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Exploring the Potential of Isoindole-1,3-Dione Derivatives as Novel Inhibitors of Aldose Reductase: An *In Silico* and *In Vitro* Insight into Therapeutic Strategies for Diabetic Complications

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This study explores the potential of isoindole-1,3-dione derivatives as novel inhibitors of aldose reductase (AR), focusing on their *in silico* and *in vitro* effects for therapeutic strategies against diabetic complications. Aldose reductase, a critical enzyme in the polyol pathway, plays a significant role in glucose metabolism and has been linked to diabetic complications. In this comprehensive study, isoindole-1,3-dione derivatives were synthesized and evaluated for their inhibitory effects on the recombinant human AR enzyme. The compounds' inhibitory activities were measured both *in vitro* and through *in silico* techniques, employing molecular docking and free binding energy calculations and ADME studies. The newly synthesized compounds demonstrated varied inhibitory effects, with ethyl and phenyl substituents at specific positions enhancing inhibitory activity. Notably, compounds with carboxylic acid derivatives exhibited potent inhibitory effects, especially compound **6** with an IC_{50} value of 1.649 μM. In conclusion, this study provides valuable insights into the inhibitory potential of isoindole-1,3-dione derivatives against AR, suggesting their potential therapeutic application in mitigating diabetic complications. The combination of experimental and computational approaches offers a comprehensive understanding of the compounds' interaction mechanisms and pharmacokinetic profiles, supporting their further exploration as antidiabetic agents.

Introduction

Aldose reductase (AR) belong to the class of oxidoreductases, utilizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor.[1,2] This enzyme facilitate the reduction of carbonyl groups in aldehydes, converting them into primary alcohols.[3] As the first and rate-limiting enzyme in the polyol pathway, AR is crucial for metabolic processes.[4–6] The polyol pathway involves a two-step conversion of glucose to fructose and is significantly implicated in the development of diabetic complications.[7] This pathway, encompassing both AR and Sorbitol Dehydrogenase (SDH) enzymes, plays a pivotal role in the pathophysiology of diabetic complications.[8,9] AR, the initial enzyme in this cascade, is activated independently of insulin in response to heightened glucose concentrations in tissues. Through the transfer of electrons from NADPH, AR catalyzes the reduction of glucose to sorbitol.

The interaction between AR and its substrate is facilitated by hydrophobic interactions.[10,11] Notably, the enzyme exhibits enhanced effectiveness in reducing carbonyl compounds when glutathione conjugates of unsaturated aldehydes are utilized as substrates, as opposed to free aldehydes. Particularly noteworthy substrates for AR include the abundant and toxic lipid-derived aldehydes, such as hexanitroethane (HNE) in conjunction with reduced glutathione (GSH)[12]. Aldose reductase, a NADPH-dependent enzyme, serves as the rate-limiting factor in the polyol pathway, primarily acting on glucose. However, its affinity for glucose is relatively low, rendering it inactive at physiological glucose concentrations. Upon the elevation of intracellular glucose levels beyond the physiological range, AR is activated, particularly under hyperglycemic conditions. Hyperglycemia leads to a decrease in the conversion rate of sorbitol to fructose due to increased AR activity and decreased SDH activity. The accumulation of sorbitol in tissues, resulting from this phenomenon, hinders effective metabolism and promotes osmotic effects, thereby causing cell edema. Such sorbitol accumulation is implicated in complications like retinopathy, neuropathy, nephropathy, and cardiovascular diseases.[13,14]

The interference with, or even suppression of, enzyme activities both *in vivo* and *in vitro* by certain compounds is termed 'inhibition,' and the agents causing this effect are referred to as 'inhibitors.' Inhibitors are typically small molecular compounds or ions, with various chemicals, drugs, and toxins exerting their functions through enzyme inhibition. To mitigate the accumulation of sorbitol in tissues during the early stages

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of hyperglycemia, aldose reductase inhibitors can be employed. Failing to regulate glucose levels in hyperglycemia can lead to irreversible neuropathy, retinopathy, and nephropathy.[15–18] Despite diabetes ranking third among global causes of death, the diseases it triggers or exacerbates (such as cardiovascular diseases and cancer) claim the top two spots.[19] Consequently, research on AR and SDH enzymes is pivotal for the development of preventive, mitigative, and delay-oriented diabetes treatments. In light of this, our study delves into the *in vitro* inhibition effects of newly synthesized isoindole-1,3-dione derivatives on AR enzymes extracted from sheep kidney tissues.

Considering the limitations of existing insulin and oral hypoglycemic agents, a demand persists for novel antidiabetic products.[20,21] Among these, certain agents incorporate isoindoline-1,3-dione derivatives (Figure 1).[22–30]

Isoindoline-1,3-dione, commonly referred to as phthalimide, finds application in the synthesis of both organic compounds and pharmaceutical agents.[28–57] Extensive research indicates that compounds featuring the phthalimide moiety 1 (as illustrated in Scheme 1) serve as building blocks for designing potential drug candidates possessing diverse biological activities. Beyond its implications in diabetes, these drugs have demonstrated relevance in addressing conditions such as tuberculosis,[30,31] AIDS,[32–34] multiple myeloma,[35–37] tumors,[38] inflammatory diseases,[39–41] hyperlipidemia,[42,43] convulsions,[44–46] depression,[47] anxiety,[48] asthma,[49] and infectious diseases.[29,50] Furthermore, certain phthalimide derivatives have been identified to exhibit inhibitory activity against specific enzymes.[28,51–53] Previous anticancer activity studies conducted on some of the 1,3-dione derivatives have also shown positive results.[54,55]

This study systematically investigates the inhibitory impacts of isoindole-1,3-dione derivatives, which were recently synthesized, on the recombinant human aldose reductase (AR) enzyme. The investigation is approached through a comprehensive integration of *in vitro* and *in silico* methodologies. The compounds employed for the analysis of AR enzyme activity were synthesized in strict accordance with the reaction scheme detailed within Scheme 1.

Materials and Method

Chemistry

*General procedure for preparation of compounds **1–5**:* This compound was prepared according to the reported method.[56–59]

*General procedure for preparation of compounds **6, 7**:* This compound was prepared according to the reported method.[52,53]

2-(4-Nitro-phenyl)-1,3-dioxo-2,3-dihydro-1H-isoindole-5-carboxylic acid (6)

Yellow powder; yield: 97 %. ^{1}H NMR (400 MHz, DMSO-D_6) δ 11.04 (s, 1H), 8.24–7.68. 9.00 (m, 7H). ^{13}C NMR (100 MHz, DMSO-D_6) δ 168.32, 166.90, 166.76, 156.26, 145.82, 132.58, 130.36, 129.12, 126.97, 125.65, 124.88, 119.89, 113.08. IR (KBr, cm^{-1}): 3484, 3244, 3223, 1603, 1589, 1505, 1482, 1471, 1396, 1184, 1134, Anal. Calcd for $C_{15}H_8N_2O_6$: C, 57.70; H, 2.58; N, 8.97; Found: C, 57.68; H, 2.62; N,8.95.

2-(3-Nitro-phenyl)-1,3-dioxo-2,3-dihydro-1H-isoindole-5-carboxylic acid (7)

Yellow powder; yield: 95 %. ^{1}H NMR (400 MHz, DMSO-D_6) δ11.05 (bs, 1H (−COOH)), 8.71–7.84 (m, 7H), ^{13}C NMR (100 MHz, DMSO-D_6) δ 170.95, 167.94, 166.35, 150.63, 148.58, 136.22, 134.35, 130.97, 124.52, 124.09, 123.44, 122.26, 120.63, 110.45, 107.87. IR (KBr, cm^{-1}): 3376, 3335, 3211, 3096, 3077, 1625, 1581, 1484, 1334, 1267, 1165, 1112,1091, 997. 932. Anal. Calcd for $C_{15}H_8N_2O_6$: C, 57.70; H, 2.58; N, 8.97; Found: C, 57.75; H, 3.01; N,8.93.

*General procedure for preparation of compounds **8–10**:* 2-(methyl/ethyl/phenyl)-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (**1**/**2**

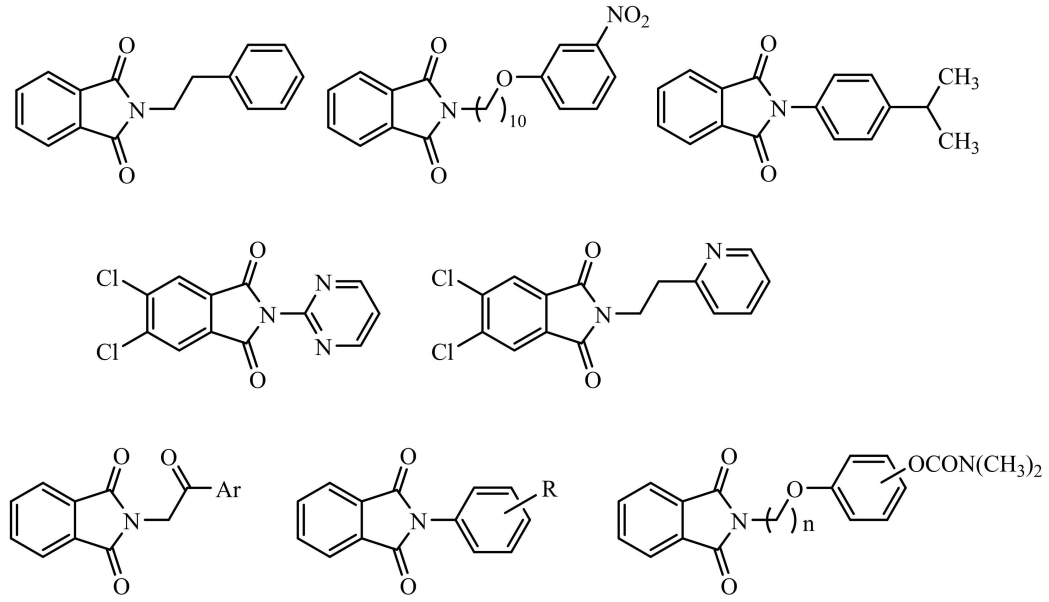
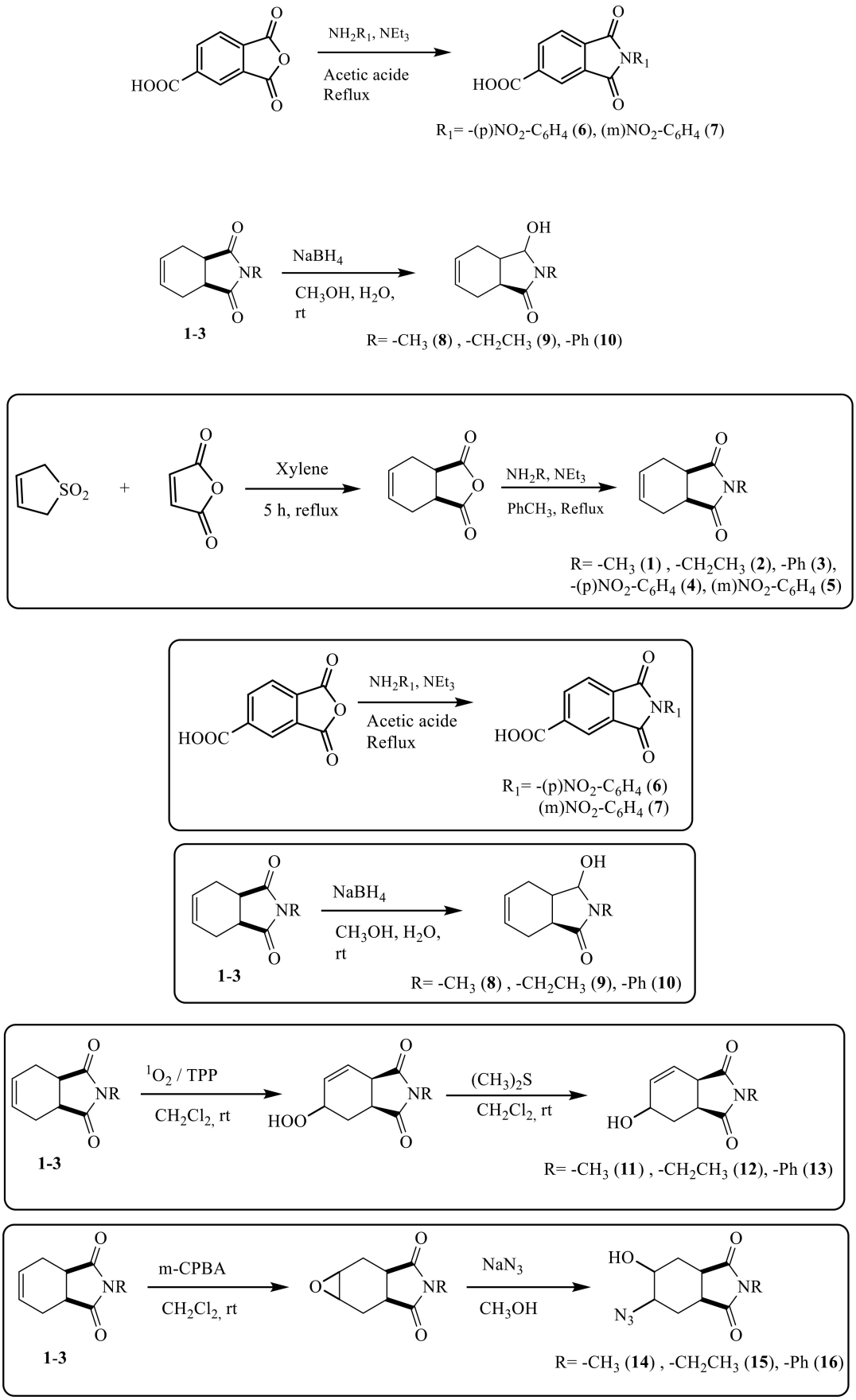


Figure 1. Some hypoglycemic and antidiabetic isoindole-1,3-dione derivatives.



Scheme 1. Synthesis scheme of isoindole-1,3-dione derivatives (**1**–**16**) for AR inhibition studies.

or **3**) (500 mg, 1 equiv.) and 20 mL of MeOH or THF in a single-necked 100 mL flask: Cooled to 0 °C with stirring in H_2O. $NaBH_4$ (338 g) (4 equiv.) was added to the reaction medium in a controlled manner. Then the reaction was brought to room temperature, was terminated after 4–5 hours by TLC control. The reaction solvent was removed in the evaporator. Saturated NH_4Cl solution (25 mL) was added to the residue and work-up was done with EtOAc (3×20 mL). Purification was done by column chromatography.

3-Hydroxy-2-methyl-2,3,3a,4,7,7a-hexahydro-isoindol-1-one (8)

Colorless crystals; yield: 92%; M.p: 103–105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.77 (m, 2H), 4.69 (dd, J = 23.7, 5.9 Hz, 2H), 2.91 (td, J = 8.3, 3.2 Hz, 1H), 2.85 (s, 3H), 2.43 (m, 2H), 2.24 (m, 2H), 1.88 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.10, 126.95, 125.61, 90.06, 39.04, 37.79, 27.27, 24.73, 22.42. IR (KBr, cm^{-1}): 3375, 3033, 2921, 2851, 1676, 1457, 1402. Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38; Found: C, 64.22; H, 7.47; N,8.23.

3-Hydroxy-2-ethyl-2,3,3a,4,7,7a-hexahydro-isoindol-1-one (9)

Colorless crystals; yield: 93%; M.p: 92–94 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00–5.80 (m, 2H), 5.11 (dd, J = 10.2, 5.8 Hz, 1H), 3.52 (m, 1H), 3.25 (m, 1H), 2.72–2.61 (m, 2H), 2.42–2.33 (m, 2H), 2.30–2.11 (m, 3H), 1.19–1.11 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.10, 127.34, 127.32, 84.96, 38.23, 35.21, 34.84, 24.24, 21.22, 13.42. IR (KBr, cm^{-1}): 3345, 3031, 2926, 2848, 1673, 1464, 1436. Anal. Calcd for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73; Found: C, 66.22; H, 7.47; N, 7.53.

3-Hydroxy-2-phenyl-2,3,3a,4,7,7a-hexahydro-isoindol-1-one (10)

Colorless crystals; yield: 95%; M.p: 108–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 7.8 Hz, 2H), 7.36 (t, J = 7.8 Hz, 2H), 7.23–7.17 (m, 1H), 5.78–5.66 (m, 2H), 5.15 (d, J = 6.7 Hz, 1H), 3.60 (dd, J = 6.8, 1.7 Hz, 1H), 3.12–3.04 (m, 1H), 2.51 (d, J = 18.8 Hz, 1H), 2.42 (q, J = 7.6 Hz, 1H), 2.33–2.15 (m, 2H), 1.85–1.75 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.43, 138.12, 129.28, 126.46, 126.06, 124.69, 122.80, 89.87, 38.32, 37.88, 24.44, 22.05. IR (KBr, cm^{-1}): 3374, 3031, 2955, 2922, 2851, 1682, 1598, 1498, 1461. Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.34, H, 6.59; N, 6.11. Found: C, 73.39; H, 7.02; N, 6.16.

*General procedure for preparation of compounds**11**–**13**:* This compound was prepared according to the reported method.[56,58]

*General procedure for preparation of compounds**14**–**16**:* This compound was prepared according to the reported method.[55]

Aldose Reductase Activity Assay and Inhibition Studies

The recombinant human AR enzyme used in the study was obtained commercially. The AR enzyme's activity was measured using a modified version of Cerelli's method. In order to measure the enzyme's activity, a 1 mL cuvette was prepared with the following components: 0.1 mL of NADPH, 0.45 mL of deionized water, 0.25 mL of Na-phosphate buffer, 0.1 mL of NADPH, 0.1 mL of enzyme solution, and 0.1 mL of DL-glyceraldehyde to start the reaction. By spectrophotometrically detecting the decrease in NADPH concentration at 340 nm, enzyme activity was assessed.[60] The research involving enzyme inhibition made use of the Optizen Pop UV/VIS Spectrophotometer. The capacity of substances **1**–**16** to inhibition the AR enzyme was examined. Each molecule was prepared in five different concentrations, and the inhibitory effects of these substances on the enzyme were evaluated. Without inhibitors, control cuvette activity was assumed to be 100%, and enzyme activity was tested at inhibitor concentrations that reduced enzyme activity by 50% (IC_{50} values). DL-Glyceraldehyde is a substrate for the AR enzyme. Table 1 lists the results of the inhibition study.

Molecular Docking Studies

The x-ray crystal structure of AR (PDB code: 2FZD) was obtained from the RCSB Protein Data Bank in pdb format with a resolution of 1.08 angstroms. The crystal structure of the receptor was then used in molecular docking studies. The Small Drug Discovery Suites package (Schrodinger 2020–3, LLC, United States) was used for molecular docking research. To repair and construct the 3D crystal structure, the Protein Preparation Wizard in Maestro 12.5 was used. After determining bond order and charges, all missing hydrogen atoms were added to the protein structure.[61] Prime was used to complete missing side chains. Using the Propka program, amino acids were ionized by establishing physiological pH. The elimination of water molecules that interacted with the protein or ligand less than three times. The OPLS3e force field was subsequently used to achieve energy minimization. By sketching 2D structures, Maestro 12.5 was used to construct 3D structures of synthesized compounds. Using Maestro 12.5's LigPrep module, a 3D structure of ligands was constructed. At a pH of 7.0 ± 2.0, the Epik module and OPLS3e force field were applied to obtain the correct molecular geometries and protonation states.

Molecular docking was used to investigate binding affinity and possible interactions between synthesized compounds and the AR receptor. Using the Receptor Grid Generation tool to choose crystalline inhibitor (tolrestat) at the binding site prior to the docking procedure's building of grid box. Extra Precision (XP) docking calculations were set by keeping the ligand flexible. After the docking technique, the types of interactions and interacting residues for the highest-scoring compounds were analyzed. Epalrestat, a standard inhibitor of the aldose reductase enzyme, was used as a positive control compound. Docking validation was performed by isolating inhibitor complexed in the crystal structure of the

Table 1. *In vitro* inhibition results of isoindole-1,3-dione derivatives.

Compound	IC_{50} (μM)	R^2
1	54.80	0.93
2	19.62	0.97
3	10.29	0.99
4	14.13	0.92
5	28.75	0.94
6	1.649	0.94
7	3.920	0.96
8	37.12	0.97
9	36.26	0.99
10	28.95	0.93
11	123.9	0.99
12	33.28	0.97
13	32.67	0.92
14	36.47	0.93
15	133.5	0.98
16	26.44	0.98
Epalrestat[64]	0.267	–

receptor prior to docking of synthesized compounds to assess the validity of the docking procedure.[62]

Analysis of ADME (Absorption, Distribution, Metabolism, and Excretion)

The ADME evaluation was performed using the QikProp module in Maestro 12.5. The results of the study provide information on the pharmacokinetic characteristics of the compounds, such as their absorption, distribution, metabolism, and excretion. Lipinski's rule of five (molecular weight, logPo/w, donorHB, and accptHB) was used to evaluate the drug-likeness of the compounds.

Binding Free Energy Calculation Utilizing Molecular Mechanics/Generalized Born Surface Area (MM-GBSA)

The electrostatic contribution of the VSGB solvation model with the OPLS3 force field was computed using the molecular mechanics-generalized Born surface area (MM-GBSA) panel. The MM-GBSA method combines molecular mechanics simulations with continuous solvation models to determine binding free energies (ΔGbind) for macromolecules.[63]

Results and Discussion

In this work, the effects of synthesized isoindole-1,3-dione derivatives on the human recombinant AR enzyme were examined. The IC_{50} values of isoindole-1,3-dione derivative compounds were found to vary between 1.649 μM and 133.5 μM. Compound **6** was found to be the most effective compound on human recombinant AR enzyme with an IC_{50} value of 1.649 μM (Table 1). Molecular docking was used to better comprehend how isoindole-1,3-dione derivatives inhibited human recombinant AR enzyme *in vitro*. Using the MM-GBSA technique, the free binding energies of enzyme-inhibitor complexes were also measured. Table 2 summarizes the docking scores and free binding energies calculated by the molecular docking and MM-GBSA techniques. A series of ADME (absorption, distribution, metabolism, and excretion) tests were conducted on isoindole-1,3-dione derivative molecules to determine their drug-like characteristics (Table 3).

The aldose reductase enzyme is a member of a superfamily of aldo–keto reductases. It is a cytosolic enzyme that is monomeric and dependant on NAD-phosphate for its activity.[65]

Table 2. Docking scores and free binding energy results of isoindole-1,3-dione derivatives with AR enzyme.

Compound	Docking score	MM-GBSA (ΔG_{bind})
1	−6.595	−38.75
2	−7.846	−42.02
3	−8.042	−50.42
4	−7.699	−49.88
5	−7.909	−46.53
6	−8.848	−49.30
7	−8.811	−46.16
8	−7.031	−45.52
9	−8.137	−51.10
10	−7.887	−50.25
11	−6.668	−37.21
12	−7.824	−35.25
13	−8.651	−49.12
14	−6.827	−40.83
15	−7.894	−38.99
16	−8.646	−45.82
Epalrestat	−6.516	−57.73

Table 3. ADME prediction findings of isoindole-1,3-dione derivatives.

Name	mol MW	donorHB	accptHB	QPlogPo/w	QPlogS	QPlogHERG	QPlogBB	QPlogKhsa	%HOA	RuleOfFive
1	165.191	0	3	1.081	−0.917	−2.427	−0.083	−0.52	89.354	0
2	179.218	0	3	1.478	−1.516	−2.648	−0.068	−0.407	93.865	0
3	227.262	0	3	2.54	−2.949	−4.169	−0.061	0.012	100	0
4	272.260	0	4	1.776	−2.964	−4.203	−0.949	−0.094	79.131	0
5	272.260	0	4	1.776	−2.964	−4.203	−0.949	−0.094	79.131	0
6	312.238	1	6	1.203	−3.556	−3.331	−2.286	−0.317	44.959	0
7	312.238	1	6	1.203	−3.556	−3.331	−2.286	−0.317	44.959	0
8	167.207	1	4.7	0.087	−0.27	−1.506	−0.111	−0.892	80.289	0
9	181.234	1	4.7	0.447	−0.594	−1.663	−0.134	−0.814	83.846	0
10	229.278	1	4.7	2.062	−2.825	−4.385	−0.078	−0.158	100	0
11	181.191	1	4.7	0.293	−1.267	−2.546	−0.498	−0.571	76.772	0
12	195.218	1	4.7	0.691	−1.511	−2.756	−0.49	−0.497	81.298	0
13	243.262	1	4.7	1.669	−2.762	−4.252	−0.515	−0.158	86.854	0
14	224.219	1	7.7	−0.908	−1.177	−2.831	−1.322	−0.871	55.147	0
15	238.246	1	7.7	−0.517	−1.406	−3.02	−1.325	−0.806	59.596	0
16	286.290	1	7.7	0.422	−2.536	−4.366	−1.366	−0.497	65.227	0

In the polyol pathway, AR catalyzes the conversion of glucose to sorbitol, which is subsequently transformed to fructose by sorbitol dehydrogenase. The accumulation of sorbitol leads to diabetes consequences including retinopathy, nephropathy, cataracts, and neuropathy.[66] In virtually all human cells, aldose reductase is a major oxidoreductase enzyme in the polyol route. It is seen in high concentrations in the lens, retina, and sciatic nerves of diabetic individuals.[65] Due to the high amount of superoxide generation associated with diabetes, aldose reductase inhibition may prevent the degradation of retinal capillaries.[66–68] However, lens epithelial cells incur apoptosis owing to GSH depletion and elevated polyol levels when they are exposed to conditions with overexpressed aldose reductase and high glucose levels.[69] Additionally, elevated AR activity results in a significant rise in NADPH consumption, which leads to oxidative stress. An important aspect of the pathophysiology of diabetic complications is increased oxidative stress.[70] Aldose reductase inhibitors (ARIs) come in a variety of forms, but none have been approved for clinical usage because of the negative adverse effects they have on patients.[71] Bioactive compounds having AR inhibiting characteristics may thus be helpful in the management and avoidance of diabetes-related complications. Determining the pharmacological and AR inhibition capabilities of isoindole-1,3-dione derivatives was the goal of this research.

In *in vitro* inhibition study on the human recombinant AR enzyme, ethyl group attachment to the 2 position of the isoindole group caused a dramatic increase in activity compared to the methyl group bound compound (**1** IC_{50} = 54,80 μM and **2** IC_{50} = 19,62 μM). However, the binding of the phenyl group to the same position (**compound 3**) caused a significant increase in activity against the AR enzyme (IC_{50} = 10,29 μM). On the other hand, adding a nitro group to the para position of the benzene ring of the **3** compound (**4**) caused a slight decrease in activity (Table 1). Although the docking scores of these four compounds largely overlap with the experimental data, the calculated free binding energies were quite successful in estimating the inhibition activity differences (Table 2). While no significant activity difference was observed between the methyl group attachment and the ethyl group attachment at the 2 position of the isoindole-1-one group, the phenyl group substitution caused an increase in the inhibition activity against the AR enzyme. The IC_{50} values of **8, 9**, and **10** compounds were calculated as 37.12 μM, 36.26 μM, and 28.95 μM, respectively (Table 1). The compound **11** obtained by attaching a methyl group to the 2 position of the 5-hydroxy isoindole-dione group showed a very low inhibition effect against the AR enzyme (IC_{50} = 123.9 μM). Substituting the methyl group with ethyl (**12**) and phenyl (**13**) functional groups led to a substantial increase in activity. Compound **11**, with a docking score of 6.665 kcal/mol against the AR enzyme, demonstrated one of the lowest docking scores, which is consistent with the experimental *in vitro* inhibition results (Table 2). While **11** compound hydrogen bonded with TYR48 and HIS110 amino acid residues, compound **12** hydrogen bonded with TYR48, HIS110, and VAL47 amino acid residues in the active site of the AR enzyme. In addition, while the compound **13** made hydrogen bonds with VAL47 and TRP111 residues and constructed a dual pi-pi interaction with TRP111 residue and single pi-pi interaction with TRP79 residue (Supplementary Figure 17). The **14** compound showed a mild inhibition effect against AR enzyme with an IC_{50} value of 36.47 μM. Although the substitution of the phenyl functional group (**16**) at the 2 position of the 4-methyl compound caused an increase in the activity, the substitution of the ethyl group (**15**) caused a dramatic decrease in the activity (Table 1). **15** is the compound with the lowest inhibitory effect against the AR enzyme among isoindole-1,3-dione derivatives, with an IC_{50} value of 133.5 μM. The main reason for this is that while the **15** compound constructed hydrogen bonds with the TYR48 and HIE110 residues in the active site of the AR enzyme, the **14** compound, in addition to these interactions, made pi-cation and salt bridge bonds thorough the azido group. Similarly, the **15** compound formed a hydrogen bond with the TRP111 residue and a dual pi-pi interaction with the TRP20 residue, while hydrogen bonded with the TRP20 residue through the azido functional group, in the active site of the AR enzyme (Supplementary Figure 18).

In vitro inhibition studies of isoindole-1,3-dione derivative compounds on the recombinant human AR enzyme have shown that the compounds with the best inhibitory effect are those containing the nitrophenyl functional group (**4, 5, 6, and 7**). However, the compounds with the best inhibitory effect were **6** and **7**, which are carboxylic acid derivatives with IC_{50} values of 1.649 μM and 3.920 μM, respectively. Compounds **6** and **7** demonstrated an inhibitory effect on the AR enzyme close to that of the standard compound epalrestat, which has a previously reported IC_{50} value of 0.267 μM.[64] The biggest and most significant aldose reductase inhibitor (ARI) class currently is carboxylic acids, which is continually expanding. The first and most extensively researched derived ARIs are carboxylic acids. In this regard, the data we obtain from our research is both very significant scientifically and consistent with the literature. While both **6** and **7** compounds made hydrogen bonds with TYR48 and TRP111 residues through the carboxylic acid functional group, they also made dual pi-pi interactions with TRP20 residue. **6** also formed a salt bridge with residue LYS77 through the carboxylic acid group (Figure 2). In addition, the calculated MM-GBSA free binding energies (ΔG_{bind}) of these compounds, which are scored very close to each other in the molecular docking study, are also quite high (Table 2). The epalrestat compound exhibited both dual π-π interactions and hydrogen bond interactions with the TRP20 amino acid residue in the active site of the AR enzyme through its carboxylic acid group, similar to compounds **6** and **7** (Supplementary Figure 19). Although the docking score of the epalrestat compound against the AR enzyme is lower than that of compounds **6** and **7**, its MM/GBSA free binding energy is superior to that of these compounds (Table 2). These results also corroborate the experimental data obtained.

Isoindole-1,3-dione derivatives' drug-likeness and pharmacokinetics were predicted using the Maestro QikProp tool. We investigated the physiochemically descriptive and pharmaceutically relevant features of isoindole-1,3-dione derivatives in order to evaluate their druggability. The molecular weight (mol MW), number of hydrogen bonds offered (donorHB), and number of

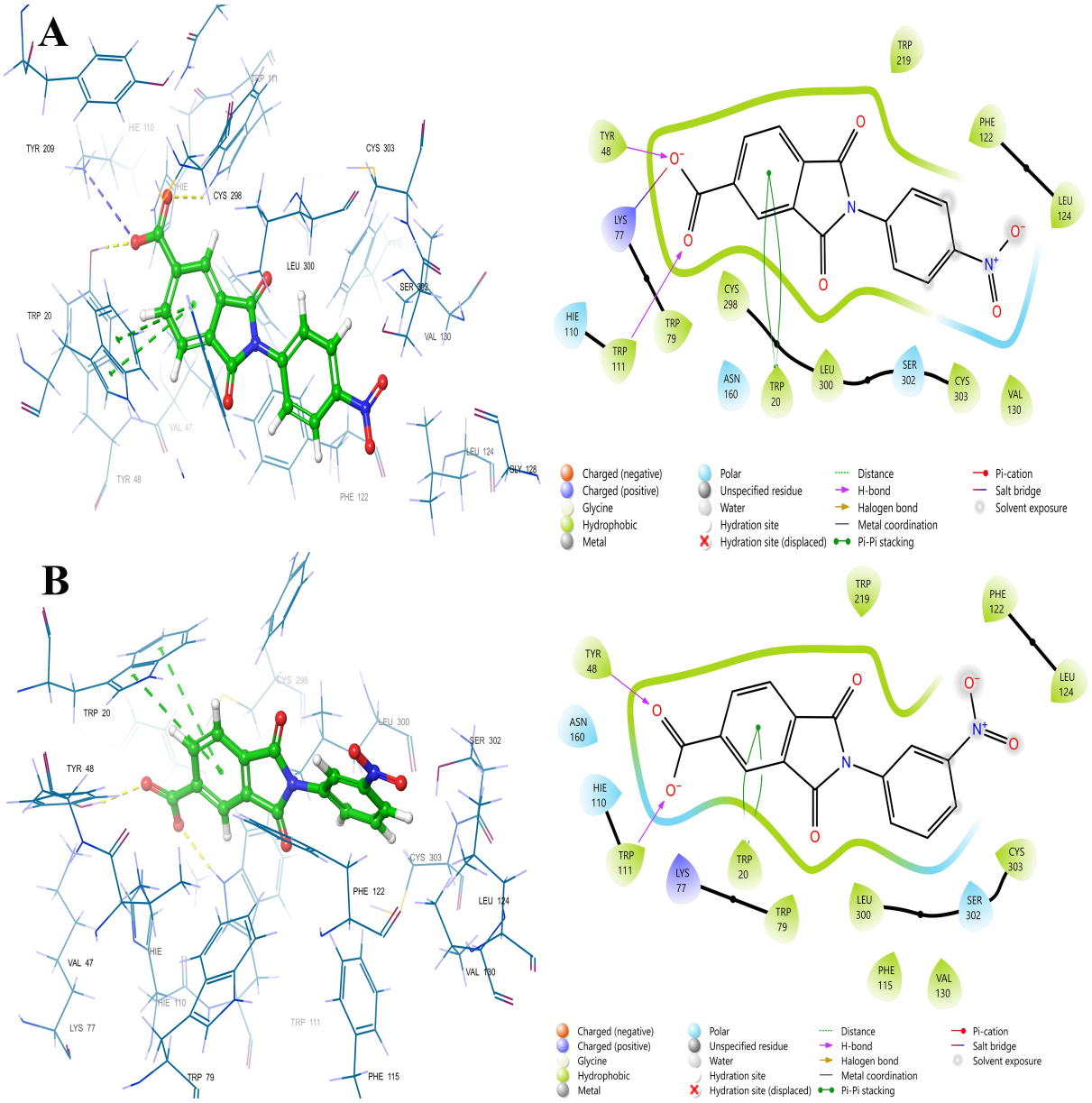


Figure 2. 3D detailed binding mode (left) and 2D ligand interaction diagram (right) of **6 (A)** and **7 (B)** for AR receptor.

hydrogen bonds accepted (accptHB) values for the isoindole-1,3-dione derivatives are all within acceptable ranges. For drug dispersion and absorption, isoindole-1,3-dione compounds demonstrated high partition coefficients (QPlogPo/w) ranging from −0.908 to 2.54. The QPlogPo/w values indicate the lipophilicity of potential drug candidates and their solubility in lipid-based environments. Water solubility (QPlogS) values range from −3.556 to −0.27, indicating acceptable water solubility of all compounds, percentage of human oral absorption (percent HOA) values range from 44.959 to 100%, and QPlogHERG (K^+ channel blockage) values are less than −5. Lipinski's 'rule of five' is a guideline used to assess drug-likeness, determining whether a chemical compound with specific pharmacological activities possesses the physical and chemical properties necessary for oral activity in humans. It was determined that all synthesized compounds, including compounds **6** and **7**, which exhibited very strong inhibitory effects against the AR enzyme, complied with Lipinski's rule of five. All of the isoindole-1,3-dione derivative compounds were determined to be therapeutically acceptable and to meet all pharmacokinetic requirements for a drug-like substance (Table 3).

Conclusions

The beta cells of the pancreatic islets of Langerhans exhibit either impaired insulin production or insulin resistance in diabetes mellitus, a metabolic condition that affects the whole body and causes elevated blood sugar levels. Due of the issues

it causes, this has turned into a problem for world health. Nephropathy, neuropathy, cardiovascular system, and retinopathy are caused by persistently high glucose levels. Aldose reductase, the pathway's rate-limiting enzyme, is a key contributor to diabetic complications because of all these metabolic impacts. Therefore, the discovery of aldose reductase enzyme inhibitors is vital for the prevention and treatment of diabetic complications. Preventing diabetic complications is essential for enhancing public health and improving quality of life. In our study, isoindole-1,3-dione derivative compounds were designed, synthesized and their *in vitro* and *in silico* inhibition effects against AR enzyme were investigated. *In vitro* experiments revealed that compounds **1**–**16** are potent AR inhibitors; with IC_{50} values ranging from 1.649 to 133.5 μM. It was determined that the docking scores of the isoindole-1,3-dione compounds ranged from −6.595 to −8.848 kcal/mol, and their free binding energies ranged from −35.25 to −51.10 kcal/mol. Also, drug-likeness studies conducted on these compounds have demonstrated that they possess excellent pharmacokinetic properties. Compound **6** was revealed to be the most promising AR inhibitor among these compounds. *In silico* studies for isoindole-1,3-dione derivative compounds, whose *in vitro* inhibition effects were investigated, also confirmed the experimental data obtained.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords: Aldose reductase · Diabetic complications · Isoindole-1,3-dione · Molecular docking

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