Bioorganic & Medicinal Chemistry 21 (2013) 4301–4310

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of organic nitrates of luteolin as a novel class of potent aldose reductase inhibitors

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ARTICLE INFO

Article history:
Received 19 March 2013
Revised 23 April 2013
Accepted 24 April 2013
Available online 3 May 2013

Keywords:
Diabetic complications
Organic nitrates
Luteolin
Aldose reductase inhibitors

ABSTRACT

Aldose reductase (AR) plays an important role in the design of drugs that prevent and treat diabetic complications. Aldose reductase inhibitors (ARIs) have received significant attentions as potent therapeutic drugs. Based on combination principles, three series of luteolin derivatives were synthesised and evaluated for their AR inhibitory activity and nitric oxide (NO)-releasing capacity in vitro. Eighteen compounds were found to be potent ARIs with IC_{50} values ranging from (0.099 ± 0.008) μM to (2.833 ± 0.102) μM. O^7-Nitrooxyethyl-$O^{3'}$,$O^{4'}$-ethylidene luteolin (**La1**) showed the most potent AR inhibitory activity [IC_{50} = (0.099 ± 0.008) μM]. All organic nitrate derivatives released low concentrations of NO in the presence of L-cysteine. Structure–activity relationship studies suggested that introduction of an NO donor, protection of the catechol structure, and the ether chain of a 2-carbon spacer as a coupling chain on the luteolin scaffold all help increase the AR inhibitory activity of the resulting compound. This class of NO-donor luteolin derivatives as efficient ARIs offer a new concept for the development and design of new drug for preventive and therapeutic drugs for diabetic complications.

1. Introduction

Diabetes mellitus (DM) is a chronic, incurable metabolic disorder defined by the dysregulation of glucose homeostasis manifesting as hyperglycaemia, abnormalities in lipid and protein metabolism, and the development of both acute and long-term complications.[1] According to International Diabetes Federation studies, approximately 366 million people worldwide were diagnosed with diabetes in 2011, and this number is expected to rise to 522 million by 2030.[2] DM is a leading cause of morbidity and mortality in the world, particularly from complications such as macrovascular complications, neuropathy, nephropathy, retinopathy, and cataractogenesis.[3,4] Increasing evidences suggest that aldose reductase may provide a common biochemical link in the pathogenesis of numerous diabetic complications and that the hyperactivity of the polyol metabolic pathway catalysed by AR in individuals with high blood glucose levels contributes to the progression of diabetic complications.[5]

AR is an aldo–keto reductase that catalyses the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of glucose to sorbitol in the first step of the polyol pathway. Sorbitol is subsequently oxidised to fructose by sorbitol dehydrogenase with concomitant reduction of NAD^+ (Fig. 1).[6] Based on these findings, AR has become an attractive molecular target for novel drug design.

ARIs have received attentions as potential therapeutic drugs for the prevention and treatment of diabetic complications.[6,7] Over the last three decades, many compounds with different structures have been reported as ARIs, including alrestatin, tolrestat, epalrestat, zopolrestat, zenarestat, ponalrestat, lidorestat, naphtho[1,2-*d*]isothiazole derivatives, sorbinil, fidarestat, and ranirestat.[8] However, except for epalrestat, none of these compounds are currently marketed. Many of the clinically evaluated ARIs have proven to be inadequate as drug candidates because of their toxic side effects or poor efficacy.[8] Therefore, scientists are exerting much effort into the development of novel ARIs with fewer side effects and excellent efficacy. Interest in flavonoids has steadily increased because of their effectiveness, mild side effects, and relatively low costs.[9–11] A thorough survey of the related literature revealed that flavonoids can modulate the activity of enzymes (such as AR), affect the behaviour of many cell systems, and produce beneficial effects in the body.[12]

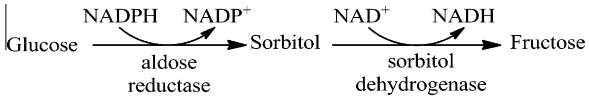

Figure 1. Polyol pathway of glucose metabolism.

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http://dx.doi.org/10.1016/j.bmc.2013.04.066

Luteolin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one), a polyphenolic compound available in food products of plant origin, belongs to the flavone subclass of flavonoids and usually appears in its glycosylated form in celery, green pepper, perilla leaf, and camomile tea.[13] Preclinical studies have shown that this flavone possesses a variety of pharmacological activities, including anti-diabetic, by reducing glucose levels, antioxidant, anti-inflammatory, and anticancer activities.[14] Previous reports have established that luteolin shows significant inhibitory activity (IC_{50} = 0.6 μM) against AR.[15–17] Therefore, luteolin, as the scaffold of ARIs, has considerable potential for the treatment of diabetic complications.

NO as a gaseous signalling molecule participates in a plethora of physiological processes, such as regulation of blood pressure, platelet aggregation, neurotransmission, and immune responses.[18,19] Considering the difficulty of performing meaningful biological studies on NO gas, its progenitors (NO donors) are typically utilised in studies that investigate such diverse effects.[18] Previous observations have shown that NO donors inhibit AR activity and sorbitol accumulation in erythrocytes.[20–22] Several studies have demonstrated that inactivation of AR occurs by modification of a hyper-reactive cysteine residue (Cys298) on the active site of AR by thiol-modifying reagents, NO donors, and nitrosothiols.[22,23] Furthermore, the vascular complications of diabetes are closely associated with a decrease in NO generation. Thus, NO donors could supply adequate amounts of NO to prevent AR activity and diabetic complications.

Our recent studies discovered that a derivative of chrysin **I** (Table 1) exhibited in vitro inhibitory activities against AR (IC_{50} = 0.290 ± 0.009 μM) and advanced glycation end-product formation.[24] This derivative of **I** was even observed to increase the glucose consumption of HepG2 cells.[24] Therefore, to study the effect of variations in the lead compound in comparison with chrysin on AR activity, luteolin derivatives were designed as analogues of compound **I**. We postulated that NO donor hybrids that incorporating the active parts of luteolin may be more potent than any of the initial compounds alone. In this study, we coupled NO donors (organic nitrates) to the 7-position of luteolin through a series of ester or ether chains of different spacers (Fig. 2). The NO-releasing capacities and AR inhibitory activities of the resulting derivatives were evaluated in vitro. We believe that this class of NO-donor luteolin compounds is worthy of further study as potential ARIs for inhibiting the polyol pathway and preventing the development of secondary diabetic complications.

2. Chemistry

All derivatives including **La1–6**, **Lb1–6**, and **Lc1–6** described in this study have been obtained by synthesis starting from luteolin, as shown in Schemes 1–4. The preparation of compounds **La1–6** were outlined in Scheme 1. Treating luteolin with 1,2-dibromoethane at 70 °C for 30 min in anhydrous DMF catalyzed by anhydrous K_2CO_3 yielded compound **1**. Compounds **2a–c** were prepared by treating compound **1** with excessive amounts of the appropriate dibromoalkane at reflux (rt) for 2–24 h in anhydrous acetone.[13] These compounds were then reacted with $AgNO_3$ producing products **La1**, **La3**, and **La5**, respectively.[24] Compounds **La2**, **La4**, and **La6** were synthesised according to the method for **2a–c**.

Compounds **Lb1–6** were synthesised in four or five steps from luteolin as shown in Scheme 2.

Compound **1** was reacted with ethyl bromoacetate to afford compound **3**. Subsequent hydrolysis of this compound and reaction with bromoalkane or dibromoalkane produced compounds **Lb2**, **Lb4**, **Lb6**, and **5a–c**. The intermediates **5a–c** were then reacted with $AgNO_3$ producing products **Lb1**, **Lb3**, and **Lb5**, respectively.

The synthetic route for compounds **Lc1**, **Lc3**, and **Lc5** were summarized in Scheme 3. Luteolin was heated with dichlorodiphenylmethane in diphenyl ether at 175 °C for 30 min yielded compound **6**.[25] Compounds **7a–c** were synthesised according to the method for **2a–c**. Intermediates **7a–c** were then reacted with $AgNO_3$ producing compounds **8a–c**. Subsequent cleavage of the diphenylmethyl group of **8a–c** with a mixture of acetic acid and water (4:1) gave the corresponding nitrate derivatives **Lc1**, **Lc3**, and **Lc5**.[26]

Luteolin was treated with 0.5 equiv bromoalkane and anhydrous K_2CO_3 to producing compounds **Lc2**, **Lc4**, and **Lc6**, respectively (Scheme 4).

3. Results and discussion

3.1. Measurement of nitric oxide

Griess assay is the most popular method for the analysis of NO because of its low costs, simple execution, and straightforward data analysis.[27,28] The capacity of thiol-induced NO generation of organic nitrates of luteolin was evaluated after incubation for 1 h in the presence of L-cysteine. The effectiveness of the synthesised compounds was determined with respect to sodium nitroprusside (SNP) as an NO donor. These results are summarised in Table 1.

The percentages of released NO, which varied from 1.018 ± 0.046% to 4.637 ± 0.040%, were equivalent to those of organic nitrates of chrysin.[24] However, the capacity of NO released from SNP was substantially higher (10.42 ± 1.80%) than organic nitrates of luteolin. These results should be evaluated based on the actual additional amount of NO required by the body. The concentrations of NO required to mediate primarily protective effects are extremely low (picomolar to nanomolar range).[24] In the present study, the release of adequate amounts of NO required to protect the body were balanced with the concentration range demanded for the sufficient activity of luteolin derivatives.

3.2. Aldose reductase inhibitory activity of the target compounds

All newly synthesised derivatives of luteolin were evaluated for their potential inhibitory effect on AR isolated from bovine lenses using quercetin as a reference drug. The assay was based on the spectrophotometric monitoring of NADPH oxidation, which has proven to be a reliable method, with DL-glyceraldehyde as the substrate and NADPH as the cofactor.[24,29] In Table 1, results of the current study were compared with the results previously reported[24] for **I** in a similar assay.

All of the luteolin derivatives exhibited moderate or significant in vitro inhibitory activities on AR with IC_{50} values ranging from (0.099 ± 0.008) μM to (2.833 ± 0.102) μM. Compare with chrysin,[24] the 7-hydroxyl and catechol moiety at the B ring of luteolin could interacts with more AR binding site, therefore, luteolin [(0.754 ± 0.062) μM] exhibited the strong activity. Among the tested compounds, **La1** was the most active ARI, with an IC_{50} value of (0.099 ± 0.008) μM. **La1** was 7.5-fold more potent than luteolin and 28.5-fold more active than quercetin [(2.850 ± 0.040) μM]. These results indicate that replacement of the lead compound with luteolin, as in compounds **La3**, **La1**, and **Lc1**, could improve AR inhibitory activities. **La3** [(0.127 ± 0.011) μM] was 2.3-fold more effective than compound **I** [(0.290 ± 0.009) μM] under the same conditions (Table 1).

Figure 3 shows the AR inhibitory potency of the newly synthesised derivatives and a possible mechanism that explains the structure–activity relationships (SARs) described in the follow section. Figure 3A shows that the AR inhibitory activity of compounds **I**,