



Molecular modeling of alkaloids bouchardatine and orirenierine binding to sirtuin-1 (SIRT1)

Gérard Vergoten^a, Christian Bailly^{b*}

^a. Inserm, INFINITE - U1286, Institut de Chimie Pharmaceutique Albert Lespagnol (ICPAL), Faculty of Pharmacy, University of Lille, Lille 59006, France

^b. Scientific Consulting Office, OncoWitan, Lille 59290, France

ARTICLE INFO

Article history

Received 31 May 2022

Accepted 22 July 2022

Available online 25 September 2022

Keywords

Sirtuin 1 (SIRT1)

Alkaloids

Bouchardatine

Orirenierine

Natural products

Cancer

Inflammatory diseases

Molecular modeling

Drug design

ABSTRACT

Objective Bouchardatine (**1**) is a β -indoloquinazoline alkaloid isolated from the plant *Bouchardatia neurococca*, acting as a modulator of adipogenesis and lipogenesis, and as an anticancer agent. The natural product functions as an activator of proteins adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and sirtuin 1 (SIRT1). We used molecular modeling to investigate the SIRT1-binding capacity of compound **1** and various structural analogues, such as orirenierine A (**2**) and orirenierine B (**3**) isolated from the medicinal plant *Oricia renieri*.

Methods We investigated the binding to human SIRT1 (hSIRT1) of 25 natural products including the β -indoloquinazoline alkaloids **1** – **3** and analogues, in comparison with the reference product sirtinol (*R* and *S* isomers). A sirtinol binding model was elaborated starting from the closed and open state conformations of the catalytic domain of hSIRT1 (PDB structures 4KXQ and 4IG9). For each compound bound to SIRT1, the empirical energy of interaction (ΔE) was calculated and compared to that of sirtinol.

Results In our model, compound **1** was found to bind modestly to the sirtinol site of SIRT1. In contrast, the presence of a phenolic OH group at position 7 on the quinazolinone moiety conferred a much higher binding capacity. Compound **2** provided SIRT1 protein complexes as stable as those observed with sirtinol. The replacement of the hydroxy substituent (**2**) with a methoxy group (**3**) reduced the SIRT1 binding capacity. Other SIRT1-binding natural products were identified, such as the alkaloids orisuvaeolines A and B. Structure-binding relationships were discussed.

Conclusion The study underlines the capacity of β -indoloquinazoline alkaloids to interact with SIRT1. This deacetylase enzyme could represent a molecular target for the alkaloid **2**. This compound merits further attention for the design of drugs active against SIRT1-dependent pathologies.

1 Introduction

The alkaloid bouchardatine (**1**) was isolated twenty years ago from the aerial parts of the plant *Bouchardatia*

neurococca (F. Muell.) Baill. (Rutaceae) collected in the state forest of Polmailly (Queensland, Australia). It is a small rainforest tree commonly known as “union nut” that is endemic to eastern Australia. The name

*Corresponding author: Christian Bailly, Professor, E-mail: christian.bailly@oncowitan.com.

Peer review under the responsibility of Hunan University of Chinese Medicine.

DOI: 10.1016/j.dcmcd.2022.10.004

Citation: VERGOTEN G, BAILLY C. Molecular modeling of alkaloids bouchardatine and orirenierine binding to sirtuin-1 (SIRT1). Digital Chinese Medicine, 2022, 5(3): 276–285.

Bouchardatia was given after the French pharmacist Apollinaire Bouchardat (1806 – 1886). The term *neurococca* derives from the Greek “neuron” (nerve) and “cocos” (a berry), referring to the ribbed carpels or cocci. There is no mention of the use of this plant in traditional medicine, unlike other related Rutaceae species. Chinese medicinal materials originated from Rutaceae family are frequently used to treat eczema, rheumatism, animal bites, and other health problems [1, 2]. Diverse bioactive natural products have been isolated from *Bouchardatia neurococca*, including limonoids such as veprisonic and isoveprisonic acid, and alkaloids such as bouchardatine, dictamnine, rutaecarpine and their derivatives [3]. Sesquiterpenes have been isolated from the plant leaf oil so far, while the phytochemical content of this species has been little investigated [4].

Bouchardatine (**1**) comprises a quinazolin-4-one core linked to an indole unit equipped with a formyl group (Figure 1). The alkaloid can be isolated from a methanol extract of the bark of *Bouchardatia neurococca* after chromatography. Alternatively, total syntheses of the compound have been described [5]. An efficient 5-step synthesis from anthranilamide afforded gram-quantities of

compound **1** for pharmacological studies [6]. The product has revealed interesting properties in treating metabolic diseases and cancer. A lipid-lowering effect was initially described using cultured 3T3-L1 adipocytes. Compound **1** was found to reduce dose-dependently the proliferation of adipocytes and the accumulation of lipids by suppressing the expression of adipogenic factors such as peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT enhancer-binding proteins (C/EBP) $\alpha/\beta/\delta$. The lipid-lowering effect of compound **1** was linked to an activation of the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway [6]. Further studies on the effects of compound **1** on adipogenesis and lipogenesis led to a better understanding of the mechanism of action of the natural product. RAO et al. [7] found that compound **1** increased the activity of the protein sir-tuin 1 (SIRT1) to facilitate activation of AMPK by the enzyme liver kinase B1 (LKB1). Activation of the SIRT1-LKB1-AMPK signaling pathway by compound **1** in adipose tissue and liver is at the origin of the anti-obesity effect. The capacity of compound **1** to reduce obesity was demonstrated using a specific high-fat diet model in mice [7].

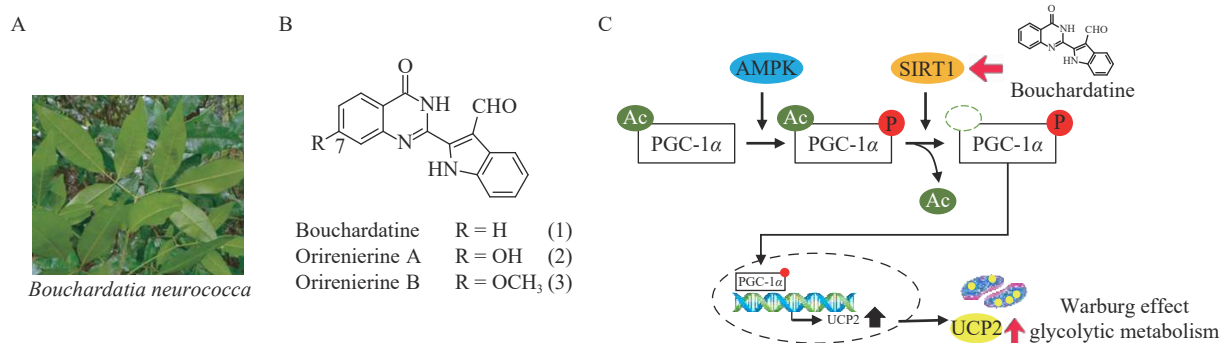


Figure 1 Bouchardatine, plant origin, and mechanism of action

A, the aerial part of the plant *Bouchardatia neurococca* (F. Muell.) Baill. (<https://alphitonia.com/EditSpeciesE.cshtml?id=555>). B, structures of the alkaloids bouchardatine (**1**), orienierine A (**2**), and orienierine B (**3**). C, a schematic illustration of the mechanism of compound **1** as an activator of the deacetylase SIRT1 to upregulate the expression of uncoupling protein 2 (UCP2) implicated in the glycolytic metabolism of cancer cells.

The interest towards compound **1** has been underlined in a recent study of the anticancer activity of the product in a murine model for rectal cancer [8]. The growth of a xenografted HCT-116 tumor in mice was reduced markedly upon daily administration of compound **1** at the dose of 50 mg/kg (i.p. injection). More importantly, the authors demonstrated that compound **1** activated the metabolic regulator SIRT1, leading to the subsequent activation of peroxisome proliferators activated receptor γ coactivator 1 α (PGC-1 α), coupled to a downstream upregulation of uncoupling protein 2 (UCP2) and an alteration of the cancer cell metabolism, as depicted in Figure 1 [8]. UCP2 is an antioxidant mitochondrial protein involved in the maintenance of the Warburg effect in

cancer cells. Most importantly, this study demonstrated that activation of the SIRT1-PGC1 α -UCP2 axis by compound **1** was at the origin of the anticancer effect. A mutation (H355A) in the enzymatic site of SIRT1 abolished the stimulation effect of compound **1**, suggesting that this protein could be a direct target for the natural product [8].

Based on these considerations, we attempted to evaluate the capacity of compound **1** to interact with SIRT1 using molecular modeling. The protein functions as a nicotinamide adenine dinucleotide (NAD⁺)-dependent class III histone deacetylase (HDAC). The tridimensional structure of the catalytic domain of human SIRT1 has been solved by X-ray crystallography [9]. We used the HDAC domain of human SIRT1 (hSIRT1, PDB: 4KXQ and

4IG9) to perform a docking study to locate the potential binding site for compound **1**, using the known hSIRT1 inhibitor sirtinol as a reference. The same model has been used successfully by different research groups to identify new inhibitors of hSIRT1 [10, 11]. An *in silico* model of the bouchardatine-hSIRT1 complex was elaborated, and then used to compare the binding capacity of over 20 alkaloids, including orirenierine A and B (compounds **2** and **3**, respectively), orisuaveoline A and B, luotonins A – F, and other products.

2 Materials and methods

2.1 Molecular structures and software

Two tridimensional structures of hSIRT1 in a closed (4KXQ) and an open (4IG9) state conformation were retrieved from the Protein Data Bank (www.rcsb.org) [9]. Docking experiments were performed using the GOLD software (GOLD 5.3 release, Cambridge Crystallographic Data Centre, UK). Before starting the docking procedure, the structure of the ligands had been optimized using a classical Monte Carlo (MC) conformational searching procedure as described in the BOSS software [12]. Potential GSK-3 -binding sites for the different products were searched using the web server Computed Atlas of Surface Topography of proteins (CASTp) 3.0, and visualized with the molecular modeling software Chimera 1.15 [13, 14].

2.2 In silico molecular docking procedure

With each structure, the drug binding site and surrounding amino acids were defined based on shape complementarity criteria. Shape complementarity and geometry considerations were in favor of a docking grid centered in the volume defined by the central amino acid. Within the binding site, side chains of specific amino acids were considered fully flexible. For structure 4KXQ, the flexible amino acids were Phe273, Phe297, Ile316, Tyr317, Ile347, His363, Ser365, Phe366, Val412, and Phe413. The ligand was always defined as flexible during the docking procedure. Up to 100 poses that were energetically reasonable were kept while searching for the correct binding mode of the ligand. The decision to keep a trial pose was based on ranked poses, using the Picewise Liner Potential (PLP) fitness scoring function (which is the default in GOLD version 5.3 used here) [15]. The same procedure was used to establish molecular models for the various natural products (Table 1) and the reference compounds. The empirical potential energy of interaction (ΔE) for the ranked complexes was evaluated using the simple expression $\Delta E (\text{interaction}) = E (\text{complex}) - [E (\text{protein}) + E (\text{ligand})]$. For that purpose, the Spectroscopic Empirical Potential Energy function Spectroscopic Potential Algorithm for Simulating Biomolecular conformational Adaptability

(SPASIBA) and the corresponding parameters were used. Free energies of hydration (ΔG) were estimated using the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) model in MC simulations within the BOSS software [16]. The stability of the receptor-ligand complex was evaluated based on the empirical potential energy of interaction [17, 18]. The MM/GBSA procedure was used to evaluate free energies of hydration (within the BOSS program) [12] in relation to aqueous solubility. Molecular graphics were created and analyzed using Discovery Studio Visualizer, Biovia 2020 (Dassault Syst mes BIOVIA Discovery Studio Visualizer 2020, San Diego, Dassault Syst mes, 2020).

Table 1 Calculated potential energy of interaction (ΔE , kcal/mol) and free energy of hydration (ΔG_{hyd} , kcal/mol) for the interaction of indoloquinazolinone alkaloids (and selected natural products) with SIRT1 (PDB: 4KXQ)

Compound	CID ^a	ΔE	ΔG_{hyd}
(R)-Sirtinol	1376646	– 75.70	– 32.60
(S)-Sirtinol	1376645	– 74.50	– 29.60
Bouchardatine	135800540	– 49.50	– 21.70
Orirenierine A	–	– 71.40	– 23.70
Orirenierine B	–	– 56.90	– 25.40
Orisuaveoline A	135953334	– 65.40	– 18.70
Orisuaveoline B	25157868	– 64.40	– 26.15
Ketoyobirine	275187	– 62.90	– 20.70
Hortiacine	378227	– 54.00	– 18.70
Evodiamine	442088	– 52.30	– 17.30
Hydroxyevodiamine	56967381	– 63.90	– 18.80
Angustine	441983	– 58.40	– 1.40
Angustoline	3084765	– 48.00	– 3.40
Nauclefine	5320037	– 49.40	– 21.90
Rhetsinine	99652	– 54.20	– 17.30
Tephcalostan	11013898	– 59.10	– 25.90
Tephcalostan B	25243039	– 56.65	– 22.15
Tephcalostan C	–	– 58.80	– 27.80
Tephcalostan D	102283894	– 63.10	– 21.80
Luotonin A	10334120	– 48.95	– 18.50
Luotonin B	10017730	– 42.55	– 12.60
Luotonin C	102369825	– 44.80	– 19.10
Luotonin D	102369826	– 55.30	– 17.00
Luotonin E	11483874	– 45.95	– 23.20
Luotonin F	13545779	– 53.55	– 13.10

^aCompound IDentity number, as defined in PubChem (<https://pubchem.ncbi.nlm.nih.gov>). “–” represents that these compounds are not listed in the PubChem databank.

2.3 MC simulations

The two most widely used methods to investigate protein-ligand stability and affinity are Molecular Dynamics (MD) and MC simulations. MD simulation of proteins is a challenge that requires careful consideration of the 1st

law of thermodynamics [19]. Both methods employ an empirical force field to control the total energy (MC, energy minimization) and forces (MD, Newton equations of motion). To use MD simulations confidently, a force field parameterized for dynamical properties is required. The development of a reliable and accurate force field for conformational analysis remains a concern. It requires accuracy of the force field over the whole potential surface, rather than in the region of the global minimum [20]. The most used academic force fields, such as Chemistry at HARvard Macromolecular Mechanics (CHARMM), Assisted Model Building with Energy Refinement (AMBER), and GRONingen MOlecular Simulation (GROMOS), do not exhibit the required vibrational spectroscopic quality. Minimization of a protein structure with normal modes can result in the calculation of imaginary wavenumbers corresponding to maxima in the potential energy (transition states, mainly due to inadequate barriers to internal rotation). The spectroscopic SPASIBA force field has been specifically developed to provide refined empirical molecular mechanics force field parameters, as described in other studies [17, 21]. For this reason, we adopted MC simulations rather than MD, which requires a substantial increase in computing time to achieve the same level of convergence [22].

3 Results

3.1 Construction of the sirtinol-SIRT1 binding model

We started our analysis with two structural models of SIRT1. The PDB structures 4KXQ and 4IJ9 refer to a closed and an open state conformation of the catalytic domain of hSIRT1, respectively [9]. The two structures were very similar, as shown from the superimposed model in Figure 2A. The position of the adenosine 5'-diphosphoribose (ADP-ribose) ligand was identical for the two structures; the secondary structure of the binding site was preserved. The only significant difference was around the loop Phe273-Ile279, which seemed to act as a gate, closing or opening the site, as represented in Figure 2B, though this gate did not affect sirtinol binding around site I347. To locate the drug binding site, we used the web server CASTp 3.0, which was specifically designed for the analysis of protein topography. This tool is mostly used in identifying drug binding sites and quantifying the volume of the binding pocket [14]. A view of the drug-binding pocket defined with CASTp using the open state conformation (4IJ9) is shown in Figure 2B. Two potential binding sites were identified for the reference inhibitor sirtinol. The best site was located near residue Ile347 ($\Delta E = -71.30$ kcal/mol and $\Delta G = -30.40$ kcal/mol) and a secondary site near residue Ile316 ($\Delta E = -55.40$ kcal/mol and $\Delta G = -20.00$ kcal/mol). The main sirtinol binding site Ile347 was juxtaposed to the ADP-ribose binding site

(Figure 2C). This site Ile347 was defined as the potential binding site for the other ligands tested in our docking analysis, and was juxtaposed to the C-pocket, where the nicotinamide moiety of NAD^+ binds and the hydrolysis takes place. Drug binding to this site is known to block the transformation of NAD^+ to productive conformation and to inhibit the deacetylase activity [23, 24]. The secondary weak site (Ile316) has never been mentioned in other studies; it is considered too weak and irrelevant. Our analysis was thus focused on the primary site.

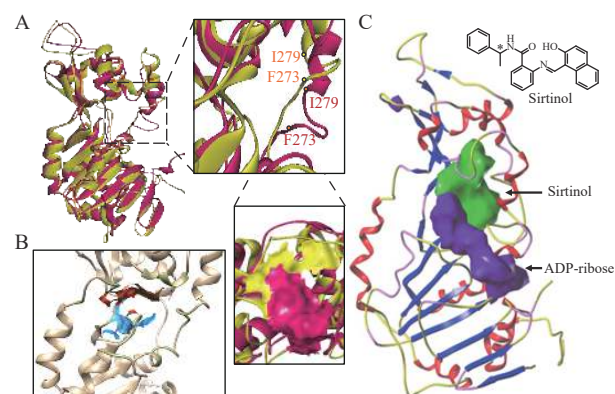


Figure 2 Molecular model of sirtinol bound to SIRT1

A, superimposition of the open state (4IJ9, magenta) and closed state (4KXQ, yellow) conformations of SIRT1. A closed view of the ADP-ribose binding zone is shown (inset) to underline the different positions of the F273-I279 flexible loop, closed or opened. B, a view of the sirtinol binding zone (as defined by CASTp 3.0) delimited by the hydrophilic (blue) and hydrophobic (red) areas. C, global view of the catalytic domain of hSIRT1 with the ADP-ribose (violet) and sirtinol (green) binding regions highlighted. The structure of sirtinol is shown (with the asymmetry center *).

We defined the pocket around residue Ile347 as the sirtinol binding site and compared the two isomers (*R*)-sirtinol and (*S*)-sirtinol, but no significant difference was found between the two products: both produced stable complexes with SIRT1 as indicated from the ΔE values collated in Table 1. As expected, there is no enantioselective inhibitory effect toward SIRT1. It has been shown that (*R*)- and (*S*)-sirtinol have similar inhibitory effects on the yeast and human SIRT1 enzymes [25]. Altogether, our sirtinol-SIRT1 model falls in good agreement with models previously published. We kept the model (4KXQ) for the subsequent docking analysis.

3.2 Interaction of bouchardatine with SIRT1

A docking model of compound **1** bound to SIRT1 was elaborated, as shown in Figure 3, where compound **1** sits in the small cavity close to the ADP-ribose binding site. Several interactions stabilized the drug-protein complex, including a π -stacking interaction with Phe273 and an H-bond between NH-indole and Ile347. Additional van der Waals contacts contributed to maintaining the

the molecule to ADP-ribose whereas the 7-OCH₃ group of compound **3** pulled apart the molecule from the ADP-ribose (Figure 5B).

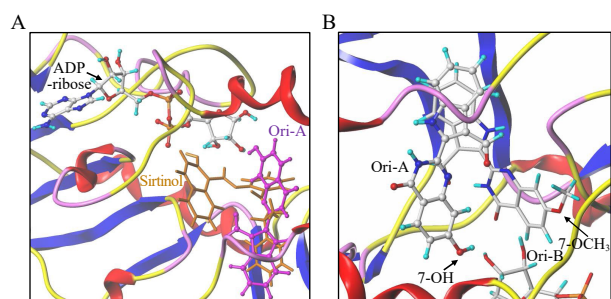


Figure 5 Compounds orientation in the binding site A, detailed view of the drug-binding pocket of SIRT1 showing the distinct orientation of orirenierine A (Ori-A) and sirtinol. The two compounds are facing the ADP-ribose ligand. B, superimposed view of orirenierine A (Ori-A) and orirenierine B (Ori-B) bound to SIRT1. Note the distinct orientations of the 7-hydroxy (Ori-A) and 7-methoxy group (Ori-B) (arrows).

3.4 Other alkaloids

The identification of orirenierine A (**2**) as a potential SIRT1 binder prompted us to look for other indoloquinazolinone alkaloids susceptible to forming stable complexes with the protein. We identified a dozen natural products bearing an indoloquinazolinone unit and extended the search to 20 compounds, including quinazolinocarboline alkaloids like evodiamine, coumestan derivatives like tephcalostans, and pyrroloquinolinequinoline alkaloids like luotonin A and its derivatives (Figure 6). However, in most cases these compounds were contained in an extended pentacyclic core system, quite distinct from the bi-unit system of compounds **1** – **3**. For

example, the two alkaloids orisuaveolines A and B isolated from the plant *Oricia suaveolens* [30] bore the same β -indoloquinazolinone core as compounds **1** – **3** but the quinazolinone and indole unit were fused to form a penta (orisuaveoline A) or hexa (orisuaveoline B) cyclic rigid system. SIRT1 docking analysis was performed with all compounds (Table 1). We did not identify a natural product superior to orirenierine A for binding to SIRT1. However, useful information was obtained.

Orisuaveolines A and B bore the same -OH or -OCH₃ as orirenierine A (**2**) and orirenierine B (**3**), respectively. These two compounds were superior to compound **1**, thus providing another piece of evidence for the key role of the phenolic OH group in protein binding. However, the rigid polyaromatic system was apparently less favorable than the bis-unit of compounds **1** – **3**. The ΔE value calculated with orisuaveoline A was only 10% higher than that of orirenierine A. The anti-inflammatory action of this compound would be worth investigating, which has not been studied thus far.

The quinolone alkaloid evodiamine (isolated from the fruit of *Evodia rutaecarpa* and other plants) displayed a large range of biological activities, including antitumor, anti-inflammation, and antimicrobial effects [31, 32]. It represented a lead structure for the design of anticancer compounds [33], with its anticancer activity being considered as SIRT1-mediated [34]. SIRT1 was likely to play a significant role in the capacity of evodiamine to inhibit the migration and invasion of cancer cells [35], but our calculations suggest that SIRT1 is not a direct binding target for evodiamine. At least, we can underline that the compound cannot form highly stable complexes upon binding to the sirtinol site of the protein. However, it is

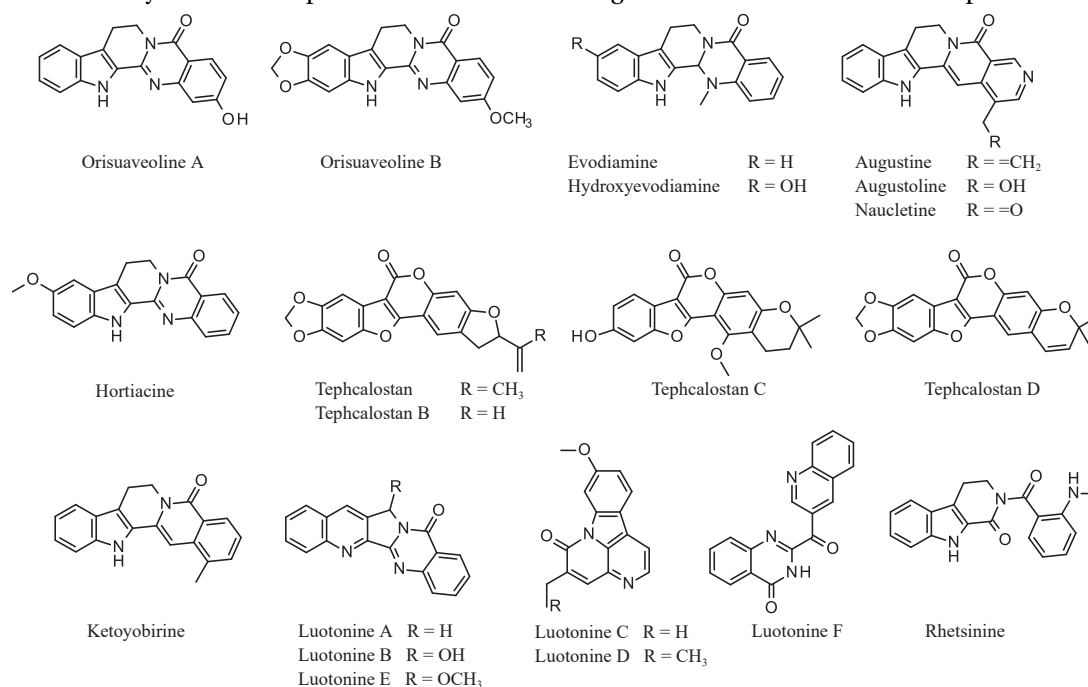


Figure 6 Structure of the other natural products evaluated as potential SIRT1 binders (as indicated in Table 1)

interesting to note that 10-hydroxyevodiamine demonstrated a better SIRT1 binding capacity than that of evodiamine (Table 1). The OH-group significantly reinforced the stability of the alkaloid-protein complex, with a gain of ΔE value of about 15% ($\Delta E = -52.30$ and -63.90 kcal/mol for evodiamine and hydroxyevodiamine, respectively). This is interesting as 10-hydroxyevodiamine has been used to design fluorescent probes and tumor-active water-soluble derivatives [36, 37]. The 10-OH group can be exploited to reinforce evodiamine binding to SIRT1, whereas the N-methyl group is apparently unessential. The little-known alkaloid ketoyobirine, which is rarely investigated [38], is as efficient as evodiamine in binding to SIRT1. In short, several compounds could be tested but only one stands out as a promising SIRT1 binder: orirenierine A.

4 Discussion

More than 150 naturally occurring quinazolinone alkaloids have been identified by now [39, 40]. Some of them have been thoroughly investigated, such as the luotonins [41], while others have received little attention, such as the quinazolinocarboline alkaloid hortiacine [42]. This is also the case for the alkaloids orirenierines A and B, and orisuaveolines A and B isolated from *Oricia renieri* and *Oricia suaveolens*, respectively, by the same research group [26, 30]. These alkaloids have been described but their mechanisms of action remain unknown. In contrast, the quinazolinone alkaloid bouchardatine (1) has received attention owing to its potential in the treatment of obesity-related metabolic disorders and cancers. The signaling pathways modulated by this natural product have been characterized, notably the SIRT1-PGC1 α -UCP2 axis, and a recent study suggested that SIRT1 could be a direct target for the natural product [8]. Based on this study, and considering the structural analogy between compound 1 and orirenierines A and B (2 and 3), we established a docking study to investigate this topic.

The analysis indicated the superior capacity of the related alkaloid compound 2 compared to compound 1 to bind to hSIRT1. This alkaloid 2, which is structurally close to compound 1 (Figure 1), showed better adaptability to form stable complexes with hSIRT1 than compound 3 and all the other polycyclic indoloquinazolinone alkaloids tested. The observation is of great importance from a medicinal point of view because compound 2 derives from the Cameroonian plant *Oricia renieri* (Rutaceae) and is used locally to treat different types of infections and cancers [26, 30]. Our analysis suggests that SIRT1 may effectively represent a protein target for these alkaloids. They have the capacity to bind to the sirtinol-binding site, in the pocket facing the ADP-ribose ligand. Compound 1 emerged as a relatively weak SIRT1 binder compared to the reference sirtinol, whereas compound 2 is

quasi-equipotent to sirtinol, at least in terms of binding energy (ΔE). The phenolic OH which distinguishes compound 1 from 2 is a prime element for protein binding, and the capping of this group in the form of a methoxy (compound 3) reduces the binding interaction. This OH group is undoubtedly a key protein binding element. It is noteworthy that the potency of the three compounds in terms of SIRT1 binding is comparable to their potency as cytotoxic agents against cancer cells. Compound 2 was found to be more cytotoxic toward PC3 prostate cancer cells compared with compounds 3 and 1, with IC₅₀ values of 23.2, 36.5, and 39.4 μ mol, respectively [26]. In other words, the higher capacity of 2 to interact with SIRT1 correlates with its higher cytotoxic potential. This compound merits further attention. It is an important component of extracts of *Oricia renieri* [27], and for the first time, a molecular target for it is proposed.

SIRT1 binders were actively searched because these compounds showed potential in the treatment of cancer and other human diseases, including autoimmune diseases [43-45]. It is therefore interesting to propose a new scaffold β -indoloquinazoline for the design of SIRT1-interacting drugs. This scaffold has been little investigated thus far. However, there are two noticeable exceptions. Recently, the drug IQZ23 has been proposed as a candidate for the treatment of obesity and obesity-related metabolic disorders. This β -indoloquinazoline derivative was found to activate the AMPK pathway but its molecular target is not precisely known [46]. The preclinical development of IQZ23 has been initiated [47]. It would be interesting to determine its capacity to modulate the activity of SIRT1. Similarly, compounds 1 – 3 present a structural analogy with the anticancer dihydroquinazolinone derivative MHY2245 recently identified as a potent sirtuin inhibitor [48, 49]. This reinforces the idea that orirenierines can be used as templates to design SIRT1 modulators.

The present work can also guide the future design of bouchardatine analogues. Various synthetic analogues of compound 1 have been designed as inhibitors of adipogenesis/lipogenesis [50]. For example, the lipid-lowering activity of compound 1 can be considerably enhanced by replacing the quinazolin-4-one with a quinazolin-4-amine group. Potent activators of AMPK have been obtained, with compounds active *in vivo*, of interest in the treatment of metabolic diseases and non-alcoholic fatty liver disease (NAFLD) [51, 52]. Based on our observations, new compounds bearing the key 7-OH group of compound 2 could be now envisioned to reinforce SIRT1 binding and potentially their activity against different metabolic diseases.

As an important protein target, SIRT1 is associated with the activity of different Chinese medicine prescriptions, such as Taohuajing (桃花精) treating diabetic cardiomyopathy [53], Yunpi Heluo Decoction (运脾和络方) treating type 2 diabetes [54], Zhibai Dihuang Decoction (知

柏地黄汤) treating oral ulcers [55]. The identification of novel SIRT1 binders may help further to better understand the molecular basis of the bioactivity of different Chinese medicine products.

This computational study is useful to select and prioritize compounds for further laboratory experiments. Docking analysis such as those reported here have limited predictability, due to the complexity of the protein folding process and its dynamic [56]. Molecular docking represents a useful aid in the process of drug discovery and development [57, 58], but its predictive value is limited. We do not underestimate the necessity to carry out wet-lab validations for the predicted SIRT1-binding candidates identified here.

5 Conclusion

In summary, SIRT1 is an NAD⁺-dependent deacetylase implicated in various human pathologies. Small molecule modulators of SIRT1 were searched, including SIRT1 inhibitors and activators. Here we found that alkaloids bearing an unfused β -indoloquinazoline ring system can provide potential SIRT1 binders. We characterized the interaction of the alkaloid orirenierine A (**2**) with human SIRT1 at the sirtinol-binding site. The specific contribution of the phenolic OH group of compound **2** was underlined. The use of various structural analogues enables the definition of structure-binding relationships.

6 Highlights

- (i) The plant alkaloid bouchardatine (**1**) has been found to activate the enzyme SIRT1.
- (ii) Molecular docking indicates that analogue orirenierine A (**2**) presents a high capacity to bind to the sirtinol site of SIRT1.
- (iii) A few other indoloquinazoline alkaloids interacting at the sirtinol site of SIRT1 have been identified.
- (iv) Structure-binding elements have been defined in the chemical series.
- (v) The β -indoloquinazoline unit can be exploited to design SIRT1-binding drugs active against cancers.

Competing interests

The authors declare no conflict of interest.

References

- [1] FU XL, ZHOU J, TANG WW, et al. Study on the compatibility effect and active constituents of *Atractylodes Rhizoma* in Ermiao Wan against acute gouty arthritis. *Journal of Ethnopharmacology*, 2021, 279: 114353.
- [2] YAO Q, GAO Y, LAI C, et al. The phytochemistry, pharmacology and applications of *Melicope pteleifolia*: a review. *Journal of Ethnopharmacology*, 2020, 251: 112546.
- [3] WATTANAPIROMSAKUL C, FORSTER PI, WATERMAN PG. Alkaloids and limonoids from *Bouchardatia neurococca*: systematic significance. *Phytochemistry*, 2003, 64(2): 609-615.
- [4] PEREIRA LPLA, RIBEIRO ECG, BRITO MCA, et al. Essential oils as molluscicidal agents against schistosomiasis transmitting snails - a review. *Acta Tropica*, 2020, 209: 105489.
- [5] KIM K, KIM HY, OH K. *ortho*-Naphthoquinone-catalyzed aerobic oxidation of amines to fused pyrimidin-4(3*H*)-ones: a convergent synthetic route to bouchardatine and sildenafil. *RSC Advances*, 2020, 10(52): 31101-31105.
- [6] RAO Y, LIU H, GAO L, et al. Discovery of natural alkaloid bouchardatine as a novel inhibitor of adipogenesis/lipogenesis in 3T3-L1 adipocytes. *Bioorganic and Medicinal Chemistry*, 2015, 23(15): 4719-4727.
- [7] RAO Y, YU H, GAO L, et al. Natural alkaloid bouchardatine ameliorates metabolic disorders in high-fat diet-fed mice by stimulating the sirtuin 1/liver kinase B-1/AMPK axis. *British Journal of Pharmacology*, 2017, 174(15): 2457-2470.
- [8] XU YH, SONG QQ, LI C, et al. Bouchardatine suppresses rectal cancer in mice by disrupting its metabolic pathways via activating the SIRT1-PGC-1 α -UCP2 axis. *European Journal of Pharmacology*, 2019, 854: 328-337.
- [9] DAVENPORT AM, HUBER FM, HOELZ A. Structural and functional analysis of human SIRT1. *Journal of Molecular Biology*, 2014, 426(3): 526-541.
- [10] PADMANABHAN B, RAMU M, MATHUR S, et al. Identification of new inhibitors for human SIRT1: an in-silico approach. *Medicinal Chemistry*, 2016, 12(4): 347-361.
- [11] RAMPOGU S, RAMPOGU LM. Network based approach in the establishment of the relationship between type 2 diabetes mellitus and its complications at the molecular level coupled with molecular docking mechanism. *BioMed Research International*, 2016, 2016: 6068437.
- [12] JORGENSEN WL, TIRADO-RIVES J. Molecular modeling of organic and biomolecular systems using BOSS and MCPRO. *Journal of Computational Chemistry*, 2005, 26(16): 1689-1700.
- [13] PETTERSEN EF, GODDARD TD, HUANG CC, et al. UCSF Chimera-a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 2004, 25(13): 1605-1612.
- [14] TIAN W, CHEN C, LEI X, et al. CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Research*, 2018, 46(W1): 363-367.
- [15] JONES G, WILLETT P, GLEN RC, et al. Development and validation of a genetic algorithm for flexible docking. *Journal of Molecular Biology*, 1997, 267(3): 727-748.
- [16] JORGENSEN WL, ULMSCHNEIDER JP, TIRADO-RIVES J. Free energies of hydration from a generalized born model and an ALL-atom force field. *Journal of Physical Chemistry B*, 2004, 108(41): 16264-16270.
- [17] VERGOTEN G, MAZUR I, LAGANT P, et al. The SPASIBA force field as an essential tool for studying the structure and dynamics of saccharides. *Biochimie*, 2003, 85(1-2): 65-73.
- [18] LAGANT P, NOLDE D, STOTE R, et al. Increasing normal modes analysis accuracy: the SPASIBA spectroscopic force field introduced into the CHARMM program. *Journal of Physical Chemistry A*, 2004, 108(18): 4019-4029.
- [19] TORSHIN IY, NAMIOT VA, ESIPOVA NG, et al. Numeric

- analysis of reversibility of classic movement equations and constructive criteria of estimating quality of molecular dynamic simulations. *Journal of Biomolecular Structure and Dynamics*, 2021, 39: 4066–4076.
- [20] HOMANS SW. A molecular mechanical force field for the conformational analysis of oligosaccharides: comparison of theoretical and crystal structures of Man α 1-3Man β 1-4GlcNAc. *Biochemistry*, 1990, 29(39): 9110–9118.
- [21] MEZIANE-TANI M, LAGANT P, SEMMOUD A, et al. The SPASIBA force field for chondroitin sulfate: vibrational analysis of D-glucuronic and N-acetyl-D-galactosamine 4-sulfate sodium salts. *Journal of Physical Chemistry A*, 2006, 110(39): 11359–11370.
- [22] JORGENSEN WL, TIRADO-RIVES J. Monte Carlo versus molecular dynamics for conformational sampling. *Journal of Physical Chemistry*, 1996, 100(34): 14508–14513.
- [23] WU J, LI Y, CHEN K, et al. Identification of benzofuran-3-yl(phenyl)methanones as novel SIRT1 inhibitors: binding mode, inhibitory mechanism and biological action. *European Journal of Medicinal Chemistry*, 2013, 60: 441–450.
- [24] LIN MH, ISLAM A, LIU YH, et al. Antibiotic heliomycin and its water-soluble 4-aminomethylated derivative provoke cell death in T24 bladder cancer cells by targeting sirtuin 1 (SIRT1). *American Journal of Cancer Research*, 2022, 12(3): 1042–1055.
- [25] MAI A, MASSA S, LAVU S, et al. Design, synthesis, and biological evaluation of sirtinol analogues as class III histone/protein deacetylase (Sirtuin) inhibitors. *Journal of Medicinal Chemistry*, 2005, 48(24): 7789–7795.
- [26] WANSI JD, HAPPI EN, BAVOUA JL, et al. Bioactive β -indoloquinazoline alkaloids from *Oricia renieri*. *Planta Medica*, 2012, 78(1): 71–75.
- [27] NOUGA AB, NDOM JC, MPONDO EM, et al. New furoquinoline alkaloid and flavanone glycoside derivatives from the leaves of *Oricia suaveolens* and *Oricia renieri* (Rutaceae). *Natural Product Research*, 2016, 30(3): 305–310.
- [28] FONG Y, LIN YC, WU CY, et al. The antiproliferative and apoptotic effects of sirtinol, a sirtuin inhibitor on human lung cancer cells by modulating Akt/ β -catenin-Foxo3a axis. *The Scientific World Journal*, 2014, 2014: 937051.
- [29] TSAI YF, YU HP, CHANG WY, et al. Sirtinol inhibits neutrophil elastase activity and attenuates lipopolysaccharide-mediated acute lung injury in mice. *Scientific Reports*, 2015, 5: 8347.
- [30] WANSI JD, MESAİK MA, CHIOZEM DD, et al. Oxidative burst inhibitory and cytotoxic indoloquinazoline and furoquinoline alkaloids from *Oricia suaveolens*. *Journal of Natural Products*, 2008, 71(11): 1942–1945.
- [31] LUO C, AI J, REN E, et al. Research progress on evodiamine, a bioactive alkaloid of *Evodiae fructus*: focus on its anti-cancer activity and bioavailability (review). *Experimental and Therapeutic Medicine*, 2021, 22(5): 1327.
- [32] LI DP, LI Y, JIANG XW, et al. Evodiamine-A privileged structure with Broad-Ranging biological activities. *Mini Reviews in Medicinal Chemistry*, 2022, 22(21): 2680–2701.
- [33] FAN M, YAO L. The synthesis, structural modification and mode of anticancer action of evodiamine: a review. *Recent Patents in Anticancer Drug Discovery*, 2022, 17(3): 284–296.
- [34] WANG C, WANG MW, TASHIRO S, et al. Roles of SIRT1 and phosphoinositide 3-OH kinase/protein kinase C pathways in evodiamine-induced human melanoma A375-S2 cell death. *Journal of Pharmacological Sciences*, 2005, 97(4): 494–500.
- [35] ZHOU P, LI XP, JIANG R, et al. Evodiamine inhibits migration and invasion by Sirt1-mediated post-translational modulations in colorectal cancer. *Anticancer Drugs*, 2019, 30(6): 611–617.
- [36] CHEN S, DONG G, WU S, et al. Novel fluorescent probes of 10-hydroxyevodiamine: autophagy and apoptosis-inducing anticancer mechanisms. *Acta Pharmaceutica Sinica B*, 2019, 9(1): 144–156.
- [37] CHEN S, BI K, WU S, et al. Water-soluble derivatives of evodiamine: discovery of evodiamine-10-phosphate as an orally active antitumor lead compound. *European Journal of Medicinal Chemistry*, 2021, 220: 113544.
- [38] WOODWARD RB, WITKOP B. The structure of ketoyobyrine. *Journal of the American Chemical Society*, 1948, 70(7): 2409–2412.
- [39] KSHIRSAGAR UA. Recent developments in the chemistry of quinazolinone alkaloids. *Organic & Biomolecular Chemistry*, 2015, 13(36): 9336–9352.
- [40] HAMEED A, AL-RASHIDA M, UROOS M, et al. Quinazoline and quinazolinone as important medicinal scaffolds: a comparative patent review (2011-2016). *Expert Opinion on Therapeutic Patents*, 2018, 28(4): 281–297.
- [41] LIANG JL, CHA HC, JAHNG Y. Recent advances in the studies on luotonins. *Molecules*, 2011, 16(6): 4861–4883.
- [42] MOHANTA PK, KIM K. A short synthesis of quinazolinocarboline alkaloids rutaecarpine, hortiacine, euxylophoricine A and euxylophoricine D from methyl *N*-(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene) anthranilates. *Tetrahedron Letters*, 2002, 43(22): 3993–3996.
- [43] CUYAS E, VERDURA S, LLORACH-PARES L, et al. Metformin is a direct SIRT1-Activating compound: computational modeling and experimental validation. *Frontiers in Endocrinology (Lausanne)*, 2018, 9: 657.
- [44] SHEN P, DENG X, CHEN Z, et al. SIRT1: a potential therapeutic target in autoimmune diseases. *Frontiers in Immunology*, 2021, 12: 779177.
- [45] HUANG C, JIANG S, GAO S, et al. Sirtuins: research advances on the therapeutic role in acute kidney injury. *Phytomedicine*, 2022, 101: 154122.
- [46] RAO Y, XU Z, HU YT, et al. Discovery of a promising agent IQZ23 for the treatment of obesity and related metabolic disorders. *European Journal of Medicinal Chemistry*, 2020, 192: 112172.
- [47] JIANG F, ZHENG J, RAO Y, et al. Determination of IQZ23 in rat plasma using LC-MS/MS: consideration for matrix effect and internal standard interference. *Bioanalysis*, 2022, 14(8): 455–465.
- [48] TAE IH, SON JY, LEE SH, et al. A new SIRT1 inhibitor, MHY2245, induces autophagy and inhibits energy metabolism via PKM2/mTOR pathway in human ovarian cancer cells. *International Journal of Biological Sciences*, 2020, 16(11): 1901–1916.
- [49] KANG YJ, JANG JY, KWON YH, et al. MHY2245, a Sirtuin inhibitor, induces cell cycle arrest and apoptosis in HCT116 human colorectal cancer cells. *International Journal of Molecular Sciences*, 2022, 23(3): 1590.
- [50] RAO Y, LIU H, GAO L, et al. Synthesis and biological evaluation of novel bouchardatine derivatives as potential adipogenesis/lipogenesis inhibitors for antiobesity treatment. *Journal of Medicinal Chemistry*, 2015, 58(23): 9395–9413.
- [51] GAO L, XU Z, RAO Y, et al. Design, synthesis and biological evaluation of novel bouchardatine analogs as potential

- inhibitors of adipogenesis/lipogenesis in 3T3-L1 adipocytes. *European Journal of Medicinal Chemistry*, 2018, 147: 90–101.
- [52] RAO Y, LU YT, LI C, et al. Bouchardatine analogue alleviates non-alcoholic hepatic fatty liver disease/non-alcoholic steatohepatitis in high-fat fed mice by inhibiting ATP synthase activity. *British Journal of Pharmacology*, 2019, 176(16): 2877–2893.
- [53] YAO R, CAO Y, WANG C, et al. Taohuajing reduces oxidative stress and inflammation in diabetic cardiomyopathy through the sirtuin 1/nucleotide-binding oligomerization domain-like receptor protein 3 pathway. *BMC Complementary Medicine and Therapies*, 2021, 21(1): 78.
- [54] MAO Z, LIU S, YU T, et al. Yunpi Heluo Decoction reduces ectopic deposition of lipids by regulating the SIRT1-FoxO1 autophagy pathway in diabetic rats. *Pharmaceutical Biology*, 2022, 60(1): 579–588.
- [55] SHAO Y, DING B, JI J, et al. The anti-inflammatory effect of Zhibaidihuang Decoction on recurrent oral ulcer with Sirt1 as the key regulatory target. *Evidence-Based Complementary Alternative Medicine*, 2021, 2021: 8886699.
- [56] GUPTA CL, AKHTAR S, BAJPAI P. *In silico* protein modeling: possibilities and limitations. *EXCLI Journal*, 2014, 13: 513–515.
- [57] KAUR T, MADGULKAR A, BHALEKAR M, et al. Molecular docking in formulation and development. *Current Drug Discovery Technologies*, 2019, 16(1): 30–39.
- [58] SACAN A, EKINS S, KORTAGERE S. Applications and limitations of *in silico* models in drug discovery. *Methods in Molecular Biology*, 2012, 910: 87–124.

生物碱 bouchardatine 及 orirenierine 与组蛋白去乙酰化酶 1 (SIRT1) 结合的分子建模研究

Gérard Vergoten^a, Christian Bailly^{b*}

a. Inserm, INFINITE - U1286, Institut de Chimie Pharmaceutique Albert Lespagnol (ICPAL),

Faculty of Pharmacy, University of Lille, Lille 59006, France

b. Scientific Consulting Office, OncoWitan, Lille 59290, France

【摘要】目的 Bouchardatine(**1**)是从植物 *Bouchardatia neurococca* 中分离得到的一种 β -吡啶喹啉生物碱,可作为脂肪生成的一种调节剂及一种抗癌药。天然产物作为蛋白腺苷 5'-单磷酸 (AMP) 活化蛋白激酶 (AMPK) 和组蛋白去乙酰化酶 1 (SIRT1) 的激活剂发挥作用。我们利用分子模型研究了化合物 **1** 和各种结构类似物的 SIRT1 结合能力,如从药用植物 *Oriciarenieri* 中分离得到的 orirenierine A(**2**)和 orirenierine B(**3**)。**方法** 我们研究了包括 β -吡啶喹啉生物碱 **1–3** 和类似物在内的 25 种天然产物与人源组蛋白去乙酰化酶 1 (hSIRT1) 的结合,并与参比产物去乙酰化酶(R和S异构体)进行了比较。从 hSIRT1 催化结构域的闭合和开放状态构象(PDB 结构: 4KXQ 和 4IG9)开始阐述去乙酰化酶结合模型。对于与 SIRT1 结合的每种化合物,我们计算了相互作用的经验能量 (ΔE),并与去乙酰化酶进行比较。**结果** 在我们的模型中,发现化合物 **1** 与 SIRT1 的去乙酰化酶位点适度结合。相反,喹啉酮部分 7 位酚羟基表现出更高的结合能力。化合物 **2** 提供的 SIRT1 蛋白复合物与去乙酰化酶观察到的一样稳定。用甲氧基(**3**)取代羟基取代基(**2**)降低了 SIRT1 的结合能力。我们还鉴定了其他 SIRT1 结合的天然产物,如生物碱 orisuaveolines A 和 B,并讨论了结构结合关系。**结论** 本研究强调了 β -吡啶喹啉生物碱与 SIRT1 相互作用的能力。这种去乙酰化酶可以代表生物碱 **2** 的分子靶标。这种化合物在设计对 SIRT1 依赖性病理有活性的药物研究方面值得进一步关注。

【关键词】组蛋白去乙酰化酶 1; 生物碱; Bouchardatine; Orirenierine; 天然产物; 癌症; 炎症性疾病; 分子建模; 药物设计