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Identification of pentacyclic triterpenes derivatives as potent inhibitors against glycogen phosphorylase based on 3D-QSAR studies

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ABSTRACT

Naturally occurring pentacyclic triterpenes (PT), a novel class of inhibitors against glycogen phosphorylase (GP), hold promise for the treatment of type-2 diabetes and other diseases with disorders in glycogen metabolism. To identify novel and more potent GP inhibitors, the receptor-based comparative molecular field analysis (CoMFA) and comparative molecular similarity analysis (CoMSIA) approaches were performed to investigate the quantitative structure–activity relationships (QSAR) among 106 PT analogues. The validated models demonstrated that the elongated or bulky substitutions in C17 position and/or C2, C3 positions are favorable. Then based on the structural information extracted from these models, 56 derivatives were synthesized and biochemically tested in this study. The IC_{50} value of the most potent compound **P50** was found to be 1.1 μM.

1. Introduction

Increased hepatic glucose production by glycogenolysis and gluconeogenesis is a contributing factor to the hyperglycaemia in type-2 diabetes. In humans, glucose is stored as glycogen in high concentrations in liver tissues as well as skeletal muscle. Glycogen phosphorylases (GP), which catalyze the first step of glycogen breakdown, play a key role in glucose metabolism, especially in glycogenolytic pathway. Due to its crucial role in modulation of glycogen metabolism, pharmacological inhibition of GP may afford a useful therapeutic approach to type-2 diabetes [1]. In addition, several structural classes of GP inhibitors have been reported and claimed to have pharmaceutical applications in the treatment of cardiovascular diseases and tumors, including glucose analogues at the active site, azasugar inhibitors, lactone at the allosteric (AMP) site, indole-2-carboxamide inhibitors at the indole binding site, caffeine at the purine nucleoside site and cyclodextrins at the glycogen storage site [2–8]. Pentacyclic triterpenes (PT), which possess various pharmacological properties such as antitumor, anti-HSV, anti-HIV, and anti-inflammatory activities [9,10], were found as a novel scaffold of inhibitors of GP, and the result of X-ray crystallographic studies disclosed the molecular basis of the PT binding to GPb at the allosteric site [11].

In our previous studies, we synthesized a series of PT derivatives and evaluated their bioactivity [11–23], which paved the way for further QSAR analysis. In this study, in order to explore the inhibition mechanism of PT derivatives and then to guide lead optimization, a 3D-QSAR study of PT derivatives by CoMFA and CoMSIA methods was performed. These QSAR models were first developed based on a training set of 80 compounds and validated by a test set of 26 compounds with structural and bioactivity diversities. The total 106 compounds in the training and test sets were first docked into the allosteric site of GPb [11] to identify their binding conformations and orientations. With the receptor-based ligand-alignment, the QSAR (CoMFA and CoMSIA) models were constructed and the topology of the allosteric site was mapped which revealed the match of contour maps by QSAR models with the neighboring key

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residues in the allosteric binding site. Based on the structural information derived from our models, another 56 compounds were synthesized (Tables 1–5) and among them, more potent inhibitors were identified, which demonstrated our QSAR models are rational and predictive. Taken together, our 3D-QSAR models would light up the road for the optimization of GP inhibitors with chemically more accessible structures.

The syntheses of 43 compounds (**P01**–**P19**, **P29**–**P36**, **P38**–**P44**, **P48**–**P56**) presented in this study have been described in our previous reports [11–23] and another 4 compounds (**P25**, **P45**–**P47**) will be reported in due course. The compounds were biochemically evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa). The activity of RMGPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis [24].

2. Results and discussion

2.1. The binding mode analysis of asiatic acid

To explore the molecular basis of GP inhibition by PT, we have investigated the binding mode of asiatic acid to GPb based on the crystal structure of the complex. As previously reported [11], the crucial residues involving in the H-bond and Van der Waals interactions with asiatic acid reside at the junction of GPb dimer. Considering the H-bond interactions, as shown in Fig. 1, the hydroxyls in the C2, C3 and C23 positions H-bond with the $N^{\delta 2}$ of Asn44′, $O^{\delta 1}$ and $O^{\delta 2}$ of Asp42′ respectively. Besides, the carboxyl in the C17 position H-bonds with the N^{ϵ} and N^{η} of Arg310. Moreover, the charged residues Arg81, Arg242, Asp306 and Arg309 around this region afford good environment for H-bond interactions. Overall, these H-bonds lead asiatic acid to adopt a conformation appropriate in this large binding pocket and also provide ideas for further inhibitor optimization. As for the shape of the binding pocket, it indicates the space of the allosteric site affords a few modifications on the scaffold for inhibitor optimization (the cavity volume is 1091.65 $Å^3$ and the surface area is 778.30 $Å^2$), shown in Fig. 1. The R1 region neighboring C19 and C20 positions is solution oriented, which affords the modification with bulky hydrophilic substituents here. As the hydrophilic substituents here probably make the derivatives more accessible to the appropriate orientations. Meanwhile, in C17 position of the scaffold, due to the long channel-like space in R2 region, the derivatives of the elongated or bulky substituents in this region may increase the inhibitor binding affinity. In addition, another minor channel-like space exists in the interface of two monomers around the C2 and C3 positions (R3 region). Therefore, the derivatives of the elongated substituents in the R3 position may also increase the inhibitor binding affinity. Briefly, the H-bond interactions and the space of the binding pocket analysis afford a few useful hints for the design of more potent inhibitors against GPb.

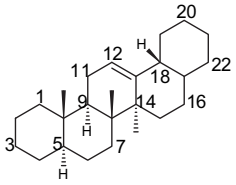
2.2. The receptor-based ligand-alignment

Molecular alignment is crucial for the construction of the QSAR models. Here the receptor-based alignment was performed by using molecular docking method, which would be more rational and reliable than ligand-based QSAR method for taking into account the receptor binding information. Meanwhile, the crystal structure of GP in complex with asiatic acid affords hints for the docking results [25]. In this study, 80 molecules among 106 compounds from our previous studies [11–23] as the training set were docked into the GPb dimer protein by AutoDock3.0.3 [26]. Furthermore, the remaining 26 compounds as the test set were docked into the receptor by the same protocol as to get appropriate conformations for the validation of the QSAR models. As a result, the conformations in the training set derivative from molecular docking were well aligned. The numbering system on the PT skeleton is shown in Fig. 2. Therefore, it provides us a good platform for the further receptor-based QSAR study.

2.3. QSAR models

Statistical models of QSAR are widely used to evaluate various theoretical properties of chemical structures. Here, we established the receptor-based CoMFA and CoMSIA models to design more

Table 1
Structures of compounds (**P01-P13**) designed and synthesized with the guide of the QSAR models. (IC_{50} μM) ($PIC_{50} = -\log(IC_{50}\ M)$).



Cpd no.	2	3	Substituents		19	20	Bioactivity	
			4	17			IC_{50}	PIC_{50}
P01	=O		$(CH_3)_2$	$CO_2CH_2C_6H_5$		$(CH_3)_2$	3.2	5.49
P02	OH		$(CH_3)_2$	$CO_2CH_2C_6H_5$		$(CH_3)_2$	4.2	5.38
P03	OH		$(CH_3)_2$	CO_2CH_3		$(CH_3)_2$	28.1	4.55
P04	OH		$(CH_3)_2$	$CO_2C_2H_5$		$(CH_3)_2$	15.3	4.82
P05	OH		$(CH_3)_2$	$CO_2C_3H_7$		$(CH_3)_2$	27.3	4.56
P06	OH		$(CH_3)_2$	$CO_2CH_2CHCH_2$		$(CH_3)_2$	23.1	4.64
P07	OH		$(CH_3)_2$	$CO_2C_4H_9$		$(CH_3)_2$	13.8	4.86
P08	OH		$(CH_3)_2$	$CO_2C_2H_4Br$		$(CH_3)_2$	25.4	4.60
P09	OH		$(CH_3)_2$	$CO_2CH_2CO_2C_2H_5$		$(CH_3)_2$	22.5	4.65
P10	=N–OH		$(CH_3)_2$	$CO_2CH_2C_6H_5$		$(CH_3)_2$	16.3	4.79
P11	=O		$(CH_3)_2$	$CO_2CH_2C_6H_5$	CH_3	CH_3	24.2	4.62
P12	OH		$(CH_3)_2$	$CO_2CH_2C_6H_5$	CH_3	CH_3	22.3	4.65
P13	=N–OH		$(CH_3)_2$	$CO_2CH_2C_6H_5$	CH_3	CH_3	20.2	4.69