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Synthesis, inhibitory activities, and QSAR study of xanthone derivatives as α-glucosidase inhibitors

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Dedicated to the honor and memory of late Professor Botao Fan in recognition of his encouragement.

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ABSTRACT

Xanthones and their derivatives have been reported to exhibit strong inhibitory activities toward α-glucosidase. To provide deep insight into the correlation between inhibitory activities and structures of xanthones, multiple linear regression (MLR) method was employed to establish QSAR models for 43 xanthone derivatives that have diverse structures. Among the 38 typical descriptors investigated, Hs (number of H-bond forming substituents), N_π (number of aromatic rings), and S (softness value) can be utilized to model the inhibitory activity. Thus, inhibitory activities of xanthone derivatives can be regulated by H-bond forming substituents, π-stacking-forming aromatic rings and softness values on the xanthone skeleton. The accuracy and predictive power of the proposed QSAR model were verified by LOO validation, Y-randomization, and test group validation with newly synthesized xanthone derivatives.

1. Introduction

Modern diseases, such as diabetes, HIV, and cancers are increasingly threatening the public health, therefore finding efficient therapy for these diseases is becoming one of the major goals in modern medicinal chemistry. It is well known that α-glucosidase (EC 3.2.1.20) is a key enzyme that is involved in these diseases,[1–6] and therefore many efforts have been made in the design and synthesis of agents that are capable of inhibiting α-glucosidase.[7–15] Such agents would have potential applications, for example, in developing chemotherapeutic agents for clinic use in the treatment of these diseases. However, traditional trials and error approaches that are used in the discovery of new drugs are time-consuming and costly. To ameliorate this, one practical approach that may be used to rapidly discover more effective drug candidates is to structurally modify natural products whose activities are well established, and then to apply statistic analytical methods (e.g., the well-known quantitative–structure activity relationship (QSAR) study) to establish correlations between chemical structures and the corresponding biological activities.[16–18]

As part of our projects to construct small organic molecules targeting biomacromolecules, we have keenly become interested in the design and synthesis of xanthone-based α-glucosidase inhibitors. Xanthones represent a class of naturally occurring compounds that are widely distributed in nature[19–21] and exhibit various pharmacological properties such as antioxidant,[22,23] antimalarial[24] and anti-inflammatory activities,[25] inhibition of a variety of tumor cell lines' growth,[26–28] and modulation of PKC isoforms. [29] Noticeably, recent studies by us[30,31] and others[32–35] have indicated that xanthones and their synthetic derivatives are capable of inhibiting α-glucosidase. We found that the inhibitory activities toward α-glucosidase of synthetic xanthones could be largely improved by attaching H-bond forming substituents or extending the π-conjugated systems.[30,31] More recently, Seo et al reported that naturally occurring xanthones from *Cudrania tricuspidata* displayed potent α-glucosidase inhibition.[35] These studies have indicated that xanthones are attractive as a versatile platform for the development of a new class of α-glucosidase inhibitors, and also spurred major efforts aimed at clarifying the structure–activity correlation that can be used to guide the discovery of potent inhibitors for medical use.

Herein, we describe an unprecedented QSAR study on a series of xanthone derivatives $\mathbf{X_{1-43}}$ (Chart 1) with the aim to provide comprehensive insight into the correlation between structures and inhibitory activities of xanthones. Specifically, multiple linear regression (MLR) method is employed to establish QSAR models for a training set of compounds $\mathbf{X_{1-34}}$ that have known

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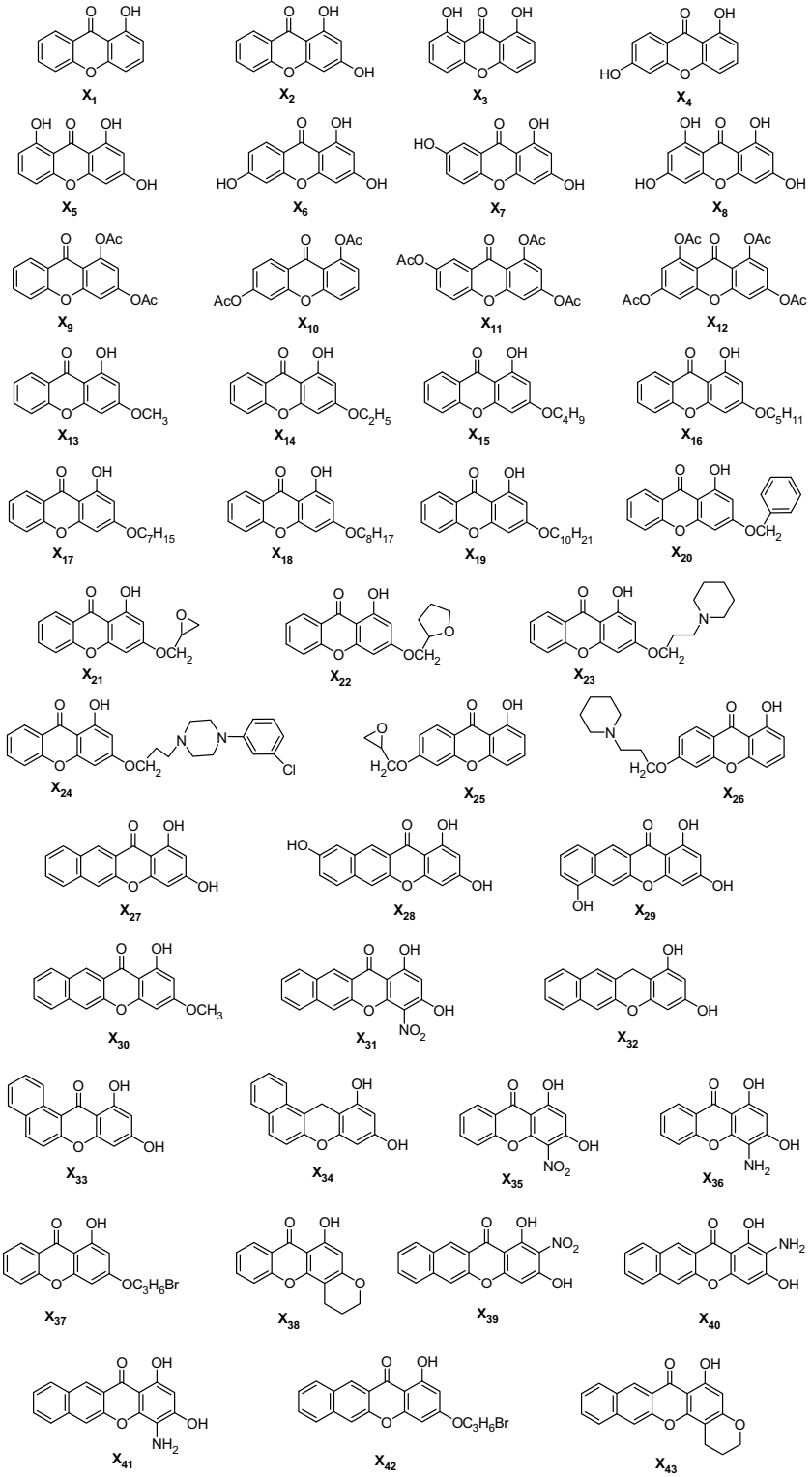


Chart 1.

inhibitory activities, and then the QSAR model is used to predict the inhibitory activities of $\mathbf{X_{35-43}}$ that will be synthesized as test group. The potential of the QSAR model in guiding future effort in the rational design and synthesis of xanthone derivatives that potentially have high-inhibitory activities toward α-glucosidase is briefly discussed.

2. Material and method

2.1. Dataset

In this QSAR study, compounds $\mathbf{X_{1-34}}$ from our recent work[30,31] serve as training set to build the QSAR models, whereas newly syn-

Table 1
Electronic, constitutional, steric, topological, and physicochemical descriptors

ID	Description	Notation
1	LUMO energy	LUMO
2	HOMO energy	HOMO
3	Total energy	E
4	Hardness	η
5	Softness	S
6	Electronegativity	χ
7	Electrophilicity	ω
8	Dipole moment	Dp
9	Melting point	MP
10	Boiling point	BP
11	Critical pressure	Pc
12	Critical temperature	Tc
13	Critical volume	Vc
14	Principal moment of inertia X	PMIX
15	Principal moment of inertia Y	PMIY
16	Principal moment of inertia Z	PMIZ
17	Molar refractivity	MR
18	Henry's law constant	HLC
19	Molecular weight	MW
20	log$P$	log$P$
21	Partition coefficient (octanol water)	Clog$P$
22	Number of H-bonding substituents	Hs
23	number of aromatic rings	N_π
24	Heat of formation	HOF
25	Gibbs energy	G
26	Repulsion energy	Er
27	Balaban index	BIndx
28	Cluster count	ClsC
29	Diameter	Diam
30	Molecular topological index	TIndx
31	Radius	Rad
32	Shape attribute	ShpA
33	Shape coefficient	ShpC
34	Sum of degrees	SDe
35	Sum of valence degrees	SVDe
36	Total connectivity	Tcon
37	Total valence connectivity	TVCon
38	Wiener index	Windx

thesized $\mathbf{X_{35-43}}$ act as test set to evaluate the predictive ability of the established QSAR models. In order to model and predict the inhibitory activities with accuracy, 38 descriptors (Table 1) including electronic, constitutional, steric, topological, and physicochemical parameters were taken into account as inputs to the model building.

Electronic descriptors **1–7** were obtained from quantum chemical calculations. These global electronic descriptors that are defined on the basis of density functional theory, such as hardness (η), chemical potential (μ), softness (S), electrophilicity index (ω), and electronegativity (χ)[36–38] have been widely used in SAR/QSAR investigations.[39–41] All the compounds were fully optimized at the density functional theory (DFT)/B3LYP level of theory,[42,43] together with the 6-31G* basis set. The most stable geometries of each compound were confirmed by frequency analysis, in which no imaginary frequency was found for all the minima. All the calculations were performed by using the Gaussian 03 package of programs.[44]Using Koopmans' theorem for closed-shell molecules, these electronic descriptors were obtained from the following equations:

$$I = -\varepsilon_{\mathrm{HOMO}} \quad \text{and} \quad A = -\varepsilon_{\mathrm{LUMO}} \tag{1}$$

$$\eta \approx \frac{1}{2}(\varepsilon_{\mathrm{LUMO}} - \varepsilon_{\mathrm{HOMO}}) = \frac{1}{2}(I - A) \tag{2}$$

$$\mu \approx \frac{1}{2}(\varepsilon_{\mathrm{LUMO}} + \varepsilon_{\mathrm{HOMO}}) = -\frac{1}{2}(I + A) \tag{3}$$

$$S = 1/(2\eta) \tag{4}$$

$$\chi = (I + A)/2 \tag{5}$$

$$\omega = \mu^2/(2\eta) \tag{6}$$

In addition, we have shown in the previous study[30,31] that (1) xanthone derivatives having extended π-conjugated systems show greatly improved inhibitory activities most probably through enhanced π-stacking interaction, and (2) H-bond donating/accepting substituents make greater contribution to the inhibition process than those that can only act as H-bonding acceptors. Therefore, we introduced herein two constitutional descriptors: (1) N_π, the number of aromatic rings in the skeleton of the xanthones to evaluate the weight of the possible π-stacking interaction; and (2) Hs, the number of H-bonding substituents, which is defined as the following equation:

$$\mathrm{Hs} = m + n/2 \tag{7}$$

wherein m and n are the numbers of H-bond donating/accepting and accepting substituents, respectively. All the other representative 29 descriptors, including steric, topological, and other physicochemical parameters, were calculated with Chem3D Ultra (version 8.0) built-in models.

2.2. Stepwise multiple linear regression

The elimination selection-stepwise regression (ES-SWR) variable selection method was used to select the most appropriate descriptors. By the combination of forward selection and backward elimination, the independent variables were individually added to or deleted from the model at each step of the regression based on three criteria: correlation coefficient (R^2), Fisher ratio value (F), and standard deviation (SD). The QSAR models are obtained with standardized data of descriptors.

2.3. Cross-validation technique

Since a high-correlation coefficient only indicates how well the equations fit the data, cross-validation procedure was carried out in order to explore the reliability of the proposed models. In this aspect, the well-known "leave-one-out" (LOO) approach was used in which a number of models were developed with one sample ignored each time. Then, the ignored data were predicted by each model and the differences between predicted and observed activity values were evaluated. The LOO cross-validation coefficient Q^2 that is given by Eq. 8 was used as an indicator of the predictive performance and stability of a model. In general, LOO cross-validated coefficient Q^2 being higher than 0.5 can be considered as a statistical proof of the high-predictive ability.[45]

$$Q^2 = 1 - \frac{\sum_{i=1}^{n}(y_{\mathrm{exp}} - y_{\mathrm{pred}})^2}{\sum_{i=1}^{n}(y_{\mathrm{exp}} - \bar{y})^2} \tag{8}$$

wherein y_{exp} and y_{pred} are the observed and predicted values for the dependent variables, respectively, and $\bar{y}$ is the average observed value.

2.4. Y-Randomization test

A widely used approach to establish the robustness of a given QSAR model is the so-called Y-randomization.[46] In this approach, dependent variable vector (inhibitory activity in this study) is randomly shuffled and a new QSAR model is built using the original independent variables. If the new QSAR models have lower R^2 and Q^2 values for several trials, then the given QSAR model is thought to be robust.

2.5. Estimation of the predictive ability of a QSAR model

As indicated in the literatures,[45,47,48] high value of cross-validation regression coefficient appears to be necessary but not the

sufficient criteria to confirm the high-predictive power of a QSAR model. Instead, it can only be estimated by an external test group of compounds that are not used in building of the QSAR model. The external R^2_{CVext}, defined in Eq. 9 by Tropsha and coworkers,[45] is a convenient criteria to estimate the predictive power of a QSAR model

$$R^2_{CVext} = 1 - \frac{\sum_{i=1}^{test}(y_{exp} - y_{pred})^2}{\sum_{i=1}^{test}(y_{exp} - \bar{y}_{train})^2} \quad (9)$$

where $\bar{y}_{train}$ is the averaged value for the dependent variable for the training set.

All the following criteria should be met for a given QSAR model[45]:

$$R^2_{ext} > 0.6 \quad \text{and} \quad R^2_{CVext} > 0.5 \quad (10)$$

$$\frac{(R^2_{ext} - R^2_o)}{R^2_{ext}} < 0.1 \quad \text{or} \quad \frac{(R^2_{ext} - R'^2_o)}{R^2_{ext}} < 0.1 \quad (11)$$

$$0.85 \leqslant k \leqslant 1.15 \quad \text{or} \quad 0.85 \leqslant k' \leqslant 1.15 \quad (12)$$

where R^2_{ext} refers to the correlation coefficient between the predicted and observed activities of external compounds.

2.6. Synthesis and biological activities of test group

Compounds $\mathbf{X_{35-43}}$ were synthesized to serve as an external test group for the QSAR analysis. The synthetic routes are depicted in Scheme 1. Nitration[19,25] of compounds $\mathbf{X_2}$ or $\mathbf{X_{27}}$ with 70% HNO_3 in acetic acid gave compounds $\mathbf{X_{35}}$, $\mathbf{X_{39}}$, and $\mathbf{X_{31}}$,[49] respectively, which were then hydrogenated[35,50] in the presence of 10% Pd–C to give $\mathbf{X_{36}}$, $\mathbf{X_{40}}$, and $\mathbf{X_{41}}$, respectively. Alkylation[30,31] of $\mathbf{X_2}$ and $\mathbf{X_{27}}$ with 1,3-dibromopropane in acetone afforded $\mathbf{X_{37}}$ and $\mathbf{X_{42}}$, respectively, treatment of which with K_2CO_3 in DMF gave compounds $\mathbf{X_{38}}$ and $\mathbf{X_{43}}$, respectively. All these xanthone derivatives were characterized by NMR, mass, IR, and elemental analyses (see Section 5). Compounds $\mathbf{X_{35-43}}$ statistically represent the distribution of data sets, among which $\mathbf{X_{35}}$–$\mathbf{X_{38}}$ have three conjugated fused rings; $\mathbf{X_{39}}$–$\mathbf{X_{41}}$, $\mathbf{X_{42}}$, and $\mathbf{X_{43}}$ have four extended and conjugated fused rings; $\mathbf{X_{38}}$ and $\mathbf{X_{43}}$ have an additional unconjugated ring; $\mathbf{X_{37}}$ and $\mathbf{X_{42}}$ have a side chain; $\mathbf{X_{35}}$ and $\mathbf{X_{39}}$ have an additional H-bond accepting substituent NO_2 and $\mathbf{X_{36}}$, $\mathbf{X_{40}}$, and $\mathbf{X_{41}}$ have an additional H-bond donating/accepting substituent NH_2.

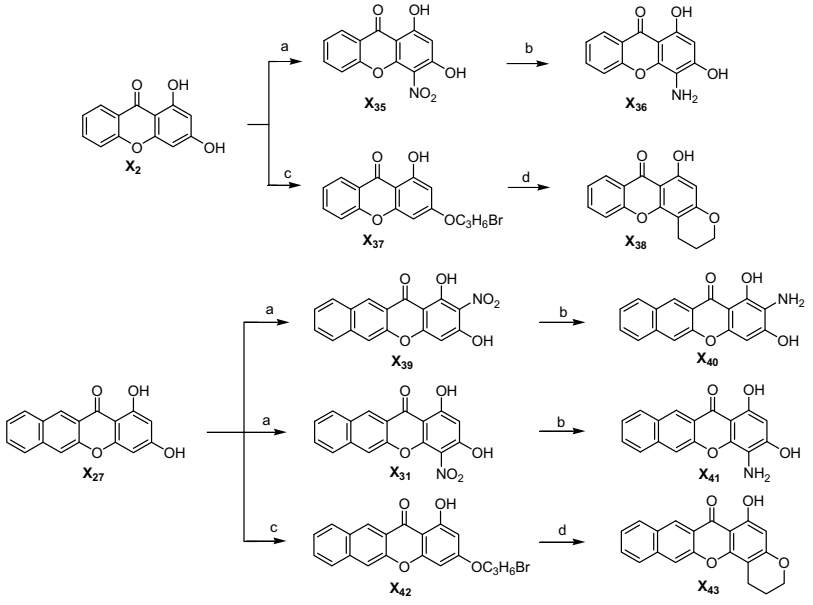
The inhibitory activities of compounds $\mathbf{X_{35-43}}$ toward yeast's α-glucosidase were evaluated by using similar methods as described in previous studies.[30,31] The obtained IC_{50} values together with those of compounds $\mathbf{X_{1-34}}$ are summarized in Table 2. Among the test sets, compound $\mathbf{X_{39}}$ has the highest inhibitory activity with IC_{50} being 5.9 μM, whereas $\mathbf{X_{35}}$ has the lowest inhibitory activity with IC_{50} value of 235 μM. Thus, given the structural diversity and activity range, the design and synthesis of $\mathbf{X_{35-43}}$ as the test set was reasonable.

3. Results and discussion

In order to select the predominant descriptors that will affect the inhibitory activities of these compounds, correlation analysis was performed with statistical software SPSS,[51] taking every calculated descriptor as an independent variable and $\log(1/IC_{50})$ as a dependent variable. Based on the correlation analysis, the aforementioned stepwise multiple linear regression technique was used to establish the QSAR model

$$\begin{aligned} &\log(1/IC_{50}) = 0.239Hs + 0.090S + 0.160N_\pi - 1.750 \\ &n = 34, \quad R^2 = 0.790, \quad Q^2 = 0.733, \quad SD = 0.195, \\ &F = 37.524, \quad p < 0.00001 \end{aligned} \quad (13)$$

wherein n is the number of compounds of training set, compounds $\mathbf{X_{1-34}}$; R^2, the correlation coefficient; Q^2, the LOO cross-validated coefficient; SD, the standard deviation; F, the Fisher's F-value, and p is the p-value (calculated from F statistics). The predicted values of $\log(1/IC_{50})$ from Eq. 13, together with the corresponding residual values are listed in Table 2. It can be seen that $\mathbf{X_9}$ has much higher residual value (0.64) than all the other compounds in training set. Further analysis has indicated that its standardized residual is the highest and greater than two, suggesting that $\mathbf{X_9}$ is an outlier based on the commonly accepted hypothesis that values of standardized residual above two are characteristic of an outlier. Thus, compound $\mathbf{X_9}$ was not considered during the course of exploratory data analysis. Correspondingly, an alternative QSAR model for the remaining 33 compounds was obtained



Scheme 1. Synthetic route for xanthone derivatives $\mathbf{X_{35-43}}$. Reagents and conditions: (a) 70% HNO_3, AcOH, 60–70 °C; (b) H_2, 10% Pd–C, THF; (c) BrC_3H_6Br, K_2CO_3/acetone, reflux; (d) K_2CO_3/DMF, 60–80 °C.

Table 2
Values of the selected most important descriptors, the experimental/predicted log(1/IC_{50}) values and their corresponding residual values for the training and test set[a]

Compound	IC_{50}	Hs	N_π	S	Exp. log(1/IC_{50})	Pred. log(1/IC_{50}) for Eq. 13	Residual values for Eq. 13	Pred. log(1/IC_{50}) for Eq. 14		Residual values for Eqs. 14	
$\mathbf{X_1}$	177.4	0.5	2	0.124	−2.25	−2.21	−0.04	−2.25		0.00	
$\mathbf{X_2}$	160.8	1.5	2	0.118	−2.21	−1.88	−0.33	−1.91		−0.30	
$\mathbf{X_3}$	91.5	1.0	2	0.126	−1.96	−1.98	0.02	−1.99		0.03	
$\mathbf{X_4}$	131.4	1.5	2	0.120	−2.12	−1.86	−0.26	−1.88		−0.24	
$\mathbf{X_5}$	81.8	2.0	2	0.121	−1.91	−1.64	−0.27	−1.64		−0.27	
$\mathbf{X_6}$	41.5	2.5	2	0.115	−1.62	−1.52	−0.10	−1.53		−0.08	
$\mathbf{X_7}$	14.7	2.5	2	0.124	−1.17	−1.41	0.24	−1.38		0.21	
$\mathbf{X_8}$	17.1	3.0	2	0.115	−1.23	−1.31	0.08	−1.31		0.07	
$\mathbf{X_9}$	31.9	1.0	2	0.112	*−1.50*[b]	*−2.14*[b]	*0.64*[b]	–	–	–	–
$\mathbf{X_{10}}$	138.9	1.0	2	0.112	−2.14	−2.14	0.00	−2.21		0.07	
$\mathbf{X_{11}}$	46.5	1.5	2	0.115	−1.67	−1.91	0.24	−1.95		0.29	
$\mathbf{X_{12}}$	49.7	2.0	2	0.112	−1.70	−1.74	0.05	−1.78		0.09	
$\mathbf{X_{13}}$	172.9	1.0	2	0.120	−2.24	−2.06	−0.18	−2.10		−0.14	
$\mathbf{X_{14}}$	110.8	1.0	2	0.120	−2.04	−2.05	0.01	−2.10		0.05	
$\mathbf{X_{15}}$	130.1	1.0	2	0.120	−2.11	−2.05	−0.06	−2.09		−0.02	
$\mathbf{X_{16}}$	120.9	1.0	2	0.120	−2.08	−2.05	−0.03	−2.09		0.01	
$\mathbf{X_{17}}$	113.8	1.0	2	0.120	−2.06	−2.05	0.00	−2.09		0.04	
$\mathbf{X_{18}}$	123.7	1.0	2	0.120	−2.09	−2.05	−0.04	−2.09		0.00	
$\mathbf{X_{19}}$	115.6	1.0	2	0.120	−2.06	−2.05	−0.01	−2.09		0.03	
$\mathbf{X_{20}}$	98.2	1.0	2	0.120	−1.99	−2.05	0.06	−2.09		0.10	
$\mathbf{X_{21}}$	66.6	1.5	2	0.119	−1.82	−1.86	0.04	−1.89		0.06	
$\mathbf{X_{22}}$	53.0	1.5	2	0.120	−1.72	−1.86	0.13	−1.88		0.16	
$\mathbf{X_{23}}$	115.4	1.0	2	0.131	−2.06	−1.93	−0.14	−1.92		−0.14	
$\mathbf{X_{24}}$	61.8	1.0	2	0.133	−1.79	−1.90	0.11	−1.88		0.09	
$\mathbf{X_{25}}$	63.5	1.5	2	0.120	−1.80	−1.85	0.05	−1.88		0.07	
$\mathbf{X_{26}}$	132.7	1.0	2	0.130	−2.12	−1.93	−0.19	−1.93		−0.20	
$\mathbf{X_{27}}$	9.3	1.5	3	0.133	−0.97	−1.33	0.36	−1.33		0.36	
$\mathbf{X_{28}}$	5.8	2.5	3	0.141	−0.76	−0.84	0.08	−0.77		0.00	
$\mathbf{X_{29}}$	8.0	2.5	3	0.139	−0.90	−0.87	−0.03	−0.80		−0.10	
$\mathbf{X_{30}}$	31.3	1.0	3	0.134	−1.50	−1.52	0.02	−1.52		0.03	
$\mathbf{X_{31}}$	20.1	1.5	3	0.134	−1.30	−1.32	0.02	−1.31		0.01	
$\mathbf{X_{32}}$	27.8	2.0	3	0.113	−1.44	−1.36	−0.08	−1.43		−0.02	
$\mathbf{X_{33}}$	39.9	1.5	3	0.126	−1.60	−1.41	−0.19	−1.44		−0.16	
$\mathbf{X_{34}}$	34.9	2.0	3	0.113	−1.54	−1.36	−0.18	−1.42		−0.12	
$\mathbf{X_{35}}$	235.2	1.5	2	0.119	−2.37	–	–	–	−1.89	–	−0.48
$\mathbf{X_{36}}$	102.3	2.0	2	0.120	−2.01	–	–	–	−1.66	–	−0.35
$\mathbf{X_{37}}$	146.6	1.0	2	0.119	−2.17	–	–	–	−2.11	–	−0.06
$\mathbf{X_{38}}$	198.1	1.0	2	0.124	−2.30	–	–	–	−2.03	–	−0.27
$\mathbf{X_{39}}$	5.9	1.5	3	0.132	−0.77	–	–	–	−1.34	–	0.57
$\mathbf{X_{40}}$	6.3	2.5	3	0.133	−0.80	–	–	–	−0.89	–	0.09
$\mathbf{X_{41}}$	8.3	2.0	3	0.133	−0.92	–	–	–	−1.11	–	0.19
$\mathbf{X_{42}}$	29.7	1.0	3	0.134	−1.47	–	–	–	−1.52	–	0.05
$\mathbf{X_{43}}$	67.3	1.0	3	0.136	−1.83	–	–	–	−1.49	–	−0.34

[a] The IC_{50} value of each compound was determined against yeast's α-glucosidase in 50 mM phosphate buffer (pH 6.8) containing 5% v/v DMSO at 37 °C. The experiments were performed in triplicate and repeated at least three times, and the mean values were taken. The data for compounds $\mathbf{X_{1-25}}$ and $\mathbf{X_{26-34}}$ were selected from Refs. 30,31 respectively.
[b] Compound $\mathbf{X_9}$ has the highest standardized residual that is greater than two, and thus was regarded as an outlier.

$$\log(1/IC_{50}) = 0.261Hs + 0.122S + 0.152N_\pi - 1.758$$
$$n = 33, \quad R^2 = 0.872, \quad Q^2 = 0.839, \quad SD = 0.154, \quad (14)$$
$$F = 65.912, \quad p < 0.00001$$

Generally, a good QSAR model has the feature of large F, small SD, very small p-value, and R^2 and Q^2 values that are close to one. Both Eqs. 13 and 14 meet these criteria and thus both are statistically acceptable, however, Eq. 14 has much higher correlation coefficient R^2 (0.872) and LOO cross-validated coefficient Q^2 (0.839) that is close to R^2. Therefore, Eq. 14 is statistically better, and thus all the discussions that follows will be based on Eq. 14.

To establish the robustness, Eq. 14 was further validated by applying the Y-randomization. Several random shuffles of the Y vector were performed and the obtained lower R^2 and Q^2 values excluded the possibility of chance correlation or structural dependency of the training set.

As indicated in Eq. 14, the most significant descriptors that affect the inhibitory activity are: Hs, the number of H-bonding substituents; S, the softness value and N_π, the number of aromatic rings in the skeleton of the xanthones. Their values for all the compounds, the predicted values of log(1/IC_{50}), and their corresponding residual values, are listed in Table 2. In addition, in order to avoid internal correlations, we also performed a correlation analysis on these selected descriptors. The obtained correlation matrix (Table 3) clearly indicates that the selected descriptors in the QSAR model are in low correlation.

The predictive power of the selected descriptors was explored by external compounds $\mathbf{X_{35-43}}$ that were synthesized according to their structural characteristics and biological activity range. With the validation analysis using these compounds, the proposed QSAR model (Eq. 14) was proved to meet all the required criteria that are defined in Eqs. 10–12, that is, $R^2_{ext} = 0.825$, $R^2_{CVext} = 0.735$, $(R^2_{ext} - R^2_o)/R^2_{ext} = -0.123$, $(R^2_{ext} - R'^2_o)/R^2_{ext} = -0.136$, $k = 1.065$, and $k' = 0.911$. The observed versus predicted values for all the training and test sets are shown graphically in Figure 1.

The aforementioned QSAR model (Eq. 14) has clearly indicated that all the three descriptors Hs, N_π, and S, have positive correlation with the inhibitory activity. This is an interesting observation

Table 3
Correlation matrix for the selected descriptors in Eqs. 13 and 14

	Hs	N_π	S
Hs	1		
N_π	0.311	1	
S	−0.0273	0.472	1

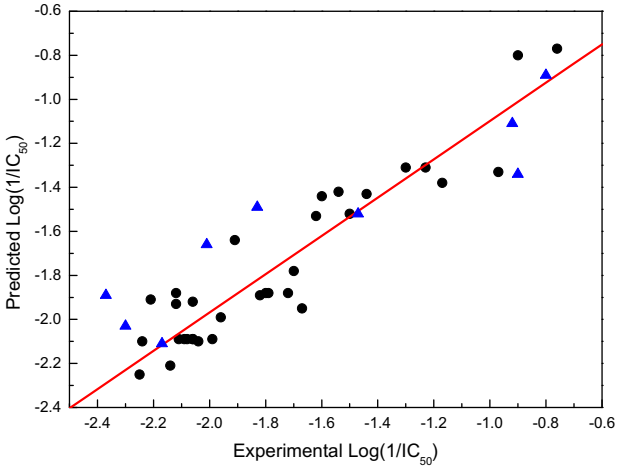


Figure 1. Plot of predicted inhibitory activities [log(1/IC$_{50}$)] of training set (•) and test set (▲) against the observed values for QSAR model by Eq. 14.

and may be used to guide future effort in the rational design and synthesis of xanthone derivatives having potentially potent inhibitory activity. Thus, as already shown in our previous studies, [30,31] it may be constantly a practical approach to improve the inhibitory activity by introducing more H-bond forming substituents (such as hydroxyl and amino groups) and/or more aromatic rings onto the skeleton. It is noteworthy that softness value is positively correlated with the inhibitory activity. This may be rationalized by taking into account that the good correlation between softness and polarizability makes it easier for a substrate that has higher softness value to deform its electronic cloud,[52–54] hence leading to stronger binding affinity toward the enzyme. If that is the case, the inhibitory activity would be expected to be improved by introducing some hetero atoms with high softness onto the skeleton of xanthones. This is currently under investigation, which will be reported in due course.

4. Concluding remarks

Strong QSAR model has been successfully established by using MLR for 34 xanthone derivatives having inhibitory activities toward α-glucosidase. Its predictive power was validated by screening nine external xanthone derivatives that have diverse structures and wide-ranged activity. This, together with the LOO validation and Y-randomization has unambiguously demonstrated the robustness of the QSAR model itself and its power of predicting external data with accuracy. Thus, this QSAR model may be used as an efficient tool to predict the inhibitory activities of xanthone derivatives. Among 38 typical descriptors, three descriptors, Hs, S, and N_π can be utilized to model the inhibitory activity. That is, inhibitory activities of xanthone derivatives can be significantly improved by increasing the number of H-bonding substituents, aromatic rings in the skeleton of the xanthone derivatives and softness values onto the xanthone skeleton. Rational design and synthesis of new xanthone derivatives in search of new α-glucosidase inhibitors, guided by the QSAR model, is actively under progress in our laboratories.

5. Experimental

NMR spectra were recorded on a Varian INOVA 300 MB NMR spectrometer in either $CDCl_3$ or acetone-d_6 or DMSO-d_6, and tetramethylsilane was used as an internal standard. Mass spectra were measured on a DSQ low resolution mass spectrometer. IR spectra were obtained on a Bruker EQUINOX55 Fourier transformation infrared spectrometer. Elemental analyses were carried out on an Elementar Vario EL series elemental analyzer. Melting points were determined on a WRS-1B digital melting point apparatus and were uncorrected. UV spectra were recorded on a Shimadzu UV-3150 scanning spectrophotometer.

p-Nitrophenyl (PNP) glycoside and α-glucosidase (from baker's yeast) used in this study were purchased from Sigma (St. Louis, MO, USA). All the other reagents were of analytical quality and used as received.

5.1. Synthesis of compounds X_{35} and X_{39}

General procedures: Nitric acid (70%, 0.5 mL) in acetic acid (5 mL) was slowly added to a solution of compound **X$_2$** or **X$_{27}$** (2 mmol) in acetic acid (20 mL). The mixture was stirred at 60 °C for 1–4 h and then poured into ice-cooled water (200 mL). The formed precipitates were filtered, washed with water, and recrystallized from ethanol to give **X$_{35}$** and **X$_{39}$** as yellow solids, respectively.

5.1.1. 1,3-Dihydroxy-4-nitro-9*H*-xanthen-9-one (X_{35})

Yield 61% from compound **X$_2$** in 1 h. Mp 227 °C (dec); IR (KBr): 3427, 2940, 1652, 1589, 1510, 1454, 1348, 1297, 1205, 1160, 1029, 824, 756, 632 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ: 13.44 (s, 1H, ArOH), 11.34 (br s, 1H, ArOH), 8.25 (dd, J = 8.1, 1.8 Hz, 1H, ArH), 7.99–7.93 (m, 1H, ArH), 7.67 (d, J = 8.1 Hz, 1H, ArH), 7.62–7.56 (m, 1H, ArH), 6.46 (s, 1H, ArH); EI-MS m/z(%): 273 ([M]$^+$, 100). Anal. Calcd for $C_{13}H_7NO_6$: C, 57.15; H, 2.58; N, 5.13. Found: C, 57.20; H, 2.45; N, 5.22.

5.1.2. 1,3-Dihydroxy-2-nitro-12*H*-benzo[*b*]xanthen-12-one (X_{39})

Yield 42% from compound **X$_{27}$** in 4 h. Mp 281–283 °C; IR (KBr): 3421, 3044, 1641, 1592, 1491, 1411, 1351, 1308, 1187, 876, 744 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 13.76 (s, 2H, ArOH), 8.85 (s, 1H, ArH), 8.25 (d, J = 8.1 Hz, 1H, ArH), 8.11 (s, 1H, ArH), 8.06 (d, J = 8.4 Hz, 1H, ArH), 7.72 (t, J = 8.1 Hz, 1H, ArH), 7.59 (t, J = 8.3 Hz, 1H, ArH), 6.54 (s, 1H, ArH); EI-MS m/z (%): 323 ([M]$^+$, 100). Anal. Calcd for $C_{17}H_9NO_6$: C, 63.16; H, 2.81; N, 4.33. Found: C, 63.01; H, 2.82; N, 4.09.

5.2. Synthesis of compounds X_{36}, X_{40}, and X_{41}

General procedures: A solution of **X$_{35}$**, **X$_{39}$**, or **X$_{31}$** (1 mmol) in THF (20 mL) was hydrogenated in the presence of 10% palladium on charcoal (20 mg) at room temperature for 10 h. The catalysts were removed by filtration and the filtrates were concentrated to give **X$_{36}$**, **X$_{40}$**, and **X$_{41}$**, respectively, as yellow powders.

5.2.1. 4-Amino-1,3-dihydroxy-9*H*-xanthen-9-one (X_{36})

Yield 81% from compound **X$_{35}$**. Mp 237 °C (dec); IR (KBr): 3345, 3277, 3073, 2922, 2851, 2668, 2544, 1665, 1614, 1521, 1465, 1393, 1340, 1286, 1198, 1145, 1058, 940, 900, 822, 754, 645 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ: 8.20 (dd, J = 8.4, 1.5 Hz, 1H, ArH), 7.85–7.79 (m, 1H, ArH), 7.52 (d, J = 8.4 Hz, 1H, ArH), 7.48–7.42 (m, 1H, ArH), 6.34 (s, 1H, ArH); EI-MS m/z(%): 243 ([M]$^+$, 100). Anal. Calcd for $C_{13}H_9NO_4$: C, 64.20; H, 3.73; N, 5.76. Found: C, 64.45; H, 3.91; N, 5.59.

5.2.2. 2-Amino-1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one (X_{40})

Yield 59% from compound X_{39}. Mp 283–285 °C; IR (KBr): 3345, 3270, 3073, 2922, 2668, 1661, 1611, 1523, 1462, 1395, 1343, 1266, 1197, 1140, 1058, 940, 903, 821, 754 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ: 13.78 (s, 2H, ArOH), 8.89 (s, 1H, ArH), 8.25 (d, J = 7.8 Hz, 1H, ArH), 8.11 (s, 1H, ArH), 8.07 (d, J = 7.8 Hz, 1H, ArH), 7.75–7.62 (m, 1H, ArH), 7.57–7.46 (m, 1H, ArH), 6.61 (s, 1H, ArH); EI-MS m/z(%): 293 ([M]$^+$, 100). Anal. Calcd for $C_{17}H_{11}NO_4$: C, 69.62; H, 3.78; N, 4.78. Found: C, 69.41; H, 3.65; N, 4.97.

5.2.3. 4-Amino-1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one (X_{41})

Yield 75% from compound X_{31}. Mp 265–267 °C; IR (KBr): 3345, 3277, 3073, 2922, 2851, 2668, 2544, 1665, 1614, 1521, 1465, 1393, 1340, 1286, 1198, 1145, 1058, 940, 900, 822, 754, 645 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ: 13.07(s, 2H, ArOH), 8.85 (s, 1H, ArH), 8.25 (d, J = 7.8 Hz, 1H, ArH), 8.11 (s, 1H, ArH), 8.07 (d, J = 7.8 Hz, 1H, ArH), 7.76–7.63 (m, 1H, ArH), 7.55–7.48 (m, 1H, ArH), 6.33 (s, 1H, ArH); EI-MS m/z(%): 293 ([M]$^+$, 100). Anal. Calcd for $C_{17}H_{11}NO_4$: C, 69.62; H, 3.78; N, 4.78. Found: C, 69.91; H, 3.63; N, 4.77.

5.3. Synthesis of compounds X_{37} and X_{42}

General procedures: To a solution of X_2 or X_{27} (0.2 mmol) and 1,3-dibromopropane (2 mmol) in acetone (20 mL) was added K_2CO_3 (0.25 mmol). The mixture was refluxed under stirring for 2–4 h. After cooling, the mixture was filtered and the organic filtrate was concentrated. The crude products were purified by chromatography on a silica gel column to afford X_{37} and X_{42}, respectively, as yellow solids.

5.3.1. 3-(3-Bromopropoxy)-1-hydroxy-9*H*-xanthen-9-one (X_{37})

Yield 91% from compound X_2. Mp 124–126 °C; IR (KBr): 3430, 3091, 2924, 1661, 1610, 1570, 1507, 1470, 1437, 1364, 1303, 1223, 1162, 1082, 825, 794, 761, 673 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 12.83 (s, 1H, OH), 8.22 (dd, J = 1.5 Hz, 1.8 Hz, 1H, ArH), 7.73–7.66 (m, 1H, ArH), 7.42–7.33 (m, 2H, ArH), 6.42 (d, J = 2.1 Hz, 1H, ArH), 6.35 (d, J = 2.1 Hz, 1H, ArH), 4.20 (t, J = 6.0 Hz, 2H, OCH_2), 3.61 (t, J = 6.3 Hz, 2H, $-CH_2Br$), 2.41–2.32 (m, 2H, CH_2); EI-MS m/z: 348 ([M]$^+$, 100), 350 ([M+2]$^+$, 100). Anal. Calcd. for $C_{16}H_{13}BrO_4$: C, 55.04; H, 3.75. Found: C, 55.21; H, 3.88.

5.3.2. 3-(3-Bromopropoxy)-1-hydroxy-12*H*-benzo[*b*] xanthen-12-one (X_{42})

Yield 73% from compound X_{27}. Mp 190–192 °C; IR (KBr): 3431, 2941, 1646, 1595, 1509, 1470, 1447, 1346, 1252, 1166, 1034, 821, 741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 12.90 (s, 1H, ArOH), 8.84 (s, 1H, ArH), 8.04 (d, J = 8.7 Hz, 1H, ArH), 7.90 (d, J = 8.7 Hz, 1H, ArH), 7.81 (s, 1H, ArH), 7.65–7.59 (m, 1H, ArH), 7.53–7.47 (m, 1H, ArH), 6.45 (d, J = 2.4 Hz, 1H, ArH), 6.34 (d, J = 2.4 Hz, 1H, ArH), 4.24 (t, J = 6.0 Hz, 2H, OCH_2), 3.63 (t, J = 6.0 Hz, 2H, $-CH_2Br$), 2.43–2.34 (m, 2H, CH_2); EI-MS m/z: 398 ([M]$^+$, 100), 400 ([M+2]$^+$, 100). Anal. Calcd. for $C_{20}H_{15}BrO_4$: C, 60.17; H, 3.79. Found: C, 60.29; H, 3.78.

5.4. Synthesis of compounds X_{38} and X_{43}

General procedures: To a solution of X_{37} or X_{42} (0.5 mmol) in DMF (30 mL) was added K_2CO_3 (2.0 mmol). The mixture was stirred at 60–80 °C for 0.5–2 h. The reaction mixture was poured into ice-water and then extracted with $CHCl_3$. The crude products obtained after removal of the solvent were purified by chromatography on a silica gel column to afford X_{38} and X_{43}, respectively, as yellow solids.

5.4.1. 6-Hydroxy-2,3-dihydro-1*H*-pyrano[2,3-*c*] xanthen-7-one (X_{38})

Yield 27% from compound X_{37}. Mp 182–184 °C; IR (KBr): 3430, 3076, 2925, 1660, 1614, 1569, 1465, 1403, 1370, 1310, 1227, 1148, 1098, 953, 828, 758 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 13.18 (s, 1H, OH), 8.25 (dd, J = 8.1 Hz, 1.8 Hz, 1H, ArH), 7.72–7.66 (m, 1H, ArH), 7.43–7.32 (m, 2H, ArH), 6.37 (s, 1H, ArH), 4.28 (t, J = 5.1 Hz, 2H, OCH_2), 2.76 (t, J = 6.3 Hz, 2H, Ar-CH_2), 2.10–2.01 (m, 2H, CH_2); EI-MS m/z: 268 ([M]$^+$, 100). Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.75; H, 4.60.

5.4.2. 6-Hydroxy-2,3-dihydro-1*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (X_{43})

Yield 28% from compound X_{42}. Mp 241–243 °C; IR (KBr): 3430, 2924, 1641, 1605, 1470, 1444, 1353, 1293, 1251, 1174, 1112, 958, 829, 750, 655 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 13.25 (s, 1H, ArOH), 8.82 (s, 1H, ArH), 8.03 (d, J = 8.7 Hz, 1H, ArH), 7.88 (d, J = 8.7 Hz, 1H, ArH), 7.78 (s, 1H, ArH), 7.60 (t, J = 8.7 Hz, 1H, ArH), 7.48 (t, J = 8.7 Hz, 1H, ArH), 6.37 (s, 1H, ArH), 4.29 (t, J = 5.4 Hz, 2H, OCH_2), 2.76 (t, J = 6.3 Hz, 2H, Ar-CH_2), 2.10–2.02 (m, 2H, CH_2); EI-MS m/z: 318 ([M]$^+$, 100). Anal. Calcd for $C_{20}H_{14}O_4$: C, 75.46; H, 4.43. Found: C, 75.45; H, 4.62.

5.5. Enzyme assays

The inhibitory activities of all the xanthone derivatives were measured by using the methods similar to those described previously.[30,31] Typically, α-glucosidase activity was assayed in 50 mM phosphate buffer (pH 6.8) containing 5% v/v dimethylsulfoxide and the PNP glycoside was used as a substrate. The inhibitors were pre-incubated with the enzyme at 37 °C for 0.5 h. The substrate was then added and the enzymatic reaction was carried out at 37 °C for 60 min. The reaction was monitored spectrophotometrically by measuring the absorbance at 400 nm. The assay was performed in triplicate with five different concentrations around the IC_{50} values that were roughly estimated in the first round of experiments, and the mean values were adopted.

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