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Synthesis, evaluation and quantitative structure–activity relationship (QSAR) analysis of Wogonin derivatives as cytotoxic agents

Jinlei Bian [1], Tinghan Li [1], Tianwei Weng, Jubo Wang, Yu Chen, Zhiyu Li *

Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

A novel series of 49 wogonin derivatives were synthesized by introducing group at 7-, 8- or B ring of wogonin. The cytotoxic activities against HepG2, A549 and BCG-823 cancer cell lines were also investigated *in vitro*. Several of them showed obvious cytotoxic activities and compound **3h** possessed the highest potency against HepG2, A549, and BCG-823 with IC_{50} values of 1.07 μM, 1.74 μM and 0.98 μM, respectively. A quantitative structure-activity relationship (QSAR) study of these synthetic derivatives as well as wogonin indicated that high solubility and low octanol/water partition coefficient are favorable, and excessive electrostatic properties and refractivity are unfavorable for the cytotoxic activities of these wogonin derivatives. These findings and results provide a base for further investigations.

Cancer has been the second common cause of human death in the developed world.[1,2] Natural products have long been a source of antitumor drugs and new natural products are reasonable to be expected as promising leads for antitumor drug discovery.[1,3,4] Among natural products, flavonoid is a group of polyphenolic compounds present in plants and they show diverse biological activities such as anti-inflammatory, neuroprotection and antitumor activity.[4,5] Epidemiological studies have been shown that the flavones derived from *Scutellaria* possessed cytostatic and cytotoxic activities against many human cancer cell lines with little to no toxicity to normal cells.[5,6] As one of the representative flavonoid, the natural product wogonin (5,7-dihydroxy-8-methoxyflavone, **1**) and its derivatives have been recently reported to possess inhibitory activities against cancer.[7] Great efforts have been devoted to explore the mechanism of its antitumor activity and many results suggested that **1** possessed potent antitumor activities both *in vitro* and *in vivo*.[7–9] However, little is known about their structure-activity relationship (SAR), by far.

Recently, our laboratory has been committed to develop derivatives of wogonin as cytotoxic drugs, and forty-nine wogonin derivatives have been designed and synthesized. In this work, we mainly reported part of our work which focused on investigating the substitutions at 7-, 8-position and B ring (Fig. 1), and compounds investigated on other position would be reported in our further research. The cytotoxic activities of all these compounds were also evaluated. For a detailed understanding of SAR of these compounds and thus guiding our ongoing research to further develop potent cytotoxic wogonin derivatives, we also carried the quantitative structure-activity relationship (QSAR) studies. QSAR is one of the main research fields in drug design and the aim of it is to develop some quantitative models to predict activity of a compound which can help to reduce the research time of new drugs.[10–13] Many biological activity correlates greatly with the structures and conformations of the compounds. QSAR model used molecular descriptors to represent the characteristics of one molecule and highly correlated to the activity.[13]

The synthesis of wogonin derivatives was described in Schemes 1–3. First, 7-OH substituted derivatives (**3a-3r**) was outlined in Scheme 1. Starting from wogonin (**1**), a three or four-carbon linker was introduced to 7-OH to form the intermediate **2**, which was subsequently reacted with several structurally diverse amines to provide compounds **3a-3o**. Compounds **3p-3r** were obtained directly from **1** with corresponding chloroheterocycle by reflux in acetonitrile.

Scheme 2 described the synthesis of 8-OH substituted derivatives (**10a-10v**). These compounds were synthesized in six steps from chrysin (**4**). Tetramethylammonium hydroxide reacted with **4** resulted in the intermediate **5** in 30% yield. Then benzylation of **5** was conducted by reaction with benzyl bromide in the presence of potassium carbonate to afford intermediate **6**. Compound **7** was obtained by acidified **6** in the presence of hydrochloric acid. Followed by introduced a two or three or four-carbon linker to 8-OH to form compound **8**, which then reacted with various amines

* Corresponding author.
E-mail address: zhiyuli@cpu.edu.cn (Z. Li).
[1] These two authors contributed equally to this work.

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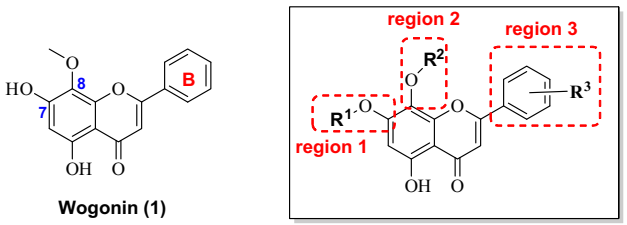
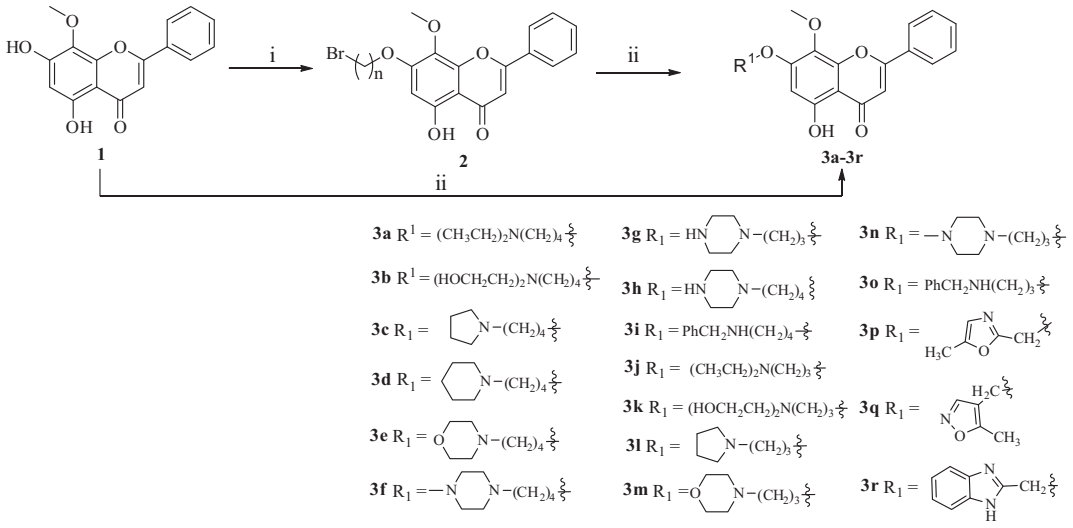


Fig. 1. The structure of wogonin (**1**) and the designed wogonin derivatives.

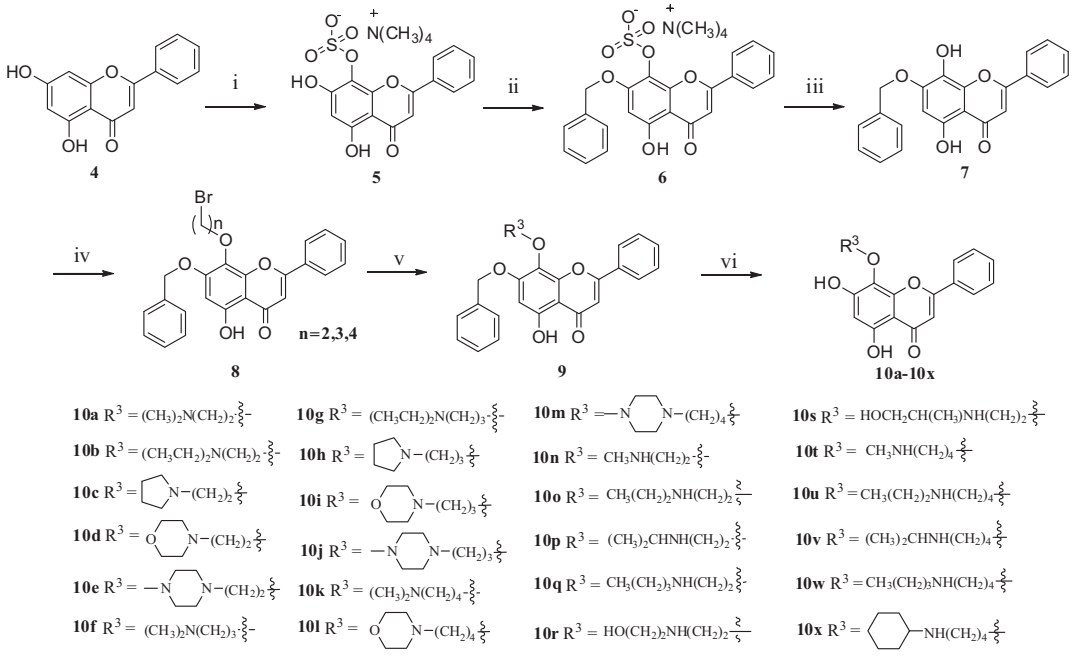
to give key intermediates **9**. Debenzylation of **9a-9v** give rise to the target compounds **10a-10x**.

The synthetic routes for compounds **19a-19g** were outlined in Scheme 3. Benzylation of **11** was conducted by reaction with benzyl bromide in K_2CO_3 to afford intermediate **12**. Oxidation of **12** using 65% HNO_3 provided the quinone **13**, which was reduced by sodium thiosulfate to the phenol **14**. Methylation of **14** with dimethylsulfate obtained intermediate **15**, which then debenzylation to give compound **16**. Followed by Friedel-Crafts acylation with cinnamoyl chloride derivatives using BF_3-Et_2O as catalyst provided the intermediate **1a** and **17a-17g**. Subsequently, **1a** and **17a-17g** were cyclized to the corresponding flavones (**1b** and **18a-18g**) mediated by iodine at 120 °C. Finally, the target compounds **1** and **19a-19g** were obtained through demethylation of intermediate **1b** and **18a-18g**. All of the target compounds were confirmed by ^{1}H NMR, IR and HRMS (ESI) spectra, and representative structure **10o** was also confirmed by ^{13}C NMR spectrum.

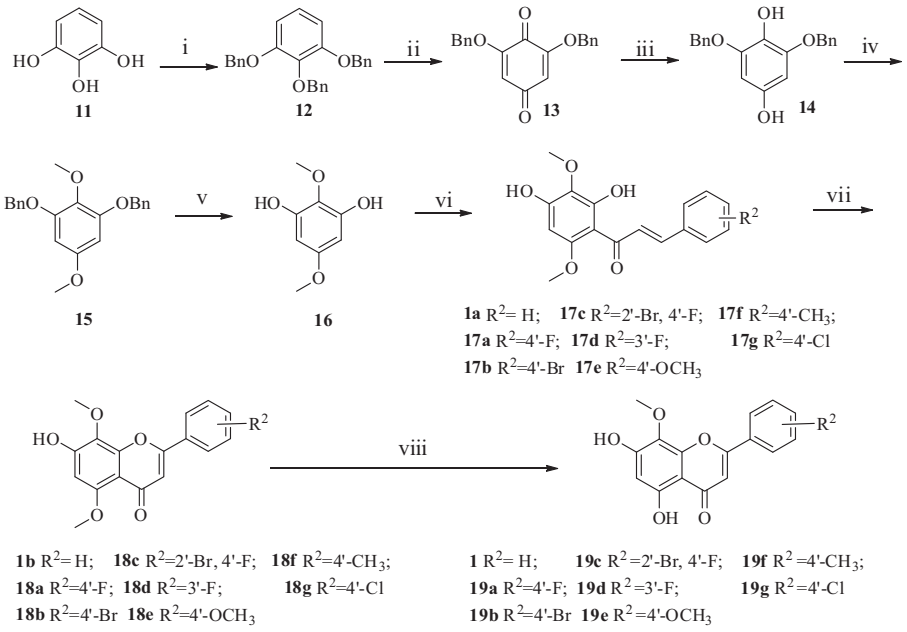
Cytotoxicity studies were performed on the derivatives of wogonin with cell survival being determined by the MTT assay



Scheme 1. Synthesis of compounds **3a-3r**. Reagents and conditions: (i) $Br(CH_2)_nBr$, K_2CO_3, acetone, reflux, 8 h, 70%; (ii) substituted amino derivatives, CH_3CN, reflux, 1–5 h, 30–60%.



Scheme 2. Synthesis of compounds **10a-10v**. Reagents and conditions: (i) $K_2S_2O_8$, $(CH_3)_4NOH$, H_2O, r.t., 7 h, 30%; (ii) $PhCH_2Br$, K_2CO_3, DMF, 50 °C, 6 h; (iii) 6 M HCl, r.t., 8 h, 40%; (iv) $Br(CH_2)_nBr$, K_2CO_3, acetone, reflux, 8 h, 75%; (v) substituted amino derivatives, CH_3CN, reflux, 1–5 h, 30–60%; (vi) H_2, Pd/C, THF, r.t., 8 h, 80%.



Scheme 3. Synthesis of compounds **19a-19h**. Regents and conditions: (i) $PhCH_2Br$, K_2CO_3, acetone, reflux, 48 h, 87%; (ii) AcOH, 65% HNO_3, 40 °C, 4 h, 45%; (iii) $Na_2S_2O_4$, $CH_3COOC_2H_5/H_2O$, r.t., 1 h, 37%; (iv) $(CH_3)_2SO_4$, NaOH, C_2H_5OH, r.t., 3 h, 85%; (v) H_2, Pd/C, MeOH, r.t., 8 h, 90%; (vi) Cinnamoyl chloride derivatives, BF_3-Et_2O, $CHCl_3$, reflux, 1.5–6 h, 35–80%; (vii) I_2, DMSO, 120 °C, 5–8 h, 30–40%; (viii) $AlCl_3$, CH_3CN, reflux, 8 h, 65–85%.

against the human liver carcinoma cell lines HepG2, human non-small cell lung cancer (NSCLC) cell lines A549, and human gastric carcinoma cell lines BCG-833. 5-Fu, a standard cytotoxic drug, was selected as the positive control to compare the potency of cytotoxicity of the tested compounds under the same experimental condition. In addition, wogonin was also selected as the control. These compounds were treated over a range of concentrations from 0.1 to 100 μM for 48 h and the results of cytotoxic activity *in vitro* were expressed as the IC_{50} which represents the concentration of a drug that is required for 50% inhibition. All of the derivatives were evaluated for their activity towards HepG2 cell lines and some of them were tested against the other cell lines (Table 1).

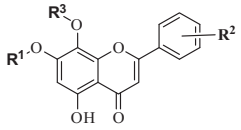
As shown in Table 1, part of these compounds showed moderate to potent cytotoxicity against all the three cell lines. Some of these compounds, such as compounds **3a-3h**, **3j-3k**, **3m**, **3o** with substitution at 7-position, even showed more potent against all the three cell lines compared with the control 5-Fu and wogonin. Especially, compound **3h** was found to be the most potent, with IC_{50} values of 1.07 μM (HepG2), 1.74 μM (A549) and 0.98 μM (BCG-823), over ten-fold more potent than the control 5-Fu and wogonin. However, compounds **10a-10x** and **19a-19g** with substitutions at 8-position or B-ring were generally less potent than the control. Through the observed cytotoxic efficacy of the synthesized wogonin derivatives, a general SAR rule could be attained, the activity was enhanced when the 7-position of wogonin was occupied by aliphatic amines rather than the case when 8-position or B-ring was substituted, as shown in all of the tested derivatives. In order to further explore the observed pharmacological properties and determining the main controlling factors governing the activities, QSAR study was carried.

The dataset of 50 synthetic wogonin derivatives (including wogonin) was randomly divided into a training set of 42 compounds and a test set of 8 compounds (see Schemes 1–3 for full set of compound structures). Both sets represent equally well the chemical and biological properties of the whole data set. The test set met the standards that it must include no less than five compounds, whose activities and structures must cover the range of activities and structures of compounds from the training set.[13] The measurement of cytotoxic activity was expressed as pIC_{50} ($pIC_{50} = -\log (IC_{50} \times 10^{-6})$), where the IC_{50} values were experimental values determined against HepG2 cancer cell lines.

The structures of these compounds were sketched using the molecular builder module of MOE software (version 2015.10) and minimized for energy via steepest descent, conjugate, and truncated Newton method in sequence employing MMFF94 as force field with energy tolerance value of root mean square gradient 0.01 kcal/mol. The conformation search of energy-minimized structure was performed using stochastic method. Relative pIC_{50} value of each compound was entered in database manually opposite to molecules.

The QSAR model was generated using AutoQSAR packed in MOE. QSAR model generation in MOE was different from other software. MOE involves the descriptor based methodology and gave two type of value (predicted pIC_{50} and the residual value). Our model was developed choosing the HepG2 inhibitory activities as dependent variable and constructed based on the partial least squares (PLS) method using more than 300 descriptors as model fields built in MOE.[14] Regression analysis was performed for the training data set and the quality was evaluated using a squared correlation coefficient (r^2), root mean square error (RMSE), and cross validation squared correlation coefficient (q^2).[15,16] Assessment of QSAR model is done by noting q^2 value which was considered as an indicator of the predictive performance and stability.[11,12] The coefficient r^2 of the model and the test indicated how well the equation fits the data. During the AutoQSAR analysis, compounds **3** and **15** showed high deviation in their residue values and produced negative influence on the predictive ability of the model, thus we took **3** and **15** as outliers. Therefore, 40 compounds were used in QSAR modelling and the excellent established QSAR equations including 8 descriptors for 40 compounds in training set were summarized in Eq. (1) along with their statistical parameters. The 8 descriptors used in these two models were belonging

Table 1
In vitro cytotoxic activity of wogonin derivatives.



Entry	No.	HepG2	A549	BCG-823	pIC_{50}	Calculated value	Residual value
1[a]	**3a**	1.72	1.00	6.17	5.76	5.51	0.25
2	**3b**	2.64	1.26	5.89	5.58	5.41	0.17
3	**3c**	3.61	1.53	6.34	5.44	5.41	0.03
4	**3d**	1.97	1.15	18.20	5.71	5.57	0.14
5	**3e**	3.30	1.52	5.30	5.48	5.69	−0.21
6	**3f**	1.12	1.50	5.30	5.95	5.91	0.04
7	**3g**	9.14	10.10	12.00	5.04	5.09	−0.05
8	**3h**	1.07	1.74	0.98	5.97	5.52	0.45
9	**3i**	45.70	4.59	48.10	4.34	4.71	−0.37
10	**3j**	3.33	1.83	13.30	5.48	5.30	0.18
11	**3k**	3.79	3.00	6.45	5.42	5.29	0.13
12	**3l**	15.40	1.39	17.90	4.81	5.22	0.59
13	**3m**	6.50	2.30	3.70	5.19	5.54	−0.35
14	**3n**	31.60	9.90	52.70	4.50	5.60	−1.10
15	**3o**	8.12	1.57	9.93	5.09	4.73	0.36
16[a]	**3p**	20.25	>100	ND	4.69	4.80	−0.11
17	**3q**	21.66	>100	ND	4.66	4.66	0
18	**3r**	29.44	>100	ND	4.53	4.52	0.01
19	**10a**	39.98	>100	ND	4.40	4.40	0
20	**10b**	68.07	>100	ND	4.17	4.22	−0.05
21	**10c**	68.47	67.68	ND	4.16	4.16	0
22	**10d**	39.89	>100	ND	4.40	4.38	0.02
23[a]	**10e**	56.56	>100	ND	4.25	4.09	0.16
24	**10f**	41.78	>100	ND	4.38	4.30	0.08
25[a]	**10g**	67.53	>100	ND	4.17	4.05	0.12
26	**10h**	58.51	>100	ND	4.23	4.07	0.15
27	**10i**	21.51	>100	ND	4.68	4.38	0.30
28	**10j**	42.64	>100	ND	4.37	4.46	−0.09
29	**10k**	35.69	>100	ND	4.45	4.49	−0.04
30	**10l**	15.12	82.29	ND	4.82	4.54	0.28
31	**10m**	15.12	>100	ND	4.82	4.77	0.05
32	**10n**	64.16	ND	59.92	4.19	4.11	0.08
33	**10o**	98.40	ND	62.99	4.01	4.09	−0.08
34	**10p**	14.12	ND	>100	4.85	4.61	0.24
35	**10q**	57.18	ND	72.42	4.24	4.40	−0.16
36[a]	**10r**	56.96	ND	59.82	4.24	4.10	0.14
37	**10s**	43.53	ND	92.23	4.36	4.63	−0.27
38	**10t**	97.03	ND	>100	4.01	4.06	−0.05
39	**10u**	9.99	ND	51.02	5.00	4.05	0.95
40	**10v**	29.92	ND	71.24	4.52	4.58	−0.06
41[a]	**10w**	38.89	ND	61.88	4.41	4.26	0.15
42	**10x**	10.93	ND	48.57	4.96	4.86	0.10
43	**19a**	43.90	ND	ND	4.36	4.40	−0.04
44[a]	**19b**	38.20	ND	ND	4.42	4.46	−0.04
45	**19c**	33.50	ND	ND	4.47	4.43	0.04
46[a]	**19d**	59.40	ND	ND	4.23	4.72	−0.49
47	**19e**	42.40	ND	ND	4.37	4.37	0
48	**19f**	24.90	ND	ND	4.60	4.50	0.10
49	**19g**	34.20	ND	ND	4.47	4.56	−0.09
50	**1**	19.0	15.8	16.5	4.72	4.80	−0.08
51	**5-Fu**	17.2	16.1	10.1	–	–	–

[a] Compounds were randomly selected as test set.

to two categories: physical-chemical properties (h_log D, h_log P, log (*o*/*w*), SlogP_VSA9, SMR_VSA7 and TPSA), and partial charge descriptors (PEOE_VSA + 1 and Q_VSA_NEG).

Physical-chemical properties descriptor h_log D represents the oxtanol/water distribution coefficient at pH 7; h_log P was obtained from log of the octanol/water partition coefficient using an 8 parameter model based on Hueckel Theory using 1836 molecules[17]; Log (*o*/*w*) represents log of the octanol/water partition coefficient (including implicit hydrogen); SlogP represents log of the octanol/water partition coefficient and SlogP_VSA9 can be defined as the sum of van der Waals surface area (v_i) such that the contribution to logP(o/w) for atom i as calculated in the SlogP descriptor (L_i) is in the range (>0.40) divided by the total surface area; SMR is the descriptor of molecular refractivity and SMR_VSA7

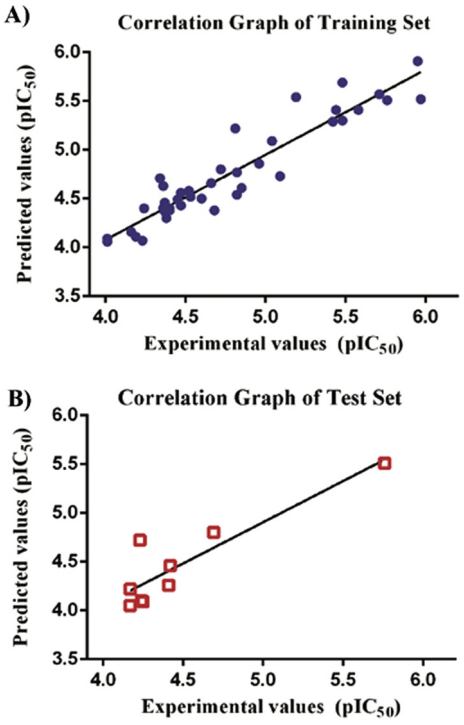


Fig. 2. The correlation plot between experimental and the predicted pIC_{50} values of compounds from (A) training set and (B) test set.

can be defined as the sum of van der Waals surface area (v_i) such that molecular refractivity (R_i) is in the range (>0.56) divided by the total surface area; and TPSA represents polar surface area (Å^2) which is calculated using group contributions to approximate the polar surface area from connection table information only.

Partial charge descriptor PEOE_VSA + 1 represents the partial charge which can be defined as the sum of van der Waals surface area (v_i) such that partial charge (q_i) is in the range (0.05, 0.10) divided by the total surface area; Q_VSA_NEG represents total negative van der Walls surface area.

$$\begin{aligned} pIC_{50} = &-2.56632 - 0.66452(\text{h\_log D}) + 1.31371(\log S) \\ &- 1.03041(\log(o/w)) - 0.04310(\text{PEOE\_VSA} + 1) \\ &- 0.15864(\text{SlogP\_VSA9}) - 0.11972(\text{TPSA}) \end{aligned} \quad (1)$$

n = 40, r^2 = 0.882, RMSE = 0.315, q^2 = 0.786.

According to the reference, a reliable QSAR model is indicated by $r^2 > 0.80$ and $q^2 > 0.50$. As shown in Eq. (1) and Fig. 2, the model exhibited good regression between the experimental and predicted activity with r^2 = 0.882 and q^2 = 0.786. Subsequently, the model was used for the validation of test set and predicted the activity of test set compounds with r^2 = 0.807 (Fig. 2). As the Eq. (1) showed, the physical-chemical properties h_log *D*, log *S*, and log (*o*/*w*) showed positive, positive and negative sign in the equation, respectively, which indicated that low octanol/water partition coefficients and high solubility were favorable for the cytotoxic activity. Other descriptors (including PEOE_VSA + 1, Q_VSA_NEG and SMR) indicated that the van der Waals surface areas combination with the electrostatic properties and refractivity were important factors influencing the activity of these wogonin derivatives as cytotoxic drugs. And the negative descriptor SMR (molecular refractivity) probably mean that saturated substituents were more favorable than unsaturated.

In this work, the bioactive natural product wogonin and its 49 derivatives were synthesized and evaluated their cytotoxic activities in cellular proliferation assay. Several compounds even showed more potent cytotoxic activity than wogonin. Especially compound **3h** improved the cytotoxic activity against HepG2, A549 and BCG-823 cancer cell lines by approximately 18-fold, 9-fold and 17-fold, respectively. QSAR study has been performed to explore the structural features reasonable for the cytotoxic activity. The model showed significant prediction ability with q^2 = 0.786 and the predicted activities of test set compounds were reliable with r^2 = 0.882. Eight descriptors are likely to influence the cytotoxic activities of these compounds. The descriptors mainly related with two factors: physical-chemical properties (high solubility and low octanol/water partition coefficient are favorable), and partial charge descriptors (excessive electrostatic properties and refractivity are unfavorable). These QSAR model could be applicable for next work, which might facilitate the discovery of improved compounds. In addition, further investigation about the mechanism of cellular activity and other biological activities of these wogonin derivatives are underway by our laboratory.

Acknowledgments

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.076.

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