Journal of Medicinal Plants Research Vol. 5(31), pp. 6731-6737, 23 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.713

Full Length Research Paper

Ocotillol-type derivatives (II) synthesis and protective effects on cultured anoxia/reoxygen injury myocardiocytes

Yi Bi[1], Jingwei Tian[2], Chunmei Ji[3], Jiangfeng Zhang[1], Nan Wang[1], Naicai Jiang[1], Haijun Sun[1] and Qingguo Meng[1]*

[1]Department of Medicinal Chemistry, School of Pharmacy, Yantai University, 32 Qingquan Road, Yantai 264005, China.
[2]Department of pharmacology, School of Pharmacy, Yantai University, 32 Qingquan Road, Yantai 264005, China.
[3]Weifang Peoele's Hospital, Weifang 261041, China.

Accepted 30 November, 2011

The ocotillol-type derivatives (3β, 12β)-12, 25-dihydroxy-20S, 24R-epoxy-dammar-24-en-3-yl-2-O-β-D-glucopyranosyl-β-D-glucopyranoside and (3β, 6α, 12β)-3, 12, 25- trihydroxy- 20S, 24R- epoxy- dammar-24-en-6-yl-2-O-[6-deoxy-α-L-mannopyranosyl]-β-D-glucopyranoside (3a and b) had been designed and synthesized from 20 (S)-Rg3 and 20 (S)-Rg2 which structures were confirmed by electrospray ionization mass spectrometry (ESI-MS), Hydrogen- Nuclear magnetic resonance (^{1}H-NMR) and carbon- Nuclear magnetic resonance (^{13}C-NMR). 20(S)-Rg3, 20(S)-Rg2, 3a and b were evaluated *In vitro* for its protective effect on cultured myocardiocytes with anoxia /reoxygen injury. The pharmaceutical data appeared that these four compounds had protective effect and 3a showed the most potent bioactivity among them.

Key words: Ocotillol-type derivative, synthesis, protective effect of cultured myocardiocytes, 20(S)-Rg2, 20(S)-Rg3.

INTRODUCTION

Panax ginseng is a traditional Chinese medicine which can treat many diseases, such as cardiovascular diseases and stroke. *P. ginseng* contains numbers of ginsenosides (Shibata et al., 1985; Seiji et al., 1995). Ocotillol is one of the main components of *Panax quinquefolium L* and has been proved to play a role in protective effect on myocardial ischemia injury. (Ma et al., 2005; Wang et al., 2007; Yu et al., 2007) In our previous study, many ocotillol-type derivatives of gensenosides had been synthesized and proved to have protective effect of myocardial ischemia. (Wang et al., 2010; Han et al., 2011; Bi et al., 2011) Now we design to synthesize the ocotillol-type derivatives of 20(S)-Rg2 and 20(S)-Rg3 to obtain the lead compounds with protective effect on cultured anoxia/reoxygen injury myocardiocytes. The lead compounds will further carry on the experiment of protective effect of myocardial ischemia *In vivo*.

*Corresponding author. E-mail: qinggmeng@163.com.

MATERIALS AND METHODS

General

Melting points were determined using a digitzing melting point apparatus (WRS-1B) and are reported directly. All of the compounds synthesized were purified by column chromatography (CC) on silica gel (200 to 300 mesh) and thin-layer chromatography (TLC) on silica gel GF254 plates (Yantai Chemical Industry Research Institute, China). Subsequently, they were routinely analyzed by ^{1}H-NMR (Bruker VANCE-400), MS (Applied Biosystoms Mariner spectrometer),

Synthesis of (3β, 12β)-12-acetyl-20-hydroxydammar- 24- en- 3-yl - 2-O-β-D-(3',4',6'-tri-acetyl)-glucopyranosyl-β-D-(2'',3'',4'',6''-tetra-acetyl)-glucopyranoside (1a)

Acetic anhydride (14.1 ml, 12.27 mmol) was added to a stirred solution of 20(S)-Rg3 (4.80 g, 6.12 mmol), *N*, *N*-dimethylamino-pyridine (0.32 g and 2.61 mmol) in pyridine (80 ml). The mixture was stirred for 24 h at room temperature. The solvent was removed in vacuous and the residue taken up in ethyl acetate and dilute hydrochloric acid. The organic phase was separated and washed with water and brine, dried over Na_2SO_4 and then concentrated to

Table 1. ^{13}C NMR date of compounds **1a** and **2a** (C_6D_5N, δ, ppm).

No	1a	2a
1	39.5	40.1
2	27.2	27.1
3	88.8	91.1
4	39.2	41.0
5	56.2	57.4
6	18.5	19.1
7	35.4	36.0
8	37.0	37.9
9	50.5	51.2
10	39.7	40.0
11	32.1	33.4
12	71.0	71.4
13	48.5	49.0
14	51.7	52.0
15	31.4	32.6
16	26.7	26.9
17	54.8	54.9
18	16.5	16.7
19	15.8	15.9
20	73.9	73.2
21	26.8	26.9
22	35.8	35.5
23	23.0	23.2
24	126.2	126.5
25	130.0	130.8
26	25.8	25.8
27	17.7	17.6
28	28.1	28.3
29	16.6	16.0
30	17.0	17.3
3-glc-1’	105.1	104.4
2’	83.5	81.0
3’	78.2	78.2
4’	71.7	71.8
5’	77.9	77.5
6’	62.6	62.7
2”- glc-1”	106.1	105.3
2”	83.5	76.2
3”	78.4	78.5
4”	71.6	71.8
5”	77.1	77.7
6”	62.8	63.0

yield a semi-solid. Flash chromatography (petroleum ether: ethyl acetate=1:1) to give the product as white solid (4.71 g, 70.1% yield, mp 222-223°C). ESI-MS, *m/z*: 1143.55 $[M + Na]^+$.

Synthesis of (3β, 6α, 12β)-3, 12-diacetyl-20-hydroxydammar-24- en- 6- yl -2-O-[(3’,4’,6’-tri-acetyl)-6-deoxy-α-L-mannopyranosyl]-β-D-(2”,3”,4”- tri-acetyl)-glucopyranoside (1b)

The prepared method was the same as compound 1a. Acetic anhydride (32.5 ml and 28.29 mmol), 20(S)-Rg2 (9.28 g, 11.82 mmol), *N*, *N*-dimethylamino- pyridine (0.64 g and 5.22 mmol), pyridine (150 ml). Yield: 8.91 g (67.7%) as a white solid, mp 185 to 186ºC. ESI-MS, *m/z*: 1117.53 $[M + H]^+$.

Synthesis of (3β, 12β)-12-acetyl-20S,24R-epoxy-dammar- 24-en- 3- yl - 2-O-β-D-(3’,4’,6’-tri-acetyl)-glucopyranosyl-β-D-(2”,3”,4”,6”-tetra-acetyl)-glucopyranoside (2a)

A solution of 1a (2.23 g and 1.99 mmol) in dichloromethane (20 ml) was cooled to -3°C. Then a solution of meta-chloroperbenzoic acid (*m*-CPBA) (0.82 g, 4.75 mmol) in dichloromethane (10 ml) was added drop wise and stirred for 1 h. The organic solution was washed with water and brine and dried over Na_2SO_4. The dichloromethane was evaporated in vacuo and yield a white solid. The residue was chromatographied over silica gel (petroleum ether: ethyl acetate= 1:1) and crystallized from ethyl acetate to get white powder (1.14 g, 51.1% yield and mp 191 to 192°C). ESI-MS, *m/z*: 1159.61$[M + Na]^+$.

Synthesis of (3β, 6α, 12β)-3, 12- diacetyl- 25- hydroxy- 20S, 24R- epoxy- dammar-24-en-6-yl-2-O-[(3’,4’,6’-tri-acetyl)-6-deoxy-α-L-mannopyranosyl]-β-D-(2”,3”,4”-tri-acetyl)-glucopyranoside (2b)

The prepared method was the same as compound 2a and 1b (7.11 g and 6.37 mmol) in dichloromethane (100 ml), *m*-CPBA (3.30 g and 19.13 mmol). Yield: 3.49 g (49.1%) as a white solid, mp 187 to 188°C. ESI-MS, *m/z*: 1159.57 $[M + Na]^+$.

Synthesis of (3β, 12β)-12, 25-dihydroxy-20S, 24R-epoxy-dammar-24-en-3-yl-2-O-β-D-glucopyranosyl-β-D-glucopyranoside (3a)

Sodium hydroxide (1.55 g and 38.75 mmol) was added to the solution of 2a (1.14 g and 1.00 mmol) in methanol (20 ml) and water (5 ml). The resulting mixture was stirred at room temperature for 2 h. The solvent was diluted with water (200 ml). After filtration, the solid was washed with water and dried in vacuo. The residue was chromatographied over silica gel (ethyl acetate) and crystallized from ethyl acetate-methanol to yield compound 3a as white solid (0.20 g, 17.5% yield, mp 185 to 186°C). ESI-MS, *m/z*: 801.2$[M + H]^+$, 823.2$[M + Na]^+$.

Synthesis of (3β, 6α, 12β)-3, 12, 25- trihydroxy- 20S, 24R-epoxy- dammar-24-en-6-yl-2-O-[6-deoxy-α-L-mannopyranosyl]-β-D-glucopyranoside (3b)

The prepared method was the same as compound 3a. Sodium hydroxide (2.52 g and 63.00 mmol), 2b (2.79 g and 2.46 mmol) in methanol (30 ml) and water (10 ml). Yield: 0.61 g (31.2%) as a white solid, mp 224 to 225°C. The ^{13}C NMR data of compound 1a and 2a were shown in Table 1. The H NMR data of compound 3a was shown in Table 2. The ^{13}C NMR data of compound 1b and 2b were shown in Table 3. The H NMR data of compound 3b was shown in Table 4.

Biological activity

H9C2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 5% equine serum. When the cultures reached 80% confluence, cells were sub-cultured using trypsin and were seeded in 96 well culture plates at a density of 8×10^3~1×10^4 cells/well (control group). To induce oxygen and glucose-deprivation, the culture media were replaced with glucose-free Earle’s solution 100 uL for 30 min, and then added sodium dithionite ($Na_2S_2O_4$) with final concentration of 2 mmol/L. After 1 h, $Na_2S_2O_4$ solution were removed (model group), and the compound of interest were added with the final concentration of

Table 2. The NMR data and 13C-1H correlation of **3a** (in C_6D_5N).

δc correlated proton					
No 20(S)-Rg3(s) **3a** *HSQC HMBC* **1H-1H COSY**					
1	39.3	39.2	1.52 (1e), 0.79 (1a)	19	1e, 2a
2	26.9	26.7	2.18 (2e), 1.82 (2a)		1a, 3, 2e
3	89.1	88.7	3.36 (dd, J= 4.4, 4.3 Hz)	3-glc-1,28,29	2a, 2e
4	39.9	40.0	0.74	28,29	
5	56.6	56.5	0.66(d, J=10.0 Hz)	28,29,19	6a
6	18.6	18.4	1.51(6e), 1.39(6a)		5,7a
7	35.4	35.2	1.37(7e), 1.24(7a)	18	6e, 6a
8	40.0	39.7		18,30	
9	50.6	50.7	1.42	18,19	11e
10	37.1	37.0		19	
11	32.2	32.1	2.01(11e), 1.39 (11a)	30	9,13,12
12	71.2	70.3	3.75 (m)		11a, 13
13	48.8	48.4	1.86 (m)	30	11,12,17
14	51.9	52.2	1.46	18,30	
15	31.5	32.6	1.51(15e), 1.09 (15a)	30	16
16	27.0	28.1	1.94(16e), 1.49 (16a)	17	17,15a
17	55.0	48.4	2.30(m)	21	13,16
18	16.0	15.5	0.97(s)	7	
19	16.5	16.5	0.83(s)		
20	73.1	86.7		21	
21	28.3	28.8	1.27(s)		
22	36.1	32.4	1.92(22e), 1.70 (22a)	21	23a, 23e
23	23.2	26.7	2.16(23e), 1.84(23a)	27	22a, 22e
24	126.5	88.8	4.16(dd, J =7.0, 7.0 Hz)	26,27	23a, 23e
25	130.9	70.3		26,27	
26	25.9	25.5	1.30 (s)		
27	17.2	28.7	1.45 (s)		
28	28.3	28.0	1.28 (s)	29,3	
29	16.8	15.5	1.11 (s)	28,5	
30	17.8	18.2	0.91 (s)		
3-glc-1’	105.3	105.1	4.95 (d, J =8.0 Hz)	3	3-glc-2’
2’	83.7	83.5	4.04	2”- glc-1”	3-glc-1’
3’	78.1	78.1	4.23		
4’	71.8	71.7	4.25		
5’	78.4	78.1	4.00		
6’	63.1	62.7	4.59,4.62		
2”- glc-1”	106.2	106.1	5.37 (d, J =7.5Hz)	3-glc-2’	2”- glc-2”
2”	77.3	77.1	4.16		2”- glc-1”
3”	78.5	78.2	4.32		
4”	71.9	71.6	4.35		
5”	78.2	78.0	3.94		
6”	62.9	62.9	4.50,4.49		

1 ug/mL for 24 h at 37°C in a humidified 5% CO_2 incubator (20(S)-Rg2, 20(S)-Rg3, 3a and b group). After 24 h, 25 µL precooling 50% trichloroacetic acid (TCA) (w/v) (final concentration of 10% TCA) was added into each well, and the mixtures was kept at 4°C for 1 h, then discarded the fixing solution was washed with deionized water for 5 times. After drying, 100 µL Solid Rocket Boosters (SRB) was added into each well to stain the cells. After 10 min of staining, discarded the staining solution, washed the wells with 1% acetic

Table 3. ^{13}C NMR date of compounds 1b and 2b (C_6D_5N,δ, ppm).

No	1b	2b
1	39.56	39.55
2	27.56	27.07
3	77.12	77.13
4	40.48	40.50
5	39.56	39.30
6	61.41	61.39
7	74.81	74.79
8	41.85	41.81
9	49.63	49.64
10	39.56	40.27
11	32.00	32.02
12	70.65	70.66
13	48.35	48.36
14	50.26	50.12
15	32.02	32.21
16	25.96	25.70
17	53.44	53.44
18	17.61	17.63
19	17.28	17.28
20	72.34	87.01
21	26.9	28.05
22	35.70	32.21
23	22.45	27.07
24	126.19	84.77
25	132.01	70.66
26	25.78	25.70
27	17.07	27.94
28	32.11	28.05
29	16.64	17.28
30	16.83	18.15
6-glc-1’	100.75	100.74
2’	76.31	76.28
3’	77.12	77.12
4’	73.15	73.13
5’	78.56	78.10
6’	63.51	63.50
2”-	98.34	98.32
Rha-1”	72.34	72.11
2”	71.30	71.30
3”	74.81	74.79
4”	69.42	69.64
5”	18.11	17.83
6”		

acid for 5 times and dried the samples in the air. At the last step, bound stain is solubilized with 150 uL Tris solution in each well so that the samples were ready for cell density measurement (OD 540) and calculate the cell viability (Zhu et al., 2009; Jian et al., 2009; Yu et al., 2010).

Cell Viability=OD/OD (control group)

RESULTS

Chemistry

Compounds 3a and b were prepared as shown in Scheme 1. 20(S)-Rg3 and 20(S)-Rg2 were converted to the intermediate 1a and b with *N*, *N*-dimethylaminopyridine and acetic anhydride in pyridine. Through epoxidation of 1a and b with m-CPBA, the compound 2a and b were obtained. Then 2a and b were degradated with potassium hydroxide to give compound 3a and b.

Biological activity

The synthesized compounds 3a and b were evaluated for their protective effect on cultured myocardiocytes in anoxia/reoxygen injury. The results reported in Figure 1

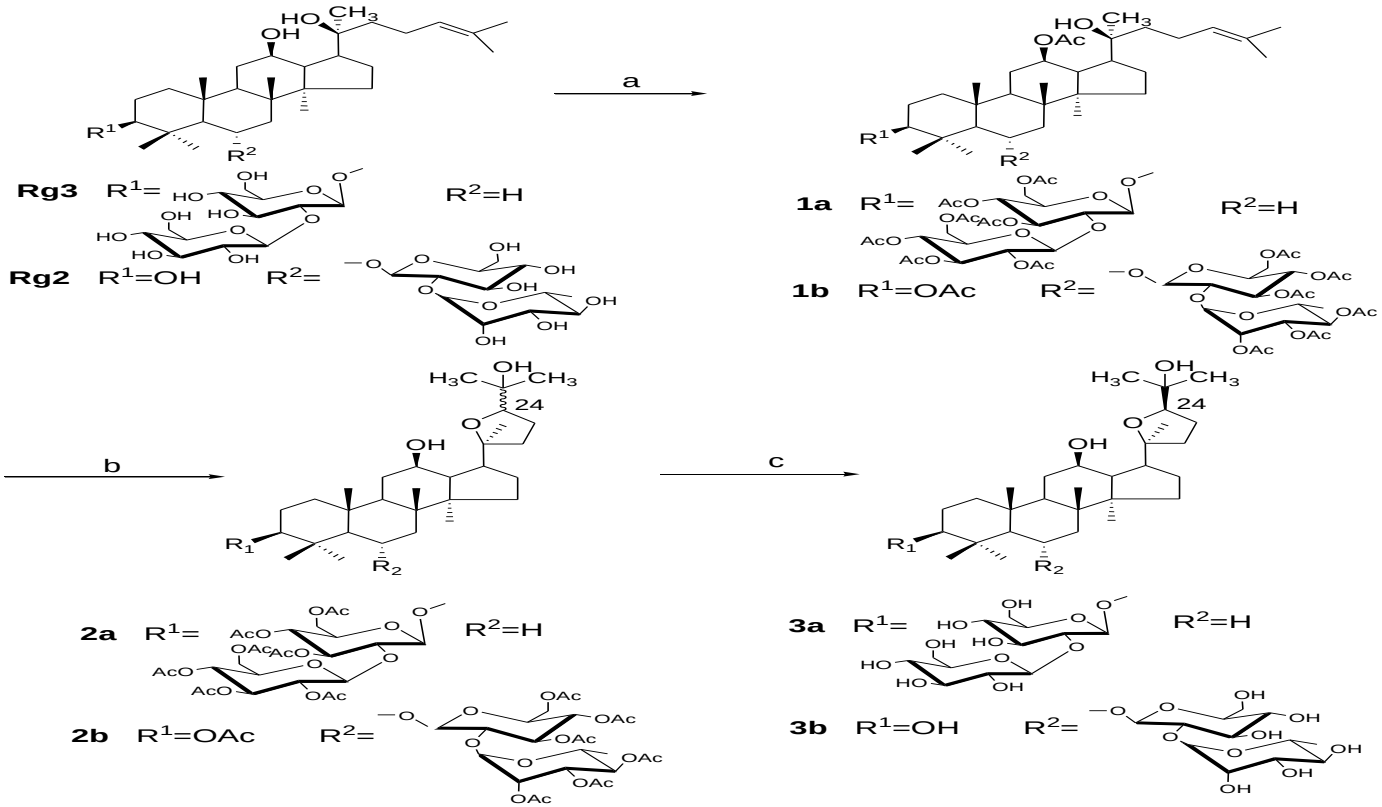
Table 4. The NMR data and 13C-1H correlation of 3b (in C_6D_5N).

δc Correlated proton				
No	20(S)-Rg2	3b	HSQC	HMBC
1	39.52	39.51	1.41 (m), 0.82(m)	19
2	27.59	27.74	1.70 (m), 1.82(m)	19
3	78.53	78.54	3.47(dd, J= 7.5, 11.3 Hz)	28, 29
4	39.91	40.00		28, 29
5	60.75	60.91	1.39 (d, J=11.0 Hz)	28, 29,19
6	74.33	74.30	4.66 (dd, J= 3.0, 9.5 Hz)	28, 29
7	45.97	45.99	1.98, 2.5	18
8	41.06	41.11		18, 30
9	49.68	49.42	1.54 (d,7.0)	18,19
10	39.28	39.30		19
11	31.20	31.69	1.93 (m),1.98(m)	18,19
12	70.96	69.43	3.69 (m)	
13	48.07	48.29	1.54 (m)	30
14	51.61	52.14	1.46	18, 30
15	31.94	32.42	1.26 (m), 1.60 (m)	18, 30
16	26.75	25.44	1.45 (m), 1.74 (m)	
17	54.58	50.10	2.08 (m)	21
18	17.63	17.81	1.00 (s)	
19	17.54	17.51	0.83 (s)	28, 29
20	72.49	86.69		21
21	26.90	26.92	1.27(s)	
22	35.72	32.74	1.92 (m), 1.06 (m)	21
23	22.91	28.75	2.16 (m), 1.84 (m)	26, 27
24	126.26	85.61	4.16 (dd, J =7.0, 7.0 Hz)	26, 27
25	130.76	70.27		26, 27
26	25.78	32.74	1.30 (s)	27
27	17.07	27.14	1.45 (s)	26
28	32.11	32.10	1.28 (s)	29,19
29	17.63	18.15	1.11 (s)	28
30	16.83	16.89	0.91 (s)	
6-glc-1’	101.87	101.92	4.95 (d, J =8.0 Hz)	
2’	78.31	78.32	4.04	
3’	79.30	79.30	4.26	
4’	72.96	72.66	4.25	
5’	78.31	78.37	4.00	
6’	63.00	63.16	4.56,4.39	
2”- rham-1”	101.73	101.78	6.51 (s)	
2”	72.34	72.42	4.79 (m)	
3”	72.20	72.28	4.69 (m)	
4”	74.07	74.19	4.32 (m)	
5”	69.42	71.13	4.97 (m)	
6”	18.68	18.71	1.79 (d,6.0)	

showed that 20(S)-Rg2, 20(S)-Rg3, 3a and b exhibited potent protective effect on cardiac muscle cells apoptosis. This effect of 3a was superior to 20(S)-Rg3 and the effect of 3b was weaker than 20(S)-Rg2.

DISCUSSION

Compound 3a and b had been designed and synthesized and had been proved to have protective effect on cultured



Scheme 1. synthetic route for the preparation of ocotillol-type derivatives. Reagents: a) $(CH_3CO)_2O$, DMAP. pyridine; b) *m*-CPBA, CH_2Cl_2; c) NaOH, CH_3OH, H_2O.

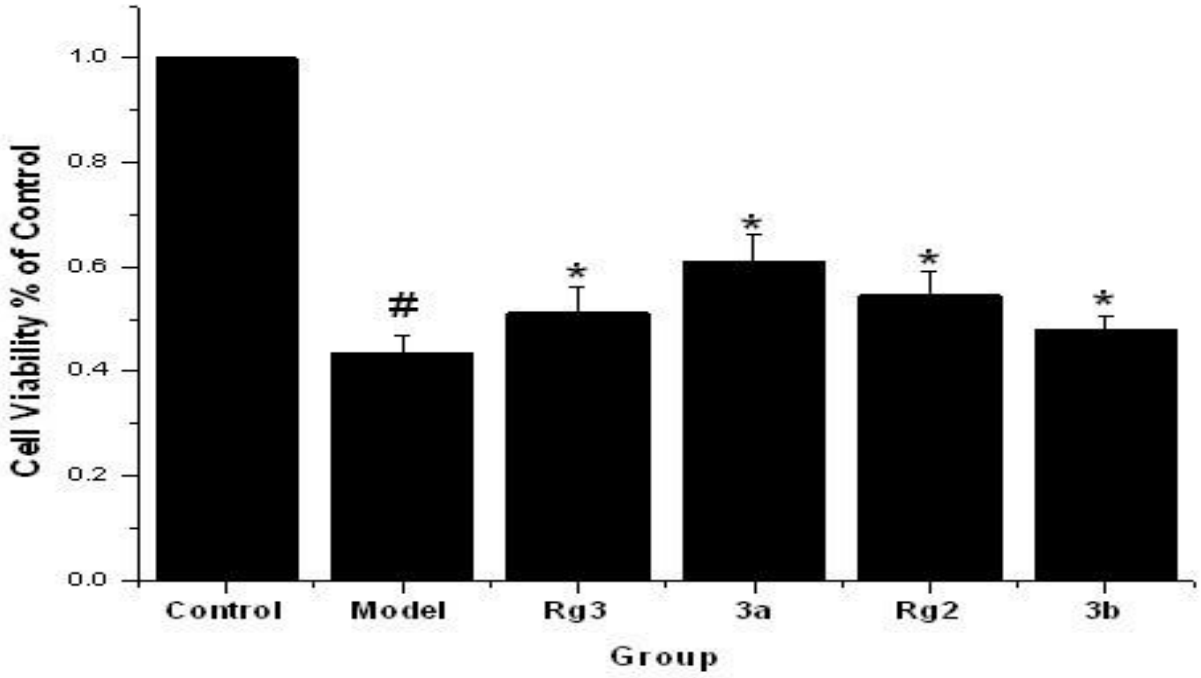


Figure 1. Protective activity of 20(S)-Rg2, 20(S)-Rg3, compounds **3a** and **3b** on cultured anoxia/reoxygen injury myocardiocytes. Data was expressed as the mean ± S.D. (n = 6 to 8), Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. # *p<0.01* compared with control group, **p<0.01* compared with model group.

myocardiocytes with anoxia/reoxygen injury. Compound 3a was the most potent derivative. Further design, synthesis and systemic acquired resistance (SAR) studies of ocotillol-type ginsenosides derivatives are ongoing in our laboratory and the results will be reported in due course.

ACKNOWLEDGEMENTS

The authors are grateful to National Natural Science Foundation of China (No. 81001358), A Project of Shandong Province Higher Educational Science and Technology Program (No. BS2010YY073), Scientific Research Foundation of the Higher Education Institutions of Shandong Province, China (No. J07WE26), Shandong Provincial Natural Science Foundation, China (No. Y2007C138) and Taishan Scholar Project for financial support.

REFERENCES

Bi Y, Tian JW, Wang L, Zhao FL, Zhang JF, Wang N, Sun Haijun, Meng QG (2011). Synthesis, structure determination and protective effects on cultured anoxia/ reoxygen injury myocardiocytes of ocotillol-type derivatives. J. Med. Plants Res., 5: 2424-2429.

Han B, Meng QG, Li Q, Zhang JF, Bi Y, Jiang NC (2011). Effect of 20 (S)-protopanaxatriol and its epimeric derivatives on myocardial injury induced by isoproterenol. Arzneimittelforschung, 61: 148-152.

Jian J, Liu X, Huang RB, Jiang WZ (2009). Protection and itsmechanism of two flavonemorphons from Yulangsan on hypoxia-reoxygenation induced injury inmyocardial cells. Chin. Pharmacol. Bull., 25: 942-945.

Ma SG, Jiang YT, Song SJ, Wang ZH, Bai J, Xu SX, Liu K (2005). Alkaline-degradation products of ginsenosides from leaves and stems of Panax quinquefolium. Acta Pharmaceutica Sinica., 40: 924-930.

Seiji F, Ryoji K, Kazuhiro O, Kazuo Y, Chiu MH, Nie RL, Osamu T (1995). Dammarane Glycosides from aerial part of Neoalsomitra Integrifoliola .Phytochemistry, 39: 591-602.

Shibata S, Tanaka L, Shoji L, Saito H (1985). Chemistry and pharmacology of panax. Econ. Med. Res., 1: 217-284.

Wang L, Wang YT, Xu SQ, Sun CH, Yan S, Liu JY, Wang YY, Hou W, Jin YP (2007). A review on studies of the components and pharmacological activity of Panax quinquefolium L. Special Wild Econ. Anim. Plant Res., 3: 73-77.

Wang T, Meng QG, Zhang JF, Bi Y, Jiang NC (2010). Study on the structure-function relationship of 20(S)-panaxadiol and its epimeric derivatives in myocardial injury induced by isoproterenol. Fitoterapia, 81: 783-787.

Yu C, Fu FH, Yu X, Han B, Zhu M (2007). Cardioprotective effect of ocotillol, a derivate of pseudoginsenoside F11 on myocardial injury induced by isoproterenol in rats. Arzneimittelforschung, 57: 568-572.

Yu W, Li L, Yao S, Wu JL (2010). Protective effects of garlic polysaccharide on cultured myocardiocytes in anoxia/ reoxygenation injury. Chin. Pharmacol. Bull., 26: 697-699.

Zhu XJ, Liang F, Wang XH, Zhao WM, Gao X (2009). Protective effect of COPP on hypoxia/reoxygenation injury of H9C2 myocytes. Chin. Pharmacol. Bull., 25: 352-356.