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POM analysis and computational interactions of 8-hydroxydiospyrin inside active site of protein tyrosine phosphatase 1B

Saud BAWAZER[1]; Asghar KHAN[2]; Abdur RAUF[3,*]; Taibi Ben HADDA[4]; Yahya S. AL-AWTHAN[5,6]; Omar BAHATTAB[5]; Umer RASHID[7]; Inamullah KHAN[8]; Muhammad Asif NAWAZ[9]; Md Sahab UDDIN[10,11]; Olatunde AHMED[12]; Mohammad Ali SHARIATI[13]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 21955, Saudi Arabia
[2] Department of Medicine Khyber Teaching Hospital, Peshawar, 25000, Pakistan
[3] Department of Chemistry, University of Swabi, Swabi, 23430, Pakistan
[4] LCM Laboratory, Mohammed First University, Faculty of Sciences, Oujda, 60000, Morocco
[5] Biology Department, Faculty of Sciences, University of Tabuk, Tabuk, 1144, Saudi Arabia
[6] Department of Biology, Faculty of Science, Ibb University, Ibb, Yemen
[7] Department of Chemistry, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, 22060, Pakistan
[8] Department of Pharmacy, University of Peshawar, Peshawar, 25120, Pakistan
[9] Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, 14400, Pakistan
[10] Department of Pharmacy, Southeast University, Dhaka, 1213, Bangladesh
[11] Pharmakon Neuroscience Research Network, Dhaka, 1207, Bangladesh
[12] Department of Biochemistry, Abubakar Tafawa Balewa University, Bauchi, 740272, Nigeria
[13] Moscow State University of Technologies and Management (The First Cossack University), Moscow, 119991, Russian Federation

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Abstract: Protein tyrosine phosphatase 1B (PTP1B) inhibition is considered as a potential therapeutic for the treatment of cancer, type 2 diabetes, and obesity. In our present work, we investigated the anti-diabetic potential of 8-hydroxydiospyrin (8-HDN) from *D. lotus* against the PTP1B enzyme. It showed significant inhibitory activity of PTP1B with an IC_{50} value of 18.37 ± 0.02 μM. A detailed molecular docking study was carried out to analyze the binding orientation, binding energy, and mechanism of inhibition. A comparative investigation of 8-HDN in the catalytic, as well as the allosteric site of PTP1B, was performed. Binding energy data showed that compound 8-HDN is more selective for the allosteric site and hence avoids the problems associated with catalytic site inhibition. The inhibition mechanism of 8-HDN can be further investigated as an active lead compound against PTP1B by using *in vitro* and *in vivo* models.

Abbreviations

8-HDN:	8-hydroxydiospyrin
PTP1B:	Protein tyrosine phosphatase 1B
T2D:	Type 2 diabetes

Introduction

Native to the tropics, *Diospyros*, known as data plum, is a genus of shrubs and evergreen trees. About 500 forms of the plant are known globally, of which 24 species are mostly found in India (Uddin *et al.*, 2011a). The importance of different species might refer to either as their dark timber called ebony trees or their fruit called persimmon trees. *Diospyros lotus* L. (*Ebenaceae*), a deciduous tree, is extensively cultivated in tropical zones of Asia and Southeast Europe due to its resistance to drought. Its fruit has been shown to have anti-tumor and anti-diabetic competency (Hamedia and Shojaosadati, 2019), antiseptic and febrifuge, as well as a medicating agent of constipation (Rauf *et al.*, 2015). In addition, a number of papers have highlighted the various applications of *D. lotus* including its nutritional content (Glew *et al.*, 2005), being employed as medical agents (Loizzo *et al.*, 2009; Rauf *et al.*, 2017; Rauf *et al.*, 2015), antidiarrheal activity (Rauf *et al.*, 2014), mitigating oxidative stress (OS) through scavenging free

*Address correspondence to: Abdur Rauf, mashaljcs@yahoo.com
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radicals (Rauf *et al.*, 2017), protecting cisplatin-induced OS (Cho *et al.*, 2016; Saral *et al.*, 2016), pro-inflammatory mediators (Cho *et al.*, 2016). Protein tyrosine phosphates are referred to as a diverse family of enzymes that mostly disaccord the regular activities accomplished by protein tyrosine kinases (Gurzov *et al.*, 2015). Several recent studies revealed that the PTP enzyme plays critical roles in signaling pathways. In this light, controlling the level of protein tyrosine phosphorylation is considered as a rampant mechanism that involves fundamentally in intracellular activities such as transcription, differentiation, and migration (Tonks, 2006; Nagata *et al.*, 2012; Li *et al.*, 2013). Among PTP family members, PTP1B is found in the cell and it is a type of PTP that is not associated with receptors. It is an interesting target for several disorders such as obesity and type 2 diabetes (T2D) (Kennedy and Ramachandran, 2000). Also, PTP1B contributes to the negative regulation of leptin- and insulin-receptor as reported by some genetic and biochemical studies (Koren and Fantus, 2007). In support of this, elevated insulin sensitivity, increased glycemic regulation, and resistance to obesity induced by diet were recorded in PTP1B-knockout experimental mice (Ali *et al.*, 2009). Thus, PTP1B inhibition can serve as a novel target in the control of obesity and type 2 diabetes mellitus. Consistently, scientists have intensified their efforts to isolate novel and natural PTP1B inhibitors globally.Therefore, in this study, 8-hydroxydiospyrin (8-HDN) (Fig. 1) from *D. lotus* was screened for PTP1B inhibiting activity. By molecular docking model, to display the molecular interaction between PTP1B and the 8-HDN.

Materials and Methods

Plant material (Diospyros lotus)

Diospyros lotus L. roots were obtained from Toormang Razagram, Pakistan. The root samples of the plant were authenticated by Dr. Abdur Rashid of the Department of Botany, University of Peshawar, Pakistan. The voucher specimen number, RF/01, was deposited at the Herbarium of the institution.

Extraction of the plant sample and isolation of the compound

The air-dried root samples of *D. lotus* were pulverized using an electric blender, after which 14 kg of the pulverized sample was weighted into another container. MeOH was added to the container containing the pulverized root sample of the plant and allowed to stand for 6 days. Thereafter, the mixture of the plant with the MeOH was filtrated and the filtrate was concentrated through the process of evaporation using a rotary vacuum evaporator at 50°C and reduced pressure. After the extraction process, the extract obtained was weighed (202 g) was defatted with hexane to remove color and dyes. The crude extract was subjected to various solvents to obtain various fractions such as hexane, chloroform, and ethyl acetate. The chloroform fraction (20 g) was subjected to repeat normal phase column chromatographically, which afforded 8-HDN (1.24 g).

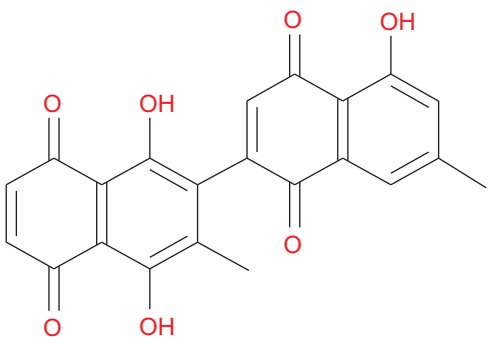

FIGURE 1. Chemical structure of 8-HDN.

PTP1B inhibitory activity

The enzyme (PTP1B) inhibition analysis was performed in 96-well plates in the presence of 3,3-dimethyl glutarate buffer, pH = 7.0. The reaction mixture includes *p*-nitrophenol phosphate (*p*NPP) at a concentration of 1 mM, PTB1B at a concentration of 10 mM, and various concentrations of 8-HDN as per our recently published method (Bawazeer *et al.*, 2019). Following the incubation period at 27°C for 40 min, the absorbance at 405 nm of the released *p*NPP was recorded. The procedure was performed in triplicates, and IC_{50} values were evaluated.

Molecular docking analysis

In this study, Molecular Operating Environment software, (version 2016.0802) was used to dock 8-HDN (Alhumaydhi *et al.*, 2021). The three-dimensional (3-D) structure of enzyme PTP1B in complex with catalytic inhibitor was retrieved from protein data Bank (PDB ID = 1NNY). While the 3-D crystal structure of PTP1B with allosteric inhibitor was obtained from PDB (ID = 1T49). Preparation of ligands (ursolic acid and 8-HDN) and downloaded proteins (3D protonation, energy minimization, and determination of binding site were carried out by our previously reported methods (Jan *et al.*, 2020; Tanoli *et al.*, 2019; Iftikhar *et al.*, 2018). All the ligand structures were drawn using the Builder option in MOE. A database of compounds was built as ligand.mdb. The compounds were then energy minimized up to 0.001 Gradient using MMFF94X forcefield. The enzyme structure was opened in the MOE window. The 3D protonation was done for all atoms in an implicit solvated environment at pH = 7, temperature = 300 K, and salt concentration of 0.1. The complete structure was energy minimized using MMFF94X forcefield. Finally, all the compounds were docked into the binding sites of the prepared enzymes. Default docking parameters were set, and ten different conformations were generated for each compound. The lowest binding energy ligand enzyme complexes were analyzed by the MOE ligand interaction module. While, for the 3-D interaction plot, a discovery studio visualizer was used (Biovia Systems 2017). While the surface model was created using Chimera (2020-09-08) (Pettersen *et al.*, 2004)

Results

PTP1B inhibitory activity

The chemical structure of 8-HDN was characterized by using physical and spectroscopic data recently published by our group (Uddin *et al.*, 2013; Ullah *et al.*, 2015). The chloroform fraction (20 g) was subjected to repeat normal phase column chromatographically, which afforded 8-HDN

TABLE 1

Protein tyrosine phosphatase 1B (PTP1B) inhibition activity of 8-HDN and ursolic acid

S. No.	Compound	IC_{50} (μM ± SEM)
1	8-HDN	18.37 ± 0.02
2	Ursolic acid	3.21 ± 0.02

(1.24 g). The inhibitory activity of the compound (8-HDN) on PTP1B gave an IC_{50} value of 18.37 ± 0.02 μM. The IC_{50} value was greater than the value obtained for ursolic acid (control), which has an IC_{50} value of 3.21 ± 0.02 μM (Tab. 1).

Molecular docking

We have performed a detailed molecular docking study to analyze the binding orientation, binding energy, and mechanism of inhibition. For the mode of inhibition, we carried out a comparative investigation of 8-HDN in catalytic as well as the allosteric site of protein tyrosine phosphatase 1B (PTP1B). It has been reported in the literature that the inhibition of the catalytic domain could result in off-target undesirable side effects. While the allosteric site is not well conserved among phosphatases and hence avoids the problems associated with catalytic site inhibition. For the current study, the 3-D structure of enzyme PTP1B in complex with catalytic inhibitor was retrieved from protein data Bank (PDB ID = 1NNY). While the 3-D crystal structure of PTP1B with allosteric inhibitor was obtained from PDB (ID = 1T49). Ribbon and surface superimposed models of the two retrieved proteins are shown in Figs. 2a and 2b. The catalytic site is centered at Cys215. It includes a WPD loop (Trp179, Pro180, and Asp181). While the allosteric site is located nearly 20 Å away from Cys215 (Fig. 2a).

Before docking of 8HDN and control (ursolic acid), we validated the docking protocol by using the redock method. The computed root-mean-square deviation (RMSD) for redocking of ligands from both studied proteins showed the reliability of the docking algorithm (RMSD for 1NNY = 0.86 Å; RMSD for 1T49 = 0.93 Å). The superimposed ribbon and surface diagram of 8-HDN and native catalytic/allosteric site inhibitors are shown in Figs. 3a and 3b. Three-dimensional interaction plot into the binding site of catalytic site (PDB ID INNY) revealed that the compound under study forms three hydrogen bond interactions with Arg24, Arg254 and Gln262. Met258 forms π-sulfur interactions. A weak π-alkyl interaction also helps to stabilize the ligand-enzyme complex (Fig. 4a). A two-dimensional (2-D) interaction plot of the compound into the catalytic site is shown in Fig. 4b. The computed binding energy for compound 8-HDN in the catalytic site is −5.3659 kcal/mol.

A three-dimensional interaction plot into the allosteric binding site (ID = 1T49) revealed that the affinity of the compound is favored by three hydrogen bonds and five hydrophobic interactions. Ser187 and Asn193 form hydrogen bond interactions with carbonyl oxygen. While Glu276 forms hydrogen bond interactions with the hydroxyl group. Leu192, Phe196 and Phe280 forms π-π stacking interactions with 5,8-dihydronaphthalen rings (Fig. 5a). 2-D interaction plot of the compound into the allosteric site is shown in Fig. 5b. The computed binding energy for compound 8-HDN in the allosteric site is −6.2109 kcal/mol. While for control (Ursolic acid) is −4.8945 kcal/mol (Tab. 2).

The binding energy computed for ursolic acid (positive control) in the catalytic site is −6.7216 kcal/mol (Tab. 1). It forms four hydrogen bond interactions with Arg24, Arg254, and Gly259 (Figs. 6a and 6b).

POM analyses

A potential drug candidate must have a good pharmacological profile with pharmacokinetic properties. Among *in silico* prediction tools. Petra, Osiris and Molinspiration (POM) calculations have been developed and documented for years to access the pharmacokinetic profile (Hakkou *et al.*, 2017;

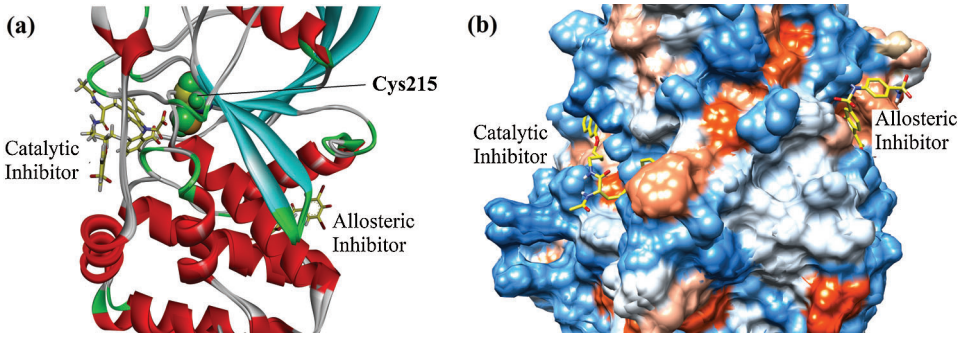

FIGURE 2. Superimposed ribbon (a) and surface (b) diagram of natives into the catalytic and allosteric binding site of protein tyrosine phosphatase 1B (PTP1B).
The two retrieved enzymes are superimposed by using discovery studio visualized.

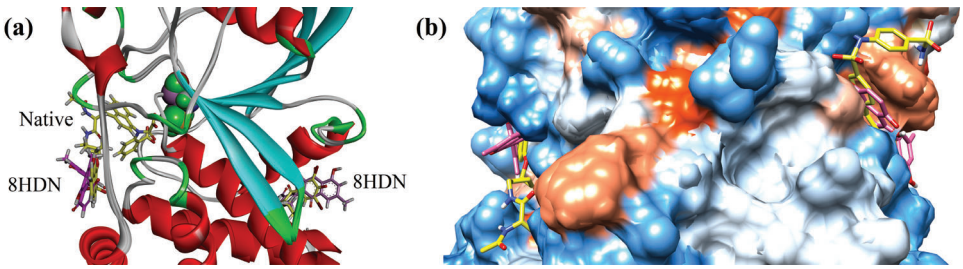

FIGURE 3. Superimposed ribbon (a) and surface diagram of compound 8-HDN (pink stick) and natives (yellow) into the catalytic and allosteric binding site.
The two retrieved enzymes are superimposed by using discovery studio visualized.

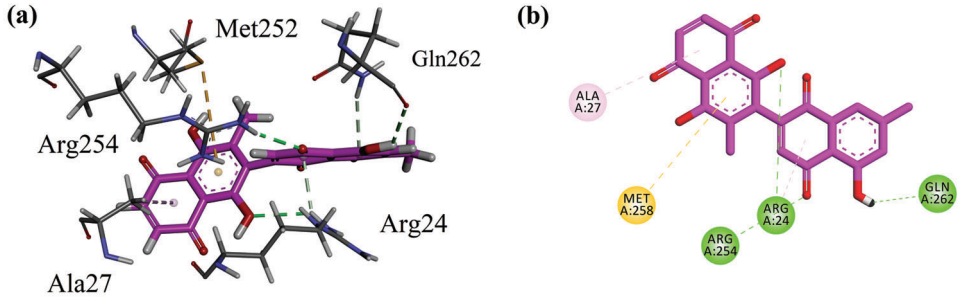

FIGURE 4. (a) Close-up 3-D interaction plot of the compound 8-HDN into the catalytic binding site of protein tyrosine phosphatase 1B (PTP1B, PDB ID = 1NNY) (b) 2-D interaction plot.

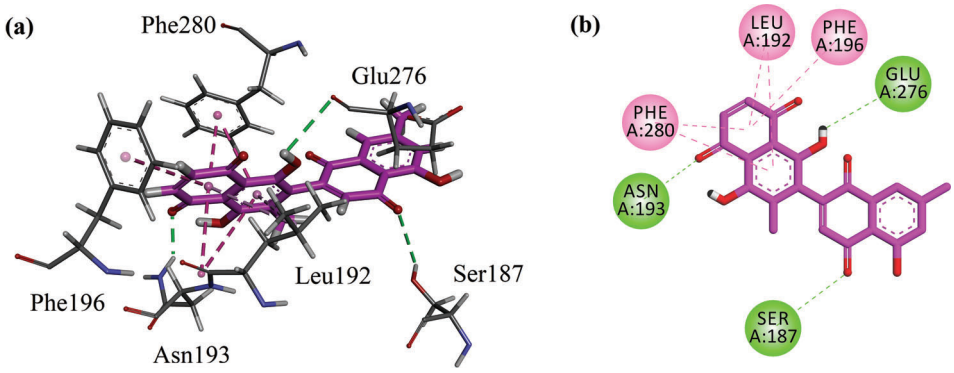

FIGURE 5. (a) Close-up 3-D interaction plot of the compound 8-HDN into the allosteric binding site of protein tyrosine phosphatase 1B (PTP1B, PDB ID = 1T49) (b) 2-D interaction plot.

TABLE 2

Binding energy values (in kcal/mol) computed via MOE docking into the binding site of catalytic and allosteric domains of PTP1B

Compounds	Binding energy (kcal/mol)	
	Catalytic Domain (PDB = 1NNY)	Allosteric domain (PDB = 1T49)
8-HDN	−5.3659	−6.2109
Ursolic acid (Control)	−6.7216	−4.8945

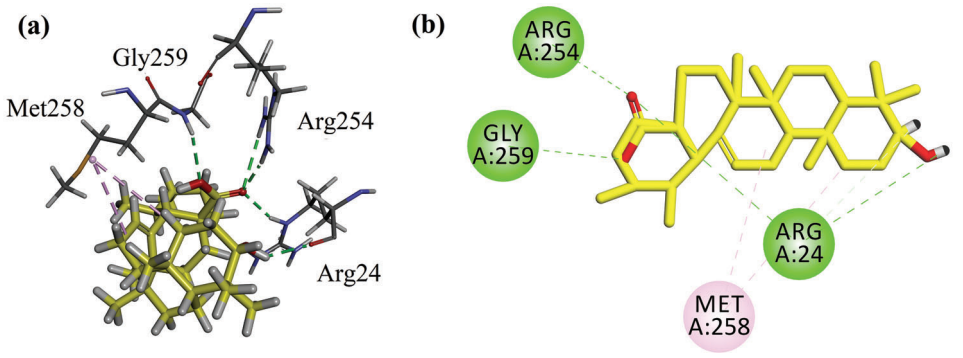

FIGURE 6. (a) Close-up 3-D interaction plot of the compound ursolic acid (positive control) into the catalytic binding site of protein tyrosine phosphatase 1B (PTP1B, PDB ID = 1NNY) (b) 2-D interaction plot.

Mabkhot *et al.*, 2016; Rauf *et al.*, 2015; Tighadouni *et al.*, 2016; Sajid *et al.*, 2016; Abdelhady *et al.*, 2015; Header *et al.*, 2015; Ben Hadda, 2015) to form sets of pharmacologically and diverse important conformers and tautomer, which can be used within 2D pharmacophore search procedures to elevate the number of meaningful hits of such test. These POM analyses give some information about the general limitations in the area of 2D structure and conformers/tautomer generation. The results of POM calculations are briefly described and discussed, and some outcomes obtained with the different tools are given. The results of the analysis are shown in Tab. 3.

Discussion

Current research indicates the promising potential of 8-HDN to be further explored and developed as a novel compound targeting PTP1B, especially in diabetes and cancer. Recently, enzyme inhibitory activity of similar compound diospyrin has been reported on DNA gyrase of *Mycobacterium*

TABLE 3

Molinspiration calculations of molecular properties and bioactivity scores of 8-HDN

Calculation of molecular properties		Calculation of bioactivity scores	
miLogP	3.02	GPCR ligand	−0.08
TPSA	129	Ion channel modulator	−0.14
nOHNH	3	Kinase inhibitor	0.06
nviolations	0	Nuclear receptor ligand	0.08
nrotb	1	Protease inhibitor	−0.07
Volume	321	Enzyme inhibitor	0.34

tuberculosis (Karkare *et al.*, 2013), as well as anticancer and antiparasitic activities of its derivatives and analogs (Dev *et al.*, 2012; Kumar *et al.*, 2012).

Docking studies

The design or identification of protein tyrosine phosphatase 1B (PTP1B) is an attractive area of research for medicinal/drug discovery researchers. However, there are a few challenges in developing PTP1B inhibitors. The catalytic site and its surrounding sub-sites have highly conserved polar architecture resulting in low bioavailability and off-target side effects. A few strategies have been developed to address these challenges (Kumar *et al.*, 2018; Wiesmann *et al.*, 2004). Wiesmann *et al.* (2004) discovered a druggable and non-conserved allosteric pocket (20 Å away from the active site) formed by more hydrophobic Leu192, Phe196 and Phe280. Asn193, Glu276 and Trp291 of α3 and α6 helices also interact with the inhibitors. There are a number of studies reported in the literature about the inhibition mechanism of PTP1B. These studies were carried out *via* inhibition kinetics and docking simulations. In a study carried out by Na *et al.* (2007), they revealed that naturally occurring amentoflavone from *Selaginella tamariscina* showed allosteric inhibition of PTB1B. Cai *et al.* (2015) reported *in-vitro* inhibition of PTP1B and docking studies of fifteen identified constituents from *Anoectochilus chapaensis*. The IC_{50} values of the nine active compounds were found in the range of 1.16–6.21 μM. Docking studies were carried out on the catalytic site of 1NNY, and the computed binding energy values were found between −7.4 to −8.5 kcal/mol. The tested compounds showed interactions with catalytic domain residues. Recently, Mphahlele *et al.* (2020) presented *in-vitro* and docking studies of 5-acetyl-2-aryl-6-hydroxybenzo[b]furans. The IC_{50} values of the nine active compounds were found in the range of 11.9–31.88 μM. Mechanism of inhibition was also investigated catalytic (PDB = 1NNY) as well as the allosteric site (PDB = 1T49) of protein tyrosine phosphatase 1B (PTP1B) *via* docking simulations. The computed binding energy values were found between −5.35 to −7.81 kcal/mol for catalytic inhibition. While for allosteric inhibition, the binding energies range from −6.82 to −11.20 kcal/mol. They concluded that the studied compounds are more selective for the allosteric site. Paudel *et al.* (2018) evaluated the PTP1B inhibitory potential of three principal components: mulberrofuran G, albanol B, and kuwanon G in *M. alba* rootbark. The studied compounds showed allosteric PTP1B inhibition *via* Asn193 and Glu276. While for catalytic inhibition, their mode of inhibition was through Arg24, Tyr46, Asp48, and Arg254.

In the current study, we investigated the mechanism of PTP1B inhibition by 8-HDN. Catalytic site inhibition by 8-HDN with a binding energy value of −5.3659 kcal/mol established three hydrogen bond interactions, a π-sulfur interaction, and a weak π-alkyl interaction. While the computed binding energy for positive control was −6.7216 kcal/mol. The PTP1B complex with 8-HDN allosteric site showed a binding energy value of −6.2109 kcal/mol. Binding energy data showed that compound 8-HDN is more selective for the allosteric site.

Pi-charge calculation and molecular structure optimization

The charge repetition of 8-HDN shows an important combined antibacterial/antifungal and antiviral O, O, O-pharmacophore site (Figs. 7 and 8), which deserves a separate supplementary antiviral/antiparasite screening. Thus, we have started this compound, and other achievements can be made. Our previous experience with similar flavonoids molecules indicates that a subtle change in pharmacophore can lead us to more efficient antioxidant and antinociceptive and anti-inflammatory agents (Ben Hadda *et al.*, 2013; Rauf *et al.*, 2016).

Osiris calculations

The theoretical toxicity risks determination for the 8-HDN using the Osiris program indicated that this flavonoid (Fig. 9) causes fewer side effects compared to the standard clinical drugs. It also revealed that 8-HDN can serve as an antibiotic with some pharmacomodulation (DS = 0.52). From the data estimated in Fig. 9, the structure is not supposed to mutagenic when analyzed via the mutagenicity assessment of the molecular system. Based on the reproductive and irritating effects, 8-HDN is at low risk compared with the control. The hydrophilicity character of the compound has been shown in terms of the cLog*P* value. It has been established that the permeation or absorption is highly affected by the hydrophilicity (cLog$P$ < 5).

POM analysis

Petra, Osiris and Molinspiration (POM) analysis is a well-known bioinformatics tool to identify the pharmacophore

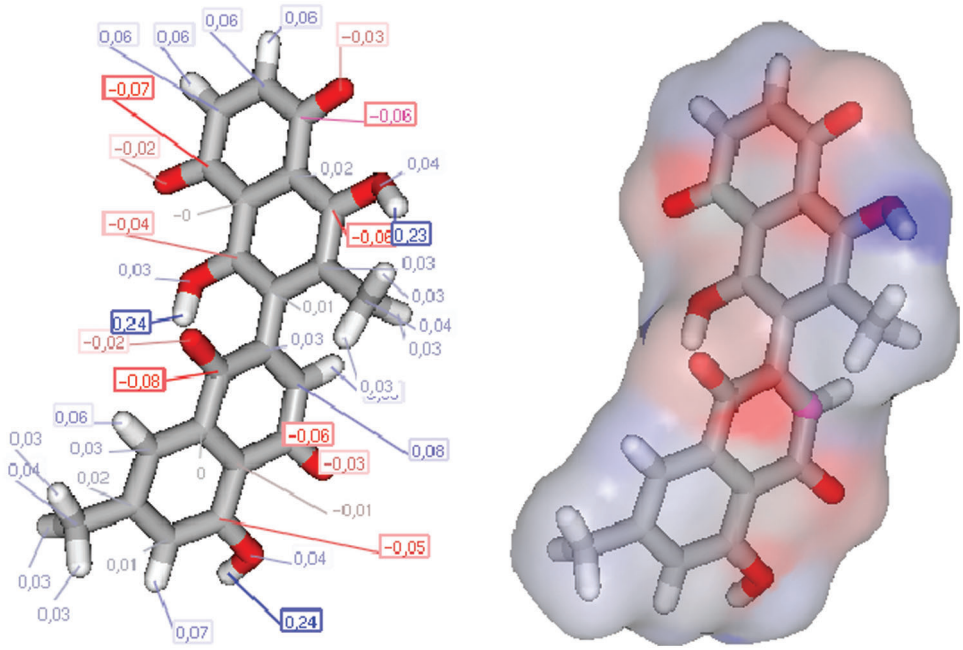

FIGURE 7. Charges and molecular structure optimization of 8-HDN.

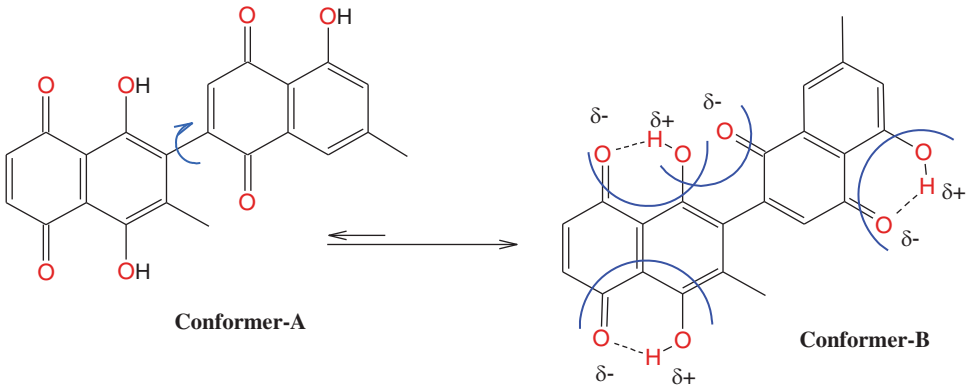

FIGURE 8. Conformation of 8-HDN.

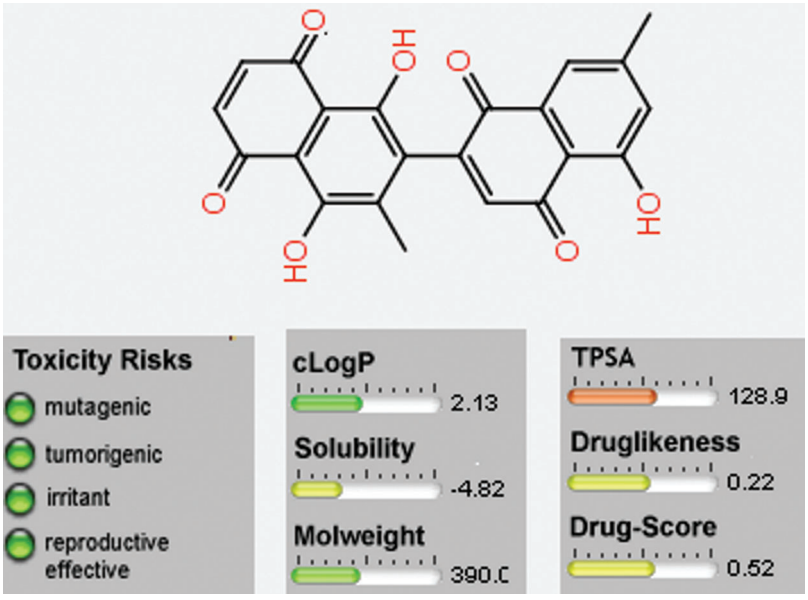

FIGURE 9. Osiris calculations of molecular properties of 8-HDN.

sites and predict the biological activities of molecules on the basis of steric/electrostatic properties. According to Molinspiration calculation, when miLog*P* is greater than 5, the permeation or absorption reduces. As a result of this, the compound (8-HDN) has a miLog*P* value within the acceptable criteria, but other vital indices should be

considered. This is linked to the geometrical configuration of the pharmacophore site (Fig. 5) because it is flexible for 8-HDN. We have calculated the molecular properties (TPSA, number of violations and volume) for the compound, and we have noted that they could be used as potential hits. Theoretical drug scores calculated via the online Molinspiration program are presented in Tab. 3. The calculation of bioactivity scores combines ion channel modulator, nuclear receptor ligand, kinase inhibitor, GPCR ligand, enzyme inhibitor and protease inhibitor in five separate values that may be employed to investigate the 8-HDN's total ability to qualify as a drug. The studied compound showed a promising ability to act as an enzyme inhibitor (Tab. 2).

Conclusions

In conclusion, to the best of our knowledge, no report is available on the antidiabetic potential of hydroxydiospyrin and *D. lotus*; our results deserve attention. Further studies, based on *in vivo* models, are needed to further elucidate this relevant biological activity. Docking studies were carried out to investigate the mechanism of inhibition. Binding orientation and binding energy data were computed into the catalytic (−5.3659 kcal/mol) and allosteric (−6.2109 kcal/mol) binding site of PTP1B. Binding energy data showed that compound 8-HDN is more selective for the allosteric site. 8-HDN can be further screened as an active lead compound against PTP1B by using *in vitro* and *in vivo* models. Overall, we found that 8-HDN structure optimizations based on the performed POM analysis.

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Authors Contributions: The authors confirm contribution to the paper as follows: Study conception and design: S. Bawazer, A. Khan, A. Rauf; data collection: T. B. Hadda, Y. S. Al-awthan; analysis and interpretation of results: O. Bahattab, U. Rashid, I. Khan; M. A. Nawaz draft manuscript preparation: M. S. Uddin, O. Ahmed, M. A. Shariati. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data including complete spectroscopic data of 8-hydroxydiospyrin (8-HDN) associated materials used to support the Research of this study are available from the corresponding authors upon request.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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