Quinazolinone-based rhodanine-3-acetic acids as potent aldose reductase inhibitors: synthesis, functional evaluation and molecular modeling study

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Abstract

A series of quinazolinone-based rhodanine-3-acetic acids was synthesized and tested for in vitro aldose reductase inhibitory activity. All the target compounds displayed nanomolar activity against the target enzyme. Compounds **3a**, **3b**, and **3e** exhibited almost 3-fold higher activity as compared to the only marketed reference drug epalrestat. Structure-activity relationship studies indicated that bulky substituents at the 3-phenyl ring of the quinazolinone moiety are generally not tolerated in the active site of the enzyme. Insertion of a methoxy group on the central benzylidene ring was found to have a variable effect on ALR-2 activity depending on the nature of peripheral quinazolinone ring substituents. Removal of the acetic acid moiety led to inactive or weakly active target compounds. Docking and Molecular dynamic simulations of the most active 2,4-thiazolidinedione-3-acetic acid derivatives were also carried out, to provide the basis for further structure-guided design of novel inhibitors

Key words: Rhodanine-3-acetic acids; aldose reductase inhibitors; molecular modeling.

The incidence of diabetes mellitus has markedly increased over the last century due to changes in human life style and behavior.[1] Estimates indicate that about 400 million patient worldwide are affected by diabetes and by the year 2030, about 10% of world population will suffer from this chronic disease.[2] More importantly, the management of the debilitating complications associated with diabetes costs at least 10% of overall healthcare cost in many countries.[3] For these reasons, the discovery and development of drugs for prevention and treatment of diabetic complications will remain a major challenge to medicinal chemists. In this context, there has been a growing interest over the last few decades in aldose reductase inhibitors as therapeutic candidates for diabetic

complications.[4-26] Aldose reductase (ALR-2) is the key enzyme of the polyol pathway through which glucose is metabolized under conditions of hyperglycemia associated with diabetes. In the polyol pathway, glucose is reduced by aldose reductase to sorbitol with the associated oxidation of NADPH to NADP. The accumulation of sorbitol in cells leads to osmotic stress. The second step in the polyol pathway involves the oxidation of sorbitol to fructose by the enzyme sorbitol dehydrogenase using NAD^+ as a cofactor. Alteration of the proportion of cytosolic NADH to NAD^+ results in reductive stress which is associated with reduced intracellular concentrations of glutathione, activation of protein kinase C, and non-enzymatic glycation.[27,28] Aldose reductase inhibitors are categorized into two major classes: acetic acid derivatives and the cyclic imides. The acetic acid derivatives currently available include epalrestat, tolrestat, zenarestat, and ponalrestat. The cyclic imides include the hydantoins and their bioisosteres such as the rhodanines, 2,4-thiazolidinediones, and the succinimides. Sorbinil, fidarestat, and imirestat are hydantoins whereas, ranirestat and minalrestat belong to the succinimides. (**Chart 1**). Unfortunately, many of these agents were unsuccessful in clinical trials due to poor pharmacokinetics, adverse effects, or low efficacy. Only epalrestat, which is a rhodanineacetic acid derivative, was approved for clinical use in Japan, China and India.[29] Long-term studies have revealed that epalrestat is generally well tolerated with only mild side effects such as nausea, vomiting, and elevation of liver enzyme levels.[16,30-34] These findings urged us to address novel rhodanineacetic acid derivatives as potential useful therapeutic candidates for the management of diabetic complications. In the present investigation, a series of quinazolinone-based rhodanineacetic acid derivatives was designed, synthesized, and tested for *in vitro* aldose

reductase inhibitory activity. Another series of representative rhodanines lacking the acetic acid moiety was also tested for comparison purposes.

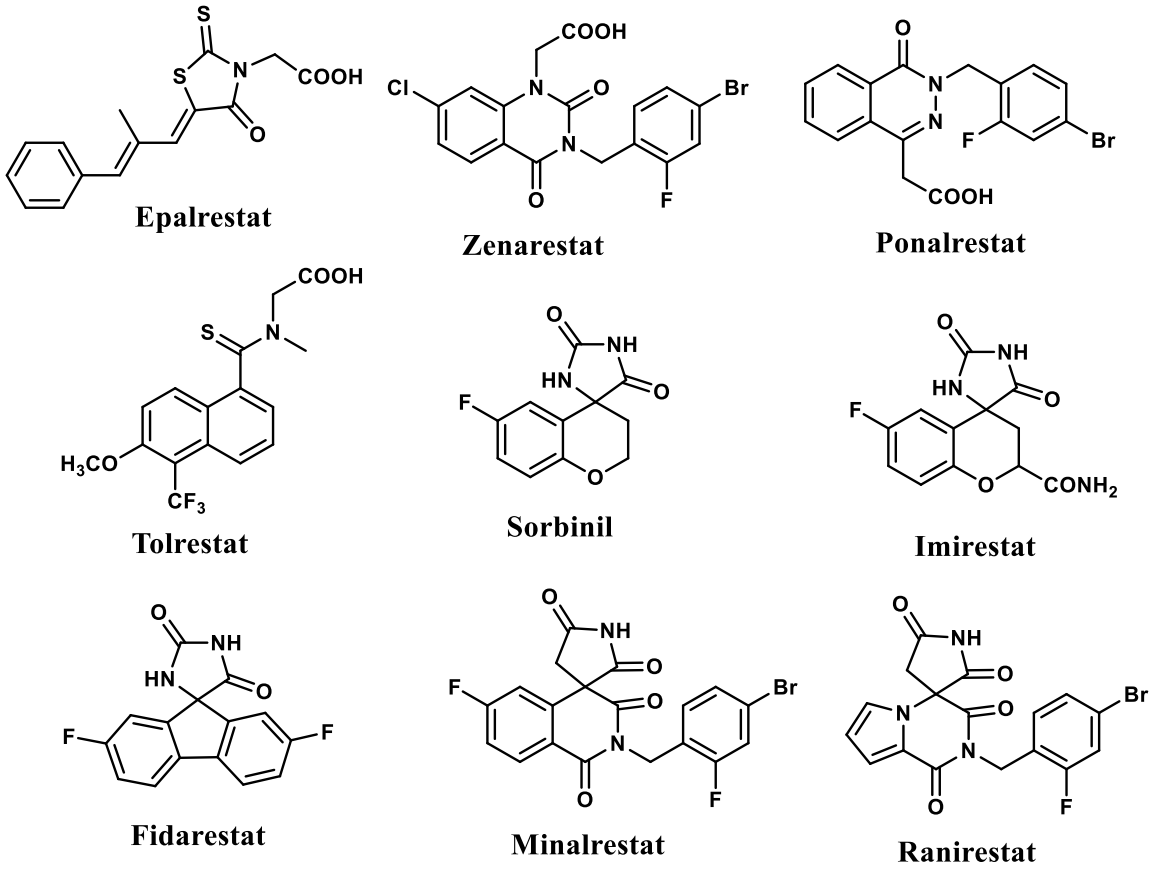
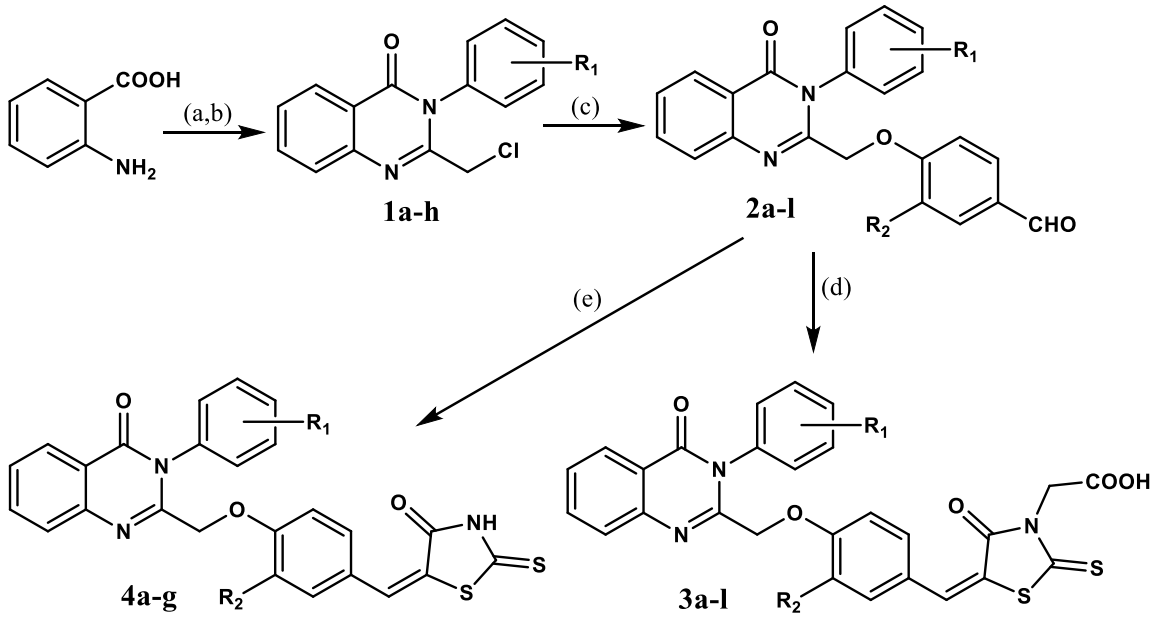


Chart 1. Chemical structure of available aldose reductase inhibitors.

A straightforward synthetic pathway was adopted to synthesize the target compounds as outlined in **Scheme 1**. The starting chloromethylquinazolinones (**1a-h**) were synthesized from anthranilic acid in two steps following reported procedures.[35-38] The first step involves chloroacetylation of anthranilic acid using chloroacetyl chloride in dry benzene under reflux conditions. In the second step, cyclization to the desired chloromethylquinazolinones was achieved by direct reaction of the N-chloroacetyl anthranilic acid derivatives with the appropriate anilines in presence of phosphorous oxychloride as a condensing agent in dry toluene. Subsequently, 4-hydroxybenzaldehyde

or vanillin was alkylated with the chloromethylquinazolinones (**1a-h**) in refluxing acetonitrile under the basic conditions of potassium carbonate and in the presence of potassium iodide to afford the aldehydes (**2a-l**) in good yields as previously reported by us.[39] The title rhodanineacetic acids (**3a-l**) were obtained in fair yields via Knoevenagel-type condensation of the aldehydes with rhodanine-3-acetic acid in refluxing acetic acid using β-alanine as a condensing agent. On the other hand, compounds 4a-g were obtained by condensation of the appropriate aldehyde with rhodanine in the presence of sodium acetate. Structures of all the target compounds were fully characterized by means of ^{1}H and ^{13}C NMR spectroscopy and their purity were satisfactorily confirmed by elemental analysis.

Scheme 1:

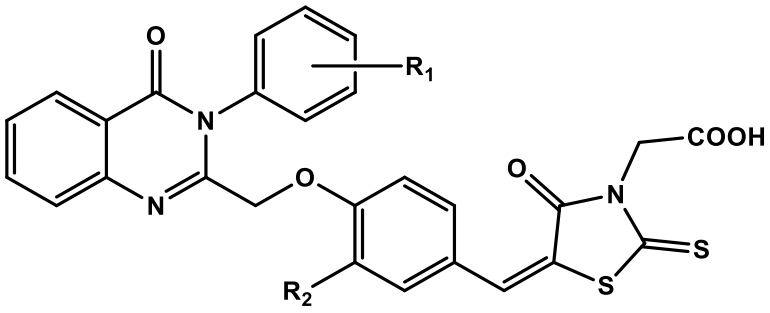


<u>Reagents and conditions</u>:

(a) Chloroacetyl chloride, benzene, reflux, 3h. (b) Substituted aniline, $POCl_3$, toluene, 115 ºC, 3h. (c) 4-Hydroxybenzaldehyde or vanillin, K_2CO_3, KI, acetonitrile, reflux, 3h. (d) Rhodanine-3-acetic acid, β-alanine, glacial acetic acid, 100 ºC, 3h. (e) Rhodanine, sodium acetate, glacial acetic acid, reflux, 24-48 h.

All the novel synthesized compounds were tested *in vitro* for their aldose reductase inhibitory activity following standard protocols.[40-43] The obtained IC_{50} values are reported in **Table 1**. Our investigation started by synthesizing and testing four compounds of the benzylidene series, namely **3a**-**3d**. Their functional evaluation indicated that, moving from the hit **3a** (IC_{50} 60.9 nM), the presence of the electron-withdrawing fluoro atom on the 3-phenyl ring of the quinazolinone core, as in **3b** (IC_{50} 55.6 nM), was tolerated while the presence of the bulkier bromo atom, as in **3c** (IC_{50} 711 nM), resulted in an almost 10-fold decrease of activity. Similarly, compound **3d** (IC_{50} 574 nM), having an electron-donating 3-methyl substituent, turned out to be less active than the unsubstituted parent compound, **3a**. We than examined the effect on ALR-2 activity by inserting a 3-methoxy group on the central benzylidene ring. The hit compound of the novel series, namely **3e** (IC_{50} 49.7 nM) turned out to be slightly more active than the unsubstituted counterpart **3a** (IC_{50} 60.9 nM). On this basis, additional compounds of the 3-methoxybenzylidene series were synthesized and tested, bearing different electron-withdrawing and electron-donating substituents on the distal phenyl ring of the quinazolinone core. The observed functional data indicated that, regardless of both the type of the substituent and its position on the ring, peripheral substituents have a negative impact on ALR-2 activity. Actually, compared to the unsubstituted hit **3e** (IC_{50} 49.7 nM), all the substituted compounds (**3f-l)** showed IC_{50} values in the low micromolar range, as shown in **Table 1**. To test the importance of the presence of an acetic acid moiety, a series of representative rhodanines **4a-g** was synthesized as well, and tested for their ALR-2 activity. All the compounds of the novel series displayed no or weak activity against the target enzyme, as shown in **Table 2**.

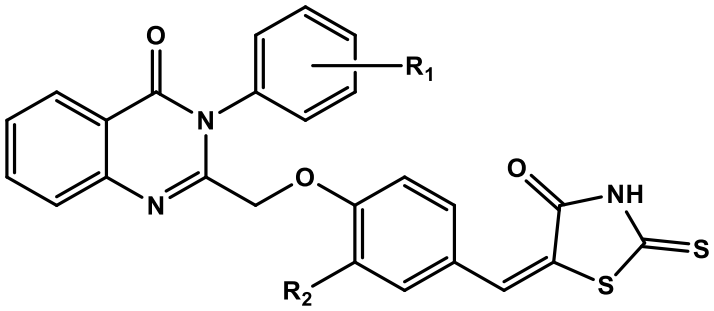
Table 1. ALR2 inhibitory activities of rhodanine-3-acetic acid derivatives.



Ms Code	R_1	R_2	IC_{50} (nM)[a]
3a	H	H	60.9
3b	4-F	H	55.6
3c	4-Br	H	711
3d	3-CH_3	H	574
3e	H	OCH_3	49.7
3f	4-F	OCH_3	358
3g	3-Cl	OCH_3	419
3h	4-Br	OCH_3	309
3i	3-Br	OCH_3	401
3j	4-CH_3	OCH_3	512
3k	3-CH_3	OCH_3	329
3l	4-CF_3	OCH_3	346
Epalrestat	-	-	170

[a]IC_{50} values, means, represent the concentration required to produce 50% enzyme inhibition. Errors ± 5% (from 3 different assays, data not shown).

Table 2. Percentage ALR2 inhibitory activities of rhodanine derivatives (**4a-g**).



Code	R_1	R_2	Percent inhibition[a]
4a	H	H	48.5%
4b	4-F	H	39.2%
4c	4-Cl	H	n.a.[b]
4d	4-Br	H	n.a.[b]
4e	4-CH_3	H	n.a.[b]
4f	4-OCH_3	H	52.7%
4g	H	OCH_3	62.9%

[a] The inhibitory effect was estimated at a concentration of 10 µM. [b] n.a = not active.

To propose a structural binding mode of ALR2 when bound to the rhodanine-3-acetic acid derivatives, docking experiments were carried out on the newly synthesized compounds starting from the most active, **3e**. It has been clearly proven that ALR2 can adopt different binding site conformations, depending on the structural characteristics of an inhibitor. To combat any computational bias, the target compound was docked into the binding pocket of the human ALR2/$NADP^+$-IDD594 complex using the automated Autodock Vina plugin package in UCSF chimera.[44,45]

Visual inspection of the docked pose with the best binding affinity (-11.05 k.cal/mol) (**Figure 1**), revealed **3e** to bind favorably into the polar anion-binding pocket. Upon assessment of the residue-ligand interaction network, stabilizing hydrogen bond

interactions were illustrated with residue Tyr48 and strong electrostatic interactions between **3e** and the nicotinamide moiety of the $NADP^+$ cofactor. The presence of the rhodanine ring is also shown to well orient the planar and aromatic quinazolinone fragments into the "specificity pocket", thus creating favorable hydrophobic bonds with Leu300.

These results validate the docked pose of the ALR2-3e complex in comparison to the docked complex of the ALR2-4c complex of which the 4c compound docked completely out of the binding pocket of ALR2. This concludes to that the removal of the acetic acid moiety leads to inactive or weakly active target compounds.

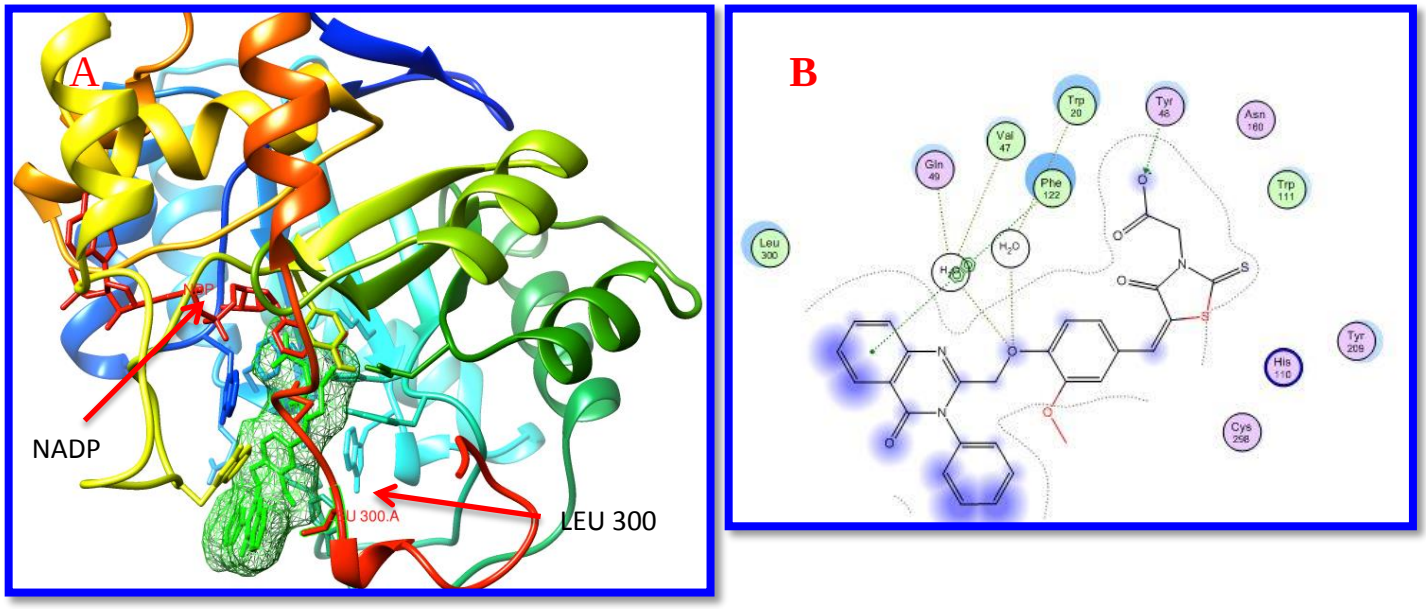


Figure 1. Docked conformation of **3e** in the active site of ALR2. **A**) Ligand carbon atoms are displayed in green and $NADP^+$ in red. **B**) Ligand interaction and the binding mode of compound **3e** with aldose reductase receptor. The complex shows strong hydrogen bonds with TYR 48, HOH 2062, HOH2131 at distance 2.08,3.07,2.8 A°.

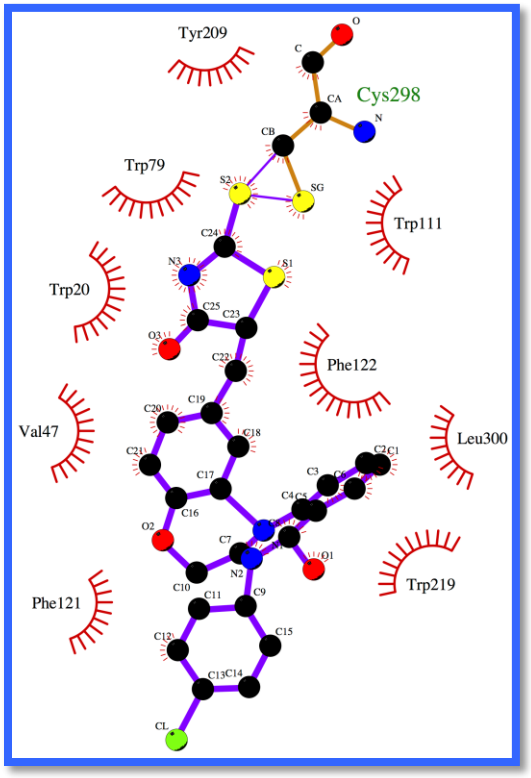


Figure 2. Ligand interactions of compound **4c** in complex with aldose reductase. The complex shows hydrogen bond interactions with HOH 2062, HOH2131 at distance 2.94, 3.22, 2.24A°.

Molecular dynamics study is a significant tool to understand the stability and the binding mode of the ligand-receptor interactions. It also helps to understand the dynamics of the protein, which represent the true nature of protein better than ordinary docking, which does not completely consider the dynamic of protein.[46]

The thermodynamic energy contribution of **3e** to the total binding free energy of the complex surmounts to the stability of **3e** in the ALR2 binding pocket and thus the stability

of the complex during the simulation. Table 3 summarizes the free binding energy of the system.

Table 3: The free binding energy calculations of compound **3e** Aldose Reductase complex during MD Using the MM-PBSA Method.

	Δ E_{vdW}	**ΔE_{elec}**	**ΔG_{gas}**	**ΔG_{solv}**	**ΔG_{bind}**
Total Energy	-79.26±0.148	-27.95±0.23	-107.22± 0.25	46.21±0.149	-61.00± 0.196

In conclusion, we described in this work a novel series of quinazolinone-based rhodanine-3-acetic acid derivatives, developed as aldose reductase inhibitors. Tested in vitro against the target enzyme, they all proved to be active, showing IC_{50} values in the submicromolar/nanomolar range. Significantly, three out of the synthesized compounds, namely **3a**, **3b**, and **3e**, turned out to be almost 3-fold more active than the reference drug, epalrestat. The acetic acid moiety of the compounds proved to be a key functional element as its removal, as in compounds **4a-g**, resulted in a remarkable decrease of inhibitory efficacy. Docking studies performed on the most active compound helped to understand the binding mode of the synthesized derivatives, thus providing the basis for further structure-guided design hypotheses of novel analogues with higher efficacy.

Acknowledgements

The authors are grateful to the Faculty of Pharmacy, Zagazig University for partial financial support of this work. C.L.M acknowledges Regione Toscana for the financial support (IDARA Project, DD650/2014).

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