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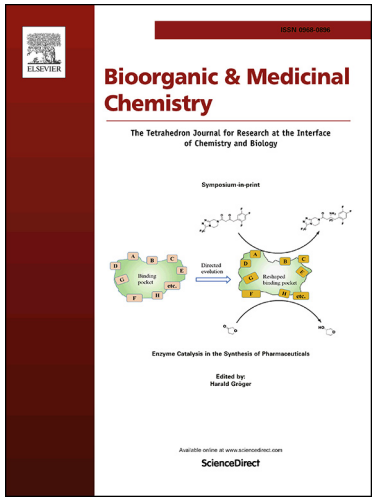

Synthesis and molecular modeling of novel non-sulfonylureas as hypoglycemic agents and selective ALR2 inhibitors

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Synthesis and molecular modeling of novel non-sulfonylureas as hypoglycemic agents and selective ALR2 inhibitors

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Abstract

Novel non-sulfonylureas derivatives bearing an acetamide linker between a spirohydantoin scaffold and a phenyl ring were prepared and their hypoglycemic activity was estimated *in vivo*. Their abilities to discriminate *in vitro* between aldehyde reductase (ALR1) and aldose reductase (ALR2) were determined. The molecular docking and the *in silico* prediction studies were performed to rationalize the obtained biological results and to predict the physicochemical properties and drug-likeness scores of the new compounds. *N*-(2,4-Dichlorophenyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (**3e**) displayed an 84% reduction in blood glucose level superior to that of repaglinide 66% and showed an IC_{50} value of 0.37 μM against ALR2 that is superior to that of sorbinil 3.14 μM. Compound (**3e**) was selective 96 fold towards ALR2 which is closely related to serious diabetic complications. Based on the identification of this hit candidate, a new generation of safe and effective antidiabetic agents could be designed.

1. Introduction

Meglitinides are non-sulfonylureas antidiabetic agents that help the pancreatic beta cells to release insulin properly.[1,2] One major example of these agents is repaglinide, which is mainly metabolized in the liver rather than the kidney. Therefore, repaglinide can be used safely in the treatment of patients with renal insufficiency.[1-3] Repaglinide is characterized by a rapid onset of action, a short duration of action and a low risk of hypoglycemia.[4,5] It binds to site B of sulfonylurea receptor 1 (SUR1) in the pancreatic beta cells (**Figure 1**). On the other hand, sulfonylureas, such as tolazamide, are known to bind to site A (**Figure 1**). They are actually believed to develop hypoglycemia and to accelerate pancreatic beta cells exhaustion and apoptosis.[6] Thus, non-sulfonylureas have many advantages superior to sulfonylureas antidiabetic agents. It is well known that the minimum pharmacophoric requirements for enhancing the antidiabetic activity of non-sulfonylureas include the presence of an amide bridge in between two lipophilic moieties (**Figure 2**).[7]

Unfortunately, despite of the availability of many sophisticated antidiabetic agents, the patients still suffer from serious complications that are responsible for a high rate of mortality.[8] These complications are hypoglycemia, hyperglycemic crises, diabetes ketoacidosis (DKA) and hyperglycemic hyperosmolar state (HHS).[9] It is worth to mention that ALR2 is responsible for these diabetic complications while ALR1 is responsible for an essential detoxification mechanism in liver.[10,11] Aldose reductase ALR2 inhibitors are known to overcome these complications.[12] Therefore, selective inhibition of ALR2 is essential. A large number of structurally diverse compounds have been observed to inhibit ALR2, such as sorbinil (**Figure 2**) [9]. All ALR2 inhibitors interact at a common site, known as 'inhibitor site'.[13] Most notably, the hydantoin ring of sorbinil is responsible for essential hydrogen bonding interactions with the key

amino acids in ALR2 active site (**Figure 2**).[14] Kador and coauthors proposed that the minimum requirements for enhancing the selective inhibition of ALR2 include a primary lipophilic region and a carbonyl group.[14,15] In addition, the introduction of a secondary lipophilic moiety coplanar with the primary lipophilic one enhances binding with ALR2 specificity pocket (**Figure 2**). We previously published the synthesis, ALR2 inhibition as well as hypoglycemic activity of sulfonylureas derivatives.[16] In the present study, our tactic is to synthesize a new generation of non-sulfonylureas analogues keeping their advantages as hypoglycemic agents and to modulate their aldose reductase inhibitory activity to overcome the diabetic complications. The design of our target molecules is based on the presence of an acetamide linker between a spirohydantoin scaffold and a phenyl ring (**Figure 2**). These molecules are expected to fulfill all the minimum requirements for enhancing both hypoglycemic activity and selective ALR2 inhibition according to the mixed pharmacophore theory.[15,17,18] In attempt to improve the physicochemical properties of our target molecules, two diversity points were generated (**Figure 2**). The first point was the incorporation of various substituents on the phenyl ring. The second point was based on modulating the lipophilic ring attached to the spirohydantoin scaffold.

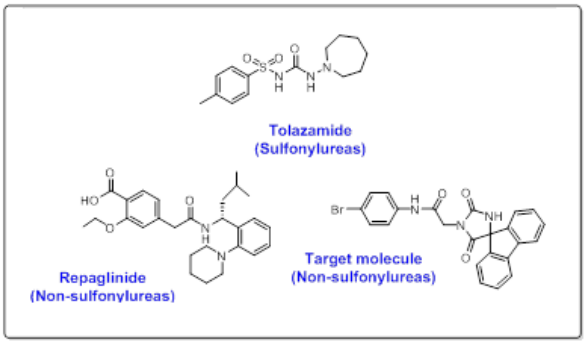


Figure 1. The structures of sulfonylurea and non-sulfonylurea derivatives.

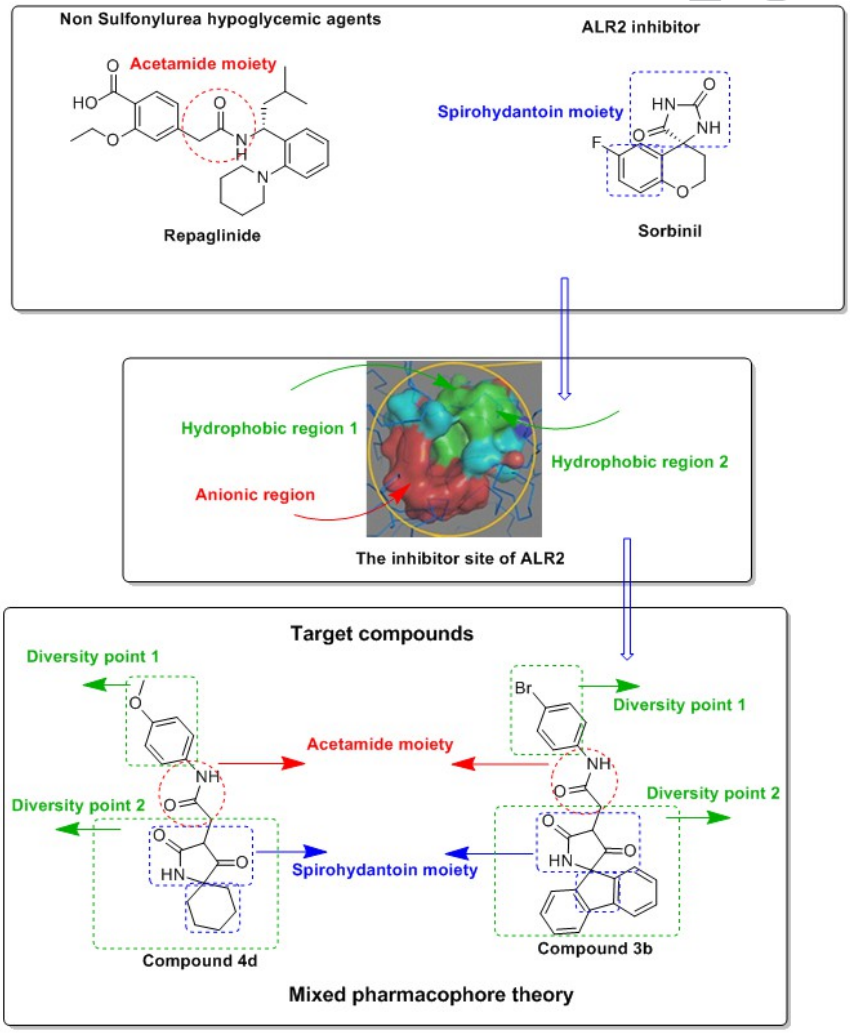


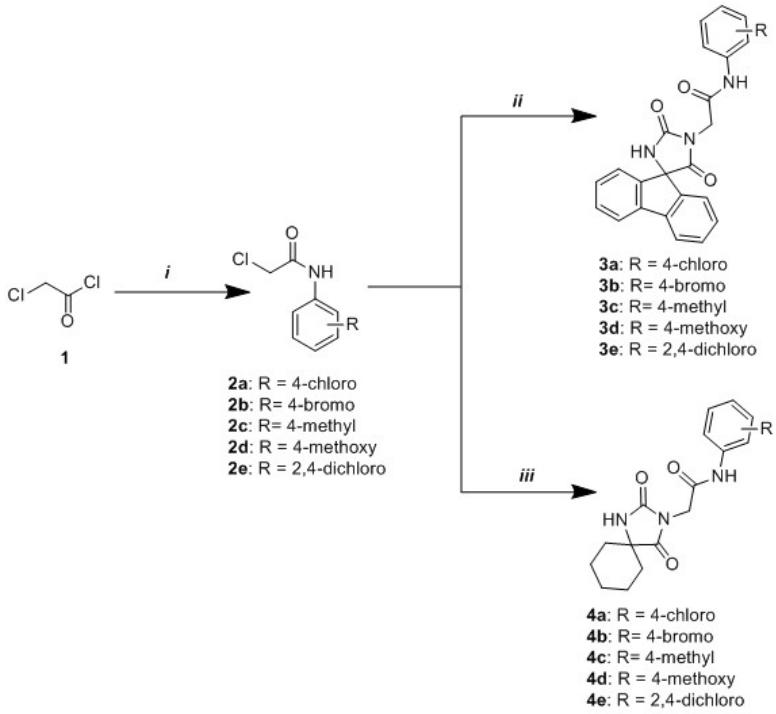
Figure 2. The design of novel non-sulfonylurea derivatives as hypoglycemic agents and aldose reductase inhibitors.

2. Results and discussion

2.1. Chemistry

The synthesis of different acetamide derivatives is shown in **scheme 1**. 2-Chloro-N-phenylacetamide derivatives (**2a-e**) were prepared through reaction of the appropriate aniline (4-chloro, 4-bromo, 4-methyl, 4-methoxy or 2,4-dichloroaniline) with chloroacetyl chloride (**1**) in glacial acetic acid.[19,20] Spiro-imidazolidinedione derivatives (spirohydantoin or cyclohexanonyl moieties) were prepared as previously reported.[15,21] Reaction of (**2a-e**) with spiro-imidazolidinediones in the presence of sodium bicarbonate ($NaHCO_3$) and sodium iodide (NaI) resulted in the formation of the newly synthesized target compounds (**3a-e**) and (**4a-e**).[22]

The structures of the synthesized compounds (**3a-e**) and (**4a-e**) were approved with elemental analyses and spectral data (IR, ^{1}H-NMR,^{13}C-NMR and EI-MS). IR spectra showed collectively secondary amine signals at 3200-3400 cm^{-1} that indicated the coupling step. Besides, the appearance of (C-H sp^3) signals at around 2850-3000 cm^{-1}. In the ^{1}H NMR spectra of these compounds, the appearance of one upfield NH signal in the range the range 5.70-6.00 ppm confirmed the nucleophilic substitution reaction that occurred at the desheilded NH of spiroimidazolidine-2,4-dione nucleus. Another singlet signal appeared downfield at 9.76-10.10 that referred to NH group of the secondary aromatic amine. Moreover, a characteristic singlet peak for CH_2 protons appeared at 3.82-4.25 ppm. The aromatic protons of the fluorenonyl ring were displayed downfield as multiplet signals at 7.15-8.08 ppm. The aliphatic protons of the cyclohexanonyl ring were displayed upfield as multiplet signals at 1.39-2.08 ppm. The ^{13}C-NMR spectra showed signals for fluorenonyl, cyclohexanyl, imidazolidinedionyl, phenyl rings as well as acetamide moiety at the expected regions.



Scheme 1. Synthesis of acetamide derivatives of spiro-imidazolidinediones. Reagents and conditions: (*i*) aniline derivatives (4-chloro, 4-bromo, 4-methyl, 4-methoxy or 2,4-dichloroaniline), glacial acetic acid, 100 °C; (*ii*) $NaHCO_3$, NaI and spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (*iii*) $NaHCO_3$, NaI and spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione.

2.2. Biological activity

The antidiabetic activities of the newly synthesized compounds were tested *in vivo*. Moreover, their inhibitory activities against aldehyde reductase (ALR1, EC 1.1.1.2) and aldose reductase (ALR2, EC 1.1.1.21) were evaluated *in vitro*. The selectivity indexes of the new compounds were determined.

2.2.1. *In vivo* hypoglycemic activity

Diabetic BALB/c mice were used to perform *in vivo* study. The blood glucose levels were measured by collecting the blood samples at 0 h followed by 2, 4, 6 and 8 h.[15] Most of the new compounds were found to be active hypoglycemic agents (**Table 1**). Compounds (**3e** and **4b**) were the most active compounds with 84.3 and 85.1% reduction in blood glucose, respectively. They were more potent than the standard drug repaglinide with 66.1% reduction. Both compounds have a halogen atom (Cl or Br) at the phenyl ring. It was observed that the presence of two halogen atoms at the *ortho* and *para* positions on the phenyl ring increases the activity of the compound. Compounds (**3a**, **3b** and **4b**) possess moderate activities (80.4%, 81.1% and 79.2% reduction, respectively) but they are still more potent than the standard drug repaglinide. On the other hand, compound (**3d**) did not lower the plasma glucose level.

Comp.	Blood glucose concentration ± SEM[a] (mg/dl) basal	Blood glucose concentration ± SEM[a] (mg/dl) After 2h	Blood glucose concentration ± SEM[a] (mg/dl) After 4h	Blood glucose concentration ± SEM[a] (mg/dl) After 6h	Blood glucose concentration ± SEM[a] (mg/dl) After 8h	Inhibition after 8 h (%)
3a	460 ± 3.56	350 ± 5.53	234 ± 4.76	189 ± 6.78	90 ± 2.87	80.4
3b	452 ± 4.87	331 ± 4.52	218 ± 3.82	173 ± 6.33	85 ± 2.87	81.1
3c	358 ± 6.98	314 ±6.84	283 ± 7.11	211 ± 4.71	167 ± 5.45	53.4
3d	320 ± 5.67	258 ± 3.98	237 ± 5.54	279 ± 5.67	370 ± 3.91	≥ 200
3e	398 ± 3.81	290 ± 5.67	223 ± 4.61	168 ± 4.89	63 ± 3.45	84.3
4a	406 ± 5.98	347 ± 7.62	242 ± 6.56	170 ± 2.75	85 ± 5.63	79.2
4b	459 ± 3.88	386 ± 7.01	276 ± 4.54	182 ± 7.22	69 ± 4.77	85.1
4c	387 ± 7.43	305 ± 5.74	268 ± 7.71	197 ± 7.21	115 ± 5.67	70.3
4d	370 ± 2.94	322 ± 4.88	299 ± 6.53	231 ± 5.74	163 ± 6.63	56.1
4e	384 ± 7.71	319 ± 7.43	269 ± 5.44	217 ± 6.32	121 ± 4.42	68.5
Repaglinide	392 ± 6.87	313 ± 5.69	259 ± 3.81	160 ± 4.22	94 ± 1.76	66.1

a: number of trials = 3

Table 1. Blood glucose concentration (mg/dl) in diabetic mice treated with the new compounds.

2.2.2. ALR1 and ALR2 activity

The decrease in the UV absorption of NADPH at 340 nm - using UV spectrophotometer - was used as indication of the inhibitory activities of the new compounds.[23] Valproic acid was used as a positive control to evaluate ALR1 inhibitory activity.[15] On the other side, sorbinil was the positive control in case of ALR2 inhibitory activity.[24]

Compounds (**3e** and **4b**) (with IC_{50} ± SEM values of 0.37 ± 0.05 μM and 0.72 ± 0.06 μM, respectively), were found more potent inhibitors for ALR2 than the standard inhibitor, sorbinil

with IC_{50} value 3.14 ± 0.02 μM.[25] This may be assigned to the presence of chlorine atom at the *ortho* and *para* positions of the phenyl ring of compound (**3e**) and bromo atom at the *para* position of the phenyl ring of compound (**4b**). These small lipophilic halogen atoms are expected to enhance the binding character of these compounds. The presence of *para* methoxy group in compounds (**3d** and **4d**) instead, resulted in a remarkable decrease in the activity. Moreover, the new compounds exhibited moderate inhibitory activities against ALR1 and were more potent than valproic acid with IC_{50} value 57.40 ± 0.89 μM.[23] This may be due to their relative lipophilic structures compared to valproic acid that contains 2-propylpentanoic acid. IC_{50} values against ALR1 versus ALR2 was used to calculate the selectivity of the synthesized inhibitors. The selectivity index displayed that compound (**3e**) was 96 fold more selective towards ALR2 versus ALR1. In addition, compound (**4b**) was 43 fold more selective towards ALR2 (**Table 2**).

Comp.	ALR1	ALR2	S.E [a] IC_{50}[ALR1]/ IC_{50}[ALR2]
IC_{50} ± SEM (μM)[b]/(% Inhibition)[c]			
3a	22.2 ± 1.72	1.03 ± 0.33	21.60
3b	30.31 ±1.42	0.93 ± 0.03	32.60
3c	10.92 ±1.87	6.21 ± 0.77	1.76
3d	24.25 ±1.03	2.87 ± 0.18	8.45
3e	35.52 ± 1.70	0.37 ± 0.05	96.00
4a	20.67 ±2.51	1.13 ± 0.86	18.30
4b	31.20 ± 0.29	0.72 ± 0.06	43.40
4c	6.43 ± 1.13	10.2 ±2.48	0.63
4d	25.80 ± 0.68	2.76 ± 0.31	9.36
4e	32.90 ± 0.54	1.95 ± 1.06	16.90

sorbinil		3.14 ± 0.02[25]	-
Valproic acid	57.40 ± 0.89[23]		-

a: S.E = Selectivity index

b: number of trials = 3

c: % Inhibition at 0.6 μM inhibitor concentration

Table 2. In vitro ALR1 and ALR2 activities of the new compounds.

2.3. Molecular docking study

The most active compounds (**3e** and **4b**), compounds (**3a** and **4e**) with moderate activities and the least active compounds (**3c** and **4d**) were selected for performing the molecular docking studies. Our aim is to create a correlation between the found *in vitro* biological results and the probable binding modes of these compounds together with the reference drugs repaglinide, sorbinil and the native ligand 37v within the active site of ALR2 (**Figures S1a,b**, **S2a,b**, **S3a,b** and **S4a-c, supplementary information**). The X-ray crystal structure of ALR2 complexed with NADPH (PDB ID, 4QR6-ALR2) co-crystalized with (2-[(1,3-benzothiazol-2-ylmethyl)carbamoyl]-5-fluorophenoxy)acetic acid (37V) was downloaded from the Protein Data Bank (PDB). The molecular docking simulations were performed by MOE dock tool of the modeling software Molecular Operating Environment (MOE2016. 8).[26] The binding affinity between the inhibitor and the enzyme is optimum when the value of the binding score is low.

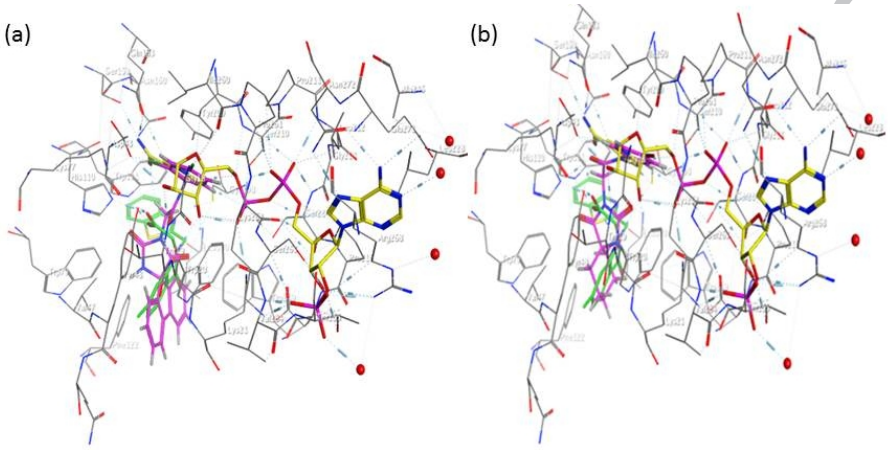
The molecular docking studies revealed that the acetamide linkers of the new compounds were extended plausibly in the active site of ALR2. These linkers orient the phenyl rings deep in the specificity pocket created by TYR 48, HIS 110, TRP 111, TRP 20, PHE 115 and SER 210 for optimum fitting. Moreover, the polar atoms of the hydantoin rings and the acetamide linkers played crucial roles in the creation of 3-6 major hydrogen bonding interactions with the key amino acids CYS 298, TRP 111, HIS 110 and TYR 48 (**Figures S1a,b, S2a,b** and **S3a,b,**

supplementary information). On the other hand, the reference drug repaglinide formed only one hydrogen bond interaction with Cys298 and displayed a relatively high binding score -5.69 (**Figure S4a, supplementary information**). This might be explained by the absence of the hydantoin scaffold in repaglinide structure. Moreover, sorbinil formed only 2 hydrogen bonding interactions with HIS 110 and TYR 48 (**Figure S4b, supplementary information**). It showed high binding score -6.92. This might be attributed to the lack of acetamide linkers in sorbinil structure. The native ligand 37v formed 3 hydrogen bonding interactions with the key amino acids HIS 110, TYR 48 and TRP 111, it displayed a binding score -7.02 (**Figure S4c, supplementary information**). These findings indicate the importance of both of the hydantoin rings and the acetamide linkers in enhancing the inhibitory activity.

The molecular docking of the most active compounds (**3e** and **4b**) with IC_{50} values 0.37 μM and 0.72 μM, respectively, showed optimum binding modes with low binding scores -7.69 and -7.63, respectively (**Figures 3a,b**). The docking studies of the compounds (**3a** and **4e**) with moderate activities and IC_{50} values 1.03 μM and 1.95 μM, respectively, revealed plausible binding modes with binding scores -7.02 and -6.87, respectively (**Figures S2a,b, supplementary information**). It is worth noting that the phenyl rings of all the previous compounds (**3a**, **3e**, **4b** and **4e**) have small lipophilic halogen atoms. While their analogues (**3c** and **4d**) were the least active compounds with IC_{50} values 6.21 μM and 2.76 μM and high binding scores -6.11 and -6.68, respectively (**Figures S3a,b, supplementary information**). This might be attributed to the steric clashes produced by the bulky methyl and methoxy groups of compounds (**3c** and **4d**) respectively.

It was observed that the hydantoin ring of standard ALR2 inhibitor (sorbinil) was superimposed with that of compound (**3e**), with RMSD value 1.5 (**Figure 4**). Moreover, the

acetamide linkers lying between the two lipophilic moieties of standard hypoglycemic agent (repaglinide) and compound (**3e**) were almost aligned, with RMSD value 1.9 (**Figure 4**). Furthermore, the lipophilic rings attached to the spirohydantoin scaffold of compound (**3e**) and sorbinil were aligned with the lipophilic rings of repaglinide (**Figure 4**). Thus, compound (**3e**) is expected to fulfill all the minimum pharmacophoric requirements essential for enhancing both hypoglycemic activity and selective ALR2 inhibition.



Figures 3a,b. 3D Docking the most active compounds (**3e** and **4b**) with target 4QR6 cocrystalized with ligand 37V, compounds (**3e** and **4b**) (**Pink),** ligand 37V **(Green**) and NADP **(yellow).**

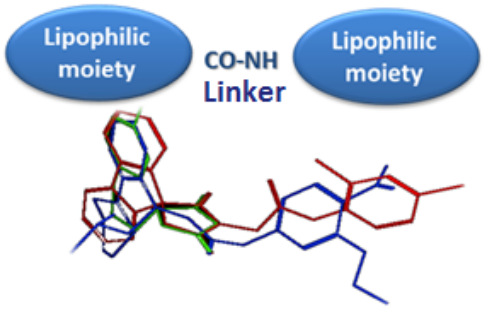


Figure 4. The alignment of the most active compound **3e** (red), sorbinil (green) and repaglinide (blue).

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

The results are presented in (**Table S1, Supplementary information**). Candidate drugs that obey the Lipinski's "rule of five" (Ro5) are expected to be promising future drugs.[27-29] Topological polar surface area TPSA values should be ≤ 140 Å^2 for prevailing drug absorption through intestine and ≤ 90 Å^2 for penetrating the blood brain barrier.[30,31] All the tested compounds showed good permeability and absorption. They possessed 2 hydrogen bond donors and 3-4 hydrogen bond acceptors (**Table S1, Supplementary information**). In addition, all tested compounds showed good permeability through cell membranes (log P ranges from 2.12 – 4.81). Veber and co-authors revealed that for controlling conformational changes and for having good oral bioavailability, the number of rotatable bond (nrotb) should be ≤ 10.[27,32] All the tested compounds had 3-4 nrotb, while repaglinide had 10 nrotb. In addition, drug-likeness scores are presented in (**Table S2, Supplementary information**). Compounds which showed positive values should be considered as drug-like molecules. All the investigated compounds displayed positive values except compounds (**3c** and **4e**) as well as the reference standard sorbinil. Moreover, compounds (**4b**, **4d**, and **3e**) showed higher drug-likeness scores 1.24, 1.01 and 1.07 respectively, than the reference drug repaglinide 0.92 (**Figure S5, Supplementary information**).

3. Experimental

3.1. Materials and methods for synthesis and analytical characterization

The commercial chemicals and solvents for the synthesis were reagent grade and used without further purification. Varian Mercury VX-300 NMR spectrometer or Jeol LA (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR) were used to measure ^{1}H- and ^{13}C-NMR Spectra. Electron impact mass spectra (EI-MS) 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 (m.p.) were determined in open capillaries using Gallenkemp melting point apparatus and are uncorrected. The progress of reactions as well as purity of all compounds were monitored through thin layer chromatography (TLC) using Merck 0.2 mm pre-coated DF-Aluminium sheets $60F_{254}$. Infrared spectra (IR) spectra were recorded on a Shimadzu FT-IR 8101 PC IR spectrophotometer (KBr pellets).

General procedure for the synthesis of 2-chloro-*N*-arylacetamide derivatives (2a-e)

The appropriate aniline (4-chloro, 4-bromo, 4-methyl, 4-methoxy or 2,4-dichloroaniline) (24.4 mmol) was dissolved in glacial acetic acid (15 mL). Chloroacetyl chloride (25.1 mmol) was added dropwise. The reaction mixture was stirred at 100 ºC for 1 h. The mixture was cooled to room temperature. Sodium acetate solution (0.4 M) was added to give a heavy precipitate. The mixture was stirred in an ice bath for 5 min. The precipitate was collected after filtration.[19] Melting points (m.p.) of the synthesized compounds were determined and were comparable with the reported values.[22,33,34]

General procedure for the synthesis of *N*-aryl-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide derivatives (3a-e)

Spiro[fluorene-9,5'-imidazolidine]-2',4'-dione (2.36 mmol) and the appropriate 2-chloro-*N*-arylacetamide derivatives (**2a-e**) (2.36 mmol) were dissolved in DMF (30 mL).[21] $NaHCO_3$ (2.36 mmol) and NaI (2.36 mmol) were added. The reaction mixture was stirred at room temperature for 36 h. The mixture was then added to water (50 mL) to afford the desired product. The precipitate was filtered and left to dry. The solid residue was recrystallized from absolute ethanol to give the corresponding acetamide derivatives.[22]

***N*-(p-Chlorophenyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (3a)**

Yield: 64%. m.p. (273-275) ºC. IR (KBr, cm^{-1}): 3390 (N-H), 1667 (amide & lactam), 1559 (C=C sp^2), 2956 (C-H sp^3). EI-MS (m/z, %): 417 (M^+), 418 (M^++1), 291, 249, 207, 179, 126. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 10.10 (s, 1H, NH-Ph), 8.11 (d, $2H_{20, 22}$ phenyl), 8.09 (d, $2H_{19, 23}$ phenyl), 7.96 (m, 8H, Ar-H), 5.79 (s, 1H, NH), 3.82 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6): δ 49.8, 76.2, 120.6, 123.4, 127.8, 129.6, 131.7, 133.4, 138.8, 141.6, 142.2, 143.1, 150.8, 169.4, 172.8. Anal.Calcd. for : $C_{23}H_{16}ClN_3O_3$: C, 66.11; H, 3.86; N,10.06. Found: C,66.31; H, 4.15; N, 9.88.

N-(p-Bromophenyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (3b)

Yield: 61%. m.p. (283-285) ºC. IR (KBr, cm^{-1}): 3331 (N-H), 1658 (amide & lactam) , 1571 (C=C sp^2), 2963 (C-H sp^3). EI-MS (m/z, %): 461 (M^+), 463 (M^++2), 291, 249, 207, 179, 169. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.92 (s, 1H, NH-Ph), 8.06 (d, $2H_{20, 22}$ phenyl),7.92 (d, $2H_{19, 23}$ phenyl), 7.27 (m, 8H, Ar-H), 5.69 (s, 1H, NH at position 1'), 3.92 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6): δ 49.3, 77.1, 121.2, 123.9, 126.3, 129.3, 130.5, 131.7, 133.4, 137.1, 140.4, 143.1, 151.4, 168.9, 172.1. Anal.Calcd. for $C_{23}H_{16}BrN_3O_3$: 59.76; H, 3.49; N, 9.09. Found: C,59.53; H, 3.77; N, 8.82.

N-(p-Tolyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (3c)

Yield: 59%. m.p. (226-228) ºC. IR (KBr, cm^{-1}): 3244 (N-H), 1653 (amide & lactam), 1561 (C=C sp^2), 2946 (C-H sp^3). EI-MS (m/z, %): 397 (M^+), 291, 249, 207, 179, 106. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 10.03 (s, 1H, NH-Ph), 7.94 (d, $2H_{20, 22}$ tolyl), 7.91 (d, $2H_{19, 23}$ tolyl), 7.77 (m, 8H, Ar-H), 5.69 (s, 1H, NH at position 1'), 4.01 (s, 2H, CH_2), 2.32 (s, 3H, CH_3). Anal.Calcd. for $C_{24}H_{19}N_3O_3$: 72.53; H, 4.82; N, 10.57. Found: C,72.81; H, 4.64; N, 10.81.

N-(p-Methoxyphenyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (3d)

Yield: 70%. m.p. (135-137) ºC. IR (KBr, cm^{-1}): 3361(N-H), 1659 (amide & lactam), 1533 (C=C sp^2), 2921 (C-H sp^3). EI-MS (m/z, %): 413 (M^+), 357, 291, 249, 207, 179, 122. ^{1}H-NMR

(400MHz) (DMSO-d_6): δ 9.76 (s, 1H, NH-Ph), 8.09 (d, $2H_{20, 22}$ phenyl),7.96 (d, $2H_{19, 23}$ phenyl), 7.30 (m, 8H, Ar-H), 5.81 (s, 1H, NH at position 1'), 4.25 (s, 2H, CH_2), 3.83 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): δ 49.8, 57.1, 76.8, 121.4, 125.1, 129.1, 130.9, 132.4, 132.9, 136.5, 140.8, 143.7, 150.6, 163, 168.9, 172.1. Anal.Calcd. for $C_{24}H_{19}N_3O_4$: C, 69.72; H, 4.63; N, 10.16. Found: C,70.04; H, 4.87; N, 9.97.

N-(2,4-Dichlorophenyl)-2-(2',4'-dioxospiro[fluorene-9,5'-imidazolidine]-3'-yl)acetamide (3e)

Yield: 56%. m.p. (210-212) ºC. IR (KBr, cm^{-1}): 3342 (N-H), 1657 (amide & lactam), 1518 (C=C sp^2), 2895 (C-H sp^3). EI-MS (m/z, %): 451 (M^+), 453 (M^++2), 291, 249, 207, 179, 159. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.78 (s, 1H, NH-Ph), 8.25 (s, $1H_{20}$ phenyl), 8.08 (d, $1H_{22}$ phenyl), 7.94 (d, $1H_{23}$ phenyl), 7.25 (m, 8H, Ar-H), 5.87 (s, 1H, NH at position 1'),4.25 (s, 2H, CH_2). Anal.Calcd. for : $C_{23}H_{15}Cl_2N_3O_3$: C, 61.08; H, 3.34; N, 9.29. Found: C,60.77; H, 3.62; N, 9.06.

General procedure for the synthesis of *N*-aryl-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide derivatives (4a-e)

Spiro[cyclohexane-1,5'-imidazolidine]-2',4'-dione was used to prepare compounds **(4a-e)** applying the same procedure used for the synthesis of compounds (**3a-e**).[21,22]

N-(p-Chlorophenyl)-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide (4a)

Yield: 75%. m.p. (223-225) ºC. IR (KBr, cm^{-1}): 3335 (N-H), 1659 (amide & lactam), 1598 (C=C sp^2), 2876 (C-H sp^3). EI-MS (m/z, %): 335 (M^+), 336 (M^++1), 249, 209, 167, 97, 85.^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.72 (s, 1H, NH- Ph), 7.72 (d, $2H_{17, 19}$ phenyl),7.50 (d, $2H_{16, 20}$ phenyl), 6.00 (s, 1H, NH at position 1'), 4.24 (s, 2H, CH_2), 2.08 (m, 4H, cyclohexanonyl), 1.89

(m, 6H, cyclohexanonyl). ^{13}C-NMR (DMSO-d_6): δ 26.2, 27.6, 29.2, 48.5, 76.8, 126.8, 130.7, 137.9, 142.7, 152.1, 170.4, 173.1. Anal.Calcd. for : $C_{16}H_{18}ClN_3O_3$: C, 57.23; H, 5.40; N, 12.51. Found: C,57.47; H, 5.21; N, 12.29.

N-(p-Bromophenyl)-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide (4b)

Yield: 63%. m.p. (252-254) ºC. IR (KBr, cm^{-1}): 3295 (N-H), 1652 (amide & lactam), 1543 (C=C sp^2), 2919 (C-H sp^3). EI-MS (m/z, %): 379 (M^+), 381 (M^++2), 249, 209, 167, 97, 129. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 10.14 (s, 1H, NH-Ph), 8.05 phenyl), 6.00 (s, 1H, NH at position 1'), 4.20 (s, 2H, CH_2), 2.02 (m,4H, cyclohexanonyl), 1.38 (m, 6H, cyclohexanonyl). Anal.Calcd. for : $C_{16}H_{18}BrN_3O_3$: C, 50.54; H, 4.77; N, 11.05. Found: C,50.28; H, 5.06; N, 11.30.

N-(p-Tolyl)-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide (4c)

Yield: 61%. m.p. (259-260) ºC. IR (KBr, cm^{-1}): 3315 (N-H), 1657 (amide & lactam), 1566 (C=C sp^2), 2930(C-H sp^3). EI-MS (m/z, %): 315 (M^+), 275, 209, 167, 97, 40. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.84 (s, 1H, NH-Ph), 7.70 (d, $2H_{17, 19}$ tolyl), 7.51 (d, $2H_{16, 20}$ tolyl), 6.10 (s, 1H, NH at position 1'), 3.92 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), 1.89 (m, 4H, cyclohexanonyl), 1.43 (m,6H, cyclohexanonyl). Anal. Calcd. for: $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C,64.81; H, 6.53; N, 13.06.

N-(p-Methoxyphenyl)-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide (4d)

Yield: 73%. m.p. (127-129) ºC. IR (KBr, cm^{-1}): 3308 (N-H), 1649 (amide & lactam), 1533 (C=C, sp^2), 2911(C-H, sp^3). EI-MS (m/z, %): 331 (M^+), 234, 209, 167, 97, 82. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.86 (s, 1H, NH- Ph), 7.78, (d, $2H_{17, 19}$ phenyl), 7.64 (d, $2H_{16, 20}$

phenyl), 5.74 (s, 1H, NH at position 1'), 4.29 (s, 2H, CH_2), 4.05 (s, 3H, OCH_3), 2.06 (m, 4H, cyclohexanonyl), 1.74 (m, 6H, cyclohexanonyl). Anal.Calcd. for : $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C,61.38; H, 6.63; N, 12.37.

N-(2,4-Dichlorophenyl)-2-(2',4'-dioxospiro[cyclohexane-1,5'-imidazolidine]-3'-yl)acetamide (4e)

Yield: 76%. m.p. (187-188) ºC. IR (KBr, cm^{-1}): 3346 (N-H), 1657 (amide & lactam), 1533 (C=C sp^2), 2956 (C-H sp^3). EI-MS (m/z, %): 369 (M^+), 371 (M^++2), 283, 209, 167, 97, 85. ^{1}H-NMR (400MHz) (DMSO-d_6): δ 9.95 (s, 1H, NH-Ph), 8.35 (s, $1H_{17}$ phenyl), 8.05 (d, $1H_{19}$ phenyl), 7.86 (d, $1H_{20}$ phenyl), 5.90 (s, 1H, NH at positi cyclohexanonyl), 1.77 (m, 6H, cyclohexanonyl). ^{13}C-NMR (DMSO-d_6): δ 25.7, 27.9, 28.3, 49.4, 76.7, 130, 131.6, 133.7, 136.2, 137.4, 142.4, 153.2, 169.4, 172.1. Anal.Calcd. for : $C_{16}H_{17}Cl_2N_3O_3$: C, 51.91; H, 4.63; N, 11.35. Found: C,52.16; H, 4.52; N, 11.59.

3.2. Biological evaluation

We previously published methods used for biological evaluation including inhibition assay of aldose and aldehyde reductase *in vitro*, induction of diabetes in experimental animals, in addition to the blood glucose level determination.[16] Both ALR1 and ALR2 were obtained from BioVision, Incorporated.[35] The Ethics Committee of the Suez Canal University approved the experimental protocols in the present study.

3.3. Molecular docking study

Molecular Operating Environment (MOE®, 8) version 2016 was used to perform Molecular docking simulation studies.[36] Target compounds optimization as well as optimization of the enzymes active site mainly followed our published procedures.[16]

3.4. Bioinformatics study

In silico molecular properties and bioactivity prediction of the new compounds were calculated using MolSoft software.[37,38]

4. Conclusion

Hydantoin core and acetamide moiety were used to design a novel series of non-sulfonylureas. The target compounds were tested for their hypoglycemic activities *in vivo* and their inhibitory activities against both ALR1 and ALR2 *in vitro*. The most active compound (**3e**) displayed an IC_{50} ± SEM value 0.37 ± 0.05 μM. The selectivity index showed that compound (**3e**) is 96 fold more selective towards ALR2. *In vivo* hypoglycemic study showed that compound (**3e**) was more potent than the commercial drug repaglinide. Docking studies indicate the importance of using both of the hydantoin ring and the acetamide moiety together in the design of target compounds. This finding could help in the discovery of a new generation of clinically useful hypoglycemic agents.

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6. References

(1) Uzu, T.; Harada, T.; Sakaguchi, M.; Kanasaki, M.; Isshiki, K.; Araki, S.; Sugiomoto, T.; Koya, D.; Haneda, M.; Kashiwagi, A. Glucocorticoid-induced diabetes mellitus: prevalence and risk factors in primary renal diseases. *Nephron Clinical Practice* **2007**, *105*, c54-c57.

(2) Guardado-Mendoza, R.; Prioletta, A.; Jiménez-Ceja, L. M.; Sosale, A.; Folli, F. The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus. *Archives of medical science: AMS* **2013**, *9*, 936.

(3) DeRuiter, J. Endocrine Pharmacotherapy Module Overview of the antidiabetic agents. *Spring* **2003**, *1*, 1-33.

(4) Han, X.; Chiang, C.; Leonard, C. E.; Bilker, W. B.; Brensinger, C. M.; Li, L.; Hennessy, S. Biomedical Informatics Approaches to Identifying Drug–Drug Interactions: Application to Insulin Secretagogues. *Epidemiology* **2017**, *28*, 459-468.

(5) Keegan, M. T. Endocrine pharmacology. In *Pharmacology and Physiology for Anesthesia*; Elsevier: **2019**, p 708-731.

(6) Del Prato, S.; Pulizzi, N. The place of sulfonylureas in the therapy for type 2 diabetes mellitus. *Metabolism-Clinical and Experimental* **2006**, *55*, S20-S27.

(7) Brown, G. R.; Foubister, A. J. Receptor binding sites of hypoglycemic sulfonylureas and related [(acylamino) alkyl] benzoic acids. *Journal of medicinal chemistry* **1984**, *27*, 79-81.

(8) Kador, P. F. The role of aldose reductase in the development of diabetic complications. *Medicinal research reviews* **1988**, *8*, 325-352.

(9) Gosmanov, A. R.; Gosmanova, E. O.; Kitabchi, A. E. Hyperglycemic crises: diabetic ketoacidosis (DKA), and hyperglycemic hyperosmolar state (HHS). In *Endotext [Internet]*; MDText. com, Inc.: **2018**.

(10) Vander Jagt, D. L.; Hunsaker, L. A. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. *Chemico-biological interactions* **2003**, *143*, 341-351.

(11) Bachur, N. R. Cytoplasmic aldo-keto reductases: a class of drug metabolizing enzymes. *Science* **1976**, *193*, 595-597.

(12) Sun, W.; Gerhardinger, C.; Dagher, Z.; Hoehn, T.; Lorenzi, M. Aspirin at Low-Intermediate Concentrations Protects Retinal Vessels in Experimental Diabetic Retinopathy Through Non–Platelet-Mediated Effects. *Diabetes* **2005**, *54*, 3418-3426.

(13) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. *Journal of medicinal chemistry* **1985**, *28*, 841-849.

(14) Lee, Y. S.; Chen, Z.; Kador, P. F. Molecular modeling studies of the binding modes of aldose reductase inhibitors at the active site of human aldose reductase. *Bioorganic & medicinal chemistry* **1998**, *6*, 1811-1819.

(15) Iqbal, Z.; Hameed, S.; Ali, S.; Tehseen, Y.; Shahid, M.; Iqbal, J. Synthesis, characterization, hypoglycemic and aldose reductase inhibition activity of arylsulfonylspiro [fluorene-9, 5′-imidazolidine]-2′, 4′-diones. *European journal of medicinal chemistry* **2015**, *98*, 127-138.

(16) Salem, M. G.; Aziz, Y. M. A.; Elewa, M.; Elshihawy, H. A.; Said, M. M. Molecular modelling and synthesis of spiroimidazolidine-2, 4-diones with dual activities as hypoglycemic agents and selective inhibitors of aldose reductase. *Bioorganic chemistry* **2018**, *79*, 131-144.

(17) Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A. M. Molecular hybridization: a useful tool in the design of new drug prototypes. *Current medicinal chemistry* **2007**, *14*, 1829-1852.

(18) Lang, V.; Light, P. E. The molecular mechanisms and pharmacotherapy of ATP-sensitive potassium channel gene mutations underlying neonatal diabetes. *Pharmacogenomics and personalized medicine* **2010**, *3*, 145.

(19) Reilly, T. J. The preparation of lidocaine. *J. Chem. Educ* **1999**, *76*, 1557.

(20) Aziz, Y. M. A.; Said, M. M.; El Shihawy, H. A.; Abouzid, K. A. Discovery of novel tricyclic pyrido [3′, 2′: 4, 5] thieno [3, 2-d] pyrimidin-4-amine derivatives as VEGFR-2 inhibitors. *Bioorganic chemistry* **2015**, *60*, 1-12.

(21) Mahmoodi, N. O.; Khodaee, Z. Evaluating the one-pot synthesis of hydantoins. *Arkivoc* **2007**, *3*, 29-36.

(22) Qandil, A. M.; Fakhouri, L. I. a-Anilinoketones, Esters and Amides: A Chemical Study. *Pharmaceuticals* **2012**, *5*, 591-612.

(23) Stefek, M.; Snirc, V.; Djoubissie, P.-O.; Majekova, M.; Demopoulos, V.; Rackova, L.; Bezakova, Z.; Karasu, C.; Carbone, V.; El-Kabbani, O. Carboxymethylated pyridoindole antioxidants as aldose reductase inhibitors: Synthesis, activity, partitioning, and molecular modeling. *Bioorganic & medicinal chemistry* **2008**, *16*, 4908-4920.

(24) Miyamoto, S. Recent advances in aldose reductase inhibitors: potential agents for the treatment of diabetic complications. *Expert Opinion on Therapeutic Patents* **2002**, *12*, 621-631.

(25) Rakowitz, D.; Maccari, R.; Ottanà, R.; Vigorita, M. G. In vitro aldose reductase inhibitory activity of 5-benzyl-2, 4-thiazolidinediones. *Bioorganic & medicinal chemistry* **2006**, *14*, 567-574.

(26) Takashita, E.; Fujisaki, S.; Shirakura, M.; Nakamura, K.; Kishida, N.; Kuwahara, T.; Shimazu, Y.; Shimomura, T.; Watanabe, S.; Odagiri, T. Influenza A (H1N1) pdm09 virus exhibiting enhanced cross-resistance to oseltamivir and peramivir due to a dual H275Y/G147R substitution, Japan, March 2016. *Eurosurveillance* **2016**, *21*.

(27) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1. *Advanced drug delivery reviews* **2001**, *46*, 3-26.

(28) Lipinski, C. A. Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies* **2004**, *1*, 337-341.

(29) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nature reviews. Drug discovery* **2007**, *6*, 881.

(30) Clark, D. E.; Pickett, S. D. Computational methods for the prediction of 'drug-likeness'. *Drug discovery today* **2000**, *5*, 49-58.

(31) Ghose, A. K.; Herbertz, T.; Hudkins, R. L.; Dorsey, B. D.; Mallamo, J. P. Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. *ACS chemical neuroscience* **2011**, *3*, 50-68.

(32) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. *Journal of medicinal chemistry* **2002**, *45*, 2615-2623.

(33) Aktürk, Z.; Kılıç, F.; Erol, K.; Pabuçcuoğlu, V. Synthesis and anticonvulsant activity of some ω-(1H-1-imidazolyl)-N-phenylalkanoic acid amide derivatives. *Il Farmaco* **2002**, *57*, 201-206.

(34) Ma, L.; Li, S.; Zheng, H.; Chen, J.; Lin, L.; Ye, X.; Chen, Z.; Xu, Q.; Chen, T.; Yang, J. Synthesis and biological activity of novel barbituric and thiobarbituric acid derivatives against non-alcoholic fatty liver disease. *European journal of medicinal chemistry* **2011**, *46*, 2003-2010.

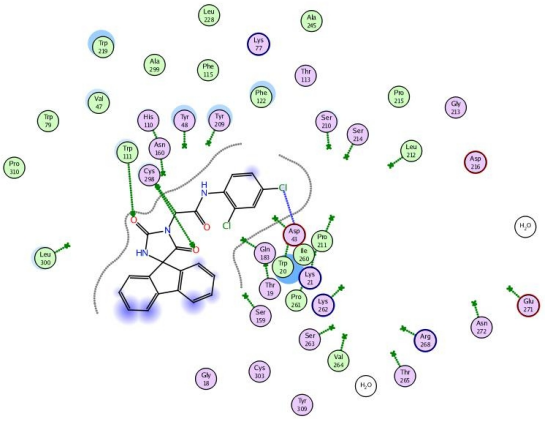
(35) BioVision, I. EZDetect™ Aldo-keto Reductase Activity Assay Kit (Colorimetric).

(36) Chemical Computing Group Inc. MO Canada. Molecualr Operating Environment (MOE). **2016**

(37) Koch, M. A.; Schuffenhauer, A.; Scheck, M.; Wetzel, S.; Casaulta, M.; Odermatt, A.; Ertl, P.; Waldmann, H. Charting biologically relevant chemical space: a structural classification of natural products (SCONP). *Proceedings of the National Academy of Sciences of the United States of America* **2005**, *102*, 17272-17277.

(38) Agnihotri, S.; Narula, R.; Joshi, K.; Rana, S.; Singh, M. In silico modeling of ligand molecule for non structural 3 (NS3) protein target of flaviviruses. *Bioinformation* **2012**, *8*, 123.

Graphical abstract



Compound (**3e**) exhibited 96 folds selective inhibitory activity against ALR2 versus ALR1, with an IC_{50} ± SEM value 0.37 ± 0.05 μM.