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Research Article
doi.org/10.1002/slct.202204050

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In Vitro Inhibitory Activity and Molecular Docking Study of Selected Natural Phenolic Compounds as AR and SDH Inhibitors**

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Polyol pathway enzymes, aldose reductase (EC 1.1.1.21; AR, ALR2), and sorbitol dehydrogenase (EC 1.1.1.14; SDH, SORD) have been widely investigated as the enzymes crucially involved in the pathogenesis of several chronic complications, including nephropathy, neuropathy, retinopathy, and cataracts associated with diabetes mellitus. Although phenolic compounds have been reported to possess many other biological activities, in continuation of our interest in designing and discovering potent inhibitors of AR and SDH, herein, we have evaluated these agents' inhibitory potential against polyol pathway enzymes. Our *in vitro* studies revealed that all the derivatives show activity against recombinant human AR (*rh*AR) and SDH (*rh*SDH), with K_i constants ranging from 9.37 ± 0.16 μM to 77.22 ± 2.49 μM and 2.51 ± 0.10 μM to 42.16 ± 1.03 μM, respectively. Among these agents, Prunetin and Phloridzin showed prominent inhibitory activity versus *rh*AR and *rh*SDH, while some were also determined to possess perfect dual activity. Moreover, *in silico* studies were also performed to rationalize binding site interactions of these agents with the target enzyme AR and SDH. According to ADME-Tox was also determined that these derivatives be agents exhibiting suitable drug-like properties. The compounds identified therapeutic potentials in this study may be promising for developing lead therapeutic agents to prevent polyol pathway complications.

Introduction

Diabetes mellitus (DM) is a severe and chronic condition brought on by an imbalance in glucose homeostasis.[1] Insulin resistance and abnormal glucose tolerance are characteristics of DM.[2] An estimated 536 million people worldwide currently have diabetes.[3] There are currently two forms of DM, and type 2 DM makes up about 90% of all cases.[4] Type 2 diabetes has been linked to hyperglycemia, or elevated blood sugar, a condition defined by an abnormal postprandial rise in blood glucose levels.[5] It is the leading contributor to complications related to metabolic syndrome, cardiovascular and neurological diseases, blindness, and renal function recession.[6]

The likelihood of acquiring severe diabetic complications in DM is decreased by therapies such as intensive insulin therapy that strictly and consistently limit glucose excursions.[7] Tight control of blood glucose levels is challenging to maintain.[8] Hence research has been done to develop new, powerful anti-diabetic medications that work through several methods.[9] Because aldose reductase (EC 1.1.1.21; AR, ALR2)[10] inhibitors (ARIs) do not affect plasma glucose,[11] they pose no danger of hypoglycemia and may be able to stop or slow the course of specific diabetic problems despite high blood sugar levels (Figure 1).[12] To date, a wide range of chemical substances with various structural characteristics have been found to be effective *in vitro* ARIs. According to their structural characteristics, the currently recognized ARIs can be categorized into four primary classes: (i) acetic acid derivatives; (ii) cyclic imides; (iii) phenolic derivatives; and (iv) phenylsulfonylnitromethane derivatives (Figure 2).[13]

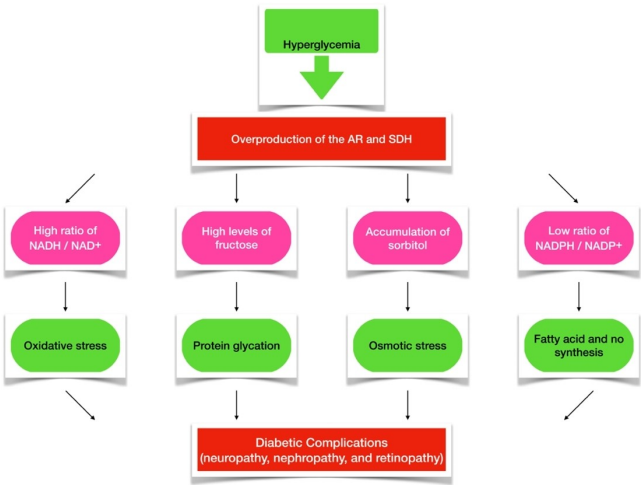

Figure 1. Schematic of the role of aldose reductase (AR) and sorbitol dehydrogenase (SDH) in the develop of some diabetic complications.[12]

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[**] *AR = aldose reductase, SDH = sorbitol dehydrogenase.*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.202204050

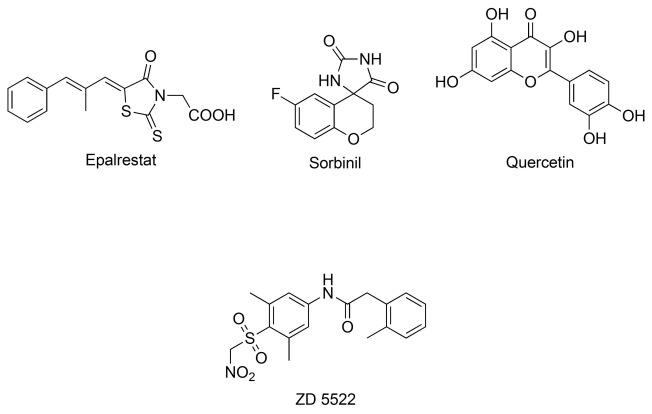

Figure 2. Well-known AR inhibitors, epalrestat (acetic acid derivative), sorbinil (cyclic imide), quercetin (phenolic derivative), and ZD 5522 (phenylsulfonylnitromethane derivative).

AR is a NAD(P)(H)-dependent cytosolic and monomeric oxidoreductase.[14] It is a member of the human Aldo-keto reductase (AKRs) superfamily[15] with numerous metabolic roles[16] and currently, 16 families, about 190 members, and many potential candidates.[17] AKRs are one of the protein superfamilies[18] that catalyze the reduction of aldehydes and ketones[19] to primary and secondary alcohols.[20] Sorbitol dehydrogenase (EC 1.1.1.14; SDH, SORD) is a cytosolic enzyme biomarker for hepatocellular toxicity.[21] It is generally presented at high levels in the liver, kidney, and testis[22] and lower amounts in multiple tissues. SDH catalyzes the interconversion of polyols and ketoses, e.g., transforms sorbitol into fructose,[23] using Zn^{2+}, and NAD^+ as cofactors[24] and plays an essential role in the polyol metabolic pathway.[25]

The polyol pathway is formed by the two-stage reaction catalyzed by AR and SDH.[26] In the first part of the reaction, AR-catalyzed glucose is reduced to sorbitol, and NADPH is converted to $NADP^+$.[27] Consumption of NADPH in this reaction leads to a decreased ratio of reduced glutathione (GSH), contributing to oxidative stress.[28] In the second part, sorbitol catalyzed by SORD converts to fructose.[29] In this reaction, NAD^+ is consumed while fructose and NADH make as products.[30] NADH is a substrate for NADH oxidase, which induces the production of superoxide anions, and, accordingly, the repetition of oxidative stress.[31] Subsequently, the fructose composed due to the catalysis of SDH is metabolized to fructose-3-phosphate and 3-deoxyglucosone, respectively.[32] This case significantly increases the formation of advanced glycation end products, which causes ROS formation at the last of the polyol pathway.[33] In addition, in the glycolytic pathway, NAD^+ is a cofactor essential for converting glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.[34] Decreased amount of NAD^+ causes increment flux into the pentose phosphate pathway in glucose metabolism.[35] Since approximately 30% of blood glucose may flux from the polyol pathway during hyperglycemia, this metabolic pathway is known to be the primary pathway regulating redox balance metabolizable between NADH and NAD^+.[36]

In the human diet, phenolic compounds are the most prevalent antioxidants and the most prevalent component of plants.[37] They are classified as secondary metabolites and perform no specific metabolic task in plant cells.[38] Phenolic compounds have at least one aromatic ring with one or more hydroxyl groups and various substituents.[39] These agents attract remarkable substantial on account of their pharmaceutical effects and are utilized in a wide range of therapeutic and pharmacological strategies.[40] Moreover, their therapeutic potentials and molecular docking mechanisms on the polyol pathway enzymes persist poorly realized, yet.[41] In the light of all this information, in this study, the potential beneficial effects of some phenolic compounds (Figure 3) were investigated on human recombinant AR (*rh*AR*)* and SDH (*rh*SDH) enzymes both *in vitro* and *in silico* conditions.

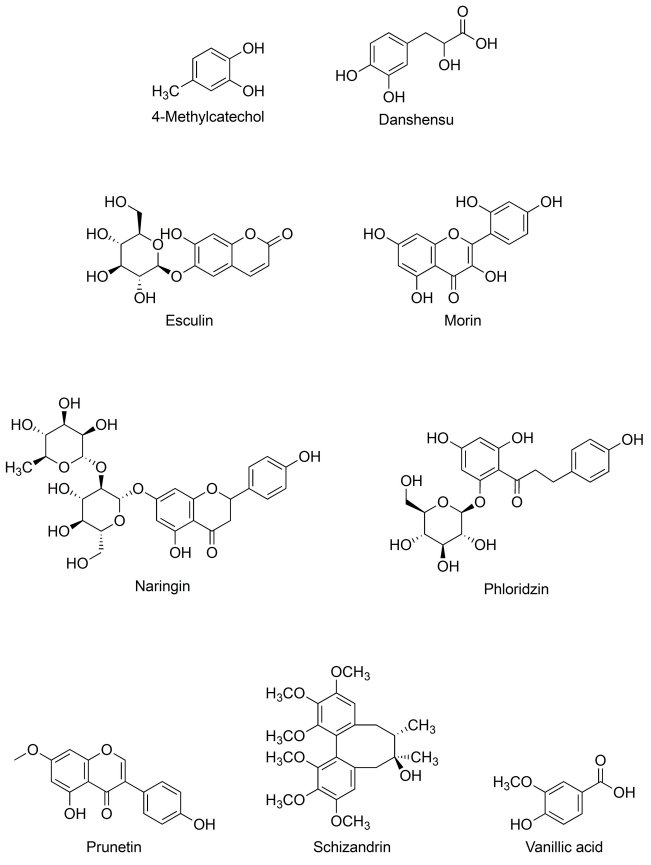

Figure 3. The chemical structures of some phenolic compounds, 4-Methylcatechol (PubChem CID: 9958), Danshensu (PubChem CID: 23693207), Esculin (PubChem CID: 16211025), Morin (PubChem CID: 16219651), Naringin (PubChem CID: 25075), Phloridzin (PubChem CID: 9912668), Prunetin (PubChem CID: 5281804), Schizandrin (PubChem CID: 3001664), Vanillic acid (PubChem CID: 8468), and Epalrestat (PubChem CID: 1549120) investigated as polyol pathway inhibitors.

Results and Discussion

Biological studies and structure-activity relationship assay

Most plant tissues, including those of fruits and vegetables, contain phytochemicals called phenolic compounds.[42–44] They have a wide range of bioactive qualities, and while not being nutrients, their dietary intake has health-protective effects.[45] Additionally, these substances are molecules with much promise for nutritional and medicinal purposes.[46] Furthermore, it is critical to keep striving to expand the knowledge base on their bioactivity and potential impact on human health. Besides, delivery techniques and doses that take therapeutic effects into account must be explicitly stated. Similar to this, physiopathological and metabolomic research on individuals whose diets are deficient in these substances is crucial for determining their therapeutic benefits and potential combinations with other phytochemicals.[47–49]

In this direction, we calculated the half-maximal inhibitory concentrations (IC_{50}) and inhibition constants (K_I) for each agents to quantify the inhibitory effect of selected phenolic compounds (Table 1). The phenolic compounds inhibited r*h*AR at low levels with IC_{50} values ranging from 7.11 ± 0.05 μM to 88.52 ± 0.89 μM and K_I constants ranging from 9.37 ± 0.16 μM to 77.22 ± 2.49 μM when compared with Epalrestat as the standard reference drug (IC_{50} = 0.29 ± 0.01 μM and K_I = 0.79 ± 0.01 μM). Esculin, Morin, and Schizandrin displayed a non-competitive inhibition effect, whereas others showed competitive inhibition. Among the compounds, Prunetin exhibits the highest inhibitory potential against r*h*AR with a K_I value of 9.37 ± 0.16 μM as a competitive inhibitor (Figure 4), while 4-Methylcatechol has the lowest potency with a K_I value of 77.22 ± 2.49 μM. The order of inhibitory activities for these compounds against r*h*AR decreased in the order of Prunetin (K_I = 9.37 ± 0.16 μM) > Phloridzin (K_I = 13.07 ± 0.33 μM) > Morin (K_I = 13.44 ± 0.23 μM) > Naringin (K_I = 14.14 ± 0.46 μM) > Esculin (K_I = 20.56 ± 0.34 μM) > Schizandrin (K_I = 27.12 ± 0.62 μM) > Vanillic acid (K_I = 33.69 ± 0.86 μM) > Danshensu (K_I = 35.50 ± 1.37 μM) > 4-Methylcatechol (K_I = 77.22 ± 2.49 μM) (Figure 5).

Regarding the inhibition of r*h*SDH, it was observed that the analyzed phenolic agents exhibited inhibition with IC_{50} values ranging between 6.24 ± 0.06 μM and 68.91 ± 0.33 μM, and K_I values ranging between 2.51 ± 0.10 μM and 42.16 ± 1.03 μM. The inhibition mechanism of Morin, Naringin, and Prunetin was noncompetitive, whereas others were competitive. Phloridzin (K_I of 2.51 ± 0.10 μM) was found to be the most potent agent as a competitive inhibitor (Figure 4), whereas Prunetin (K_I of 42.16 ± 1.03 μM) had the lowest activity against r*h*SDH. In this respect, it was found that the inhibitory strength order of these agents versus r*h*SDH was as follows: Phloridzin (K_I = 2.51 ± 0.10 μM) > Esculin (K_I = 8.48 ± 0.44 μM) > Danshensu (K_I = 11.59 ± 0.38 μM) > Morin (K_I = 13.13 ± 0.18 μM) > Schizandrin (K_I = 13.55 ± 0.66 μM) > Vanillic acid (K_I = 18.88 ± 0.68 μM) > Naringin (K_I = 33.31 ± 0.68 μM) > 4-Methylcatechol (K_I = 36.75 ± 1.84 μM) > Prunetin (K_I = 42.16 ± 1.03 μM) (Figure 5).

The studied phenolic agents developed different ratio selectivity against AR and SDH. The selectivity index (S_I) was computed by comparing the K_I for AR relative to SDH and was

Table 1. Inhibition data of selected phenolic compounds and standard drug Epalrestat.

Inhibitor	PubChem CID	r*h*AR				r*h*SDH				S_I[a]
		IC_{50} (μM)	R^2	K_I (μM)	R^2	Inhibition type	IC_{50} (μM)	R^2	K_I (μM)	R^2	Inhibition type	
4-Methylcatechol	9958	88.52 ± 0.89	0.9992	77.22 ± 2.49	0.9986	Competitive	68.91 ± 0.33	0.9998	36.75 ± 1.84	0.9975	Competitive	0.48
Danshensu	23693207	41.33 ± 0.35	0.9995	35.50 ± 1.37	0.9980	Competitive	41.88 ± 0.26	0.9996	11.59 ± 0.38	0.9990	Competitive	0.33
Esculin	16211025	16.13 ± 0.14	0.9996	20.56 ± 0.34	0.9988	Noncompetitive	18.21 ± 0.12	0.9997	8.48 ± 0.44	0.9970	Competitive	0.41
Morin	16219651	7.11 ± 0.05	0.9996	13.44 ± 0.23	0.9984	Noncompetitive	10.11 ± 0.05	0.9997	13.13 ± 0.18	0.9988	Noncompetitive	0.98
Naringin	25075	17.67 ± 0.14	0.9995	14.14 ± 0.46	0.9986	Competitive	17.02 ± 0.11	0.9997	33.31 ± 0.68	0.9986	Noncompetitive	2.36
Phloridzin	9912668	15.24 ± 0.17	0.9991	13.07 ± 0.33	0.9991	Competitive	6.24 ± 0.06	0.9995	2.51 ± 0.10	0.9983	Competitive	0.19
Prunetin	5281804	22.74 ± 0.22	0.9992	9.37 ± 0.16	0.9997	Competitive	30.83 ± 0.29	0.9992	42.16 ± 1.03	0.9975	Noncompetitive	4.50
Schizandrin	3001664	12.19 ± 0.08	0.9997	27.12 ± 0.62	0.9981	Noncompetitive	19.53 ± 0.17	0.9994	13.55 ± 0.66	0.9972	Competitive	0.50
Vanillic acid	8468	53.92 ± 0.55	0.9992	33.69 ± 0.86	0.9991	Competitive	43.68 ± 0.45	0.9993	18.88 ± 0.68	0.9986	Competitive	0.56
Epalrestat	1549120	0.29 ± 0.01	0.9971	0.79 ± 0.01	0.9992	Noncompetitive	–	–	–	–	–	

[a] Selectivity index of inhibitors for AR over off-target isoform SDH, calculated as the ratio of K_I off-target SDH/K_I target AR. A high-value ratio characterizes a potent, selective inhibitor

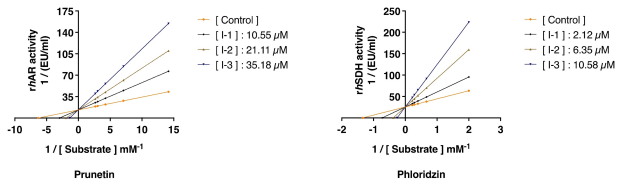

Figure 4. *In vitro* inhibition effects of Prunetin (PubChem CID: 5281804, for *rh*AR, on the left) and Phloridzin (PubChem CID: 9912668, for *rh*SDH on the right) against polyol pathway enzymes activity. Lineweaver-Burk plots were used to determine the K_I constants and inhibition types of these agents. For this aim, various Prunetin and Phloridzin concentrations were tested for five different levels of substrate.

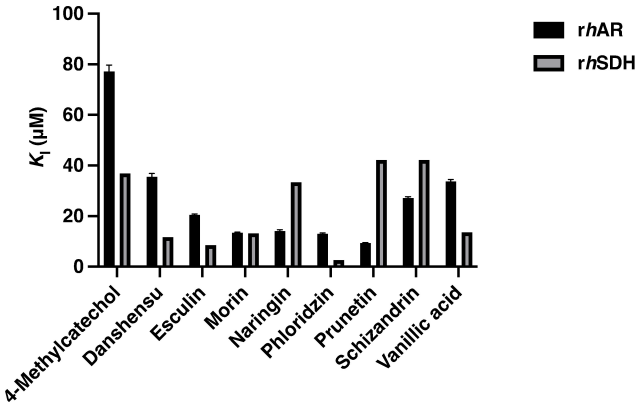

Figure 5. Inhibition activity of selected phenolic compounds. The inhibition constants (K_I) of *rh*AR and *rh*SDH were shown at Y-axis, and the compounds were oriented at X-axis.

used to indicate enzyme selectivity, as observed in Table 1. Regarding selectivity towards AR over the off-target isoform SDH, the determined S_I SDH/AR for phenolic agents ranged from 4.50 to 0.19. Prunetin and Naringin showed high selectivity towards AR with an S_I of 4.50 and 2.36, respectively, compared to other compounds. On the other hand, Danshensu and Phloridzin displayed negative selectivity against AR with the lowest S_Is of 0.33 and 0.19, respectively.

Many reports are available on polyol pathway enzymes inhibition by natural products from vegetables, medicinal plants, and food. The main group of compounds consists of phenolic compounds such as anthocyanins, coumarins, phenolic acids, flavonoids, and other bioactive compounds. For instance, Demir *et al.*[50] reported that formononetin, delphinidin chloride, genistein, tangeretin, and pelargonidin inhibited α-amylase, α-glycosidase, and AR. They found that genistein displayed the best inhibition effect against AR. Yawadio *et al.*[51] studied that black and brown rice containing peonidin-3-glucoside, ferulic acid, cyanidin-3-glucoside, and tocopherol, showed inhibition potency against recombinant AR with IC_{50} values in the range of 8.7–27.5 μg/mL. Aslan and Beydemir[52] showed that phenolic acids such as 4-hydroxybenzoic, 3,5-dihydroxybenzoic, 3,4-dihydroxybenzoic, p-coumaric, ferrulic salicylic, ellagic, caffeic, and gallic acid on AR and SDH enzyme activity. 3,4-dihydroxybenzoic acid displayed the best inhibition impact on AR, while ferrulic acid displayed the best inhibition impact on SDH. Lee *et al.*[53] isolated new lignan glycoside and phenolic biphenyl derivatives from the twigs and leaves of *Osteomeles schwerinae*. They found phenolic biphenyl derivatives markedly inhibited rat lens AR with IC_{50} values of 3.8 to 13.8 μM. In another study, Jung *et al.*[54] isolated twelve phenolic compounds (noririsflorentin, kanzakiflavone-2, sheganone, apocynin, iristectorene B, 4-hydroxybenzoic acid, tectorigenin, irigenin, 4′,7-di-*o*-methyltectorigenin, irisflorentine, iridin, and tectoridin) from *Belamcanda chinensis* and studied the inhibition effect of these compounds on AR. They reported that irigenin and tectorigenin displayed a potent AR inhibition.

In silico studies

To gain more information about the binding modes of these phenolic compounds and a comprehensive SAR study was performed to investigate the behaviors of these molecules within native ligands, EPR (PubChem CID: 1549120; (5-[(2*E*)-2-methyl-3-phenylprop-2-en-1-ylidene]-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)acetic acid) and 572 (PubChem CID: 132302; 4-[2-(hydroxymethyl)pyrimidin-4-yl]-*N*,*N*-dimethylpiperazine-1-sulfonamide) binding site of the AR and SDH, respectively. Initially, the performance of the Glide extra precision (XP) docking protocol[55] was evaluated by re-docking the co-crystallized EPR and 572 into the active sites of AR and SDH, respectively, with Schrödinger Small-Molecule Drug Discovery Suite 2022-2 for Mac (Schrödinger, LLC, NY, USA). The RMSD values between the EPR and 572 conformations and the best poses generated by these protocols was 0.1472 Å and 0.1983 Å, respectively, suggesting that the Glide XP docking algorithm was qualified for docking these agents to the AR and SDH active pockets. Later, in the present docking study, the docking patterns of EPR and 572 were compared with that of Prunetin and Phloridzin for AR (docking score of −7.394 kcal/mol), and Prunetin for SDH (docking score of −4.556 kcal/mol). MM-GBSA binding energies are approximate free energies of binding, a more negative value indicates stronger binding. The binding interactions of these agents with AR and SDH are shown in Figures 6 and 7, respectively.

A docking score of −9.307 kcal/mol and MM-GBSA value of −56.16 kcal/mol indicated that Prunetin is a tight binder for AR. Prunetin formed two H-bond with residues Tyr48 (distance of 2.12 Å) and His100 (distance of 2.01 Å), while it made a water-mediated H-bond with residue Leu300 (distance of 2.04 Å). Furthermore, it exhibited π-π stacking interactions with residues Trp20 and Phe122. However, it displayed that residues Val47, Trp79, Trp111, Phe124, Trp219, Cys298, Leu300, and Leu301 play crucial roles in the binding of Prunetin with AR (Figure 5). Also, the docking results showed that Phloridzin (docking score of −9.607 kcal/mol and MM-GBSA value of −9.55 kcal/mol) formed two water-mediated H-bond interactions with residues Tyr50 (distance of 1.80 Å) and Thr121 (distance of 1.81 Å) of SDH. It also made an H-bond with residue Arg298 (distance of 2.09 Å). Nevertheless, hydrophobic interactions were monitored between Phloridzin and residues

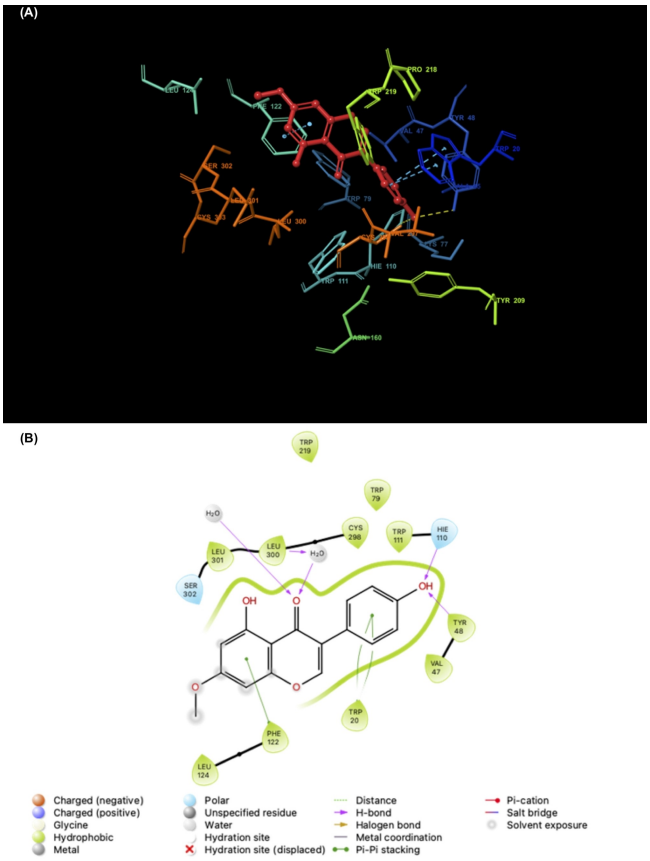

Figure 6. Molecular docking of aldose reductase enzyme (PDB code: 4JIR) with Prunetin (PubChem CID: 5281804). **(A)** 3D docking pose of Prunetin within the binding pocket of 4JIR. In the 3D panel, hydrogen bonds and π-π stacking interactions are shown in yellow and blue dashed lines, respectively. Only the interacting amino acids are demonstrated for the sake of clarity. **(B)** 2D binding interaction of 4JIR with Prunetin.

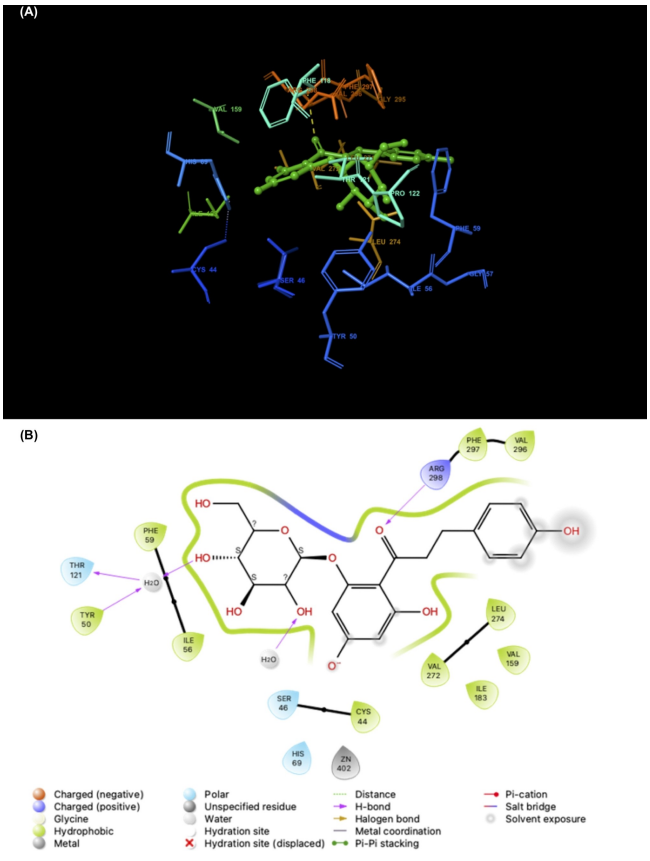

Figure 7. Molecular docking of sorbitol dehydrogenase (PDB code: 1PL6) with Phloridzin (PubChem CID: 9912668). **(A)** 3D docking pose of Phloridzin within the binding pocket of 1PL6. In the 3D panel, hydrogen bonds are shown in yellow dashed lines. Only the interacting amino acids are demonstrated for the sake of clarity. **(B)** 2D binding interaction of 1PL6 with Phloridzin.

Cys44, Ile56, Phe59, Val159, Ile183, Val272, Leu274, Val296, and Phe297 (Figure 6).

Even though ARIs can take on a wide variety of chemical configurations, a careful examination of these numerous compounds revealed that the minimal pharmacophore requirements for ARIs are two hydrophobic aromatic systems connected by a flexible chain and a polar or ionic group.[56] These results indicate that most ARIs have an acidic group interacting strongly through H-bonds with the nicotinamide moiety of NADP + and the Trp20, Tyr48, Lys77, His110, and Trp111 critical catalytic residues in the anion-binding pocket. Additionally, the inhibitors' aromatic rings can exhibit π-π stacking and other hydrophobic interactions with the backbone's Trp20, Trp79, Trp111, Pro218, Trp219, and Cys298 residues as well as Ala299, Leu300, Ser302, and Cys303 residues in the second hydrophobic selectivity pocket.[57]

All the studied phenolic agents were evaluated *in silico* using the ADME-Tox prediction program QikProp software and SwissADME platform, and the selected findings are reported in Table 2. Additionally, diagrams showing "drug-likeness" descriptors for Prunetin and Phloridzin, which are the most active derivatives in this series against AR and SDH, respectively, are given in Figure 8. All calculated parameters confirmed that

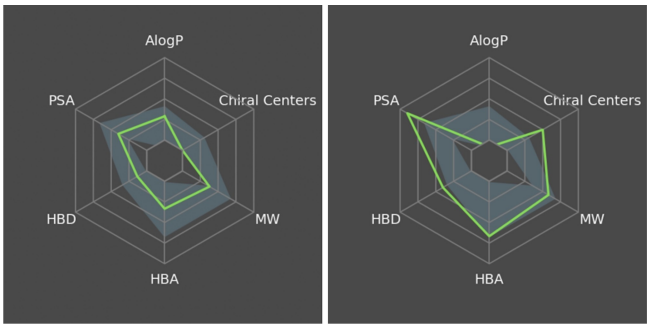

Figure 8. Diagrams show "drug-likeness" descriptors of Prunetin (PubChem CID: 5281804, on the left) and Phloridzin (PubChem CID: 9912668, on the right). (AlogP: Predicted octanol/water partition coefficient, Chiral Centers: Chiral atom count, MW: Molecular weight, HBA: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution, HBD: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, PSA: Van der Waals surface area of polar nitrogen and oxygen atoms).

Table 2. ADME-Tox related parameters of some phenolic compounds and standard drug Epalrestat.

Inhibitor	#rtvFG[a]	CNS[b]	MW[c]	Dipole[d]	Volume[e]	QPlogPoct[f]	QPlogPw[g]	QPlogPo/w[h]	QPlogS[i]	QPlogBB[j]	QPlogKp[k]	QPlogKhsa[l]	PSA[m]	Rule of Five[n]	Rule of Three[o]	PAINS[p]
4-Methylcatechol	0	0	124.14	2.52	482.99	7.59	6.04	1.00	0.00	0.00	−2.75	−0.55	44.24	0	0	1
Danshensu	0	−2	198.18	4.88	644.67	14.60	12.78	0.00	−1.14	−1.83	−5.02	−0.94	114.94	0	1	1
Esculin	2	−2	340.29	7.37	962.96	24.56	21.88	−1.57	−1.87	−2.53	−5.53	−0.99	164.96	0	1	0
Morin	0	−2	302.24	5.40	863.60	18.62	14.39	0.00	−2.84	−2.34	−5.44	−0.35	143.15	0	1	0
Naringin	2	−2	580.54	7.99	1585.22	38.93	32.00	−1.55	−3.14	−4.31	−6.57	−0.11	234.19	3	2	0
Phloridzin	1	−2	436.42	9.84	1250.99	29.16	22.83	0.00	−2.31	−3.45	−5.58	−0.83	189.14	1	2	0
Prunetin	0	−1	284.27	4.66	864.55	13.14	8.06	2.54	−3.59	−1.00	−2.60	0.89	85.84	0	0	0
Schizandrin	0	0	432.51	5.93	1316.28	17.83	6.74	5.24	−5.50	−1.00	−1.20	0.89	62.02	1	1	0
Vanillic acid	0	−1	168.15	1.76	561.73	9.55	8.10	1.05	−1.37	−1.00	−3.74	−0.75	79.70	0	0	0
Epalrestat	0	−1	319.39	6.31	982.62	14.97	8.09	3.69	−4.57	−1.00	−2.87	0.60	89.03	0	0	0

[a] Number of reactive functional groups (#rtvFG, ; 0–2). [b] Central nervous system activity (CNS; −2 (inactive), +2 (active)). [c] Molecular weight of the compound (MW; 130.00–725.00). [d] Computed dipole moment of the compound (Dipole; 1.00–12.50). [e] Total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å Radius (Volume; 500.00–2000.00). [f] Octanol/gas partition coefficient (QPlogPoct; 8.00–35.00). [g] Water/gas partition coefficient (QPlogPw; 4.00–45.00). [h] Octanol/water partition coefficient (QPlogPo/w; −2.00–6.50). [i] Aqueous solubility (QPlogS; −6.50–0.50). [j] Brain/blood partition coefficient (QPlogBB; −3.00–1.20). [k] Skin permeability (QPlogKp; −8.00–−1.00). [l] Prediction of binding to human serum albumin (QPlogKhsa; −1.50–1.50). [m] Van der Waals surface area of polar nitrogen and oxygen atoms (PSA; 7.00–200.00). [n] Number of violations of Lipinski's rule of five (max. 4). [o] Number of violations of Jorgensen's rule of three (max. 3). [p] Pan-assay interference compounds (PAINS) alert.

these compounds conformed to Lipinski's five[58] and Jorgensen's three[59] rules and were hits that exhibit similar properties to the appropriate drug.

Conclusion

Although phenolic compounds have been reported to possess many other biological activities, herein, we have studied the inhibitory potential against polyol pathway enzymes. These enzymes play a crucial role in improving complications that emerge from DM, such as cataracts, retinopathy, neuropathy, and nephropathy formation. All compounds exhibited inhibitory activity against r*h*AR and r*h*SDH with K_I constants ranging from 9.37 ± 0.16 μM to 77.22 ± 2.49 μM and 2.51 ± 0.10 μM to 42.16 ± 1.03 μM, respectively. Among them, Prunetin and Phloridzin were the most prominent and were identified as potential leads for AR and SDH inhibitors, respectively. Some of the compounds were determined to possess perfect dual activity. They may be promising compounds to evaluate and augment their activities, i.e., to inhibit AR and SDH enzymes and prevent polyol pathway complications. Moreover, molecular docking studies have also been performed to rationalize the binding site interactions of these phenolic agents. According to the *in silico* ADME-Tox study, these agents are ARIs displaying suitable drug-like properties.

Experimental Section

General information

The used materials, phenolic compounds, the chromatography media and all chemical compounds were acquired from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Ultra-pure water was used for the preparations of all test solutions.

Biological studies

In the present study, r*h*AR and r*h*SDH were expressed as a SUMO fusion protein containing 6xHis Tag. The r*h*AR and r*h*SDH were purified using Ni-NTA affinity chromatography.[60–65] The target proteins were eluted using 250 mM imidazole.[66,67] r*h*AR and r*h*SDH enzymes were obtained with 16.67 and 6.54 purification fold and with a specific activity of 0.717 and 0.333 EU/mg protein, respectively. SDS-PAGE electrophoresis technique as explained by Laemmli[68,69] was performed after the purification of the SUMO-r*h*AR and SUMO-r*h*SDH enzymes, and the molecular mass of the enzymes was found to be approximately 48 and 49 kDa, respectively. The amount of the protein was determined according to the Bradford's method,[70] a simple and quick analytical procedure at 595 nm wavelength, quantitatively as described previously.[71,72] Dimethyl sulfoxide (DMSO, PubChem CID: 679) was used to prepare solutions of the phenolic compounds and Epalrestat at an initial concentration of 1 mg/mL. The amount of DMSO in the final reaction mixture was around 1 %.[73] As in earlier studies,[74,75] kinetic tests for AR were carried out using Cerelli's method[76] at five different substrates (DL-glyceraldehyde, PubChem CID: 79014) and varying compound concentrations at 340 nm spectrophotometrically to investigate the *in vitro* inhibitory mechanisms against r*h*AR of these phenolic agents. On the other hand, r*h*SDH activity was also spectrophotometrically measured at 340 nm using substrates

(sorbitol, PubChem CID: 5780 and NAD^+, PubChem CID: 5893).[77–79] All the samples were measured three times. IC_{50} plots,[80] Michaelis-Menten curves,[81] and Lineweaver-Burk plots[82] were created, and K_I constants and types of inhibition were identified based on the observed data as in our previous work (Figures 4 and S1–S4).[83–85]

In silico studies

In silico studies were conducted employing Small-Molecule Drug Discovery Suite 2022-2 for Mac (Schrödinger, LLC, NY, USA), including the Maestro V13,[86] QikProp V7,[87] Protein Preparation Wizard,[88] SiteMap,[89] Receptor Grid Generation,[90] LigPrep,[91] Ligand Docking,[92] and Prime MM-GBSA V3[93] tools as previously reported.[94,95] Crystal structures of polyol pathway enzymes, AR (PDB ID: 4JIR; Resolution: 2.00 Å; Species: Homo sapiens)[96] and SDH (PDB ID: 1PL6; Resolution: 2.00 Å; Species: Homo sapiens)[97] were downloaded from Protein Data Bank.[98] Structural optimizations of enzymes were accomplished by the Protein Preparation Wizard tool.[99] Using drawing tools, the phenolic compounds were built in ChemDraw software V21[100] for Mac (PerkinElmer, Inc., Waltham, MA, USA). The resulting molecules were submitted to the LigPrep application for generating the most probable ionization state in the OPLS4 force field[101] at pH 7.0 ± 2.0 with Epik.[102] Energy grids were defined using the Receptor Grid Generation tool[103] with the default values. Glide docking calculations were conducted using the scoring function XP.[104–106] The MM-GBSA binding energies,[107] which predict relative binding affinities for these phenolic agents, were calculated using the VSGB energy model[108] and the OPLS4 force field.[109] The analysis of the molecular interactions was performed on the Maestro interface (Figures 6,7, S5, and S6).[110–112]

Statistical studies

Analysis of the data and drawing of graphs were realized using GraphPad Prism V9 for Mac (GraphPad Software, La Jolla California USA). The inhibition constants were calculated by SigmaPlot V12 for Windows (Systat Software, San Jose California USA). The fit of enzyme inhibition models was compared using the extra sum-of-squares F test and the AICc approach. The results were exhibited as mean ± standard error of the mean (95% confidence intervals). Differences between data sets were considered statistically significant when the *p*-value was less than 0.05.

Supporting Information Summary

IC_{50} plots and Lineweaver-Burk curves of selected phenolic compounds and molecular re-docking diagrams of aldose reductase and sorbitol dehydrogenase enzymes are provided in the Supporting Information.

Acknowledgements

This work was supported by the Research Fund of Erzincan Binali Yıldırım University (grant number FBA-2017-501) and the Research Fund of Anadolu University (grant number 2102S003).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Aldose reductase · Biological Activity · Enzymes · Inhibition · Sorbitol dehydrogenase

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Submitted: October 18, 2022
Accepted: December 7, 2022