1. Structures and stereo 3D stick model of the superposition of uzarigenin (red) and PT (blue).

Panaxtriol was prepared by acid hydrolysis of total ginsenosides derived from the stem and leaf of *panax ginseng*. Whereas PT has three hydroxyl groups at C-3, C-6 and C-12, oxidation and etherification reactions were introduced to evaluate the role of hydroxyl groups as showed in Scheme 1. PT **1** was oxidized by equivalent Dess-Martin periodinane to produce 6-keto-PT **2** and 3-keto-PT **3**, which were further etherified by different haloalkanes to give the ethers **4a-4d** and **5a-5d**, respectively. Besides, treatment of **2** with ethoxycarbonyl isothiocyanate resulted in **4e**, which was followed by a ring closure reaction to afford **4f**. ¹⁸ The 3-keto-PT **3** was converted to 3, 12-keto-PT **6** in three steps (see supporting information). Oxidized compounds **7** and **8** were accomplished by reacting **1** with Jones reagent and pyridinium chlorochromate, respectively. To explore the role of A-ring, lactones **9**, **10** and **11** were synthesized by Baeyer–Villiger reaction of **6**, **7** and **8** using 3-chloroperoxybenzoic acid, respectively.

In addition, lactam 12 was synthesized from 8 in two steps by first treating with hydroxylammonium chloride and subsequent with $SOCl_2$ through Beckmann rearrangement reaction.



Scheme 1. Reagents and conditions: (a) Dess-Martin Periodinane, DCM, rt, 3 h; (b) (i) NaH, THF, rt, 0.5 h; (ii) RBr, 70°C, 12 h; (c) (i) Ac₂O, pyridine, reflux, 12 h; (ii) Jones reagent, 3 h; (iii) KOH, MeOH/H₂O, 1 h; (d) Jones reagent, rt, 12 h; (e) PCC, rt, 6 h; (f) m-CPBA, Li₂CO₃, DCM, 12 h; (g) (i) NH₂OH·HCl, NaHCO₃, reflux, 8 h; (ii) SOCl₂, DCM, 0°C, 1 h; (h) ethoxycarbonyl isothiocyanate, CHCl₃, 60°C, 13 h; (i) NH₂OH·HCl, LiOH, EtOH, rt, 5 h.

Considering that a *seco*-A ring structure may provide an altered pattern of functionalities and new scaffold with conformational flexibility, ¹⁹ a group of A-ring opening analogs were synthesized as presented in Scheme 2. Hydrolysis of lactone **11** with p-toluenesulfonic acid in MeOH gave 3, 4-*seco* methyl ester **13a** and **13b**, while treatment of **11** with KOH in MeOH/H₂O generated **14**, which was further esterified to give ester **15**. **14** was supposed to be a carboxylic acid similar to **13a**, however it lost C-4, C-28 and C-29, which was abnormal. The fact is that a retro aldol reaction took place during the hydrolysis with the presence of KOH (mechanism was showed in Figure 2). Indole has been known to be a very important segment in biologically active molecules, thus was incorporated at A-ring of **8** to afford **16a-16c** by Fischer indole synthesis using different phenyldrazines. The structures of all synthesized compounds were elucidated by ¹H NMR, ¹³C NMR and MS (see supporting information).



Scheme 2. Reagents and conditions: (j) *p*-toluenesulfonic acid, MeOH, rt, 2 h; (k) KOH, MeOH/H₂O, rt, 1 h; (l) DCM/MeOH, H₂SO₄, 3 h; (m) phenylhydrazine derivatives, AcOH, reflux, 3 h.



Figure 2. Mechanism for the retro aldol reaction of 11 to 14.

All the synthetic derivatives were evaluated in the enzyme inhibition assay against Na⁺, K⁺-ATPase by using a known colorimetric method. ^{20, 21} Digoxin was chosen as reference compound because it is the most common drug for the treatment of HF. As can be seen in Table 1, more than half of tested PT derivatives showed increased inhibitory activities. Oxidation of hydroxyl groups of **1** on C-3 or C-6 exhibited no enhancement of inhibitory activity, while oxidation of hydroxyl group on C-12 slightly increase the inhibitory activity (**6** vs **3**). As to ethers **4a-4d** and **5a-5d**, only the monobenzyl ether derivatives, such as **4c**, **4d** and **5d**, significantly improved the inhibition.

These results revealed that ethyl ether and allyl ether were not preferable substitutions. Among them, the most potent compound (4d) was about 3.6-fold more potent than 1. Compared with 4d, 4c with a trifluoromethoxy group substituted on its C-4 position of the benzene ring showed a decrease of the potency. In addition, 4f containing an oxadiazolone group showed a slight improvement of activity (4f vs 2). Besides, the benzyl ether group at C-12 in 5c decreased the inhibitory on Na⁺, K⁺-ATPase compared with 5d, suggesting that benzyl ether group at C-12 is not beneficial to the affinity to certain enzyme site.

Inhibitory activities of PT derivatives against Na⁺, K⁺- ATPase

Table 1

	R^{1}		R^{5} R^{4} H O R^{4} H O R^{4} H O H O H O H H O H O H					
	1, 2, 3, 4a-41, 5a-5d, 0,	, 7, 8 9, 10, 11, 1	13a-D, 14,	15	104	-100		
Compound	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	X	IC ₅₀ (µM)
1	H, β OH	Η, α OH	H, β OH					1.09 ± 0.11
2	H, β OH	0	H, β OH					1.18 ± 0.11
3	0	H, α OH	H, β OH					1.14 ± 0.12
4a	H, β OC ₂ H ₅	Ο	H, β OH					1.14 ± 0.12
4 b	H, β OCH ₂ CH=CH ₂	0	H, β OH					1.05 ± 0.11
4 c	H, β O4(CF ₃ O)Bn	0	H, β OH					0.45 ± 0.03
4d	H, β OBn	0	H, β OH					0.30 ± 0.03
4e	$H, \beta \overset{3}{\overset{\circ}{\overset{\circ}{\overset{\circ}{\overset{\circ}}{\overset{\circ}{\overset{\circ}{\overset{\circ}$	0	H, β OH					1.38±0.14
4f	H, β $N \to 0$	Ο	H, β OH					0.96±0.09
5a	0	H, α OCH ₂ CH=CH ₂	H, β OCH ₂ CH=CH ₂					0.75 ± 0.08
5b	0	H, α OCH ₂ CH=CH ₂	H, β OH					0.91±0.09
5c	0	H, α OBn	H, β OBn					0.87 ± 0.08
5d	0	H, α OBn	H, β OH					0.33 ± 0.03
6	0	H, α OH	0					0.70 ± 0.06
7	0	Ο	0					0.76 ± 0.08
8	0	0	H, β OH					$0.81{\pm}0.07$
9		H, α OH	0				0	1.23±0.13
10		0	0				0	1.13±0.11
11		0	H, β OH				0	1.18 ± 0.11
12		0	H, β OH				NH	0.41 ± 0.03

13a	C(CH ₃) ₂ OH	Me		0.26±0.03
13b	CCH ₃ =CH ₂	Me		1.09 ± 0.08
14	Н	Н		$0.54{\pm}0.05$
15	Н	Me		0.41 ± 0.03
16a			Н	0.77 ± 0.07
16b			F	0.91±0.08
16c			OCF ₃	0.36±0.04
Digoxin		4		0.27 ± 0.03

When it comes to the A-ring extended compounds, lactone skeleton and lactam skeleton showed different impacts on the inhibitory activities. While lactones (9, 10, 11) showed no improvement of inhibitory activities compared with 1, lactam (12) presented a 2.6-fold inhibitory activity more potent than 1. In addition, the nearly no differences of activities between 9, 10 and 11 revealed that ketone group on C-6 and C-12 had only minimal effect on inhibition. Almost all of the seco-A ring derivatives had exhibited more potent inhibition against Na⁺, K⁺-ATPase than that of 1 except 13b. Among these seco-compounds, 13a is the most potent inhibitor (IC₅₀ = 0.26μ M), about 4.2-fold more potent than 1. The inhibitory effect of 4-hydroxyl-3-methyl ester 13a was much stronger than the 4-methylene-3-methyl ester 13b, indicating that hydroxyl at C-4 is important for binding to the enzyme in 3, 4-seco derivatives. On the other hand, the further cleavage of 3, 4-seco compounds led to a decrease in the inhibitory activity (14, 15 vs 13a, 13b). Besides, methyl esterification of carboxylic acid 14 to 15 at C-3 position brought slightly enhanced performance. For indole-fused derivatives, 16c exhibited a much better inhibitory activity than 16a and 16b, indicating that trifluoromethoxy group substituted on benzene ring is a more preferable substitution than fluorine atom and no substitution.

The arrhythmogenic activity of the most potent compound **13a** was determined in the isolated rat hearts by using a known method. ²² Digoxin was chosen as reference compound. The results showed that no arrhythmia occurred in all 4 isolated rat hearts when perfused with 0.26 μ mol/L **13a**. However, the perfusion of 0.26 μ mol/L digoxin induced arrhythmias in 2 of 4 isolated rat hearts. All these results indicated that **13a** was much safer than digoxin (see supporting information).

To understand the binding mode of the most active compound, molecular docking study of **13a** with Na⁺, K⁺-ATPase (PDB code: 4HYT) was performed with maestro v11.1. As showed in Figure 3, **13a** has a nice fit in the binding pocket. The methyl propionate chain stretched into a specific cavity, and the carbonyl group at C-3 of **13a** formed hydrogen bonds with THR-797 (2.5 Å) and ASP-121 (2.2 Å), respectively. In addition, the hydroxyl group at C-12 formed a hydrogen bond with GLU-116 in a distance of 2.2 Å.



Figure 3. Predicted binding model of **13a** to Na⁺, K⁺-ATPase. Key residues are highlighted in green and the H-bonds are represented by dashed red lines.

In summary, modification of PT by oxidation, etherification of hydroxyl groups, cleavage of A-ring and fusion of indole on A-ring, led to a series of PT derivatives. Almost a half of them showed increased inhibitory activities on Na⁺, K⁺-ATPase, and the most active compound **13a** exhibited an inhibitory activity equal to that of the standard drug digoxin. Moreover, the molecular docking study illustrated its potential binding mode. Thus, **13a** containing a 3, 4-*seco* structure provides a basis for developing novel Na⁺, K⁺-ATPase inhibitors.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 30973628) and the National Science and Technology Major Project of China (No. SQ2018ZX090301).

References and notes

1. Boxer R, Yang SX, Hager WD. Congestive heart failure and the elderly. *Conn Med.* 2003;67(8): 497.

2. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol.* 2011;8(1): 30-41.

3. Blass BE, Huang CT, Kawamoto RM, et al. Parallel synthesis and evaluation of N-(1-phenylethyl)-5-phenyl-imidazole-2-amines as Na+/K+ ATPase inhibitors. *Bioorg Med Chem Lett.* 2000;10(14): 1543-1545.

4. Smith TW. Digitalis. Mechanisms of action and clinical use. *N Engl J Med.* 1988;318(6): 358-365.

5. Schönfeld W, Menke KH, Schönfeld R, Repke KR. 5 Beta,14 beta-androstane-3 beta,14-diol binds to the digitalis receptor site on Na/K-ATPase. *J Enzyme Inhib*. 1987;2(1): 37-45.

6. Cerri A, Serra F, Ferrari P, Folpini E, Padoani G, Melloni P. Synthesis, Cardiotonic Activity, and Structure–Activity Relationships of 17β -Guanylhydrazone Derivatives of 5β -Androstane- 3β , 14β -diol Acting on the Na+,K+-ATPase Receptor. *J Med Chem.* 1997;40(21): 3484-3488.

7. Tachikawa E, Kudo K, Hasegawa H, et al. In vitro inhibition of adrenal catecholamine secretion by steroidal metabolites of ginseng saponins. *Biochem Pharmacol.* 2003;66(11): 2213-2221.

8. Wagner H, Liu X. *The International Textbook of Cardiology*. New York: Pergamon Press; 1987.

9. Lee NH, Son CG. Systematic review of randomized controlled trials evaluating the efficacy and safety of ginseng. *J Acupunct Meridian Stud.* 2011;4(2): 85-97.

10. Kim YS, Woo JY, Han CK, Chang IM. Safety Analysis of Panax Ginseng in Randomized Clinical Trials: A Systematic Review. *Medicines*. 2015;2(2): 106.

11. Yang WZ, Hu Y, Wu WY, Ye M, Guo DA. Saponins in the genus Panax L. (Araliaceae): a systematic review of their chemical diversity. *Phytochemistry*. 2014;106(10): 7.

12. Lee SW, Lee JS, Kim YH, Jin KD. Effect of ginseng saponin on the Na+, K+-ATPase of dog cardiac sarcolemma. *Arch Pharm Res.* 1986;9(1): 29-38.

13. Takino Y. Studies on the pharmacodynamics of ginsenoside-Rg1, -Rb1 and -Rb2 in rats. *Yakugaku Zasshi*. 1994;114(8): 550-564.

14. Nah SY, Kim DH, Rhim H. Ginsenosides: Are any of them candidates for drugs acting on the central nervous system? *CNS Drug Rev.* 2007;13(4): 381–404.

15. Wei Y, Ma CM, Hattori M. Anti-HIV protease triterpenoids from the acid hydrolysate of Panax ginseng. Phytochem Lett. 2009;2(2): 63-66.

16. Brown L, Thomas R, Watson T. Cardiac glycosides with non-rotating steroid to sugar linkages: tools for the study of digitalis structure-activity relationships. Naunyn-Schmiedeberg's Arch Pharmacol. 1986;332(1): 98-102.

17. Cerri A, Gobbini M. Simplified digitalis-like compounds acting on Na(+), K(+)-ATPase. J Enzyme Inhib Med Chem. 2003;18(4): 289-295.

18. Czollner L. Synthesis of new glycyrrhetinic acid derived ring A azepanone, 29-urea and 29-hydroxamic acid derivatives as selective 11β -hydroxysteroid dehydrogenase 2 inhibitors. *Biorg Med Chem.* 2011;19(6): 1866-1880.

19. Mao SW, Chen H, Yu LF, et al. Novel 3,4-seco bile acid diamides as selective anticancer proliferation and migration agents. *Eur J Med Chem.* 2016;122: 574.

20. Taussky HH, Shorr E, Kurzmann G. A microcolorimetric method for the determination of inorganic phosphorus. *J Biol Chem.* 1953;202: 675-685.

21. Matsukawa M, Mukai T, Akizawa T, et al. Isolation and Characterization of Novel Endogenous Digitalis-like Factors in the Ovary of the Giant Toad, Bufo marinus. *J Nat Prod.* 1998;61(12): 1476-1481.

22. Zhao TC, Zhang XL. Effect of activation of Na +/H + exchange system on ischemia reperfusion injury. *Chin Appl Physiol*. 1996; 12 (3): 270-272.

Highlights

- 26 panaxtriol derivatives were synthesized and evaluated for Na⁺, K⁺-ATPase inhibitory activities.
- Compound **13a** showed most inhibitory activity equal to that of the standard drug digoxin.
- Molecular docking predicted the potential binding mode.