Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

In vitro antiproliferative activity of glabridin derivatives and their *in silico* target identification

Deepak Singh Kapkoti, Shilpi Singh, Sarfaraz Alam, Feroz Khan, Suaib Luqman & Rajendra Singh Bhakuni

To cite this article: Deepak Singh Kapkoti, Shilpi Singh, Sarfaraz Alam, Feroz Khan, Suaib Luqman & Rajendra Singh Bhakuni (2018): *In vitro* antiproliferative activity of glabridin derivatives and their *in silico* target identification, Natural Product Research, DOI: 10.1080/14786419.2018.1530228

To link to this article: https://doi.org/10.1080/14786419.2018.1530228

Published online: 22 Dec 2018.

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2018.1530228

Taylor & Francis
Taylor & Francis Group

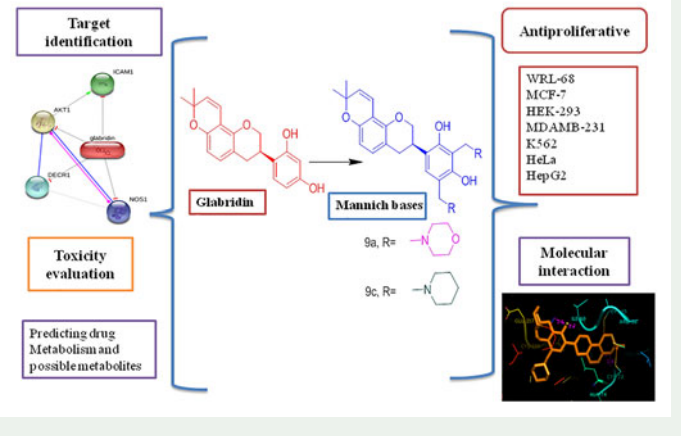
In vitro antiproliferative activity of glabridin derivatives and their *in silico* target identification

Deepak Singh Kapkoti[a], Shilpi Singh[b], Sarfaraz Alam[c], Feroz Khan[c], Suaib Luqman[b] and Rajendra Singh Bhakuni[a]

[a]Medicinal Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India; [b]Molecular Bioprospection Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India; [c]Metabolic and Structural Biology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India

ABSTRACT

Novel Mannich base derivatives of glabridin were synthesized and their antiproliferative activity were performed along with our previously reported glabridin-chalcone hybrids molecules (GCHMs) against various human cell lines MDA-MB-231 (breast adenocarcinoma), HEK-293 (embryonic kidney cell line), K562 (leukemia), MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma), HepG2 (hepatocellular carcinoma) and WRL-68 (hepatic carcinoma). The result showed that the glabridin significantly reduced cell proliferation with IC_{50} ranges from 3.67 to 58.30 μM against all the tested cell lines. The remarkable reduction in antiproliferative activity 2′,4′-dimethoxyglabridin and GCHMs compounds with phenolic OH groups protected by methoxy (OCH_3) groups suggested that the free OH groups are essential factor for the antiproliferative activity of glabridin and its derivatives. The Mannich base derivatives of glabridin showed moderate activity IC_{50} (2.20–>95.78 μM). Furthermore, *in silico* target identification analysis revealed that AKT1, DECR1 and NOS1 are the potential targets for glabridin and their derivatives.



ARTICLE HISTORY
Received 25 May 2018
Accepted 26 September 2018

KEYWORDS
Glabridin derivatives; anticancer; in silico target identification; docking; ADME

CONTACT Rajendra Singh Bhakuni bhakunirs2000@gmail.com; bhakunirs2000@yahoo.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1530228

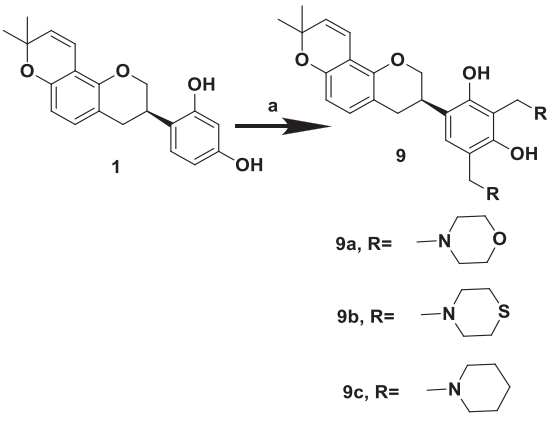
1. Introduction

Cancer is a group of diseases defined by the uncontrolled growth of the abnormal cells, their invasion into healthy cells and metastasis. It is the second leading cause of death worldwide after cardiovascular disease (GBD 2013 Mortality and Causes of Death Collaborators 2015). Although significant progress has been achieved in the field of anticancer chemotherapy but most of the drugs are in the market are toxic and having various side effects. Natural products are always being good source of lead molecules for anticancer drug discovery. It is estimated that during 1940s to 2012, out of the 175 small anticancer molecules, 131 or 74.8% are other than "S" (synthetic), in which 85 or 48.6% are actually either natural products or directly derived from them (Newman and Cragg 2012).

Glabridin is a major isoflavan isolated from the roots of *Glycrrhiza glabra* plant possesses diverse biological activities including antioxidant, antimicrobial, anti-inflammatory, estrogenic, anticancer, anti-osteoporoticetc, anti-neuroprotective (Saitoh et al. 1976; Mitscher et al. 1980; Belinky et al. 1998; Tamir et al. 2000; Fukai et al. 2002; Choi 2005; Kwon et al. 2008; Yu et al. 2008; Simmler et al. 2013). Previously glabridin has been reported to inhibit lung and breast cancer metastasis by inhibiting the Focal Adhesion Kinase (FAK)/rho signaling pathway (Hsu et al. 2011; Tsai et al. 2011). Glabridin also significantly inhibits the migration and invasion by transcriptionally inhibiting the matrix metalloproteinase 9 *via* the modulation of NF-κB and AP-1 activity in human liver cancer cells (Hsieh et al. 2014). Furthermore, it also mediates caspase activation and induces apoptosis in human promyelocytic leukemia cells (Huang et al. 2014). Introduction of aminoalkyl group into molecules *via* Mannich reaction helps to improve the solubility of the molecules hence aid to increase the bioavailability of molecules. Therefore, the Mannich base derivatives of glabridin were synthesized and evaluated for their *in vitro* antiproliferative activity. Furthermore the role of free phenolic OH groups in antiproliferative activity, we have also screened our previously reported glabridin-chalcones hybrids molecules along with 2′,4′-dimethoxyglabridin against the different human cancer cell lines (Kapkoti et al. 2016). The *in silico* target identification, molecular docking and ADMET studies have also been performed.

2. Results and discussion

Mannich reactions discovered by Carl Mannich, are the multicomponent, carbon-carbon bond forming reactions. The classical Mannich reaction involves the condensation of enolisable carbonyl compounds (aldehyde and ketones) having α-CH acidic proton/s, a primary or secondary amines and non enolisation aldehyde such as formaldehyde to form β-amino carbonyl compounds, commonly known as Mannich bases. Compounds with activated aromatic systems such as phenols, aromatic amines and electron rich heterocyclic compounds (pyrrol, furan and thiophene) also give the Mannich reaction. The Classical variant of Mannich reaction has some drawback such as formation of unwanted side-products, lack of regio- and stereo-selectivity, especially in case of primary amines. The modern variant of Mannich reaction overcomes these drawbacks by the use of preformed mannich reagents, enolates of carbonyl



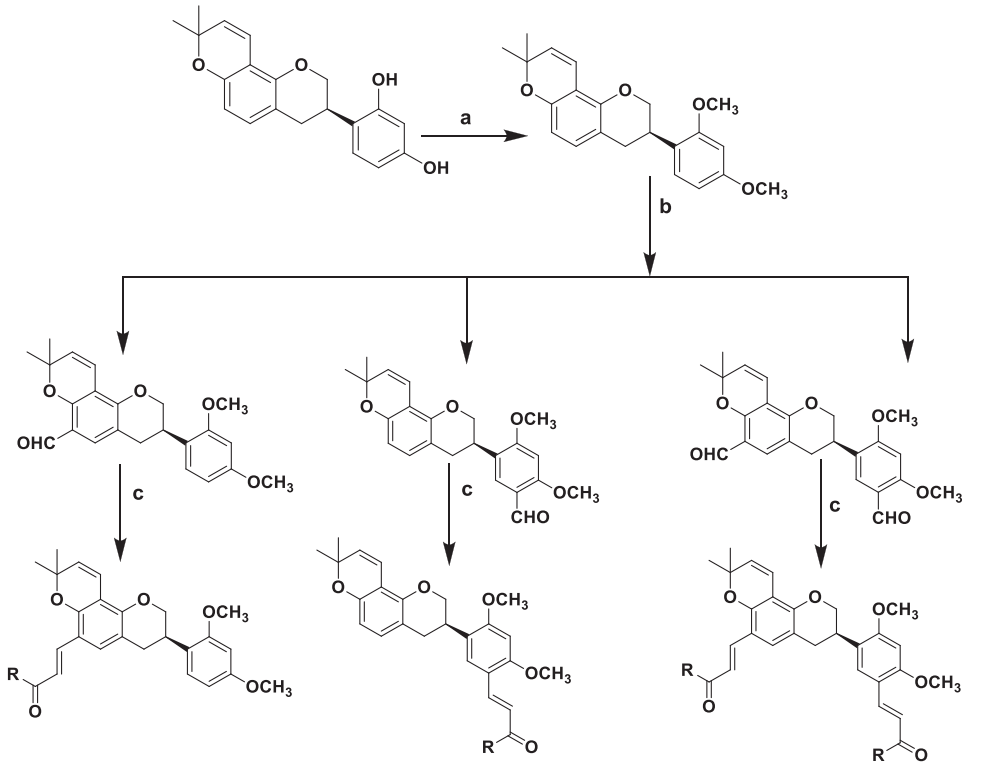
Scheme 1. Reagents and conditions: (a) HCHO, secondary amines in methanol, reflux 4 hrs.

compounds, silyl enol ethers, enolizable imines and catalysts which provide the high concentration of electrophile, activation of imines intermediate so the reaction occurred under mild condition without forming side products (Arend et al. 1998).

The catalyst generally used are the lewis acids such as Zn, ZnO, $InCl_3$, $FeCl_3$, $FePO_4$, $Bi(OTf)_3.4H_2O$, $Bi(NO_3)_3$ $Ag(OTF)_3$ etc. Enantioselective Mannich recation have been performed in the presence of chiral organocatalsyts such as *S*-proline, chiral pyrrolidine and cinchona alkaloids. Mannich reactions were also reported under both the catalysis and solvent free environment. These reactions are also performed under microwave and infrared irradiation and in ionic liquids (Filho et al. 2017).

Synthesis of mannich base derivatives of glabridin is depicted in Scheme 1. Mannich bases were synthesized by the condensation of glabridin with formaldehyde and secondary amines. The structures of final products were confirmed by their spectroscopic analysis (mass, ^{1}H and 13C NMR). Synthesis of glabridin-chalcone hybrid molecules are outline in Scheme 2 (Kapkoti et al. 2016)]. The structures of synthesized Mannich base derivatives of glabridin were assigned with the help of NMR and HRMS spectroscopy. HRMS spectrum of compound **9a** showed $[M+H]^+$ ion peak at 523.2806, suggested the molecular formula $C_{30}H_{38}N_2O_6$. Compound **9b** and **9c** showed $[M+H]^+$ ion peak at 555.2357 and 519.3210 respectively, assigned the molecular formula $C_{30}H_{38}N_2O_4S_2$ for **9b** and $C_{32}H_{42}N_2O$ for **9c**. The glabridin framework of synthesized derivatives showed similar NMR spectral value as reported for parent molecule, glabridin (Jirawattanapong et al. 2009). The two benzylic methylene (CH_2) groups gave singlets at ~3.9 ppm and ~3.6 ppm in ^{1}H NMR and showed peak at ~61.0 ppm and ~53.0 ppm in 13C NMR which was confirmed as CH_2's by DEPT-135. The nitrogen heterocyclic ring of morpholine and thiomorphline showed eight methylene groups peak and ten methylene groups peak for piperidine, confirmed by its ^{1}H, ^{13}C and DEPT-135 NMR spectra.

The antiproliferative activity of synthesized compounds were tested by MTT assay against various cell lines MDA-MB-231 (human breast adenocarcinoma), HEK-293 (human embryonic kidney cell line), K562-(Human erythromyeloblastoid leukemia), MCF-7 (human breast adenocarcinoma), HeLa (human cervix adenocarcinoma), HepG2 (human hepatocellular carcinoma) and WRL-68 (human hepatic carcinoma).



Scheme 2. Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, reflux 4 hrs (b) DMF, $POCl_3$ in acetonitrile, rt, overnight (c) $RCOCH_3$, KOH in MeOH, rt, 4–8 hrs.

Doxorubicin was used as reference compound. Compound **2**, 2′,4′-dimethoxyglabridin inhibited the proliferation of cancer cell lines up to 35.76% except hepatic and breast cancer cell lines whereas their substituted formyl derivatives (Compound **3**, **4** and **5**) inhibited the proliferation up to 48.04% excluding hepatic cell line. The glabridin-chalcone hybrid molecules were found to be moderately active with percent inhibition ranged from 2.32 to 48.81%. Compound **8f** showed maximum cytotoxicity (48.81%) at the higher tested concentration (50 μg/mL) against HepG2 (Table S1). This might be due to the absence of free OH groups, as parent molecule (glabridin) having free phenolic OH groups, inhibited the proliferation of cancer cells with IC_{50} values from 3.67 to 58.30 μM. The antiproliferative assay revealed that the Mannich bases of glabridin (**9a**–**9c**) inhibited the proliferation of the cancer cell lines. Among three compounds, **9a** and **9c** inhibited the proliferation of the cancer cell lines with an IC_{50} ranged from 16.41 to >95.78 μM except cervical cell line non-significantly in comparison to doxorubicin (Figure S1, Table 1) whereas both the compounds also inhibited the growth of normal kidney cell line (IC_{50} 2.24 μM and 2.20 μM respectively) superior to glabridin (IC_{50} 13.88 μM) and doxorubicin (IC_{50} 7.18μM). Compound **9b** showed a very weak antiproliferative activity with cytotoxicity ranges from 4.53 to 31.44% at concentration of 50 μg/mL. *In silico* analysis was performed in order to understand the molecular interaction of active compounds with targeted enzyme succinate dehydrogenase (SDH), responsible for cell viability measurement. The outcomes of docking

Table 1. IC_{50} value (μM) of compounds in selected cancer cell lines.

Compound	WRL-68	MCF-7	HEK-293	MDAMB-231	K562	HeLa	HepG2
9a	81.16 ± 1.28	73.67 ± 0.80	2.24 ± 1.74	>95.78 ± 0.97	47.79 ± 0.54	–	16.41 ± 1.89
9c	70.40 ± 1.64	55.34 ± 1.49	2.20 ± 2.54	86.19 ± 1.98	72.70 ± 1.64	–	47.50 ± 3.97
Glabridin	24.90 ± 1.88	58.30 ± 1.15	13.88 ± 0.74	42.31 ± 1.46	36.51 ± 0.62	3.67 ± 0.73	7.22 ± 1.32
Doxorubicin	4.84 ± 0.43	10.73 ± 0.32	7.18 ± 1.80	5.13 ± 0.91	12.00 ± 1.62	6.81 ± 0.79	2.22 ± 0.52

experiment expressed that the compound **9a** and **9c** were found to be least interactive with SDH with high binding energy (Table S2) whereas parent compound, glabridin showed strong binding energy (−7.07 kcal/moL) and low ki (6.61μM) as compared to malonate. Figure S2 depicted that parent compound; glabridin interacted with the active site residues CYS163, ILE159, ALA 162 and CYS 161 as compared to malonate. The interacting residues revealed the specific binding of glabridin towards SDH while compounds **9a** and **9c** did not interact with the active site residues. Therefore, it can be interrupted that inhibitory action of glabridin against SDH might be responsible for its antiproliferative behavior.

2.1. Drug target selection

There were four possible targets identified namely, AKT1, ICAM1, DECR1, and NOS1 through the STITCH 4.0 program with confidence limits of 0.71 and above (Figure S3a) which means strong association with glabridin (reference parent compound). Through STITCH 4.0, the possible protein targets for the active derivatives were also analyzed and it was found that AKT1, NOS1, ICAM1 interacted with score value 0.80, while DECR1 interacted with score value 0.71. These interacted proteins are known anticancer target. The score close to 1 was considered as a more appropriate target (Table S3).

2.2. In silico *molecular docking studies*

To better understand the ligand protein interaction and prospects of binding pattern, binding confirmations and interactive amino acid residues, the molecular docking study was performed. For docking study the protein targets are used as identified in Figure S3. Out of four different targets, docking study showed better score with three targets i.e., AKT1, DECR1 and NOS1. The docking scores of studied compounds were compared with control compound and presented in Figure S3b. The docking program produces several poses with different orientations and confirmations within the defined active site. All poses produce a different LibDock score. Higher the LibDock docking scores indicates higher the chance of ligand protein interactions. The best score was taken into account for further study. The docking results are shown in Table S4. The analysis of the protein ligand docked complexes revealed binding site residues, hydrogen bonds and docking scores. In Figure S4, best conformations and superimpositions of candidate compounds were showed along with control compound, in the binding site pocket of putative anticancer drug targets, *i.e.*, NOS1 (A), AKT1 (B), and DECR1 (C). The compounds are represented by different color such as Compound-**9a** (blue color), compound-**9c** (red color), glabridin (green color) and

control compound (yellow color). These results indicate that the compounds are bound well within the binding site pocket of putative anticancer drug targets and showed an almost similar binding pattern which are close to control compound.

2.3. In silico *ADME and toxicity studies*

The prediction of the human pharmacokinetics, bioavailabilty and toxicity screening of new chemical entities is critical during early drug development stages to determine the suitable dosing regimen. For this ADMET Predictor™ (Simulation Plus Inc., USA) was used to predict the different ADMET risk parameters and to make a comparison with the control compound glabridin. The ADMET risk was calculated for four major properties .i.e., Absorption risk, Risk with Cytochrome P450 oxidation, Risk of mutagenicity and predicted toxicity. These different properties are collectively provided in terms of risk and assessable in the form of a score with defined range limit. The candidate compound **9a** and **9c** has a molecular weight of 524.66 and 520.72, respectively, and thus violates the Lipinski rule by single molecular property.

Result showed that both the compounds have a mild absorption rate due to their size, hydrophilicity and charge, and also tend to show hepatotoxicity, and acute rat toxicity. Compound **9c** is more lipophilic in nature. Additionally compound **9a** have also the chance of mutagenicity when used for long period. The compound **9c** found to be the inhibitor of hERG which is generally considered as an anti-target. The studied compounds showed predicted toxicity of serum glutamic oxaloacetic transaminase (SGOT). High clearance rate as well as high tendency to cross the blood brain barrier was observed for both the studied compounds. Both compounds are predicted to be an inhibitor of P-glycoprotein (P-gp), a drug efflux pump and thus may not be efflux out easily. The calculated volume of distribution for compound **9a** and **9c** was 1.42 and 1.92 L/Kg, respectively. The percent unbound to blood plasma was found to be 5.41 and 5.73 for compound **9a** and **9c**, respectively. On the other hand, the blood to plasma concentration was detected as 0.98 and 0.93 for compound **9a** and **9c**, respectively. Finally studied compounds were found to be in the acceptable range of ADMET risk (Table 6). Both compounds showed no sign of reproductive and skin sensitization toxicity. The study of different enzymes, associated with human liver was also carried out, so that to check the status of adverse effects caused by the studied compounds. The results for different toxicity studies are summarized in Table S6.

2.4. *Predicting drug metabolism and possible metabolites*

Drug metabolism can produce metabolites with physicochemical and pharmacological properties that differ substantially from those of the parent drug, and consequently have important implications for both drug safety and efficacy. To tackle this, we here predict the possible metabolites and their sites of metabolism. The different metabolites as well as the sites of metabolism are provided in Figure S1. The cytochrome enzymes involved in the metabolism were CYP 2D6 and CYP 3A4. A schematic diagram which shows different metabolites are represented in Figure S5. Potency to be a substrate of Uridine 5′-diphospho-glucuronosyl transferase has also been

identified for each of the compounds, which have the potential to transform the small molecules to water soluble form.

3. Experimental

For general experimental procedures of extraction and isolation of glabridin, preparation of Mannich base derivatives, antiproliferative activity, *in silico*, see supplementary file.

4. Conclusions

In conclusion, we have prepared several derivatives of glabridin and evaluated their anticancer activity against different human cancer cell lines. Drastic decrease in antiproliferative activity of GCHMs derivatives pointed out the importance of free phenolic OH groups in their antiproliferative activity. Moderate activity of Mannich bases as compared to parent molecule (glabridin) suggested that either the introduction of aminoalkyl group on glabridin framework reduce its activity or there may be some possibility of the intramolecular hydrogen bonding between the nitrogen atom of amino group with hydrogen of phenolic OH group, so that phenolic groups are not free in mannich base which might be essential for its antiproliferative activity. The *in silico* studies revealed that AKT1, DECR1 and NOS1 are the potential targets for glabridin and their derivatives. So, it can be concluded that the glabridin is promising template for the development of potential leads for antiproliferative activity and can be further exploited by the preparation of derivatives having free phenolic OH groups.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

The authors are grateful to the Director, CSIR-CIMAP for providing necessary facilities for this work. Financial support from the National Medicinal Plant Board (NMPB, GAP-208), New Delhi, is duly acknowledged. D.S. Kapkoti is thankful to CSIR New Delhi for award of CSIR-JRF & SRF fellowship.

ORCID

Sarfaraz Alam http://orcid.org/0000-0002-7246-8687
Rajendra Singh Bhakuni http://orcid.org/0000-0002-1423-8461

References

Arend M, Westermann B, Risch N. 1998. Modern variants of the Mannich reaction. Angew Chem Int Ed Engl. 37(8):1044–1070.

Belinky PA, Aviram M, Fuhrman B, Rosenblat M, Vaya J. 1998. The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. Atherosclerosis. 137(1):49–61.

Choi E-M. 2005. The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. Biochem Pharmacol. 70(3):363–368.

Filho JFA, Lemos BC, de Souza AS, Pinheiro S, Greco SJ. 2017. Multicomponent Mannich reactions: general aspects, methodologies and applications. Tetrahedron. 73(50):6977–7004.

Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T. 2002. Antimicrobial activity of licorice flavonoids against methicillin-resistant Staphylococcus aureus. Fitoterapia. 73(6):536–539.

GBD 2013 Mortality and Causes of Death Collaborators. 2015. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Lond Engl. 385:117–171.

Hsieh M-J, Lin C-W, Yang S-F, Chen M-K, Chiou H-L. 2014. Glabridin inhibits migration and invasion by transcriptional inhibition of matrix metalloproteinase 9 through modulation of NF-κB and AP-1 activity in human liver cancer cells. Br J Pharmacol. 171(12):3037–3050.

Hsu Y-L, Wu L-Y, Hou M-F, Tsai E-M, Lee J-N, Liang H-L, Jong Y-J, Hung C-H, Kuo P-L. 2011. Glabridin, an isoflavan from licorice root, inhibits migration, invasion and angiogenesis of MDA-MB-231 human breast adenocarcinoma cells by inhibiting focal adhesion kinase/Rho signaling pathway. Mol Nutr Food Res. 55(2):318–327.

Huang H-L, Hsieh M-J, Chien M-H, Chen H-Y, Yang S-F, Hsiao P-C. 2014. Glabridin mediate caspases activation and induces apoptosis through JNK1/2 and p38 MAPK pathway in human promyelocytic leukemia cells. Ho Y-S, editor. PLoS One. 9(6):e98943.

Jirawattanapong W, Saifah E, Patarapanich C. 2009. Synthesis of glabridin derivatives as tyrosinase inhibitors. Arch Pharm Res. 32(5):647–654.

Kapkoti DS, Gupta VK, Darokar MP, Bhakuni RS. 2016. Glabridin-chalcone hybrid molecules: drug resistance reversal agent against clinical isolates of methicillin-resistant *Staphylococcus aureus*. Med Chem Commun. 7(4):693–705.

Kwon H-S, Oh S-M, Kim J-K. 2008. Glabridin, a functional compound of liquorice, attenuates colonic inflammation in mice with dextran sulphate sodium-induced colitis. Clin Exp Immunol. 151(1):165–173.

Mitscher LA, Park YH, Clark D, Beal JL. 1980. Antimicrobial agents from higher plants. Antimicrobial isoflavanoids and related substances from *Glycyrrhiza glabra* L. var. typica. J Nat Prod. 43(2):259–269.

Newman DJ, Cragg GM. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 75(3):311–335.

Saitoh T, Kinoshita T, Shibata S. 1976. New isoflavan and flavanone from licorice root. Chem Pharm Bull. 24(4):752–755.

Simmler C, Pauli GF, Chen S-N. 2013. Phytochemistry and biological properties of glabridin. Fitoterapia. 90:160–184.

Tamir S, Eizenberg M, Somjen D, Stern N, Shelach R, Kaye A, Vaya J. 2000. Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells. Cancer Res. 60(20):5704–5709.

Tsai Y-M, Yang C-J, Hsu Y-L, Wu L-Y, Tsai Y-C, Hung J-Y, Lien C-T, Huang M-S, Kuo P-L. 2011. Glabridin inhibits migration, invasion, and angiogenesis of human non-small cell lung cancer A549 cells by inhibiting the FAK/rho signaling pathway. Integr Cancer Ther. 10(4):341–349.

Yu X-Q, Xue CC, Zhou Z-W, Li C-G, Du Y-M, Liang J, Zhou S-F. 2008. In vitro and in vivo neuroprotective effect and mechanisms of glabridin, a major active isoflavan from *Glycyrrhiza glabra* (licorice). Life Sci. 82(1–2):68–78.