Bioorganic & Medicinal Chemistry Letters 24 (2014) 1334–1338

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of baicalein derivatives as potent antitumor agents

Rong Luo [a], Jubo Wang [a], Li Zhao [b], Na Lu [b], Qidong You [a], Qinglong Guo [b], Zhiyu Li [a,*]

[a] Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, People's Republic of China
[b] Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, People's Republic of China

ARTICLE INFO

Article history:
Received 1 November 2013
Revised 7 January 2014
Accepted 18 January 2014
Available online 27 January 2014

Keywords:
Flavanoid
Baicalein
Synthesis
Antitumor
Apoptosis

ABSTRACT

Baicalein (5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one), a major flavonoid extracted from the root of *Scutellaria baicalensis Georgi* (Chinese name: Huangqin), showed potent anti-proliferative activity against a broad panel of human cancer cell lines both in vitro and in vivo. A novel series of baicalein derivatives were synthesized by introducing a group to C6-OH and a nitrogen-containing hydrophilic heterocyclic ring to C7-OH via a length of 3 or 4-carbon chain in this study. The in vitro antiproliferative activities of the 30 derivatives against HepG2, A549, BCG-823 cancer cell lines were evaluated. Among them, 10 compounds exhibit more potent cytotoxicity than baicalein against the three cancer cell lines. The most potent compound **9b** possesses highest anti-proliferative potency against HepG2, A549, and BCG-823 with an IC_{50} value of 2.0 μM, 0.8 μM and 3.2 μM, respectively. Preliminary mechanism studies with compound **9b** using Annexin V/PI double-staining assay and DAPI staining assay indicated that **9b** inhibits tumor cell proliferation potentially through inducing apoptosis.

Flavonoids are a broad category of polyphenolic compounds that present ubiquitously in fruits, vegetables and beverages derived from plants such as tea and red wine. Flavonoids have been demonstrated to play an important role in human health benefits, including anti-atherosclerotic, anti-inflammatory, anticancer, antithrombogenic, antiviral and anti-osteoporotic.[1–6]

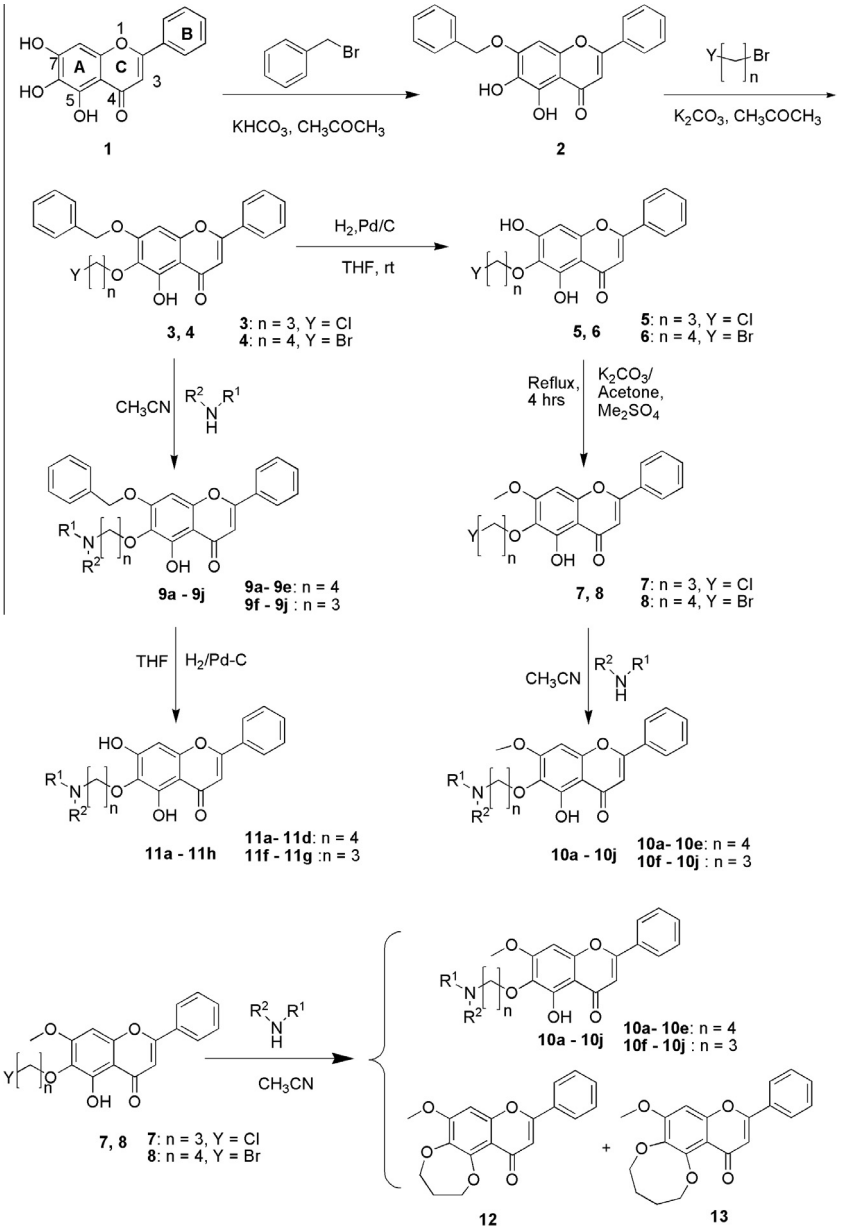
Baicalein (**1**), 5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one, is a major flavonoid from the root of a traditional Chinese herbal medicine: *Scutellaria baicalensis Georgi* (Chinese name: Huangqin).[7] It attracted lots of attention due to its cytotoxicity to various human cancer cell lines in vitro and inhibitory potency of tumor growth in vivo. Great efforts have been carried put into exploring the mechanism of the antitumor activity of **1** recent years. It has been reported that **1** induces proliferation inhibition in B16F10 melanoma cells by generating reactive oxygen species via 12-lipoxygenase.[8] Another study demonstrated that the pro-apoptotic effect of **1** in PaCa cells is mediated through reducing the expression of the pro-survival protein Mcl-1, which is highly expressed in PaCa cell lines.[9] It was shown that the anti-proliferative potency of **1** was due to cell cycle inhibition at G0/G1, also associated with suppression of cyclin D1 and D3 protein levels.[10] Chan et al. identified **1** as an agent preventing carcinogen–DNA adduct formation.[11] In addition, previous study also revealed that **1** inhibits the adhesion, proliferation, migration and invasive properties of human hepatoma cells.[12] All these results suggest that **1** possess potent antitumor activities both in vitro and in vivo and has great therapeutic potentials.

Unfortunately, some perceived disadvantages of **1** limits its clinical application. These include low aqueous solubility and poor oral bioavailability. It has been reported that two metabolites of **1** in human plasma after oral administration had been identified as baicalein 7-*O*-glucuronide (baicalin) and baicalein 6-*O*-sulfate,[13] and the metabolism would less likely happened at 5-OH position due to the intra-molecular hydrogen bond on this position. In view of these disadvantages of **1**, a nitrogen-containing hydrophilic heterocyclic ring was introduced to 6-OH via a certain length of carbon chain in order to block the metabolism of hydroxyl group of A ring. Meanwhile, methylation or benzylation of 7-OH was conducted to investigate the impact on the antitumor activity with different linking groups at 7-OH. 30 baicalein derivatives were synthesized and divided into three categories according to the linking groups at 7-OH.

The synthesis of baicalein derivatives was described in Scheme 1. Benzylation of **1** was conducted by reaction with benzyl bromide in the presence of $KHCO_3$ to afford intermediate **2**. A three or four-carbon linker was introduced to 6-OH to form intermediates **3** and **4**, which was subsequently reacted with several structurally diverse amines to provide compounds **9a**–**9j**. Debenzylation of compounds **9a**–**9j** gave rise to compounds **11a**–**11h**. Intermediates **3** and **4** were

* Corresponding author. Tel.: +86 13951678592.
E-mail address: zhiyuli@cpu.edu.cn (Z. Li).

http://dx.doi.org/10.1016/j.bmcl.2014.01.053
0960-894X/



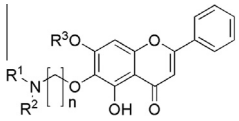
Scheme 1. Synthesis of baicalein derivatives.

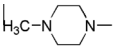
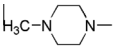
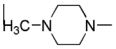
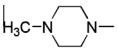
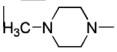
first debenzylated and then methylated at 7-position with dimethylsulfate to give the intermediates **7** and **8**, which then reacted with various amines to give the final compounds **10a**–**10j**. During the nucleophilic substitution reaction between intermediates **7**, **8** and amines, two by-products (compounds **12**, **13**) were observed, and the structures of which were identified by ^{1}H NMR and HRMS to be intramolecular substituted products (shown in Scheme 1). This side reaction could be avoided through synthesizing the nitrogen-containing side chain firstly.

The in vitro antiproliferative evaluation of all baicalein derivatives were carried out on three human cancer cell lines HepG2 (human liver carcinoma), A549 (adenocarcinomic human alveolar basal epithelial) and BCG-823(gastric carcinoma) by MTT assay with baicalein as a comparative control. All the compounds were treated over a range of concentrations from 0.1 to 100 μM for 48 h, and then the IC_{50} values were calculated, represents the concentration of a drug that is required for 50% inhibition in vitro.

From the antiproliferative data presented in Table 1, not only the length of carbon chain between the terminal heterocyclic substituent and baicalein scaffold but also the linking groups at the 5-position, 6-position and 7-position have pronounced effects on the biological activities of the molecules. Except for **9d** and **9i**, the antiproliferative activities of compounds **9a**–**9j** were particularly potent against all the three cell lines. Compound **9b** was found to be the most potent, with IC_{50} values of 2.0 μM (HepG2), 0.8 μM (A549) and 3.2 μM (BCG-823), approximately 6–16-fold more potent than baicalein. The substituent at 7-OH has great effect on its antitumor activity. The bulky benzyl group is the most preferred. For example, compounds **9a**–**9j** with a benzyl group at 7-OH were generally more active than compounds **10a**–**10j** with a methyl group. Introducing a hydrophilic hydroxyl group at 7-OH resulted in a rapid decrease in activity, for instance, compounds **11a**–**11g** were mostly inactive even at a relative high concentration of 100 μM. This result suggest that the properties of the substituents at 7-position is a crucial factor for the potency and that follows this trends: benzyloxy > methoxy ≫ hydroxyl. It may be attributed to the volume of the substituents. Among the derivatives with the 7-benzyloxy structure (**9a**–**9j**), the molecules with

Table 1
The structures and the in vitro antiproliferative activities of baicalein derivatives



Compound	*n*	R^3	R^1, R^2	Antitumor cell proliferation (IC_{50}, μM)		
				HepG2	A549	BCG-823
Baicalein	0	H	H	28.3 ± 2.4	13.0 ± 1.6	19.1 ± 2.0
9a	4	Bn–		2.2 ± 0.3	1.8 ± 0.1	4.2 ± 0.5
9b	4	Bn–		2.0 ± 0.2	0.8 ± 0.1	3.2 ± 0.4
9c	4	Bn–		2.4 ± 0.2	1.9 ± 0.3	3.0 ± 0.3
9d	4	Bn–		>100	>100	83.6 ± 4.4
9e	4	Bn–		2.5 ± 0.3	4.4 ± 0.6	7.2 ± 0.7
9f	3	Bn–		2.2 ± 0.4	2.6 ± 0.1	5.5 ± 0.5
9g	3	Bn–		4.2 ± 0.4	1.4 ± 0.2	>100
9h	3	Bn–		2.2 ± 0.3	3.10 ± 0.3	4.6 ± 0.5
9i	3	Bn–		75.8 ± 2.8	>100	51.4 ± 4.1
9j	3	Bn–		2.4 ± 0.3	1.3 ± 0.2	4.1 ± 0.5
10a	4	H_3C–		7.1 ± 0.3	35.0 ± 1.7	17.4 ± 2.3
10b	4	H_3C–		4.9 ± 0.6	13.0 ± 2.3	10.6 ± 1.7
10c	4	H_3C–		21.0 ± 3.3	34.0 ± 3.9	21.9 ± 2.9
10d	4	H_3C–		19.8 ± 2.8	25.0 ± 3.5	27.2 ± 2.1
10e	4	H_3C–		36.7 ± 2.7	32.0 ± 2.0	53.5 ± 6.7
10f	3	H_3C–		3.7 ± 0.2	4.9 ± 0.8	5.7 ± 0.9
10g	3	H_3C–		4.3 ± 0.3	6.0 ± 0.9	6.5 ± 0.8
10h	3	H_3C–		22.3 ± 2.8	17.0 ± 2.5	25.3 ± 3.9
10i	3	H_3C–		9.2 ± 0.8	14.0 ± 2.1	43.1 ± 7.0
10j	3	H_3C–		15.1 ± 1.6	18.0 ± 2.7	31.3 ± 4.1
11a	4	H		>100	51.0 ± 2.9	86.4 ± 3.7
11b	4	H		>100	75.0 ± 3.1	52.7 ± 2.8
11c	4	H		67.2 ± 8.5	>100	58.7 ± 8.0
11d	4	H		93.7 ± 6.3	>100	82.9 ± 3.4
11e	3	H		>100	>100	>100
11f	3	H		70.6 ± 8.8	>100	60.9 ± 2.1
11g	3	H		86.2 ± 5.7	>100	88.3 ± 6.4
12	3	H_3C–	–	69.0 ± 9.1	51.3 ± 3.1	>100
13	4	H_3C–	–	>100	>100	>100

The results are reported as mean value ± SEM for *n* = 3.

morpholinyl substitution at the terminal of 6-O-alkyl (**9d** and **9i**) exhibited much lower activities than that with *N*,*N*-diethylamino (**9a**), pyrrolidinyl (**9b** and **9g**), piperidyl (**9c** and **9h**), and *N*-methyl piperazinyl (**9e** and **9j**). A marked decreasing in potency is observed when morpholinyl is linking to 6-*O*-alkyl, which means the morpholinyl is a poor linking group at this position. The compounds containing a 4-carbon spacer between the terminal heterocyclic substitutes and baicalein scaffold seemed slightly more

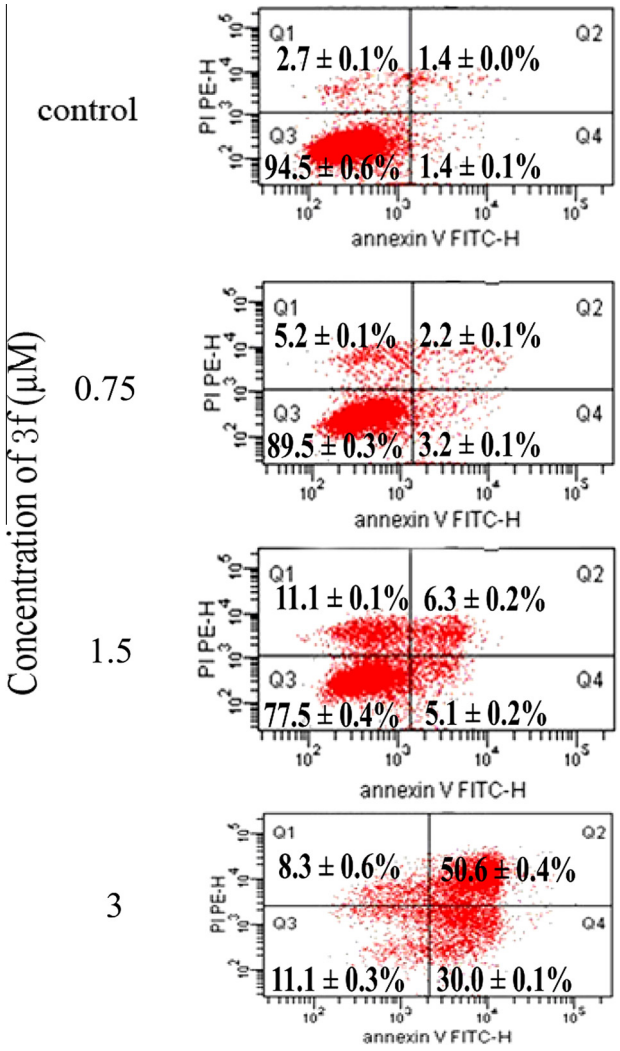


Figure 1. Compound **9b**-induced apoptosis in HepG2 cells. The cells were treated with **9b** for 72 h, and apoptosis was determined by Annexin V/PI double-staining assay.

potent than the others. This result probably indicates that the length of the linker between the terminal heterocyclic substitutes and baicalein scaffold could impact the activity as well and the 4-carbon length is optimal. Compounds (**12** and **13**) that formed through the intramolecular nucleophilic substitution between 5-OH and halogen displayed lower potency than baicalein. It revealed that the 5-phenolic hydroxyl is also crucial to the activity. In general, optimizing the structure of baicalein by introducing benzyl at 7-OH, linking amine (*N*,*N*-diethylamino, pyrrolidinyl, piperidyl or *N*-methyl piperazinyl) to 6-postion through a 4-carbon alkyl and remaining 5-OH unsubstituted proved to be able to enhance the cytotoxic activities of baicalein.

Based on our previous research, the antitumor effect of baicalein probably arose through apoptosis. In order to further characterize the potential mechanism of anticancer activities of baicalein derivatives, an apoptotic assay by flow cytometry with Annexin V and PI staining was carried out on compound **9b**, since **9b** showed the highest in vitro efficiency against HepG2 cell. Annexin V can identify the externalization of phosphatidylserine during the process of apoptosis, so it can be detected in both the early and late stages of apoptosis. PI enters the cell in late apoptosis or necrosis. Viable cells were negative for both annexin V and PI (lower left quadrant); early apoptotic cells were positive for annexin V and negative for PI (lower right quadrant); late apoptotic or necrotic cells displayed both positive annexin V and PI (upper right quadrant); non-viable cells which underwent necrosis were positive for PI and negative for annexin V (upper left quadrant).[14] As shown in Figure 1,following the treatment with 0.75, 1.5 and 3 μM of compound **9b** for 72 h in HepG2 cells, the percentage of early apoptotic cells (right low quadrant) increased (from 1.4% to 30.0%), and the percentage of late apoptotic and necrotic cells (right upper quadrant) were also increased (from 1.4% to 50.6%).

We could characterize apoptosis under a fluorescent microscope after staining the cell nucleus by DAPI because the apoptotic cell changes in morphology such as chromatin condensation and nuclear shrinking. In our experiments, HepG2 cells were incubated with 0.75 μM, 1.5 μM, 3.0 μM of compound **9b** and 0.2% DMSO as control for 72 h. As shown in Figure 2, the control cells emitted blue fluorescence with consistent intensity, indicating that the

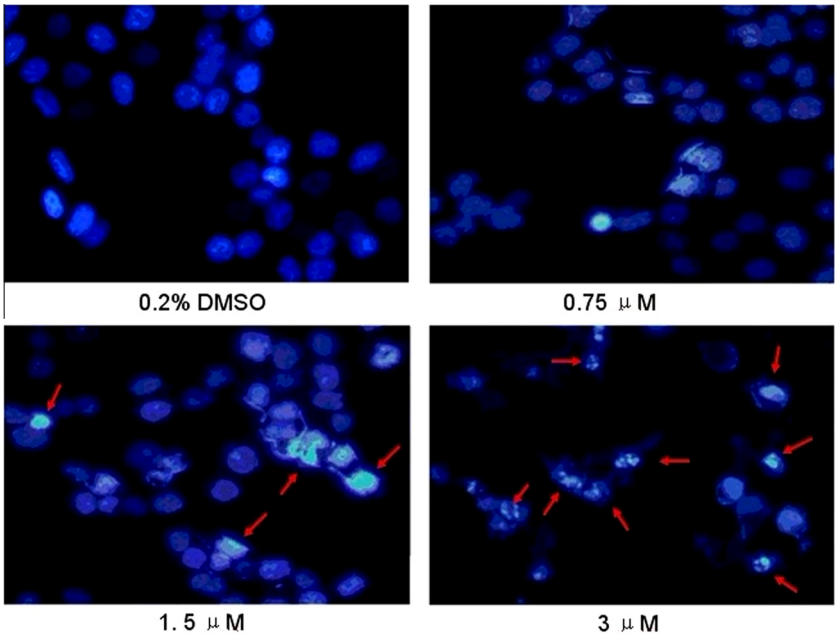


Figure 2. Fluorescent micrographs of DAPI staining (400×). The arrows indicate the apoptotic cells.

chromatin was equivalently distributed in the nuclei. The test group displayed chromatin congregated and karyopyknosis, which emitting bright fluorescence. These results indicated that compound **9b** is capable of inducing apoptosis in HepG2 cells.

In conclusion, we have synthesized 30 novel baicalein derivatives and evaluated their biological activities in cellular proliferation assay. Our work has led to the discovery of more potent compounds than baicalein. 8 compounds with the moiety of 7-benzyloxy group exhibited more potent anti-proliferation activities against three tumor cell lines than baicalein. Especially, compound **9b** was found to be 14-fold, 16-fold, and 6-fold more potent than baicalein against HepG2, A549 and BCG-823 cancer cell lines, respectively. The evidences of Annexin V/PI double-staining assay and DAPI staining assay on **9b** indicated that **9b** is a potential apoptosis-inducing agent. Further investigation about the cellular mechanism and other biological activities of the potent compounds are in progress.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Nos. 21072232 and 21372260).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.053. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Lampila, P.; van Lieshout, M.; Gremmen, B.; Lähteenmäki, L. *Food Res. Int.* **2009**, *42*, 122.
2. Hodgson, J. M.; Croft, K. D. *Mol. Aspects Med.* **2010**, *31*, 495.
3. Fang, F.; Li, J.-M.; Pan, Q.-H.; Huang, W.-D. *Food Chem.* **2007**, *101*, 428.
4. Galati, G.; O'Brien, P. J. *Free Radical Biol. Med.* **2004**, *37*, 287.
5. Steinberg, F. M.; Bearden, M. M.; Keen, C. L. *J. Am. Diet. Assoc.* **2003**, *103*, 215.
6. Peng, S.; Zhang, G.; Zhang, B. T.; Guo, B.; He, Y.; Bakker, A. J.; Pan, X.; Zhen, W.; Hung, L.; Qin, L.; Leung, W. N. *Bone* **2013**, *55*, 230.
7. Chou, D. S.; Hsiao, G.; Lai, Y. A.; Tsai, Y. J.; Sheu, J. R. *Free Radical Biol. Med.* **2009**, *46*, 1197.
8. Bednar, W.; Holzmann, K.; Marian, B. *Food Chem. Toxicol.* **2007**, *45*, 508.
9. Takahashi, H.; Chen, M. C.; Pham, H.; Angst, E.; King, J. C.; Park, J.; Brovman, E. Y.; Ishiguro, H.; Harris, D. M.; Reber, H. A.; Hines, O. J.; Gukovskaya, A. S.; Go, V. L.; Eibl, G. *Biochim. Biophys. Acta* **2011**, *1813*, 1465.
10. Pidgeon, G. P.; Kandouz, M.; Meram, A.; Honn, K. V. *Cancer Res.* **2002**, *62*, 2721.
11. Chan, H. Y.; Chen, Z. Y.; Tsang, D. S.; Leung, L. K. *Biomed. Pharmacother.* **2002**, *56*, 269.
12. Chiu, Y. W.; Lin, T. H.; Huang, W. S.; Teng, C. Y.; Liou, Y. S.; Kuo, W. H.; Lin, W. L.; Huang, H. I.; Tung, J. N.; Huang, C. Y.; Liu, J. Y.; Wang, W. H.; Hwang, J. M.; Kuo, H. C. *Toxicol. Appl. Pharmacol.* **2011**, *255*, 316.
13. Muto, R.; Motozuka, T.; Nakano, M.; Tatsumi, Y.; Sakamoto, F.; Kosaka, N. *Yakugaku Zasshi* **1998**, *118*, 79.
14. Wang, C. Z.; Li, X. L.; Wang, Q. F.; Mehendale, S. R.; Yuan, C. S. *Phytomedicine* **2010**, *17*, 63.

Web references

1. Lampila, P.; van Lieshout, M.; Gremmen, B.; Lähteenmäki, L. *Food Res. Int.* **2009**, *42*, 122. http://www.sciencedirect.com/science/article/pii/S096399690800183X.
2. Hodgson, J. M.; Croft, K. D. *Mol. Aspects Med.* **2010**, *31*, 495. http://qjmed.oxfordjournals.org/content/94/5/277.short.
3. Fang, F.; Li, J.-M.; Pan, Q.-H.; Huang, W.-D. *Food Chem.* **2007**, *101*, 428. http://www.sciencedirect.com/science/article/pii/S0308814606000045.
4. Galati, G.; O'Brien, P. J. *Free Radical Biol. Med.* **2004**, *37*, 287. http://www.sciencedirect.com/science/article/pii/S0891584904000346.
5. Steinberg, F. M.; Bearden, M. M.; Keen, C. L. *J. Am. Diet. Assoc.* **2003**, *103*, 215. http://www.sciencedirect.com/science/article/pii/S0002822302000329.
6. Peng, S.; Zhang, G.; Zhang, B. T.; Guo, B.; He, Y.; Bakker, A. J.; Pan, X.; Zhen, W.; Hung, L.; Qin, L.; Leung, W. N. *Bone* **2013**, *55*, 230. http://www.sciencedirect.com/science/article/pii/S8756328213000835.
7. Chou, D. S.; Hsiao, G.; Lai, Y. A.; Tsai, Y. J.; Sheu, J. R. *Free Radical Biol. Med.* **2009**, *46*, 1197. http://www.sciencedirect.com/science/article/pii/S0891584909000604.
8. Bednar, W.; Holzmann, K.; Marian, B. *Food Chem. Toxicol.* **2007**, *45*, 508. http://www.sciencedirect.com/science/article/pii/S0278691506002456.
9. Takahashi, H.; Chen, M. C.; Pham, H.; Angst, E.; King, J. C.; Park, J.; Brovman, E. Y.; Ishiguro, H.; Harris, D. M.; Reber, H. A.; Hines, O. J.; Gukovskaya, A. S.; Go, V. L.; Eibl, G. *Biochim. Biophys. Acta.* **2011**, *1813*, 1465. http://www.sciencedirect.com/science/article/pii/S0167488911001327.
10. Pidgeon, G. P.; Kandouz, M.; Meram, A.; Honn, K. V. *Cancer Res.* **2002**, *62*, 2721. http://cancerres.aacrjournals.org/content/62/9/2721.short.
11. Chan, H. Y.; Chen, Z. Y.; Tsang, D. S.; Leung, L. K. *Biomed. Pharmacother.* **2002**, *56*, 269. http://www.sciencedirect.com/science/article/pii/S0753332202001920.
12. Chiu, Y. W.; Lin, T. H.; Huang, W. S.; Teng, C. Y.; Liou, Y. S.; Kuo, W. H.; Lin, W. L.; Huang, H. I.; Tung, J. N.; Huang, C. Y.; Liu, J. Y.; Wang, W. H.; Hwang, J. M.; Kuo, H. C. *Toxicol. Appl. Pharmacol.* **2011**, *255*, 316. http://www.sciencedirect.com/science/article/pii/S0041008X11002687.
13. Muto, R.; Motozuka, T.; Nakano, M.; Tatsumi, Y.; Sakamoto, F.; Kosaka, N. *Yakugaku zasshi.* **1998**, *118*, 79. http://www.ncbi.nlm.nih.gov/pubmed/9549431.
14. Wang, C. Z.; Li, X. L.; Wang, Q. F.; Mehendale, S. R.; Yuan, C. S. *Phytomedicine.* **2010**, *17*, 63. http://www.sciencedirect.com/science/article/pii/S0944711309001810.