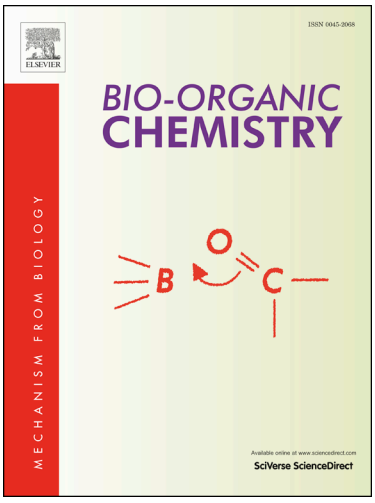

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Molecular modelling and Synthesis of Spiroimidazolidine-2,4-diones with Dual Activities as Hypoglycemic Agents and Selective Inhibitors of Aldose Reductase

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ABSTRACT

Novel derivatives of spiro-imidazolidinedione were synthesized and evaluated as hypoglycemic agents through binding to sulfonylurea receptor 1 (SUR1) in pancreatic beta-cells. Their selectivity index was calculated against both aldehyde reductase (ALR1) and aldose reductase (ALR2). Aldehyde reductase is a key enzyme in the polyol pathway that is involved in the etiology of the secondary diabetic complications. All structures were confirmed by microanalysis and by IR, ^{1}H-NMR, ^{13}C-NMR and EI-MS spectroscopy. The investigated compounds were subjected to molecular docking and an *in silico* prediction study to determine their free energy of binding (ΔG) values and predict their physicochemical properties and drug-likeness scores. Compound 1'-(5-chlorothiophene-2-ylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione showed IC_{50} 0.47 μM and 79 % reduction in blood glucose level with a selectivity index 127 for ALR2.

KEYWORDS

Spiro-imidazolidinedione, Hypoglycaemic agents, Aldose reductase, *In Silico* Prediction, Mixed pharmacophore.

1. INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by a chronic increase in blood glucose level. This is mainly attributed to impaired insulin secretion by pancreatic β cells and insulin resistance in peripheral target tissues.[1,2] Sulfonylureas are common in the design of oral hypoglycemic agents such as tolazamide 1 and glibenclamide 2.[3,4] These derivatives play a crucial role in the improvement of insulin secretion and the reduction of insulin resistance. They exert their action via several mechanisms including pancreatic ones through increasing insulin secretion from pancreatic beta-cells and the others are extra-pancreatic by improving insulin resistance in peripheral target tissues.[5,6] Despite numerous trials to control hyperglycemia, most diabetic patients still suffer from one or more of the long-term diabetic complications including retinopathy, nephropathy, neuropathy and cataract.[7,8] The enlarged glucose flux during the polyol pathway, which occurs in hyperglycemic conditions, is a potential factor in the onset and progression of such chronic complications (Figure 1).[9,10] Aldose reductase enzyme (ALR2, E.C.1.1.1.21) is the first and key enzyme involved in the polyol pathway. The main role of ALR2 is to catalyze the NADPH-dependent reduction of glucose to sorbitol. Aldose reductase inhibitors (ARIs), therefore, could be considered as a pharmacologically direct management for diabetic complications.[11]

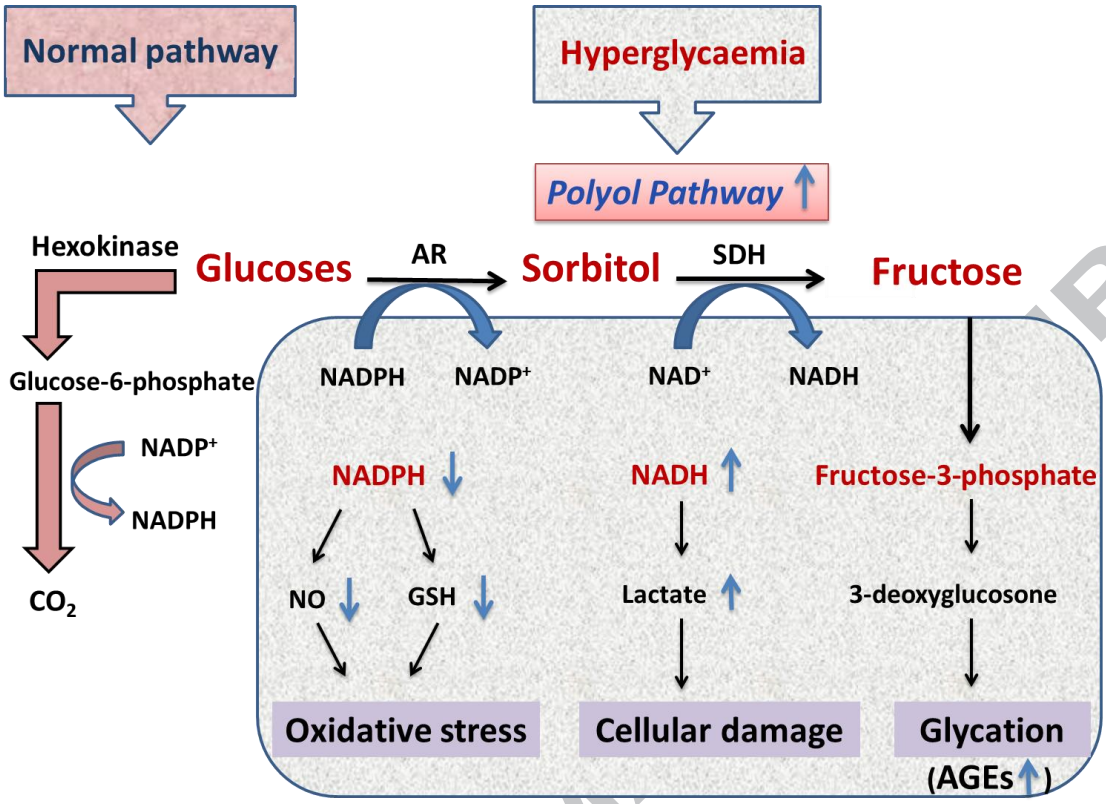


Figure 1. A schematic interaction diagram elucidating the metabolism of glucose to sorbitol in polyol pathway by aldose reductase (AR) under hyperglycemic conditions.[11] **AGE**: Advanced glycated end product; **AR**: Aldose reductase; **GSH**: Glutathione; **SDH**: Sorbitol dehydrogenase.

Various compounds have been designed to inhibit aldose reductase (AR).[12] These compounds can be classified into two main categories, the first category comprises those containing a carboxylic acid moiety, for example, 3-thiazolidineacetic acid derivative, which has been reported to be a potent ARI with IC_{50} 9 nM.[13] The second category includes those based on a cyclic imide in the form of spirohydantoin such as sorbinil **3**.[11] The carboxylic acid inhibitors were found to be less potent than the spirohydantoin inhibitors *in vivo*.[14] This might be due to the higher ability of the carboxylic acid derivatives to ionize at physiological pH. Subsequently, these derivatives could not easily cross the biological membranes. Moreover, the selectivity of a specific aldose reductase (ALR) is crucial to avoid the undesirable side effects of non-selective

inhibitors. Thus, the medication of choice should discriminate between aldehyde reductase (ALR1, EC 1.1.1.2) and aldose reductase (ALR2, EC 1.1.1.21), since, ALR1 is responsible for an important detoxification mechanism in the liver.[15,16] However, the structure of ALR1 has 65 % homology with ALR2, however some differences in their respective active sites provide the basis for their selective inhibition.[17,18] The present work has aimed to design and synthesize a single medication that can control the plasma glucose level and also reduce adjunctive secondary diabetic complications via applying a mixed pharmacophore theory (Figure 2).[19,20]

A literature survey showed that, in 2013, Iqbal et al reported the design and synthesis of 3'-(4-chlorophenylsulfonyl)spiro[cycloheptane-1,5'-imidazolidine]-2',4'-dione **4** with an IC_{50} value of 1.8 μM against ALR2.[21] In 2015, they also found that 3'-(4-chlorophenylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione **5** was 53-fold more selective toward ALR2 (with an IC_{50} value of 0.89 μM) compared with ALR1.[5] In 2017, Andleeb et al reported the design and synthesis of *(Z)*-4-((3-(2methoxyphenyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl-4-chlorobenzene-sulfonate **6** as an aldose reductase inhibitor with an IC_{50} value of 0.46 μM and 93-fold selectivity for ALR2 over ALR1 with no apparent potential for hypoglycemic activity.[14] Herein we assumed the optimization of the structure of these valuable candidates; compounds **4** and **5**. Therefore, this study has been focused on modifying spirohydantoin derivatives based on the enhancement of the binding affinity of the newly synthesized compound and improvement of its physicochemical properties according to Lipinski's "rule of five""Ro5".[22-24] This was applied through two main strategies. Strategy one was the design of various hydrophobic moieties attached to the spirohydantoin core particularly the flexible cyclohexanonyl ring and the rigid fluorenonyl one. Strategy two was studying different aryl and heteroaryl sulfonyl moieties and exploring their biological influence.

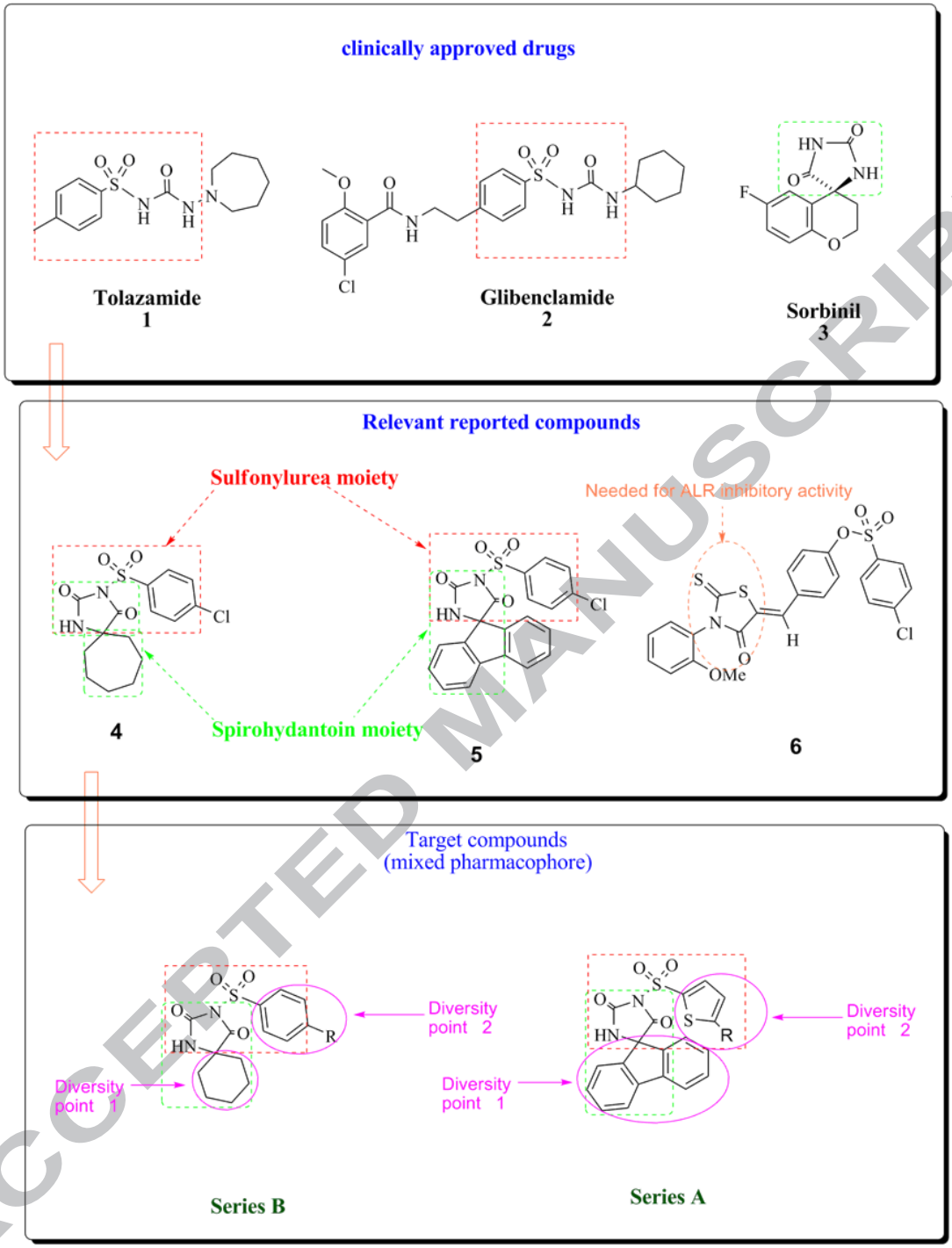
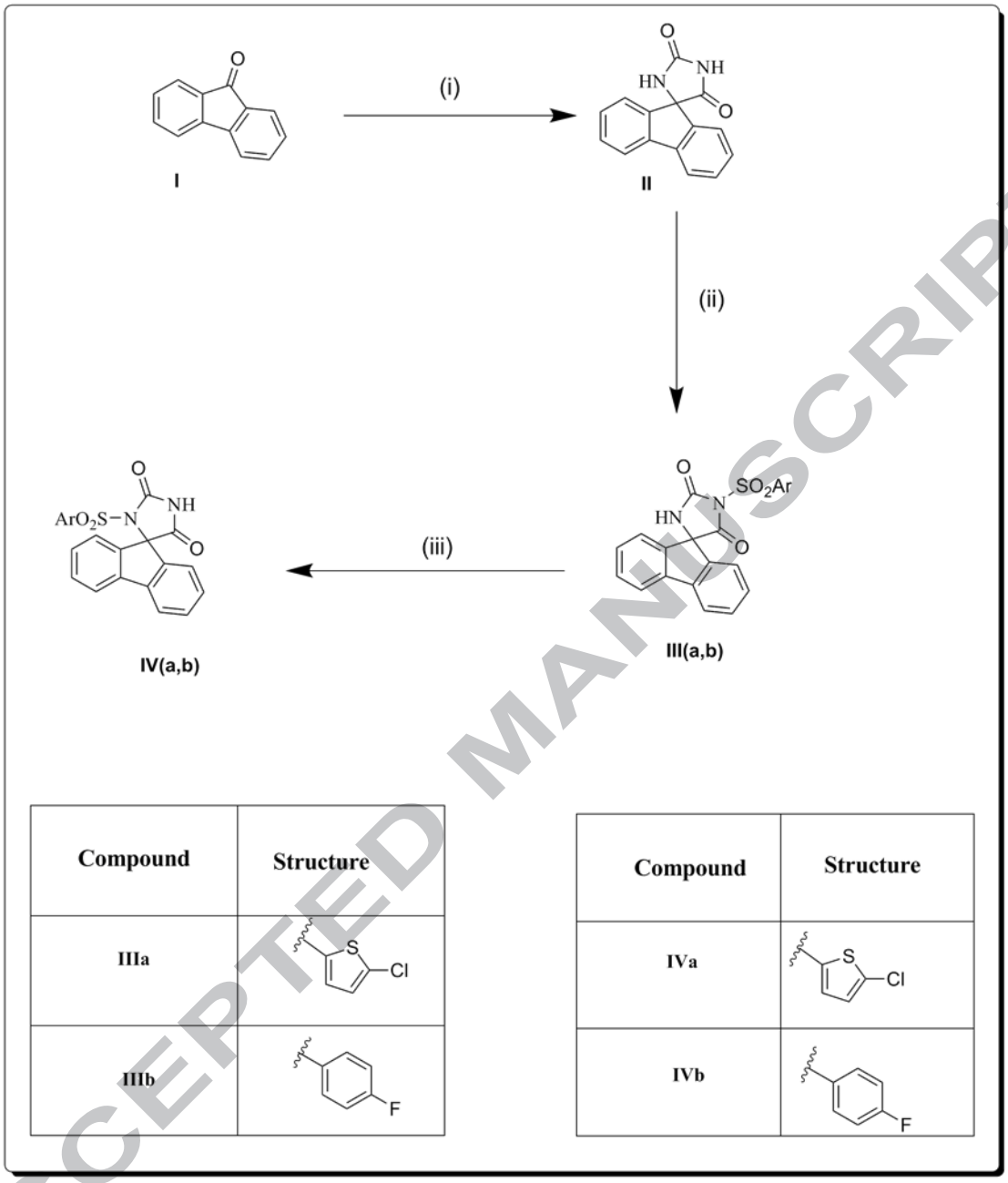


Figure 2. Mixed pharmacophore theory. The red box highlights the minimum requirements for enhanced hypoglycemic activity by sulfonylureas. The green box highlights minimal pharmacophoric requirements for an aldose reductase inhibitor. The violet rings represent the points of diversity.

2. RESULTS AND DISCUSSION

2.1. Chemistry

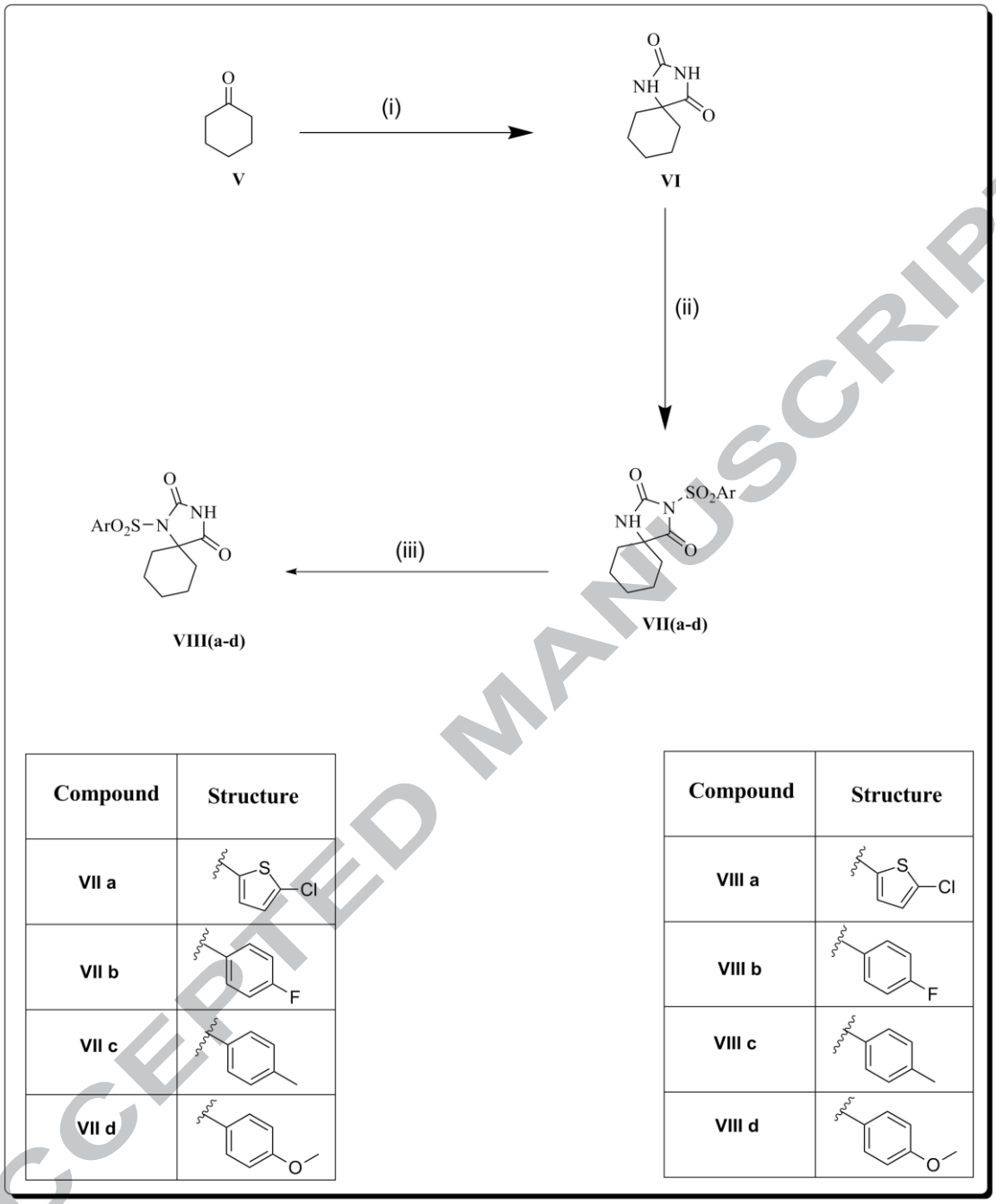
Spiro-imidazolidinedione derivatives **II** and **VI** were prepared as previously reported.[5,25] The synthetic procedures involved the reaction of 9-fluorenone **I** (scheme 1, series A) and cyclohexanone **V** (scheme 2, series B) with ammonium carbonate (NH_4CO_3) and potassium cyanide (KCN) in 50 % aq. Ethanol.[25] 3-Arylsulfonyl derivatives **IIIa** and **IIIb** (series A) and **VIIa–d** (series B), respectively, were prepared via the coupling of compounds **II** and **VI** with various arylsulfonyl chlorides ($ArSO_2Cl$) in triethylamine (Et_3N) and dimethylaminopyridine (DMAP) in CH_2Cl_2.[26] Compounds **IIIa**, **IIIb** and **VIIa–d** were further converted to their 1-arylsulfonyl analogues **IVa**, **IVb** and **VIIIa–d**, respectively using sodium hydride (NaH) under dry conditions (schemes 1 and 2).[27]



Compound	Structure
IIIa	
IIIb	

Compound	Structure
IVa	
IVb	

Scheme 1. Synthesis of spiro [fluorene-9,5'-imidazolidine]-2',4'-dione derivatives (Series A). Reagents and conditions: (i) NH_4CO_3, KCN, 55 °C and aq. ethanol; (ii) $ArSO_2Cl$, Et_3N and DMAP; (iii) NaH.



Compound	Structure
VII a	
VII b	
VII c	
VII d	

Compound	Structure
VIII a	
VIII b	
VIII c	
VIII d	

Scheme 2. Synthesis of spiro[cyclohexane- 1,5'-imidazolidine]-2',4'-dione derivatives (Series B). Reagents and conditions: (i) NH_4CO_3, KCN, 55 °C and aq. ethanol; (ii) $ArSO_2Cl$, Et_3N and DMAP; (iii) NaH.

The structures of the newly synthesized compounds were confirmed by microanalyses and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and EI-MS), which showed full agreement with their structures. The IR spectra showed bands for anti-symmetric and symmetric O=S=O absorptions in the range of 1350–1322 cm^{-1} and 1175–1140 cm^{-1}, respectively. The presence of a band for the N-H stretching in the range of 3400–3200 cm^{-1} also indicated the successful coupling between the aryl sulfonyl chloride derivatives and spirohydantoin compounds resulting in the synthesis of 3-arylsulfonylspiroimidazolidine-2,4-diones, **IIIa** and **IIIb** (series A) and **VIIa–d** (series B). The ^{1}H-NMR spectra of compounds **IIIa**, **IIIb** and **VIIa–d** displayed singlets at 5.70-6.00 ppm. This peak referred to only one imidazolidinedione NH group and confirmed the successful coupling of the other NH moiety. The characteristic multiplets assigned to fluorenonyl protons appeared downfield at 7.15-8.08 ppm and hexanonyl protons upfield at 1.39-2.08 ppm. Compounds **IIIa** and **IVa** showed doublets assigned to thienyl protons at 6.41-7.04 ppm. Moreover, compounds **IIIb** and **IVb** displayed doublets assigned to the phenyl protons at 8.09-8.20 ppm. The ^{13}C-NMR spectra showed signals for spiro[fluorene-9,5'-imidazolidine]-2',4'-dione, phenyl, thienyl and spiro[cyclohexan-1,5'-imidazolidine]-2',4'-dione rings at the expected regions. Additionally, the mass spectra of compounds **IIIa**, **IIIb**, **IVa** and **IVb** (series A) and for compounds **VIIa**, **VIIb**, **VIIIa** and **VIIIb** (series B) showed the presence of molecular ion peaks M^{+} and their isotopes $M^{+}+2$, characteristic for sulfur and halogen-containing compounds. The ^{1}H-NMR spectra of compounds **IVa** and **IVb** and compounds **VIIIa–d** confirmed the successful rearrangement by the appearance of the downfield singlets at 12.16-12.55 ppm assigned to the NH group at position 3. This was accompanied by the disappearance of the upfield singlets corresponding to the NH group at position 1 observed in the spectra of compounds **IIIa**, **IIIb** and compounds **VIIa–d**, respectively. Regarding series B, compounds **VIIb–d** and **VIIIb–d** displayed doublets assigned to the phenyl protons at 7.49-7.96 ppm. Furthermore, the ^{1}H-NMR spectra of

compounds **VIIc** and **VIIIc** are characterized by the appearance of singlets at 2.30 and 2.19 ppm, respectively, assigned to the protons of the methyl group. Concerning compounds **VIId** and **VIIId**, their ^{1}H-NMR spectra are characterized by the appearance of singlets at 3.90 and 3.80 ppm, respectively, referred to the protons of the methoxy groups.

2.2. Biological evaluation

All newly synthesized compounds were evaluated for *in vivo* antidiabetic activity and the inhibitory activity against ALR1 (EC 1.1.1.2) and ALR2 (EC 1.1.1.21). The selectivity indexes of the new compounds were determined. The results revealed that the newly synthesized compounds probably serve as a single medication with a mixed pharmacophore not only to treat diabetes but also to avoid diabetic complications.

2.2.1. *In vivo* hypoglycemic activity

In vivo study was performed on BALB/c mice after induction of diabetes and the blood glucose level was measured through collecting the blood samples at 0 h followed by 2, 4, 6 and 8 h.[5] The majority of the synthesized compounds of series A and B were found effective antidiabetic agents (Table 1). Concerning series A, compounds **IIIb** and **IVb** displayed the highest activities with 82 and 74 % reduction in blood glucose levels, respectively. They were more potent than glibenclamide **2** (63 % reduction in blood glucose level) and the reported active compound 3'-(4-chlorophenylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione **5** (72 % reduction in blood glucose level). Both of the compounds contain a halogen atom (F) at the aromatic ring (phenyl). The comparison between compounds **IIIa** and **IVa** revelled that the activity increased to a great extent (from 52 % to 70 %) after the rearrangement step. Concerning series B, the most active compound was **VIIIa** with 80 % reduction in blood glucose level. It was more potent than glibenclamide **2** (63 %) and the reported active compound 3'-(4-chlorophenylsulfonyl)spiro[cycloheptane-1,5'-imidazolidine]-2',4'-dione **4** (68 %). Compound

VIIIc was also effective in reducing blood glucose level (69 %) and was more potent than the standard drug. Regarding compound **VIIIc**, the activity was not much affected by replacing the halogen atom with methyl group. This might be attributed to the moderate size and lipophilicity of the methyl group compared with the halogen atom. Compound **VIId** was ineffective in lowering plasma glucose level. This negative effect might be attributed to the relatively lower lipophilic property of the methoxy group.

Table 1. Blood glucose concentration (mg dL^{-1}) in diabetic mice treated with series A and B compounds.

Compounds	Glucose mg/dl basal	Glucose mg/dl After 2h	Glucose mg/dl After 4h	Glucose mg/dl After 6h	Glucose mg/dl After 8h	Inhibition after 8 h (%)
Series A						
IIIa	324±4.57	255±3.78	204±5.08	187±4.62	155±2.47	52.1
IIIb	385 ± 5.69	314±3.59	284 ± 3.78	214 ± 2.74	71 ± 1.51	81.6
IVa	421±3.28	315±5.57	235±8.36	179±5.11	125±8.45	70.4
IVb	362±7.49	225±2.67	184±5.82	134±3.69	93 ± 2.43	74.3
Series B						
VIIa	451±4.56	352±4.98	274±5.43	204±1.87	154±4.78	65.9
VIIb	398±8.23	317±7.93	282±5.57	221±4.91	143±5.33	64.1
VIIc	366±4.77	276±8.41	221±3.86	198±6.78	141±2.24	61.4
VIId	321±6.75	275±7.55	194±9.36	219±2.61	254±8.45	------
VIIIa	414±7.89	278±6.34	192±5.12	125±2.76	83 ± 3.26	79.9
VIIIb	356±8.89	269±9.37	228±7.23	206±6.98	156±5.36	56.2

VIIIc	391±8.55	284±8.34	217±5.86	181±7.33	121±2.44	69.1
VIIId	408±7.93	364±7.58	261±9.22	213±4.71	161±5.89	60.5
4[a]	395 ±3.53	287 ±4.63	280 ±2.23	143 ±6.81	128±8.95[c]	67.6[d]
5[b]	245.0±4.34	232.0±4.72	160.4±5.83	78.8±2.63	68.0±1.00	72.24
Glibencla-mide	374±8.13	312±9.64	254±4.78	203±6.55	149±7.75	62.8

a: The reported results of compound 4 by Z. Iqbal et al.[21]

b: The reported results of compound 5 by Z. Iqbal et al.[5]

c: Plasma glucose concentration (mg/dl-1) After 7 h. by Z. Iqbal et al.[21]

d: Inhibition after 7 h (%).

Values represent mean ± SEM.

2.2.2. ALR1 and ALR2 activity

Inhibitory activities of the newly synthesized compounds were screened *in vitro* against ALR1 (the positive control was valproic acid)[5] and ALR2 (the positive control was sorbinil **3**)[11] based on estimating the decrease in the UV absorption by NADPH at 340 nm using UV spectrophotometer.[28] Most of the new compounds were found more potent towards ALR2 than ALR1 (Table 2). Among the compounds of series A, compound **VIIIa** was the most potent inhibitor of ALR2 with an IC_{50} value 0.47 μM. Compound **VIIIa** was more potent than the standard drug sorbinil with an IC_{50} value 3.14 μM and was also found more potent than the reported compounds 3'-(4-chlorophenylsulfonyl)spiro[cycloheptane-1,5'-imidazolidine]-2',4'-dione **4** and 3'-(4-chlorophenylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione **5** with IC_{50} values 5.59 μM and 0.89 μM, respectively. A slight decrease in the activity was observed when the thienyl group was replaced with a phenyl group in compound **IVb** with an IC_{50} value

1.62 μM. This reduction in the activity might be attributed to the relatively bigger size of the phenyl group compared to the thienyl group, in addition to the bigger size and the relative rigidity of the fluorenonyl moiety. The comparison between the newly synthesized compounds before and after rearrangements revealed that the inhibitory activity was almost better after rearrangement step. It has been shown that the arylsulfonyl group at position 1 of hydantoins affects greatly the physicochemical properties and intestinal absorption of the drugs, and they are more active than those with arylsulfonyl substituent at position 3.[29] Similarly, compounds **IIIa**, **VIIa**, **VIId** and **VIIId** exhibited a reasonable inhibition values to ALR1 and were more potent than valproic acid. This probably due to the relatively higher lipophilic characters of arylsulfonylspiroimidazolidine-2,4-diones if compared with 2-propylpentanoic acid moiety in the standard valproic acid. The selectivity for the synthesized inhibitors was calculated using their IC_{50} values against ALR1 versus ALR2. In series B, a remarkable selectivity was observed for compound **VIIIa**. The selectivity index showed that compound **VIIIa** was 127- fold more selective for ALR2 over ALR1. Compound **VIIIa** exhibited the maximum selectivity compared with the reported active compounds **4**, **5**, and **6** (Figure 2). This remarkable selectivity towards ALR2 is necessary to minimize toxicity and side effects. Therefore, compound **VIIIa** was the exception with selectivity for ALR2 over ALR1 ranging from 10.8 **VIIIc** to 0.98 **IIIb**. Compound **VIIIa** could be considered a potent and selective inhibitor with minimal side effects.

Table 2. *In vitro* ALR1 and ALR2 activities of the new compounds of series A and B.

Compounds	ALR1	ALR2	S.E
	μM IC_{50}	μM IC_{50}	IC_{50}[ALR1]/ IC_{50}[ALR2]
IC_{50} ± SEM (μM)[a]/(% Inhibition)[b]			
Series A			
IIIa	12.9 ± 0.30	3.75 ± 0.04	3.44
IIIb	10.8 ±0.05	11.1±2.07	0.98
IVa	5.54 ± 0.89	4.91±0.02	1.12
IVb	17.1± 0.98	1.62 ± 0.11	10.5
Series B			
VIIa	9.16 ±0.07	4.78 ± 0.13	1.91
VIIb	16.7 ± 0.61	7.76±0.08	2.15
VIIc	13.6 ± 0.43	8.72 ± 0.06	1.55
VIId	21.4 ± 0.06	2.53 ± 0.03	8.45
VIIIa	60.1 ± 0.72	0.47 ± 0.02	127
VIIIb	17.72 ±0.63	7.68 ± 0.82	2.31
VIIIc	38.2 ± 1.42	3.53 ± 0.06	10.8
VIIId	9.81 ±0.46	8.31 ± 0.26	1.18
4[21]	61.1 ± 0.85	5.59 ± 0.07	10.9

5[5]	47.9	0.89 ± 0.01	53.82
6[14]	43.55 %	0.468 ± 0.003	93.1
Sorbinil[c]	-	3.14 ± 0.02	-
Valproic acid[d]	57.4 ± 0.89	-	-

a: n = 3.

b: % Inhibition at 0.6 μM inhibitor concentration

c: Reported IC_{50} of 3.42 μM by Rakowitz et al.[30]

d: Reported IC_{50} of 56.1 ± 2.7 μM by Stefek et al.[28]

2.3. Molecular docking study

In an attempt to rationalize the obtained *in vitro* biological results and obtain insights into the possible binding modes of compounds **IVa**, **VIId** and **VIIIa** together with the reference drug, sorbinil **3** within the active site of ALR2. The X-ray crystal structures of ALR2 complexed with NADPH (PDB ID, 4QR6-ALR2 and 3H4G-ALR1) co-crystalized with {2-[(1,3-benzothiazol-2-ylmethyl)carbamoyl]-5-fluorophenoxy}acetic acid (37V) and (2S,4S)-2-aminoformyl-6-fluoro-spiro[chroman-4,4'-imidazolidine]-2',5'-dione (FID), respectively were download from the Protein Data Bank (PDB). The molecular docking simulations were performed by MOE dock (MOE2016. 8)[31] using Triangle matcher as placement scheme and London dG as a scoring function. The binding affinity between the ligand and the target is better when the value of ΔG is lower. The saved pose for the ligand-target complex of each compound was subjected to detailed 2D and 3D analysis for its interactions with the target. Molecular docking simulation of compounds **IVa**, **VIId** and **VIIIa** and sorbinil into ALR2 revealed that all the ligands were configured into the active site with several molecular interactions probably responsible for the

observed affinity on target enzyme ALR2 (Figures 3, 4 and 5). Binding scores and the type of interaction together with pharmacological activity are listed in (Table 3).

The energy data showed a direct correlation between the binding free energy (ΔG) values of the target compounds and their inhibitory activities[32] with ALR2. All the docked compounds showed either hydrogen bond acceptor or donor interaction with Cys298 in the active site except compound **IVa**. The structural moieties participating in such interactions involved C=O, NH or CH. Initially, the docking studies for compounds **IVa**, **VIId** and **VIIIa** on ALR2 revealed that the most active compound was **VIIIa** with an IC_{50} value 0.47 μM and with the lowest ΔG value -7.21 Kcal/mol. Its structure revealed that it has the chloro atom attached to the thienyl group at position 5 rendering it more lipophilic. The thienyl ring along with the cyclohexanonyl moiety might render the compound more flexible and smaller than its analogues with the relatively rigid and bigger fluorenonyl moiety. This comparative flexibility could increase the affinity of the compound **VIIIa** towards the active site of ALR2. All these features might result in an increased interaction with the active site of ALR2. Compound **VIIIa** made several hydrophobic and H-bonding contacts with key amino acid residues inside the active site. This active site included a hydrophobic portion that was lined with Trp20, Ala299, Trp111, Phe122, Trp219 and Leu300 and a hydrophilic portion contained Thr113, Cys298, Try48, His110, Tyr309 and cyc303. Compound **VIIIa** established strong binding affinity through a hydrophobic π-π interaction with the phenyl ring of Trp111 and H-π interaction with Thr113. The structural moiety participating in π-π interactions or H- π interactions involved the five membered ring of compound **VIIIa.** In addition, it showed a hydrogen bond donor interaction with Cys298 (Figure 3). The smaller size of both the thienyl and cyclohexanonyl moieties as well as the flexibility of its structure could increase the compound affinity towards the active site of ALR2. Therefore, all the previous features could rationalize its selective inhibition of ALR2 (Table 3 and Figure 3).

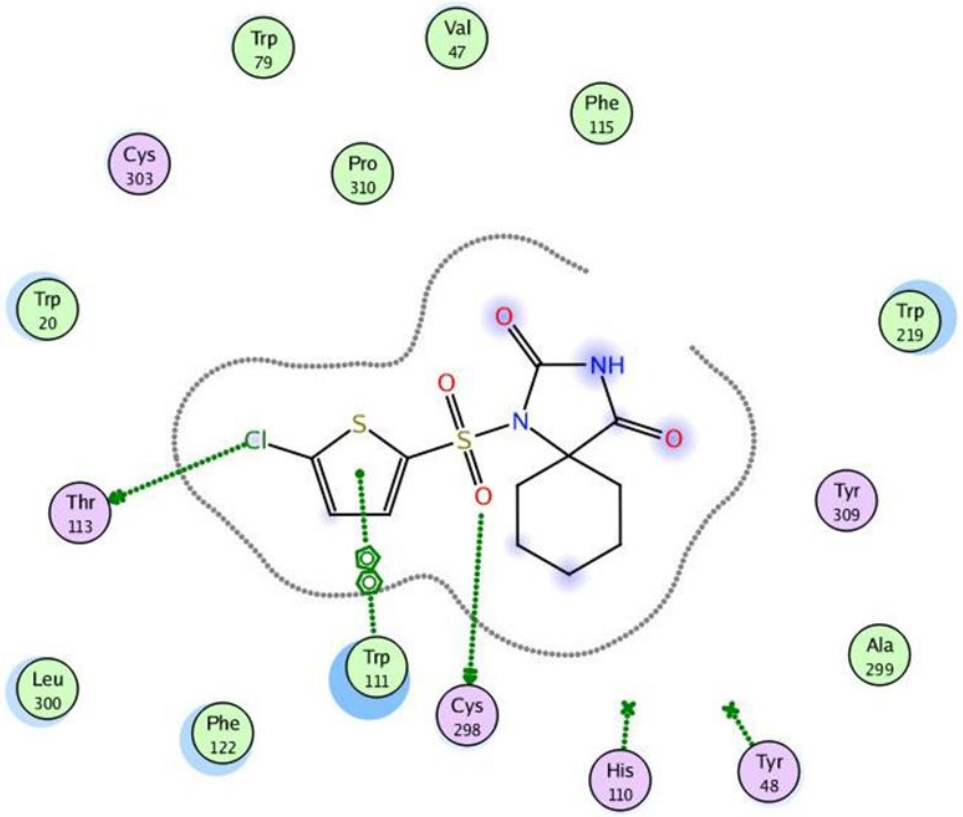


Figure 3. 2D ligand interaction of compound **VIIIa** in the inhibitor active site of ALR2.

Table 3. Inhibitory activity (μM, IC_{50}), Selectivity index (SI), Binding scores and binding interaction of compounds **IVa**, **VIId** and

Compounds	Pharmacological activity			ALR2		ALR1	
	ALR1	ALR2	SI	S (ΔG)	Binding Interaction	S(ΔG)	Binding Interaction
IVa	5.54 ±0.89	4.91±0.02	1.12	-6.304	H-donor Asp216-S H-accept.Lys262-C=**O** H-accept.Ser263-C=**O** H-accept. Thr265-C=**O** H-accept. Arg268-C=**O** pi-H Arg268-phenyl	-6.1241	H-accept. Trp114-C=**O** H-accept. His113-C=**O** H-donor Ile261-Cl
VIId	21.4 ±0.06	2.53 ± 0.03	8.45	-5.110	H-accept. Tyr48-C=**O** H-accept. Cys298-C=**O** H-accept. His110-C=**O**	-4.5874	H-accept. Tyr50-C=**O** H-accept. Trp114-C=**O** H-pi Tyr-210-N**H**
VIIIa	60.1 ± 0.72	0.47 ± 0.02	127	-7.206	H-donor Cys298-C=**O** H-donor Thr113-**Cl** pi-pi Trp111-5-mem. ring	-5.8008	H-accept. Tyr50-C=**O** H-pi Tyr-22-**C** H-accept. His113-C=**O**
Sorbinil		3.14 ± 0.02		-3.251	H-accept. Trp111-C=**O** H-pi Trp-20-**C**		

VIIIa and reference drug sorbinil with aldose reductase targets (ALR2, ALR1).

$S.I = IC_{50}[ALR1]/ IC_{50}[ALR2]$

The structures of compound **VIIIa** and sorbinil were not superimposed in the active site of ALR2 enzyme (Figure 4). Their root mean square deviations (RMSD) values were 1.54 Å and 0.69 Å, respectively. This means that these compounds were not able to generate similar docking poses. Compound **VIIIa** fits plausibly in the active site of ALR2 with a binding score -7.21. Investigation of its docking results revealed an optimum binding mode with the key amino acids. It displayed one major hydrogen bonding interaction with the key ALR2 residue Cys298. It was noticed that the thiophene ring was extended in the hydrophobic pocket created by Leu300, Alaa299 and Trp111. It was stabilized by a π-π interaction between the thiophene ring and the phenyl ring of Trp111. These binding features played a crucial role in stabilizing the three dimensional structure of the inhibitor-enzyme complex. On the other side, it was noticed that sorbinil did not exploit the pocket created by Leu300, Alaa299 and Trp111. Its core structure was shifted slightly outside the binding domain of ALR2. It showed a higher binding score -3.25, reflecting its lower binding affinity when compared to compound **VIIIa**. This probably explains the superior potency of compound **VIIIa**.

Compound **VIId** displayed the highest ΔG value -5.11 Kcal/mol. This might illustrate its low activity and reduced stability inside the active pocket of ALR2 (Table 3 and Figure 5). Compound **IVa** showed more hydrogen bond interactions with several amino acids of ALR2, which could be due to its higher Polar Surface Area TPSA 83.549 Å and rigidity. Compound **IVa** was found to bind in a different site (it might be an allosteric site) (Table 3 and Figure 6).

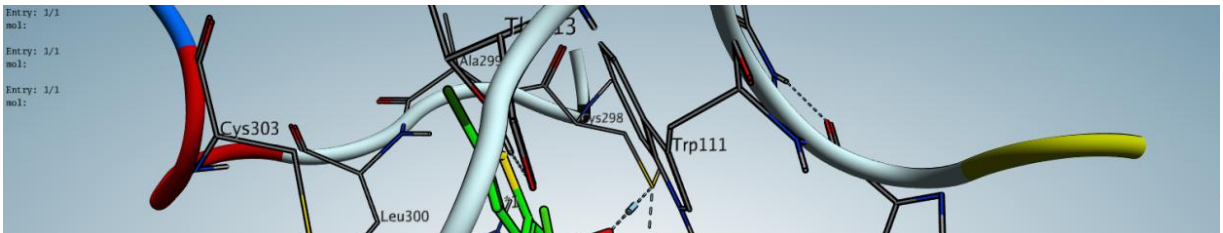


Figure 4. 3D Comparable binding interaction of compound **VIIIa** and sorbinil in the inhibitor active site of ALR2.

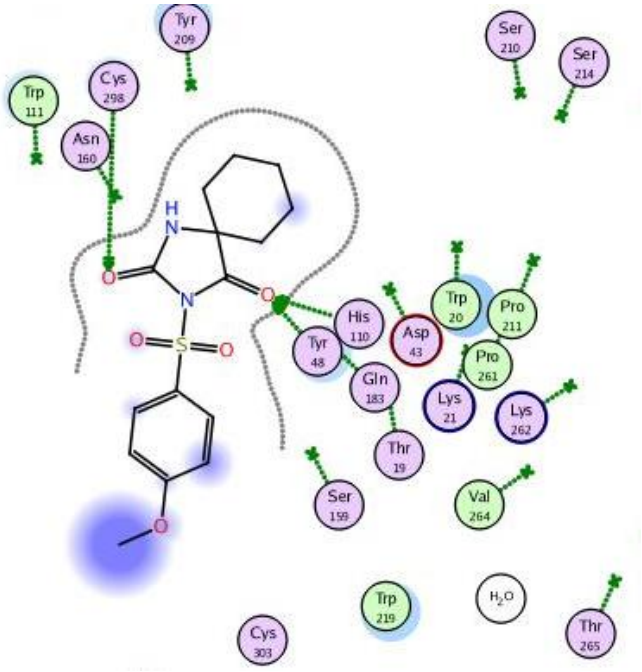


Figure 5. 2D Ligand interaction of compound **VIId** in the inhibitor active site of ALR2.

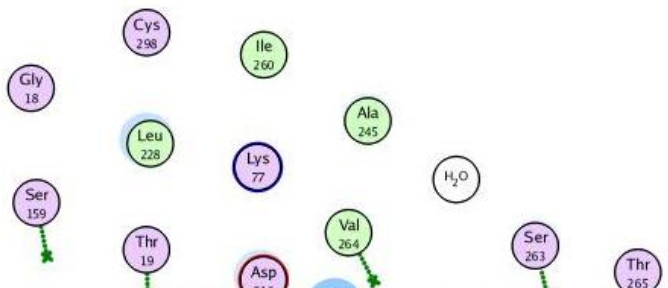


Figure 6. 2D Ligand interaction of **IVa** in the inhibitor active site of ALR2.

The compounds were then docked in the active site of ALR1 and showed either one or two hydrogen bond acceptor interaction between Tyr50 or Trp114 in the active site and the C=O moiety. Docking results of the investigated compounds revealed that compound **IVa** showed the lowest ΔG scores -6.12 Kcal/mol for ALR1 binding. The presence of chloro atom on the thienyl ring (H-donor Ile261-Cl) contributed to its good binding with ALR1 (Table 3 and Figure 7).

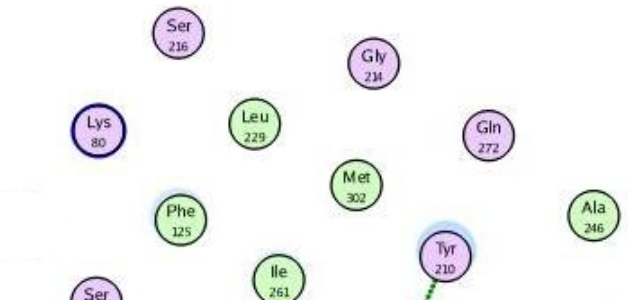


Figure 7. 2D Ligand interaction of compound **IVa** with the active site residues of ALR1.

As a result, the investigation of the binding values of compounds **IVa**, **VIId** and **VIIIa** on both targets ALR1 and ALR2, revealed that the designed compounds had inhibitory activities against both targets with greater selectivity towards ALR2 in case of **VIId** and **VIIIa**.

2.4. *In silico* prediction of physicochemical properties and drug-likeness properties

In the development of drugs intended for oral use, good drug absorption and appropriate drug delivery are very important issues.[33] Poor pharmacokinetics is responsible for the failure of 30 % of oral drugs.[34] Thus, *in silico* prediction of oral bioavailability is very important in the early stages of drug discovery in order to select the most promising compounds for further optimization. Moreover, its importance in the latter stages is to identify candidates for further clinical development.[35] Predictions of physicochemical properties and drug-likeness scores for the investigated compounds are presented in (Table 4). Candidate drugs that conform to the Lipinski's "rule of five""Ro5" tend to have lower attrition rates during clinical trials and hence

have an increased chance of reaching the market.[22-24] The rule states that those most "drug-like" molecules must have log P (logarithm of the octanol/water partition coefficient) ≤ 5, molecular weight ≤ 500 Daltons, number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10. Molecules violating more than one of these rules may have problems with oral bioavailability. The rule is called "Rule of 5", because the border values are 5, 500, 5, and 2X5. Veber et al. offered further modifications in Ro5.[36] According to Veber, it is revealed that for passing oral bioavailability criteria, the number of rotatable bond (nrotb) should be ≤10. This number (nrotb) is important for conformational changes and ultimately for binding with the amino acids in the active site of the targets.[22] Molecular or topological polar surface area TPSA is a very useful parameter for the prediction of drug transport properties. TPSA values should be ≤ 140 $Å^2$ for prevailing drug absorption through intestine and ≤ 90 $Å^2$ for molecule to penetrate the blood brain barrier (standard values).[37,38] The physicochemical properties of the tested compounds were calculated using MolSoft software[39,40] and all compounds under investigation showed good permeability and absorption. They possessed 1-2 hydrogen bond donors and 3-5 hydrogen bond acceptors (Table 4). In addition, all the compounds were showed good permeability through cell membranes (log P ranges from 2.12 – 4.81). Furthermore, the titled compounds in general had 3-4 rotatable bonds, so they fulfilled the criterion and exhibited moderate conformational flexibility for binding with both enzymes except compound **IVa** which showed some rigidity (nrobt = 1). In addition, compound **IVa** obtained relatively higher molecular weight, PSA and molecular volume MV, this made compound **IVa** approximately more rigid and affected ultimately its binding with the target and subsequently it might shift to an allosteric site. Moreover, all the tested compounds had zero violation and obeyed Lipinski's rule. Besides, the compounds had PSA values within acceptable range 78.50 – 92.78. Therefore, all the compounds obtained the ability to penetrate the blood brain barrier (except compound **VIIId,** TPSA > 90 $Å^2$)

and facilitate drug absorption through intestine. Accordingly, they are considered as drug delivery candidates. PSA were used to calculate the percentage of absorption (ABS%) using the following equation: ABS% = 109 – 0.345 PSA.[41] Generally, the tested compounds demonstrated the (ABS%) range of 76.99 – 81.91 which indicated their good oral bioavailability. Furthermore, the tested compounds were found to fulfil the requirements of solubility of more than 0.0001mg/L[42] and could be considered as drug candidates for oral absorption. Compound **VIId** and its analogues **VIIIa** exhibited higher solubility characters in comparison to compound **IVa**. The drug-likeness score (Table 4) is a combined effect of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound and is represented by a numerical value. Drug-likeness model score of compound **VIIIa** was 0.41 (Figure 8). Compounds that show positive values should be considered as drug-like candidates. The green color means non-drug like behavior and those fall under blue color area are considered as drug-like. Those compounds having negative or zero value should not be considered as drug like. Compound **VIIIa** showed positive value so it should be considered as drug-like.

Furthermore, the higher PSA for compound **VIId** was 92.78 Å versus 83.55 Å for compounds **IVa** and **VIIIa** together with the relative increase in the number of rotatable bond (nrotb =3) (Table 4) might increase its binding affinity towards the active site of ALR1. Finally, the investigated compounds showed reasonable drug-likeness scores and physicochemical characters along with non-violation of Lipinski's rule of five and acceptable pharmacokinetics (permeability, intestinal absorption). Accordingly, these compounds are suitable to be drug-like candidates.

Compounds	MWt[a] (D)	MV[a] (A^3)	Log p[a]	HBA[b] (A^o)	HBD[b] (A^o)	nrotb[a]	PSA[a] (A^2)	ABS %	Solubility[b] mg/L	nviolations[a]	Drug-likeness score[b]
IVa	430.89	320.51	4.02	5	1	1	83.55	80.17	0.01	0.0	-0.81
VIId	338.38	283.00	2.12	5	1	3	92.78	76.99	35.05	0.0	0.28
VIIIa	348.83	260.70	2.81	5	1	2	83.55	80.17	19.40	0.0	0.41
Sorbinil	236.20	189.16	0.86	3	2	0	67.43	85.73	140.11	0.0	-0.09

Table 4. Molecular properties and drug-likeness.

MWt: Molecular weight,

MV: Molecular Volume,

Log p: Log partition coefficient,

HBA: Hydrogen Bond Acceptor,

HBD: Hydrogen Bond Donor,

nrotb: number of rotatable bond,

PAS: Polar Surface Area

ABS%: Percentage of absorption

nviolations: number of violations

a: Values calculated using molinspiration 2016.10

b: values calculated using MolSoft

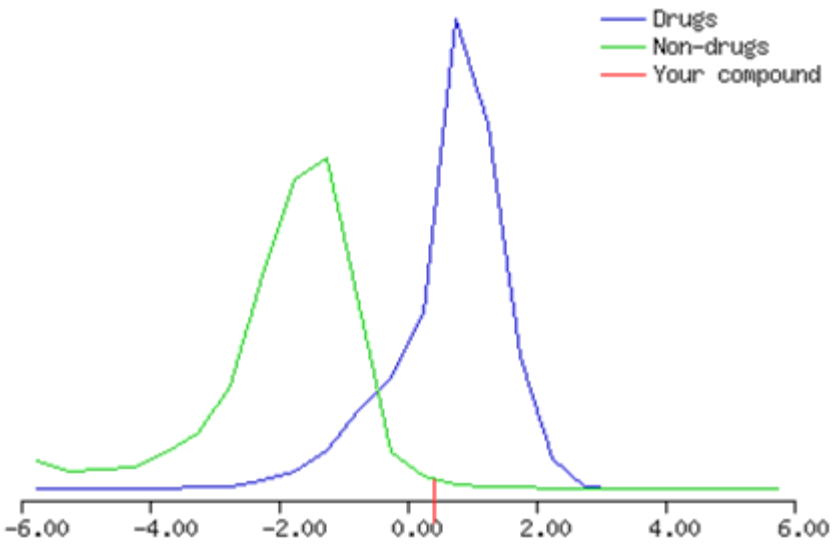


Figure 8. Drug-likeness plot of compound **VIIIa** (MolSoft). The green color means non-drug like behavior and those fall under blue color area are considered as drug-like. Those compounds having negative or zero value should not be considered as drug like. Drug-likeness model score of compound **VIIIa** was 0.41. It showed positive value so it should be considered as drug-like.

3. CONCLUSION

Based on the scaffold of the previously reported aldose reductase inhibitors with hydantoin core, a novel series of sulfonylureas were designed and tested for both ALR2 inhibition and hypoglycemic activity. Spiroimidazolidine-2,4-dione derivatives preserve all the minimum requirements of the proposed pharmacophoric models to enhance both activities. All compounds (except **VIId**) reduced the blood glucose level and all inhibited aldose reductase activity. Biological studies have led to the identification of a novel molecule with a remarkable selectivity towards ALR2 versus ALR1. The investigation showed that the most active compound is **VIIIa** with IC_{50} 0.47 μM with selectivity index 127-fold. Docking studies gave a partial explanation for

the binding mode of these novel compounds in both ALR1 and ALR2 active sites. The biological and computational data provided valuable information for the further development of this very promising lead compound.

4. EXPERIMENTAL

4.1. Chemistry

The commercial chemicals and solvents were reagent grade and used without further purification. ^{1}H- and ^{13}C-NMR Spectra were measured on a Varian Mercury VX-300 NMR spectrometer or Jeol LA (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR). Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Electron impact mass spectra were recorded on Shimadzu GCMS-QP 5050A gas chromatograph mass spectrometer (70 eV). Elemental analysis was performed at the Microanalytical Center of Cairo University, Egypt. Melting points of the compounds were determined in open capillaries using Gallenkemp melting point apparatus and are uncorrected. All the reactions were monitored through thin layer chromatography (TLC) using Merck 0.2 mm pre-coated DF-Aluminium sheets $60F_{254}$. IR spectra were recorded on a Shimadzu FT-IR 8101 PC IR spectrophotometer (KBr pellets). Values were represented in cm^{-1}. Compounds **II** and **VI** were prepared as previously described.[5,25]

4.1.1. General method for the preparation of 3'-aryl sulfonyl derivatives of spiro[fluorene-9, 5'-imidazolidine]-2',4'-dione (IIIa and IIIb)

Arylsulfonyl chloride (5.8 mmol) was added dropwise to a stirred mixture of compound **II** (4.8 mmol), triethylamine (4.8 mmol) and catalytic amount of DMAP in DCM (10 mL), and stirred for 15 h. The reaction mixture was neutralized with 1N aquous HCl and extracted with dichloromethane (4 x 25 mL). After evaporation of the solvent, the crude product was

recrystallized from ethanol-water mixture in order to obtain yellowish white semi-solid. compounds **IIIa** and **IIIb**.[26]

3'(5-Chlorothiophen-2-ylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (IIIa)

Yield 71 %; R_f = 0.35 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3255, 1643, 1562, 1352, 1168. EI-MS (m/z, %): 430 $[M]^+$, 432, 206 (100), 151. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 7.98-7.15 (m, 8H, H-1-8), 7.04 (d, J = 8Hz, 1H, H-18), 6.98 (d, J = 8 Hz, 1H, H-19), 5.71 (s, 1H, H-10); ^{13}C-NMR (DMSO-d_6): δ 76.4, 120.5, 123.4, 129.6, 131.1, 132.6, 133.5, 137.6, 141.5, 143.4, 145.1, 152.5, 169.7. Anal.Calcd. for $C_{19}H_{11}ClN_2O_4S_2$: C, 52.96; H, 2.57; N, 6.50. Found: C, 52.77; H, 2.63; N, 6.31.

3'(p-Fluorophenylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (IIIb)

Yield 69 %; R_f = 0.33 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3249, 1659, 1593, 1333, 1161. EI-MS (m/z, %): 408 $[M]^+$, 410, 206 (100), 151. ^{1}H-NMR (400MHz) (DMSO-d_6); δ 8.11-7.96 (m, 4H, H-17,18,20,21), 7.96-7.27 (m, 8H, H-1-8), 5.79 (s, 1H, H-10); ^{13}C-NMR (DMSO-d_6): δ 76.2, 120.7, 123.5, 127.1, 129.4, 131.7, 133.3, 138.4, 140.2, 141.2, 143.6, 150.8, 169.4. Anal.Calcd. for $C_{21}H_{13}FN_2O_4S$: C, 61.76; H, 3.21; N, 6.86. Found: C, 61.48; H, 3.36; N, 6.53.

4.1.2. General method for the preparation of 1'-aryl sulfonyl derivatives of spiro[fluorene-9, 5'-imidazolidine]-2',4'-dione (IVa and IVb)

Compounds **IIIa** and **IIIb** (1 mmol) were dissolved in dry benzene (15 mL) and sodium hydride (1.2 mmol) was added. The reaction mixture was refluxed under nitrogen for 2 h. The solvent was evaporated and petroleum ether was added. The precipitated sodium salt was collected by filtration and dissolved in water. The media was neutralized (PH 7) with 1 N aqueous HCl and extracted with ethyl acetate (3 x 25 mL). After evaporation of the solvent, the

rearranged product was recrystallized from ethyl acetate to obtain white crystalline solid compounds **IVa** and **IVb**.[27]

1'(5-Chlorothiophen-2-ylsulfonyl) spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (IVa)

Yield 73 %; m.p. (246-248) °C; R_f = 0.41 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3275, 1654, 1600, 1331, 1148. EI-MS (m/z, %): 430 $[M]^+$, 431, 206 (100), 151. ^{1}H-NMR (400MHz)(DMSO-d_6); δ 12.16 (s, 1H, H-12), 7.93- 7.29 (m, 8H, H-1-8), 6.85-6.88 (m, 2H, H-18,19). Anal.Calcd.for $C_{19}H_{11}ClN_2O_4S_2$: C, 52.96; H, 2.57 ; N, 6.50. Found: C, 53.28; H, 2.29 ; N, 6.79.

1'(p-Fluorophenylsulfonyl)spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (IVb)

Yield 75 %; m.p. (264-266) °C; R_f = 0.44 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm-1): 3277, 1651, 1523, 1344, 1152. EI-MS (m/z, %): 408 $[M]^+$, 410, 206 (100), 151. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 12.51 (s, 1H, H-12), 8.20-8.05 (m, 4H, H-17,18,20,21), 7.96-7.21 (m, 8H, H-1-8); ^{13}C-NMR (DMSO-d_6): δ 76.0, 120.9, 123.7, 127.3, 129.2, 131.5, 133.1, 138.2, 140.0, 141.0, 143.8, 150.5, 169.8. Anal.Calcd. for $C_{21}H_{13}FN_2O_4S$: C, 61.76; H, 3.21; N, 6.86. Found C, 61.96; H, 3.44; N, 6.66.

4.1.3. General method for the synthesis of 3'-Arylsulfonyl derivatives of spiro(cyclohexane- 1,5'-imidazolidine)-2',4'-dione (VIIa–d)

Compounds **VIIa–d** were synthesized according to the same procedures used for the synthesis of compounds **IIIa** and **IIIb**.[26]

3'-(5-Chlorothiophene-2-ylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIa)

Yield 76 %; R_f = 0.35 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm-1): 3390, 2911, 1653, 1566, 1327, 1155. EI-MS (m/z, %) 348 $[M]^+$, 350, 341, 231 (100), 167, 97. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 6.71 (d, J = 7.6 Hz, 1H, H-15), 6.68 (d, J = 7.6 Hz, 1H, H-16), 5.70 (s, 1H, H-7),

1.94-1.29 (m, 10H, H-2-6). Anal.Calcd. for $C_{12}H_{13}ClN_2O_4S_2$: C, 41.32; H, 3.76; N, 8.03. Found: C, 41.64; H, 3.42; N, 8.36.

3'-(p-Fluorophenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIb)

Yield 69 % ; m.p. (173-175)°C; R_f = 0.32 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3437, 2871, 1651, 1589; 1347, 1161. EI-MS (m/z, %): 326 $[M]^+$, 231 (100), 167, 97. ^{1}H-NMR (400MHz) (DMSO-d_6); δ 7.96-7.78 (m, 4H, H-14,15,17,18), 5.90 (s, 1H, H-7), 1.89-1.22 (m, 10H, H-2-6). Anal.Calcd. for $C_{14}H_{15}FN_2O_4S$: C, 51.53; H, 4.63; N, 8.58. Found: C, 51.91; H, 4.27; N, 8.79.

3'-(p-Methylphenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIc)

Yield 68 %; m.p. (107-109) °C; R_f = 0.37 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3387, 2945, 1651, 1597, 1344, 1159. EI-MS (m/z, %): 322 $[M]^+$, 293, 248, 231 (100), 167, 97. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 7.72 (d, J = 8.1 Hz, 2H, H-14,18), 7.50 (d, J = 8.1 Hz, 2H, H-15,17), 6.00 (s, 1H, H-7), 2.30 (s, 3H, CH_3), 2.08-1.44 (m, 10H, H-2-6). Anal.Calcd. for $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69. Found C, 55.49; H, 5.31; N, 8.41.

3'-(p-Methoxyphenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIId)

Yield 70 %; m.p. (122-124) °C; R_f = 0.31 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3377, 1653, 1537, 2923 1337, 1171. EI-MS (m/z, %): 338 $[M]^+$, 256, 231 (100), 167, 97. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 7.72 (d, J = 7.9 Hz, 2H, H-14,18), 7.49 (d, J = 7.9 Hz, 2H, H-15,17), 6.00 (s, 1H, H-7), 3.90 (s, 3H, OCH_3), 1.84-1.16 (m, 10H, H-2-6). Anal.Calcd. for $C_{15}H_{18}N_2O_5S$: C, 53.24**;** H, 5.36; N, 8.28. Found C, 53.13; H, 5.56; N, 8.13.

4.1.4. General method for the synthesis of 1'-Arylsulfonyl derivatives of spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIIa–d)

A rearrangement step was performed through the same procedure used for the preparation of compounds **IVa** and **IVb**.[27]

1'-(5-Chlorothiophene-2-ylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIIa)

Yield 80 %; m.p. (170-172) °C. R_f = 0.41 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm-1): 3315, 1649, 1554, 2943, 1328, 1163. EI-MS (m/z, %): 348 $[M]^+$, 349, 342, 231(100), 167,97. ^{1}H-NMR (400MHz, DMSO-d_6): δ12.38 (s,1H, H-9), 6.57 (d, J = 8 Hz, 1H, H-15), 6.41 (d, J = 8 Hz, 1H, H-16), 2.00-1.36 (m, 10H, H-2-6); ^{13}C-NMR (DMSO-d_6): δ25.4, 27.1, 29.8, 76.7, 131.1, 132.9, 137.8, 145.3, 151.7, 170.1. Anal.Calcd. for $C_{12}H_{13}ClN_2O_4S_2$: C, 41.32; H, 3.76; N, 8.03. Found: C, 41.12; H, 3.96; N, 8.28.

1'-(p-Fluorophenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'- dione (VIIIb)

Yield 73 %; m.p. (141-143) °C; R_f = 0.44 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3410, 2879, 1655, 1566, 1341, 1170. EI-MS (m/z, %): 326 $[M]^+$, 328, 328, 231(100), 167, 97. ^{1}H-NMR (400MHz, DMSO-d_6): δ12.30 (s, 1H, H-9), 7.79-7.58 (m, 4H, H-14,15,17,18), 1.99 (m, 10H, H-2-6); ^{13}C-NMR (DMSO-d_6): δ25.7, 27.5, 29.8, 76.4, 127.5, 131.7, 138.5.4, 140.6, 151.6, 169.8. Anal.Calcd. for $C_{14}H_{15}FN_2O_4S$: C, 51.53; H, 4.63; N, 8.58. Found: C, 51.79; H, 4.89; N, 8.22.

1'-(p-Methylphenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIIc)

Yield 66 %; m.p. (116-118) °C; R_f = 0.45 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3355, 2956, 1649, 1559, 1331, 1147. EI-MS (m/z, %): 322 $[M]^+$, 291, 248, 231(100), 167, 97. ^{1}H-NMR (400MHz, DMSO-d_6): δ12.70 (s, 1H, H-9), 7.69 (d, J = 7.9 Hz, 2H, H-14,18), 7.40 (d, J = 7.9 Hz, 2H, H-15,17), 2.19 (s, 3H, CH_3), 2.00-1.31 (m, 10H, H-2-6); ^{13}C-NMR (DMSO-d_6): δ 21.3, 25.1, 27.6, 29.3, 76.4, 127.2, 131.3, 135.6, 141.4, 151.2, 169.3. Anal. Calcd. for $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69. Found: C, 56.13; H, 5.93; N, 8.34.

1'-(p-Methoxyphenylsulfonyl)spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione (VIIId)

Yield 65 %; m.p. (133-135) °C; R_f = 0.35 (petroleum ether/ethyl acetate, 6/4); IR (KBr, cm^{-1}): 3324, 2897, 1650, 1580, 1345, 1157. EI-MS (m/z, %): 338 $[M]^+$, 256, 231(100), 167, 96. ^{1}H-NMR (400MHz, DMSO-d_6): δ12.41 (s, 1H, H-9), 7.69 (d, J = 7.7 Hz, 2H, H-14,18), 7.40 (d, J = 7.7 Hz, 2H, H-15,17), 3.80 (s, 3H, OCH_3), 1.78-1.14 (m, 10H, H-2-6). Anal. Calcd. for $C_{15}H_{18}N_2O_5S$: C, 53.24; H, 5.36; N, 8.28. Found: C, 53.41; H, 5.60; N, 8.33.

4.2. Biological evaluation

4.2.1. Inhibition assay of aldose and aldehyde reductase *in vitro*

The activity of ALR1 was determined through a reaction mixture containing 0.12 mμ of NADPH, sodium phosphate buffer (0.1 μ, pH 7.2), 0.25 ml of sodium D-glucoronate as a substrate (20 mμ) as a substrate, 20 μL of the test compound and enzyme preparation (0.4 mL) in a total volume of 1 mL. This activity was performed at 37 °C. The reaction mixture was incubated for 10 min before the addition of the substrate.

ALR2 activity was estimated in a reaction mixture containing 0.1 mμ of NADPH, sodium phosphate buffer (0.1 μ, pH 6.2), 90 μL of DL-glyceraldehyde (10 mμ) as a substrate, 20 μL of the test compound and 0.4 mL enzyme preparation in a total volume of 1 mL. This activity was performed at 33 °C. The reaction mixture was incubated for 10 min before the addition of the substrate. Both ALR1 and ALR2 were obtained from BioVision, Incorporated. [43]

The inhibition of ALR2 was examined by measuring decrease in the UV absorption by NADPH at 340 nm using UV spectrophotometer. Sorbinil and valproic acid were used as positive control for ALR2 and ALR1, respectively.[28] One unit of enzyme was defined as the amount of enzyme required to catalyze the oxidation of 1.0 mM NADPH under controlled conditions. Appropriate blanks were utilized.[44]

4.2.2. Experimental animals

The experiment was carried out on BALB/c mice (3-4 weeks old) weighing 20-37 g with a mean weight of 28.5 g. (60 mice were used and divided into twelve groups (n = 5)). The mice were kept at a temperature of 25 °C with 12 h light/12 h darkness photoperiods and fed on rodent diet. The experimental protocols in this work were approved by the Ethics Committee of the Suez Canal University.

4.2.3. Induction of diabetes

The mice were made diabetic by injecting a single intra-peritoneal dose of alloxan monohydrate, 150 mg/kg body weight, dissolved in sterile normal saline (15 %). After a fortnight, mice with hyperglycemia with blood glucose range of 200-460 mg/dl were used for this study. Blood samples were collected by tail vein puncture.

4.2.4. Glucose determination

After the application of the test compounds and the standard drug glibenclamide (10 mg/kg body weight in distilled H_2O) [5,25], the blood samples were collected at 0 h followed by 2, 4, 6 and 8 h. The blood glucose level was measured using a hand-held glucometer (Accu-Check Active, Roche).

4.3. Molecular docking study

Docking simulation study has been performed using Molecular Operating Environment (MOE®, 8) version 2016.[45] The computational software was operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory.

4.3.1. Target compounds optimization

The target compounds were constructed into a 3D model using the builder interface of the MOE program. After checking their structures and the formal charges on atoms by 2D depiction, the target compounds were subjected to a conformational search. All conformers were subjected to energy minimization. All the minimizations were performed with MOE until a RMSD gradient of 0.82806 Kcal/mole (FID) and 1.52747 Kcal/mole (37V) and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated. The obtained database was then saved as MDB (Molecular Data Base) file to be used in the docking calculations.

4.3.2. Optimization of the enzymes active site

The X-ray crystallographic structures of aldehyde reductase ALR1 (Code: 3H4G) co crystalized with native ligand (FID) and aldose reductase ALR2 (Code: 4QR6) co crystalized with native target (37V) were obtained from the RCSB-Protein data bank. The enzyme was prepared for docking studies by MOE/compute/prepare/quick prepare. Docking of the selected conformer in the prepared database was performed using MOE-Dock software. The enzyme active site file was loaded and the dock tool was initiated. The parameters were adjusted to dummy atoms as the docking site and Triangle matcher as the placement methodology. London dG as scoring parameter was used and adjusted to its default value. The MDB file of the docked ligand was loaded and dock calculations were run automatically. The obtained poses were studied and the poses showing best ligand-enzyme interactions were selected and stored for energy calculations.[45]

4.4. Bioinformatics study

In the present investigation, the newly synthesized compounds were subjected to in *silico* molecular properties and bioactivity prediction by Molinspiration online property calculation toolkit.[39] Drug—likeness and solubility parameters were calculated by MolSoft software [40] to filter and analyze their overall ability to be qualified as a potential drug, also to compare these properties with antidiabetic reference drug sorbinil.

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Highlights

- Sulfonylureas derivatives were evaluated as hypoglycemic agents and aldose reductase inhibitors.
- Compounds were tested against both ALR1 and ALR2.
- Compounds displayed antidiabetic activity.
- Compounds had inhibitory activities against both targets with higher selectivity towards ALR2.
- The most active compound (VIIIa) could be considered a potent antidiabetic agent with minimal side effects.

GRAPHICAL ABSTRACT