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Novel synthesis of Nitro-Quinoxalinone derivatives as Aldose reductase inhibitors

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[a]Abbreviations: ALR2, Aldose reductase; AKR, aldo-keto reductase; NADPH, ß-nicotinamide adenine dinucleotide phosphate reduced form; ARIs, Aldose reductase inhibitors; SAR, structure-activity relationship

Abstract

A novel, non-acid series of nitroquinoxalinone derivatives was synthesized and tested for their inhibitory activity against aldose reductase as targeting enzyme. All active compounds displayed an 8-nitro group, and showed significant activity in IC_{50} values ranging from 1.54 to 18.17 µM. Among them 6,7-dichloro-5,8-dinitro-3-phenoxyquinoxalin-2(1H)-one (**7e**), exhibited the strongest aldose reductase activity with an IC_{50} value of 1.54 µM and a good SAR (structure- activity relationship) profile.

Keywords

Quinoxalinone derivatives, Aldose Reductase inhibitors, Structure-Activity relationship

Diabetes Mellitus (DM) is a complex metabolic disorder characterized by an elevated level of blood glucose called hyperglycemia. The polyol pathway of glucose metabolism is activated in the hyperglycemia, which subsequently results in the development of long-term diabetic complications including retinopathy, nephropathy, neuropathy and cataract.[1,2,3,4] Aldose reductase (ALR2, EC I.I.I.21), a cytosolic enzyme and a member of aldo-keto reductase (AKR) superfamily, is the first and rate limiting enzyme of the polyol pathway in which ALR2 catalyzes the NADPH-dependent reduction of glucose to sorbitol, which then is converted into fructose by NAD+-dependent sorbitol dehydrogenase (Fig. 1) Several lines of evidence suggest that the primary cause of long-term diabetic complications is due to the activation of ALR2 and consequent imbalance of NADPH/ NADP+ and NAD+/ NADH coenzymes resulting in oxidative stress inside the cell along with overproduction of fructose.[5,6,7,8]

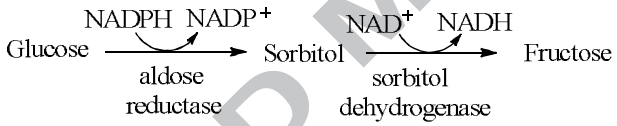


Figure 1. The polyol pathway of glucose metabolism

Therefore, ALR2 has been considered as target enzyme for suitable drug candidates to check the delay in onset, progression and further development of long-term diabetic complications.

In past years, a number of structurally different aldose reductase inhibitors (ARIs) comprising two chemical classes have been developed but the clinical efficacy and potency of these compounds still pose a challenge, and most of them have deleterious side effects. The first class comprises carboxylic acid ARIs such as, tolrestat[9], zenarestat[10] and ponalrestat[11], and the second involves cyclic imides like sorbinil[12], fidarestat[13], minalrestat[14] and ranirestat (AS-3201)[15] (Fig. 2). Currently, only epalrestat, a carboxylic acid drug is available on the market and used for the treatment of neuropathy in Japan, India and China. [16] Indeed, whereas the carboxylic acids ARIs show potent in vitro activity as aldose reductase

inhibitors, their effectiveness decreases in vivo. In their turn, the cyclic imide ARIs often develop toxicity and exhibit some side effects.[12] Therefore, development of different types of ARIs is still needed. A preferred approach to pursue the desired therapeutic and pharmacokinetic properties is to design and synthesize specific non-carboxylic acid and non-cyclic imide ARIs. Compounds with scaffolds containing nitro substituents find a wide range of applications in biological and pharmaceutical areas, and often are involved as drug candidates. [17] To this aim, we designed quinoxalinone-based nitro derivatives and evaluated them for their properties as ARIs (Aldose reductase inhibitors). The present article focuses on the syntheses of quinoxalinones containing one, two or three nitro groups and on the role of those nitro groups in the inhibition of ALR2.

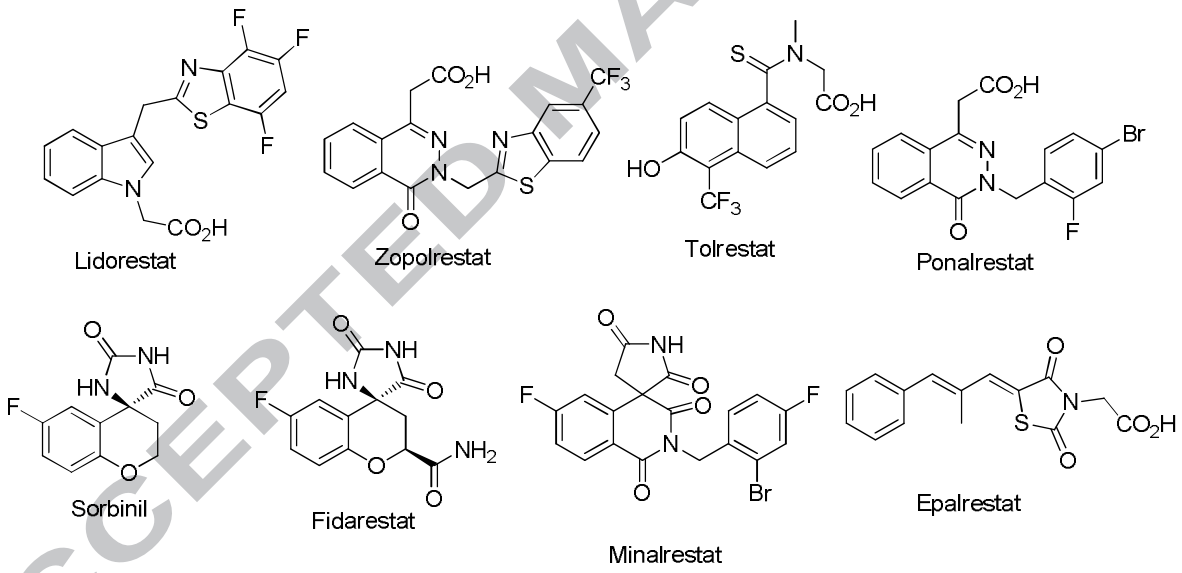
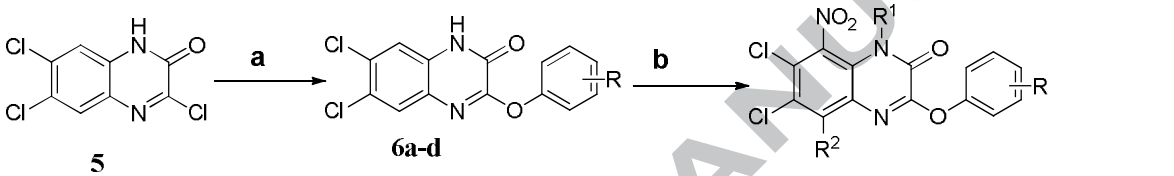


Figure 2. Chemical structures of aldose reductase Inhibitors

The preparation of starting materials dichloroquinoxalin-2(1H)-one **5** and **6-** or **7-**bromo quinoxalinone **8a,b** has been reported previously.[18] Syntheses of target compounds **7a-f** and **9a-b** were accomplished as outlined in Scheme **1** and **2**. Compound **5** reacted with various phenols produced 3-phenoxyquinoxalinone intermediates **6a-d**. Further, aromatic nitration of **6a-c** using a mixture of Cu

$(NO_3)_2$-$3H_2O$ and Ac_2O proceeded first at the C8, then at the C5 position to afford compounds **7a-e.** Finally, aromatic and N1-nitration of **6d** yielded compound **7f** as shown in scheme **1**.[19] In a similar way, aromatic nitration of 6- and 7- bromoquinoxalinone compounds **(8a, b)** yielded the corresponding two products **9a, b** as shown in scheme **2**.

Scheme 1

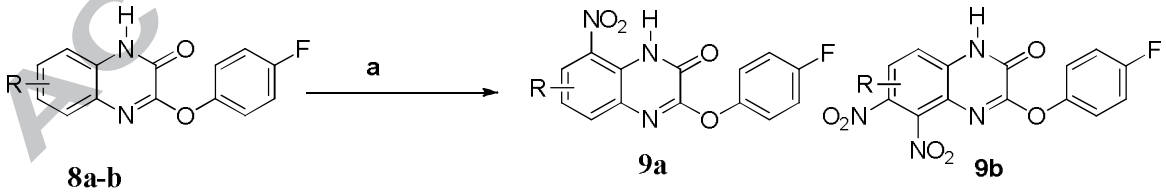


R :
6a = 4-F
6b = 4-Br
6c = H
6d = 3, 5-diMe

7a: R = 4-F, R^1 = H, R^2 = H
7b: R = 4-F, R^1 = H, R^2 = NO_2
7c: R = 4-Br, R^1 = H, R^2 = H
7d: R = H, R^1 = H, R^2 = H
7e: R = H, R^1 = H, R^2 = NO_2
7f: R = 3, 5- diMe, R^1 = NO_2, R^2 = NO_2

Scheme 1. *Reagents and conditions*: (a) Phenols, dimethylformamide (DMF), K_2CO_3, 24 h, 75 ^oC; (b) Cu $(NO_3)_2$-$3H_2O$, Ac_2O, CH_2Cl_2, 12 h, room temperature (rt)

Scheme 2



8a: R = 6-Br
8b: R = 7-Br

9a: R = 6-Br

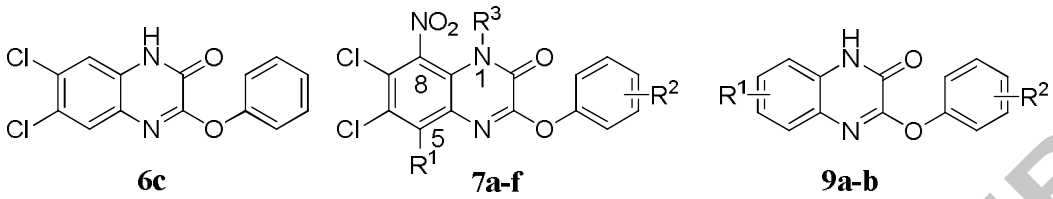
9b: R= 7-Br

Scheme 2. *Reagents and conditions*: (a) Cu $(NO_3)_2$-$3H_2O$, Ac_2O, CH_2Cl_2, 12 h, rt

The synthetic work led to a series of compounds containing one, two or three nitro groups located on the benzene ring of the quinoxalinone core and the N1 position as shown in table 1. All of the quinoxalinone derivatives were evaluated for their potential inhibitory effect on ALR2 isolated from dog lenses. The IC_{50} values were determined by linear regression analysis of the log of the concentration-response curve.[18] The in vitro assay of these derivatives showed an interesting inhibition profile with significant inhibitory activity in an IC_{50} range of 1.54-18.17 µM.

Compounds **7a-f** that had one nitro group at the C8 position, or two to three nitro groups at C5, C8 and N1 positions of the quinoxalinone framework showed significant activity in the low micromolar range. Among these, compound **7e** bearing two nitro groups at C5 and C8 positions was the most active with the IC_{50} value of 1.54 µM, whereas compound **7a** bearing a single 8-nitro group had a much lower activity. The decrease in the activity of **7a** could be attributed to the 4-fluoro substituent on the phenoxy ring as compared with **7d**.[18] However, addition of one more nitro group on the benzene ring of **7a** resulted in an increase in the activity for 5,8-dinitroquinoxalinone **7b**. This synergy reappeared in the most active compound **7e** which equally showed an enhanced activity by adding a 5-nitro group to **7d**. Replacing the 6-chloro with a bromo substituent in compound **7a** to form 6-Br-8-nitro compound **9a** also increased the activity. Compound **7f** having three nitro groups at C5, C8, and N1 showed a moderate activity with IC_{50} = 7.1 µM. The 7-Br-5,6-dinitro compound **9b** displayed the least activity. In addition, it should be noted that although both **7b** and **9b** have two nitro groups on the benzene ring of the quinoxalinone skeleton, there was a significant difference in activity between the two dinitro compounds indicating that the 8-nitro group has a positive impact on the activity. Indeed, while active compounds **7a-f** and **9**a all possess this structural feature, the second most active compound **7d** displays a single 8-nitro group (Table 1).

Table 1. Biological activity data of quinoxalinone derivatives



6c **7a-f** **9a-b**

Comp. No.	R^1	R^2	R^3	ALR2 IC_{50} [μM][a]
6c				26.67 % [b]
7a	H	4-F	H	11.81
7b	H	4-F	NO_2	4.46
7c	H	4-Br	H	7.46
7d	H	4-H	H	3.14
7e	H	4-H	NO_2	1.54
7f	NO_2	3,5-diMe	NO_2	7.1
9a	6-Br and 8-NO_2	4F		5.8
9b	7-Br and 5,6-diNO_2	4F		18.17
epalrestat				0.12

[a]IC_{50} (95% CL) values represent the concentration required to effect 50% enzyme inhibition

[b]Represents the percent inhibition of compound determined at concentration of 10 μM

To understand the mechanistic details of biological activity and the importance of nitro group at C8 position, the docking of **7d** and **7a** compounds was performed. The more active compound, **7d** was

105 docked into the binding pocket of the human ALR2/NADP+/lidorestat complex (PDB code: 1Z3N). As
106 shown in Figure **3a-b**, the results of **7d** revealed that ligand fits into the active site of the enzyme. The
107 nitro group orientate itself towards the anion-binding pocket by making two hydrogen bonds one with
108 indole N-H bond of Trp 20: one through the N-atom (3.52 Å) and the second through an O-atom of the
109 nitro group (2.68 Å). Additionally, the oxygen of the 3- phenoxy group interacts via hydrogen bonding
110 with Cys 298 (2.77 Å). The phenoxy ring is stabilized by stacking with the phenolic ring of Tyr 298 and
111 fits into the specificity pocket formed by Leu 300, Cys 303, Cys 298, Thr 113, Phe 122, and Trp 111
112 while the quinoxalinone core structure penetrates into the hydrophobic pocket formed by the side chains
113 of Trp 20, Phe 122, Trp 79 and Trp 219. Actually, both the 8-nitro group and the 3-phenoxy moiety
114 without para-substituent provide a favored conformation for interaction of **7d** with the active site,
115 resulting in an enhanced activity for **7d** compared with **7a**. In contrast, docking of **7a** (Figure **3c, d**)
116 revealed the formation of a single hydrogen bond only between an O-atom of the nitro group and the NH
117 group of Trp 20 (3.27 Å). The nitro group is oriented far away from the anion-binding pocket, and
118 similarly the phenoxy group is located slightly farther from the specificity pocket, [18] these findings
119 indicate different interaction patterns for the two compounds. The docking results provide support for
120 the observation that **7d** is a more potent inhibitor than **7a.**

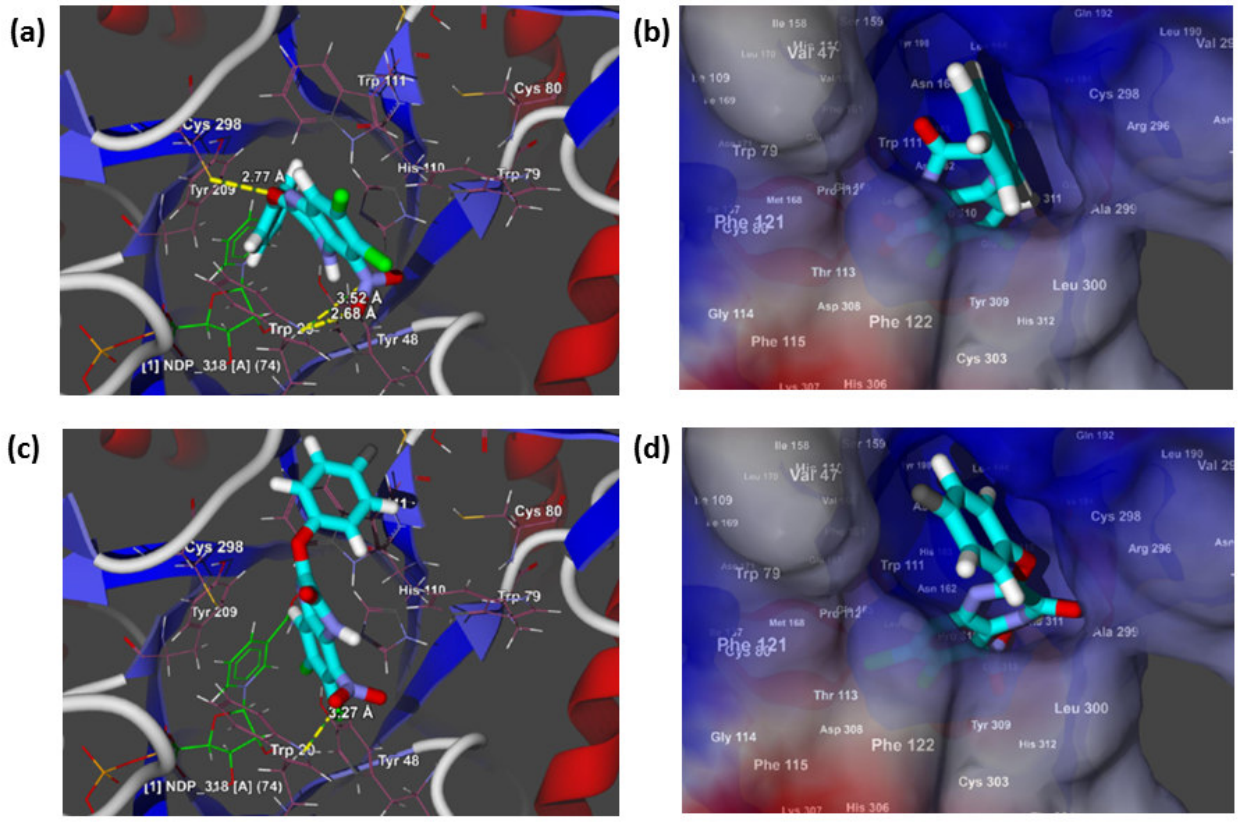


Figure 3. Docking of inhibitors into active site of ALR2; a) Docking of **7d** into active site of ALR2 in ribbon diagram; b) in surface representation. The docked position of **7d** is shown in cyan (C), red (O) and blue (N); hydrogen bonds are shown as light yellow dashed lines; c) Docking of **7a** into active site of ALR2 in ribbon diagram; d) in surface representation. The docked position of **7a** is shown in cyan (C), red (O) and blue (N); hydrogen bonds are shown as light yellow dashed lines.

In conclusion, we have developed a novel, non-acid series of ARIs consisting of nitroquinoxalinone derivatives **7a-f** and **9a, b**. These compounds showed significant activity with IC_{50} values of **1.54-18.17** µM. The most potent inhibitor was compound **7e** with two nitro groups at C5 and C8 and a 3-phenoxy substituent. Our SAR studies revealed the 8-nitro and 3-phenoxy groups as the more important features

for the activity, which is further enhanced by adding a 5-nitro substituent. However, substitution at the para-position of the 3-phenoxy moiety decreased the activity.

Acknowledgments

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References:

1. Van Heyningen, R. *Nature (London)* **1959**, 184, 194

2. Robison, W. J.; Kador, P. F.; Kinoshita, J. H. *Science* **1983**, 221, 1177

3. Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, 28, 841

4. Beyer-Mears, A.; Curz, E.; Edelist, T.; Varagiannis, E. *Pharmacology* **1986**, 32, 52

5. Kador, P.F. *Med. Res. Rev.* **1988**, 8, 325

6. Tomlinson, D. R.; Stevens, E. J.; Diemel, L.T. *Trends Phramacol. Sci.* **1994**, 15, 293

7. Yabe-Nishimura, C. *Pharmacol. Rev.* **1998**, 50, 21

8. El-Kabbani, O.; Wilson, D. K.; Petrash, M.; Quiocho, F. A. *Mol.Vision* **1998**, 4, 19

9. Sestanj, K.; Bellini, F.; Fung, S.; Abraham, N.; Treasurywala, A.; Humber. L.; Simard-Duquesne, N.; Dvornik, D. *J. Med. Chem.* **1984**, 27, 255

10. S. Ao, Y.; Kikuchi. C, Shingu.; Takano, Y.; Nomura, K.; Fujiwara, T.; Ohkubo, Y.; Notsu, Y.; Yamaguchi, I. *Metabolism* **1991**, 40, 77

11. Stribling, D.; Mirrlees D. J.; Harrison, H. E.; Earl , D .C. *Metabolism* **1985,** 34, 336

12. Costantino, L.; Rastelli, G.; Vianello, P.; Cignarella, G.; Barlocco , D. *Med. Res. Rev.* **1999,** 19, 3

13. Fukushi, S.; Merola, L. O.; Kinoshita, J. H. Invest. *Ophthalmol. Visual Sci.* **1980**, 19, 313

14. Malamas, M. S.; Hohman, T. C.; Millen, J. *J. Med. Chem.* **1994**, 37, 2043

15. Asano, T.; Saito, Y.; Kawakami, M.; Yamada, N. *J. Diabetes Complications* **2002**, 16, 133

16. Ramirez, M.A.; Borja, N. L. *Pharmacotherapy* **2008**, 28, 646

17. Liu, K.; Zhu, H. L. *Anticancer Agents Med Chem.* **2011**, 11, 687

18. Yang, Y.; Zhang , S.; Wu, B.; Chen, X.; Qin, X.; He, M.; Hussain, S.; Jing, C.; Ma, B.; Zhu, C. *ChemMedChem.* **2012**, 7, 823

19. Romea, P.; Aragones, M.; Garcia, J.; Vilarrasa, J. *J. Org. Chem.* **1991**, 56, 7038

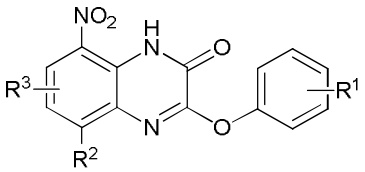
20. Motta, C.; Sartini, S.; Mugnaini, L.; Simorini, F.; Taliani, S.; Salerno, S.; Marini, A. M.; Settimo, F.; Lavecchia, A.; Novellino, E.; Cantor, M.; Failli, P.; Ciuffi, M. *J. Med. Chem.* **2007**, 50, 4917

21. Hayman, S.; Kinoshita, J. H. *J. Biol. Chem.* **1965**, 240, 877

22. Van Zandt, M. C.; Jones, M. L.; Gunn, D. E.; Geraci, L. S.; Jones, J. H.; Sawicki, D. R.; Sredy, J.; Jycot, J. L.; DiCioccio, A. T.; Petrova, T.; Mitschler, A. D, *J. Med. Chem*. **2005**, 48, 3141

170 **Graphical Abstract**

171



R^1 = H, 4-F, 4-Br and 3, 5-diMe
R^2 = H, NO_2
R^3 = diCl, H, NO_2 and Br

172 **IC_{50} = 1.54-18.17 μM**

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