Bioorganic & Medicinal Chemistry 25 (2017) 6353–6360

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure optimization of tetrahydropyridoindole-based aldose reductase inhibitors improved their efficacy and selectivity

Magdalena Majekova, Jana Ballekova, Marta Prnova, Milan Stefek *

Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dubravska cesta 9, 841 04 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 1 August 2017
Revised 29 September 2017
Accepted 5 October 2017
Available online 16 October 2017

Keywords:
Aldose reductase inhibition
Diabetic complications
Carboxymethylated tetrahydropyridoindoles
In vivo diabetic experiment
In silico modeling

ABSTRACT

In our previous study, tetrahydropyridoindoles carboxymethylated in position 8 were identified as aldose reductase (ALR2) inhibitors with mild efficacy and selectivity yet with significant antioxidant activity. In the present study we proceeded with optimization of the tetrahydropyridoindole scaffold by shifting the carboxymethyl pharmacophore from position 8 to position 5, with the aim to improve the biological activity. Commercial databases were screened for the presence of tetrahydropyridoindoles carboxymethylated in position 5 and an experimental set of eight compounds was created. Mild inhibition characterized by IC_{50} in micromolar range was recorded for compound **8** with the isopropyl substituent at the piperidine nitrogen (position 2). This alkylated tertiary nitrogen is characterized by a rather high basicity (pKa ~ 10.4) with complete protonization at physiological pH. On the other hand, ALR2 inhibition activity of the low basicity derivatives **3**–**7** with an acyl substituted nitrogen in position 2 (pKa ~ −1 to −3) was characterized with IC_{50} values in low and medium nanomolar region. Docking into the binding site of human recombinant enzyme AKR1B1 performed for **3** revealed an interaction network responsible for the high affinity and selectivity. In *ex vivo* experiment, sorbitol accumulation in isolated rat eye lenses was significantly inhibited by **3** in the presence of high glucose, starting at a concentration as low as 0.1 μM. Moreover, in streptozotocin-induced diabetic rats, compound **3** administered *intragastrically* (*i.g.,* 50 mg/kg/day) for five consecutive days significantly inhibited sorbitol accumulation in red blood cells and the sciatic nerve. Molecular obesity indices predicted along with water solubility point an excellent "lead-likeness" of compound **3**, with prospects of further structure optimizations.

1. Introduction

Diabetic complications are responsible for increased morbidity and mortality of diabetic patients. Increased flow of glucose through the polyol pathway under conditions of hyperglycemia contributes to the development of diabetic complications. Aldose reductase (ALR2), the first enzyme of the polyol pathway, thus represents an important target of a pharmacotherapy of pathologies related to glucose toxicity. Besides attenuating the activated polyol pathway under hyperglycemic conditions, inhibition of ALR2 was found to be beneficial in pathologies related to inflammation[1,2] which shifted searching for efficient aldose reductase inhibitors (ARIs) upwards to the top of health research issues. Thus in targeting long-term diabetic complications, as well as inflammatory pathologies, ARIs have been gaining increased attention.[3]

Concurrently, a multi-substrate specificity of ALR2 brought a new perspective in the designing of new ARIs. A variety of substrates may interact with the enzyme in multiple interactive modes, which offers a possibility of identifying ARIs, thus allowing to discriminate among different substrates.[4,5]

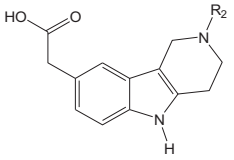
Apart from the high inhibition efficacy, there is an urgent need for other drug-like properties of novel ARIs, namely acceptable bioavailability and minimal toxicity, the properties which have caused failures in clinical trials or withdrawal from the market of several prospective ALR2 inhibitors.

In our previous study,[6] tetrahydropyridoindoles carboxymethylated in position 8 (Fig. 1) were identified as aldose reductase inhibitors with mild efficacy and selectivity yet with significant antioxidant effect as an additional biological activity. Moreover, the compounds were found interesting from the point of their acidobasic behavior. The presence of a basicity center at the tertiary nitrogen, in addition to the acidic carboxylic function, predisposes

Abbreviations: ALR2, aldose reductase; ALR1, aldehyde reductase; ARIs, aldose reductase inhibitors; AKR1B1, aldo–keto reductase family1 member1; AKR1B10, aldo–keto reductase family1 member10; Glc, glucose; Ep, epalrestat.

* Corresponding author.
E-mail address: Milan.Stefek@savba.sk (M. Stefek).

https://doi.org/10.1016/j.bmc.2017.10.005
0968-0896/



R_2: CH_3; $CH_2C_6H_5$; $CH_2CH_2C_6H_5$

Fig. 1. 8-Carboxymethylated tetrahydropyridoindoles with a mild inhibition of aldose reductase.[6]

these compounds to form double charged zwitterionic species,[7] a characteristic which favorably affected their pH-lipophilicity profile.

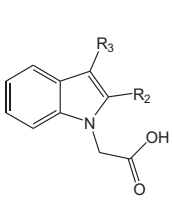
Substituted pyridoindoles represent an interesting group of compounds with a plethora of biological activities.[8–12] We proceeded with optimization of the tetrahydropyridoindole scaffold by shifting the carboxymethyl pharmacophore from position 8 to position 5, with the aim to improve aldose reductase inhibitory efficacy and selectivity. Compounds from the group of 5-carboxymethyl-1,2,3,4-tetrahydro-1*H*-pyrido[4,3-*b*]indoles used to be characterized as antagonists of prostaglandin D2 (CRTH2) receptor[13–17] and as modulators of cannabinoid (CB1) receptor.[18] They have been patented as promising remedies to treat asthma, allergy, androgenic alopecia and pain. Structurally related fused tricyclic compounds comprising indole-1-acetic acid fragment were characterized as aldose reductase inhibitors.[17,19,20]

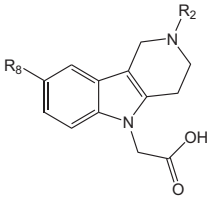
In the present study, an experimental set of 5-carboxymethyl-1,2,3,4-tetrahydro-1*H*-pyrido[4,3-*b*]indoles was created and their inhibitory potency against aldose reductase was tested. Selectivity in relation to the closely related rat kidney aldehyde reductase (ALR1) was determined. Structure–activity relationships supported by molecular docking simulations into the ALR2 binding site along with a computer-based physicochemical profiling of the compounds are discussed. For the most efficient compound, enzyme kinetics was assessed, along with its effect on sorbitol accumulation in isolated rat eye lenses incubated in the presence of high glucose as well as in selected organs in streptozotocin-induced diabetic rats *in vivo*.

2. Results and discussion

2.1. Compounds

ChemSpider database was screened for compounds embedding tetrahydropyridoindole moiety carboxymethylated at position N5. Four additional requirements were preset for final compounds: calculated log P = 0–5, up to six hydrogen bond donors, two to six hydrogen bond acceptors, and LASSO similarity value of 0.99 for ALR2 inhibition. We obtained thus a group of 78 compounds, of which the experimental set of six commercially available 5-carboxymethylated tetrahydropyridoindoles was created and subjected to experimental testing of their inhibitory potency against aldose reductase. In addition, two indole-1-acetic acid derivatives were included for comparison (Fig. 2).





	R_2	R_3
1	CH_3	CHO
2	CH_3	$COCH_3$

	R_2	R_8
3	$COOC_2H_5$	OCH_3
4	$COCH_3$	H
5		H
6	$COOC_2H_5$	COOH
7		F
8	$CH(CH_3)_2$	H

Fig. 2. Compounds **1**–**8**.

Table 1
Inhibition of rat lens ALR2 and rat kidney ALR1.

Compound	ALR2 IC_{50} (nM)	ALR1 IC_{50} (μM)	Selectivity index[a]
1	340.2 ± 37.1	11.8 ± 0.19	35
2	169.1 ± 13.6	11.2 ± 1.89	66
3	12.6 ± 2.20	9.98 ± 2.90	792
4	20.5 ± 1.67	21.9 ± 2.54	1071
5	57.5 ± 3.96	7.54 ± 0.22	131
6	12.7 ± 1.60	4.84 ± 1.46	381
7[b]	141.2 ± 53.0	43.8 ± 0.08	310
8	34 250.0 ± 4 717.2	>100	–
Epalrestat	227.0 ± 18.5	–	–
Valproic acid	–	56.1 ± 2.70	–

[a] Defined as IC_{50}(ALR1)/IC_{50}(ALR2). Results are mean values from three measurements ± SD.
[b] Reported before in Ballekova et al. (2017).[22]

2.2. ALR2 inhibition and molecular modeling

Compounds **1**–**8** were evaluated for their ability to inhibit the *in vitro* reduction of D,L-glyceraldehyde by partially purified ALR2 from rat eye lenses. Epalrestat was used as reference inhibitor (Table 1). Mild inhibition characterized by IC_{50} in micromolar range was recorded for compound **8** with the isopropyl substituent in position 2. This alkylated tertiary nitrogen is characterized by a rather high basicity (pKa ~ 10, MarvinSketch Online 2016/ChemAxon), which ensures its complete protonization at physiological pH. The presence of both basic and acidic centers predisposes these compounds to form double charged zwitterionic species, a characteristic which may remarkably improve their pH-lipophilicity profile.[7] Yet the presence of a positive charge on the tertiary nitrogen has apparently a detrimental effect on AR inhibition efficacy. Similarly, only modest AR inhibition was recorded before for structurally related zwitterionic 8-carboxy-methylated pyridoindoles[6] and amphotheric indole-1-acetic acid derivatives.[21]

On the other hand, AR inhibition activity of the low basicity derivatives **3**–**7** (pKa ~ −1 to −3) is characterized with IC_{50} values in low and medium nanomolar region (Table 1). The results obtained for this set of compounds point to the preference of the enzyme binding site for the flexible carbamate moiety of **3** and **6** contrary to the more rigid alkyl- and aryl-carbonyl substructures of **4, 5** and **7,** respectively. The replacement of the methoxy group in position 8 of compound **3** by a more polar carboxyl in compound **6** did not affect the resulting inhibition activity. Compounds **2**–**7** revealed higher inhibition efficacy in comparison with the clinically used epalrestat. Compound **7** is presently clinically tested under the name setipiprant for treatment of androgenic alopecia.[16,22] Altogether, the results establish the tetrahydropyridoindole skeleton carboxymethylated at position 5 as a prospective