Synthesis and Biological Evaluation of Novel Gigantol Derivatives as Potential Agents in Prevention of Diabetic Cataract

Jie Wu1☯, Chuanjun Lu2,3☯, Xue Li1, Hua Fang1, Wencheng Wan1, Qiaohong Yang1, Xiaosheng Sun1, Meiling Wang1, Xiaohong Hu1, C.-Y. Oliver Chen4, Xiaoyong Wei1,4*

1 School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, 510006, China, 2 College of Chemical Engineering, Zhejiang University of Technology, Hangzhou, 310014, China, 3 Institute of Drug Synthesis and Pharmaceutical Processing, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, China, 4 Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, 02111, United States of America

☯ These authors contributed equally to this work.
*
jidewowxy@163.com

Abstract

As a continuation of our efforts directed towards the development of natural anti-diabetic cataract agents, gigantol was isolated from Herba dendrobii and was found to inhibit both aldose reductase (AR) and inducible nitric oxide synthase (iNOS) activity, which play a significant role in the development and progression of diabetic cataracts. To improve its bioefficacy and facilitate use as a therapeutic agent, gigantol (compound 14f) and a series of novel analogs were designed and synthesized. Analogs were formulated to have different substituents on the phenyl ring (compounds 4, 5, 8, 14a-e), substitute the phenyl ring with a larger steric hindrance ring (compounds 10, 17c) or modify the carbon chain (compounds 17a, 17b, 21, 23, 25). All of the analogs were tested for their effect on AR and iNOS activities and on D-galactose-induced apoptosis in cultured human lens epithelial cells. Compounds 5, 10, 14a, 14b, 14d, 14e, 14f, 17b, 17c, 23, and 25 inhibited AR activity, with IC50 values ranging from 5.02 to 288.8 μM. Compounds 5, 10, 14b, and 14f inhibited iNOS activity with IC50 ranging from 432.6 to 1188.7 μM. Compounds 5, 8, 10, 14b, 14f, and 17c protected the cells from D-galactose induced apoptosis with viability ranging from 55.2 to 76.26%. Of gigantol and its analogs, compound 10 showed the greatest bioefficacy and is warranted to be developed as a therapeutic agent for diabetic cataracts.

Introduction

Gigantol (4-[2-(3-hydroxy-5-methoxyphenyl)ethyl]-2-methoxyphenol, PubChem CID: 10221179) is a naturally occurring 1,2-diphenylethene(bibenzyl) found in Herba dendrobii [1]. The literature has shown that gigantol has several bioactions, e.g. anti-carcinogenic [2–5], antioxidant [6], anti-aging [7], anti-coagulating [8], anti-mutagenic [9], antispasmodic [10–12], and anti-inflammatory [13]. Although the structure of gigantol is different from that of more
extensively studied aldose reductase (AR) inhibitors, such as carboxylic acids, spirohydantoin derivatives, and compounds with sulfonyl groups [14–16]. Previous studies have shown that gigantol extracted from dendrobii prevented and inhibited development of cataracts through its inhibitory effect on the activity of AR and inducible nitric oxide synthase (iNOS) [17].

Cataracts are the leading cause of visual impairment and blindness worldwide [18]. The development and progression of cataracts are attributed to a wide range of risk factors, e.g. aging, genetics, radiation, medications, and diseases. Among these factors, chronic hyperglycemia is understood to increase the risk of cataracts because hyperglycemic conditions increase osmotic pressure and induce oxidative damage in lenses, partially through the activation of AR and iNOS [19–22]. AR converts glucose to sorbitol, whose accumulation inside cells in turn causes fluid accumulation, elevates osmotic pressure, and induces lens swelling and degeneration of hydropic lens fibers [23–25]. All of these events enable cataract development. Furthermore, peroxynitrites are formed from superoxides and nitric oxides when iNOS expression and activity is up-regulated by the hyperglycemic condition involved in pathogenesis of cataracts [26].

Due to increasing number of patients with diabetes worldwide, the incidence of diabetic cataracts is steadily increasing [27]. Even though cataract surgery is an effective cure, this operation may not be the best option for all patients because of surgery related health concerns, complications, and costs [28, 29]. For this reason, it is necessary to develop pharmacological therapies for diabetic cataract treatment and prevention. In this context, gigantol could be a suitable drug candidate for the treatment and prevention of diabetic cataracts. However, the limited availability of gigantol from its natural source, Herba dendrobii and other plants, may limit its development and use in diabetic cataract prevention. Thus, to continue investigating applicability of gigantol in diabetic cataracts, chemical synthesis of gigantol and its analogs becomes a viable approach. In addition to serving as a therapeutic agent for diabetic cataracts, some of these analogs could be valuable drug candidates for tumor therapy, local anesthetics, antidepressants, or antipsychotics, and smooth muscle relaxants [30]. Because the bioactivity and bioefficacy of these analogs have not been assessed in diabetic cataracts, the main objective of the study was to synthesize gigantol and its analogs and then assess their effect on the development and progression of diabetic cataracts through modulation of AR and iNOS. The gigantol analogs were synthesized by using different substituents on the phenyl ring (compounds 4, 5, 8, 14a–e), substituting the phenyl ring with a larger steric hindrance ring (compounds 10, 17c), and changing the carbon chain (compounds 17a, 17b, 21, 23, 25). Their bioactions were assessed by determining their capability to inhibit AR and iNOS activity and ameliorate D-galactose-induced death of cultured human lens epithelial cells (HLECs).

Results and Discussion
Synthesis of gigantol and its analogs
The routes of synthesis of gigantol analogs are shown in Figs 1 and 2. Compounds 5 and 8 were synthesized in six steps according to previously reported procedures (Fig 1) [31]. Using commercially available 3,5-dimethoxybenzaldehyde as the starting material, compound 2 was synthesised through reduction, bromination, and reaction with triethylphosphite. Compound 2 served as the starting compound. Wittig olefination, followed by hydrogenation and demethylation, produced compounds 5 and 8. The synthesis of compounds 10, 14, and 14f was similar to that of compound 4, except that the starting material was first protected by chloromethyl methyl ether (MOMCl) and benzyl bromide, respectively (Fig 1). Compounds 17a–c were synthesized in one pot. Amine reacted with aldehyde to produce imine, and NaBH4 was then added to produce the target compounds (Fig 2). Intermediate compound 19 was synthesized by aldol condensation, followed by hydrogenation and demethylation to yield compound 21.
Fig 1. Synthesis of 4, 5, 8, 10, 14, and gigantol. Reagents and conditions: a. NaBH₄, MeOH; b. PBr₃, pyridine, 0°C; c. (OEt)₃, 120°C; d. different aldehydes, CH₃ONa, 0°C to room temperature (RT), 12 h; e. Pd/C, H₂, RT, 12 h; f. BBr₃, CH₂Cl₂, -20°C, 2 h; RT, 4 h; g. NaH, ethanol, DMF, N₂, reflux; h. MOMCl, i-Pr₂NEt, CH₂Cl₂, 0°C, 1 h; RT, 12 h; i. diethyl naphthalen-1-ylmethylphosphonate, CH₃ONa, 0°C, 1 h; rt, 12 h; j. 2 M HCl, methanol, 50°C, 1 h; k. BnBr, 18-crown-6, K₂CO₃, reflux, 9 h.

doi:10.1371/journal.pone.0141092.g001

Fig 2. Synthesis of 17, 21, 23, and 25. Reagents and conditions: a. TsOH, ethanol; 0°C, NaBH₄; b. K₂CO₃, ethanol; c. Pd/C, H₂, RT, 12 h; d. BBr₃, CH₂Cl₂, -20°C, 2 h; RT, 4 h; e. NaH, ethanol, DMF, N₂, reflux, 9 h; f. 180°C, neat, N₂.

doi:10.1371/journal.pone.0141092.g002
As shown in Fig 2, compound 23 was generated by reacting 4-methoxyaniline with 4-methylbenzene-1-sulfonyl chloride followed by the addition of BBr₃. Compound 25 was produced by the reaction of 2-(4-hydroxyphenyl)acetic acid and 2-(3,4-dimethoxyphenyl)ethanamine with stirring at 180°C without solvent under N₂. The purity of all synthesized compounds was determined by HPLC.

Biological activities of gigantol and analogs

Evaluation of AR inhibitory properties. AR has been acknowledged as a validated diabetic cataract inducer [32–36]. Thus, we tested the potential of gigantol and its analogs for prevention and treatment of diabetic cataract by assessing their capability to inhibit AR activity. Table 1 shows that compounds 14a–e were more potent than synthetic gigantol (compound 14f) except compound 14c. Results showed that most synthesized compounds were capable of inhibiting AR activity with their IC₅₀ values ranging from 5.02 to 347.35 μM in a dose-dependent manner, which were at least 5 times lower than the extracted gigantol. Among these synthetic compounds, compounds 23 (5.02 μM), 14a (17.28 μM), 14e (21.83 μM), and 10 (31.86 μM) displayed potency in AR inhibition [37, 38]. Of all tested compounds, sulfonamide compound 23 appeared to be the best inhibitor, suggesting that the N-sulfonylation link might play a critical role in the binding of the compound to the AR catalytic site because sulfonyl group has been reported as an important pharmacophore of AR inhibitors [39–42]. As synthetic gigantol (compound 14f) exhibited intermediate potency, we found that substituting one of the phenyl ring in gigantol with a larger steric hindrance naphthalene ring made the compound 9-fold more potent. In order to study the role of the 4-hydroxy-3-methoxyphenyl ring in the AR inhibition, we synthesized compounds 14a–e by keeping 4-hydroxy-3-methoxyphenyl ring and placing different substituents on the other phenyl ring, and results showed that significance of the 4-hydroxy-3-methoxyphenyl ring in AR inhibition. These results suggest that compounds 10, 14a, 14e, and 23 can be considered as lead compounds for further development of new diabetic cataract drugs.

Assessment of anti-iNOS inhibitory properties. The role of iNOS in the development of diabetic cataracts has been well documented [21]. Results showed that compounds 5, 10, 14b, and 14f inhibited iNOS in a dose-dependent manner with IC₅₀ values ranging from 432.6 to 1188.7 μM (Table 2). Although the IC₅₀ of compounds 5, 10, 14b, and 14f was larger than that of the extracted gigantol, these compounds remain good candidates for the development of diabetic cataract drugs because of their superior AR inhibitory effect.

Table 1. Inhibitory effect of gigantol and its analogs on AR activity.

| Compound | IC₅₀ (μM) | Compound | IC₅₀ (μM) |
|----------|----------|----------|----------|
| Extractive gigantol | 2516.6 ± 10.35 | 14e | 21.83 ± 5.47 |
| 4 | 347.35 ± 3.74 | 14f | 176.06 ± 3.21* |
| 5 | 288.80 ± 2.16 | 17a | NA |
| 8 | 513.38 ± 2.33 | 17b | 173.73 ± 3.38 |
| 10 | 31.17 ± 1.51 | 17c | 242.67 ± 5.67 |
| 14a | 17.28 ± 1.72 | 21 | 556.34 ± 4.37 |
| 14b | 125.94 ± 1.3 | 23 | 5.02 ± 2.57 |
| 14c | 534.35 ± 5.44 | 25 | 54.44 ± 2.39 |
| 14d | 39.20 ± 2.13 | | |

*The results are expressed as mean ± SD (n = 3).
Abbreviation: NA, no activity

*P < 0.01, vs. Extractive gigantol.

doi:10.1371/journal.pone.0141092.t001
Table 2. Inhibitory effect of gigantol and its analogs on iNOS activity.

| Compound         | IC50 (μM)       | Compound | IC50 (μM)       |
|------------------|-----------------|----------|-----------------|
| Extractive gigantol | 32.23 ± 2.61 | 14e NA   | 680.07 ± 3.28   |
| 4                | NA              | 14f NA   | NA              |
| 5                | 432.6 ± 2.37    | 17a NA   | NA              |
| 8                | NA              | 17b NA   | NA              |
| 10               | 1188.7 ± 3.31   | 17c NA   | NA              |
| 14a              | NA              | 21 NA    | NA              |
| 14b              | 433.57 ± 4.23   | 23 NA    | NA              |
| 14c              | NA              | 25 NA    | NA              |
| 14d              | NA              | NA       | NA              |

1 The results are expressed as mean ± SD (n = 3). Abbreviation: NA, no activity.

Evaluation of the effects on D-galactose-induced cell death in HLECs

Galactose toxicity causes the sequential death of different LEC populations in the lenses of galactosemic rats, starting with those in the central and peripheral mitotic zone, followed by the central (non-mitotic) LECs, and eventually the remaining LECs [43, 44]. In this study, the protective effect of the synthetic compounds was tested at concentrations 0.1, 0.5, and 1 μg·mL⁻¹ on D-galactose-induced apoptosis of the cultured HLECs. Results showed that extracted gigantol (1.0 μg·mL⁻¹, 5.28 μM) and compounds 5 (0.5 μg·mL⁻¹, 1 μM), 8 (1.0 μg·mL⁻¹, 4.39 μM), 10 (0.5 μg·mL⁻¹, 0.89 μM), 14b (0.1 μg·mL⁻¹, 0.366 μM), 14f (1.0 μg·mL⁻¹, 5.28 μM), and 17c (0.1 μg·mL⁻¹, 0.388 μM) protected HLECs from apoptosis. Of all compounds tested, compound 10 showed the most efficacious protection against apoptosis with the cell survival reaching 72.26% (P<0.05) (Fig 3). Given that apoptosis of HLEC contributes greatly to cataract formation, protecting HLECs against programmed cell death appears to

![Fig 3](https://example.com/fig3.png)

**Fig 3.** Gigantol analogs at 0.1, 0.5, and 1.0 μg mL⁻¹ on viability of HLECs treated with 250 mmol L⁻¹ D-galactose for 72 h. Cell viability was determined by the MTT assay in the absence (Con) and presence (all other groups) of D-galactose. Ext-G refers to gigantol extracted from dendrobii. Viability (mean ± SD, n = 3) is expressed as the percentage of viable cells in the treatment to those of the Con. *P < 0.01 vs. Con, **P < 0.05 vs. D-galactose.

doi:10.1371/journal.pone.0141092.g003
be one of the therapeutic strategies for cataract treatment [45]. Results show that extracted gigantol and compounds 5, 8, 10, 14b, 14f, and 17c were the most effective in the protection of LEC against D-galactose-induced apoptosis.

Conclusions

Gigantol and its synthetic analogs were evaluated for their potential to treat diabetic cataracts. Synthetic compounds were designed by placing different substituents on the phenyl ring (compounds 4, 5, 8, 14a-e), substituting the phenyl ring with a larger steric hindrance ring (compounds 10, 17c), and changing the carbon chain (compounds 17a, 17b, 21, 23, 25). We found compounds 4, 5, 10, 14b, 14d, 14e, 14f, 17c, 23, and 25 inhibited AR activity with the IC50 value ranging from 5.02 to 347.35 μM and compounds 5, 10, 14b, and 14f inhibited iNOS activity with the IC50 values ranging from 432.6 to 1188.7 μM. Furthermore, compounds 5, 8, 10, 14b, 14f, and 17c and the extracted gigantol protected HLECs from apoptosis. Among all of the test compounds, only synthesized gigantol and its analogs 5, 10, and 14b were effective to inhibited AR and iNOS activities and reduced HLEC apoptosis. Of these 3 compounds, compound 10 (3-methoxy-5-(2-(naphthalen-1-yl)ethyl)phenol) showed the most pronounced protective effect against apoptosis, and further studies are needed to test its candidacy as a diabetic cataract drug, starting with the bioavailability, efficacy, and toxicity in in vivo experiments.

Materials and Methods

Synthesis

Mass spectrometry was performed on an Agilent LC–MS 6120 system equipped with an ESI mass spectrometer. Melting points were determined using an SRS OptiMelt Automated Melting Point System. NMR spectra were generated on a Bruker Avance III spectrometer (S1 File), using tetramethylsilane (TMS) as an internal standard. The purity of synthesized compounds was verified by an HPLC system equipped with an Eclipse Plus C8 column (4.6 × 150 mm, 5 μm). Compound 12 was synthesized according to the previously reported procedure [46].

**General procedure 1.** Phosphate (1.5 equiv.) and sodium methoxide (3 equiv.) were mixed in DMF and stirred for 30 min at 0°C. Then, 1 equiv. aldehyde was added to DMF under nitrogen. The resulting mixture was stirred overnight at room temperature, quenched by addition of ice-cold water, and extracted using ethyl acetate. After the removal of organic solvents, the crude product was purified using silica gel chromatography with ethyl acetate/petroleum ether as the eluent.

**General procedure 2.** To compound dissolved in methanol, 10% Pd/C was added and the resulting mixture was stirred overnight at room temperature under hydrogen. The weight of compound to Pd/C was 10:1. After the reaction mixture was filtrated and concentrated, the crude product was purified either by recrystallization or silica gel chromatography to yield the target product.

**General procedure 3.** BBr3 (4 equiv.) was added dropwise to a 1 equiv. solution of compound in dried CH2Cl2 at -20°C under nitrogen. The resulting solution was slowly warmed to room temperature and stirred overnight. Then, ice-cold water was slowly added and the mixture filtered to yield the crude product. The final product was obtained after purification using recrystallization or silica gel chromatography.

**General procedure 4.** p-Toluenesulfonic acid (0.2 equiv) was added to a solution of amine (1.0 equiv) and aldehyde (1.0 equiv) in ethanol, and the resulting mixture was stirred at room temperature. When the raw material faded (as monitored by TLC), NaBH4 (3.0 equiv) was added at 0°C. The mixture was stirred for 1 h, followed by addition of water and extraction.
using ethyl acetate. Finally, all solvents were removed under reduced pressure to obtain the crude product, which was purified by flash chromatography on silica gel.

**\((E)-1,2\text{-bis(3,5-dimethoxyphenyl)}\text{ethane (3)}\).** Compound 3 was synthesized according to the general procedure 1. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 30:1) to yield 3 (0.51 g, 85%) as a white solid. 

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.01 (s, 2H), 6.67 (d, \(J = 2.2\) Hz, 4H), 6.41 (t, \(J = 2.2\) Hz, 2H), 3.83 (s, 12H). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 161.0, 139.2, 129.2, 104.7, 100.2, 55.4. LC/MS (ESI): 254.1 [M+H]+. HPLC purity: 97.1%.

**1,2\text{-bis(3,5-dimethoxyphenyl)}\text{ethane (4)}**. Compound 4 was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 40:1) to yield 4 (0.26 g, 87%) as a white solid. mp: 104.6 – 107.9°C. 1H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.36 – 6.35 (m, 4H), 6.33 – 6.31 (m, 2H), 3.77 (d, \(J = 1.1\) Hz, 12H), 2.85 (s, 4H). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 160.8, 144.1, 106.5, 98.0, 55.3, and 38.0. LC/MS (ESI): 303.1 [M+H]+. HPLC purity: 96.3%.

**5,5\text{-')(ethane-1, 2-diyl)dibenzene-1,3-diol**. Compound 5 was synthesized according to the general procedure 3. The crude product was purified by recrystallization with MeOH to yield 5 (0.11 g, 68%) as a white solid. mp: 245.9 – 248.1°C. 1H NMR (400 MHz, MeOD): \(\delta\) 6.15 (s, 4H), 6.09 (s, 2H), 2.69(s, 4H). \(^1\)C NMR (101 MHz, MeOD): \(\delta\) 159.3, 145.6, 108.0, 101.2, 39.0. LC/MS (ESI): 247.1 [M+H]+. HPLC purity: 99.4%.

\(3\text{-hydroxy-5-methoxybenzaldehyde (9)}\). Compound 9 was synthesized using previously reported procedures [47]. The characteristic of the compound is white solid and mp: 67 – 68°C, the yield was 53%. 1H NMR (400 MHz, MeOD) \(\delta\) 9.83 (s, 1H), 6.93 (ddd, \(J = 14.6, 2.2, 1.3\) Hz, 2H), 6.65 (t, \(J = 2.3\) Hz, 1H), 3.82 (s, 3H). \(^1\)C NMR (101 MHz, MeOD) \(\delta\) 194.0, 162.9, 160.6, 140.1, 110.0, 108.8, 106.7, 56.0.

**3\text{-methoxy-5-(2-(naphthalen-1-yl)ethyl)phenol (10)}**. Compound 10 was synthesized with compound 9 according to the general procedure 1 after protecting by MOMCl, following by deprotection and hydrogenation to produce the title product. The crude product was purified over silica gel column chromatography (petroleum ether: ethyl acetate = 2:1) yielding 10 (0.20 g, 72%) as a white solid. 1H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.08 (d, \(J = 8.1\) Hz, 1H), 7.87 (d, \(J = 7.6\) Hz, 1H), 7.73 (d, \(J = 8.1\) Hz, 1H), 7.60–7.44 (m, 2H), 7.39 (t, \(J = 7.6\) Hz, 1H), 7.30 (d, \(J = 6.9\) Hz, 1H), 6.45–6.21 (m, 3H), 4.87 (br, 1H), 3.76 (s, 3H), 3.46–3.24 (m, 2H), 3.08–2.85 (m, 2H). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 160.9, 156.8, 144.8, 137.7, 133.9, 131.8, 129.8, 126.0, 125.9, 125.6, 108.0, 99.2, 55.3, 37.1, 34.7. LC/MS (ESI): 279.1 [M+H]+. HRMS calcd for C19H18O2 [M+H]+: 279.1380, found: 279.1381. HPLC purity: 95.0%.

\(\text{(E)-2-(benzyloxy)-5-(4-(benzyloxy)-3-methoxystyryl)-1,3-dimethoxybenzen (13a)}\). Compound 13a was synthesized according to the general procedure 1. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 20:1) to yield 13a (0.25 g, 52%) as a white solid. 1H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.49 (d, \(J = 6.9\) Hz, 2H), 7.45 (d, \(J = 7.2\) Hz, 2H), 7.34 (ddd, \(J = 22.4, 13.2, 7.1\) Hz, 6H), 7.07 (d, \(J = 1.7\) Hz, 1H), 7.01–6.95 (m, 1H), 6.89 (dd, \(J = 18.3, 8.4\) Hz, 3H), 6.70 (s, 2H), 5.18 (s, 2H), 5.02 (s, 2H), 3.95 (s, 3H), 3.87 (s, 6H).
yield 13c product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 13c (0.15 g, 33%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.53–7.46 (m, 2H), 7.46–7.41 (m, 2H), 7.41–7.26 (m, 7H), 7.21 (d, J = 7.9 Hz, 1H), 7.06 (q, J = 8.0 Hz, 1H), 7.02–6.95 (m, 2H), 6.86 (dd, J = 8.8, 8.2, 6.8 Hz, 3H), 5.16 (s, 2H), 5.01 (s, 2H), 3.89 (s, 3H), 3.87 (s, 3H).

(E)-(4-(4-benzyloxy)-3-methoxystyril)-1,2-phenylene)bis(oxy)bis(methylene) dibenzene (13c).  Compound 13c was synthesized according to the general procedure 1. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 13c (0.24 g, 45%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.50–7.41 (m, 6H), 7.41–7.28 (m, 9H), 7.12 (s, 1H), 7.04 (s, 2H), 6.92 (s, 2H), 6.85 (s, 3H), 5.20 (s, 2H), 5.17 (s, 4H), 3.94 (s, 3H).

(E)-1-(benzyloxy)-4-(3,5-dimethoxystyryl)-2-methoxyphenol (14b).  Compound 14b was synthesized according to general procedure 2. The crude product was purified by column chromatography (petroleum ether: acetone = 5:1) to yield 14b (0.24 g, 45%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.41 (m, 6H), 7.41 (m, 2H), 6.85 (m, 2H), 6.62 (d, J = 7.2 Hz, 1H), 6.76 (d, J = 7.9 Hz, 1H), 6.69 (dd, J = 17.3, 9.1 Hz, 2H), 6.92–6.85 (m, 2H), 6.65 (d, J = 2.2 Hz, 2H), 6.38 (t, J = 2.2 Hz, 1H), 5.18 (s, 2H), 3.95 (s, 3H), 3.83 (s, 3H).

(E)-1-(benzyloxy)-2-(4-(benzyloxy)-3-methoxystyryl)-4-chlorobenzene (13e).  Compound 13e was synthesized according to the general procedure 1. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 13e (0.15 g, 40%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, J = 2.5 Hz, 1H), 7.44 (d, J = 7.4 Hz, 4H), 7.41–7.26 (m, 7H), 7.09 (ddd, J = 16.4, 7.9, 4.0 Hz, 3H), 7.01–6.93 (m, 1H), 6.85 (d, J = 8.5 Hz, 2H), 5.17 (s, 2H), 5.12 (s, 2H), 3.91 (s, 3H).

(4-hydroxy-3-methoxyphenethyl)-2,6-dimethoxyphenol (14a).  Compound 14a was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 14a (0.10 g, 66%) as a blue solid. mp: 78.2–79.5°C. ¹H NMR (400 MHz, CDCl₃) δ 6.83 (d, J = 7.9 Hz, 1H), 6.67 (d, J = 7.9 Hz, 1H), 6.61 (s, 1H), 6.35 (s, 2H), 5.52 (br, 2H), 3.82 (d, J = 3.6 Hz, 9H), 2.81 (s, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 146.9, 146.3, 143.8, 133.7, 132.92, 132.88, 132.11, 114.3, 111.4, 105.3, 56.3, 55.9, 38.4, 37.9. LC/MS (ESI): 305.1 [M+H]+. HPLC purity: 99.7%.

(2-hydroxy-3-methoxyphenethyl)-2-methoxyphenol (14b).  Compound 14b was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: acetone = 5:1) to yield 14b (0.09 g, 99%) as a red oil. ¹H NMR (400 MHz, CDCl₃) δ 7.50 (d, J = 8.0 Hz, 1H), 7.46 (m, 2H), 7.45 (m, 2H), 7.41 (m, 2H), 6.99 (dd, J = 16.4, 7.9, 4.0 Hz, 3H), 7.01–6.93 (m, 1H), 6.85 (d, J = 8.5 Hz, 2H), 5.17 (s, 2H), 5.12 (s, 2H), 3.91 (s, 3H).

(4-hydroxy-3-methoxyphenethyl)benzene-1,2-diol (14c).  Compound 14c was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: acetone = 5:1) to yield 14c (0.06 g, 53%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) δ 6.82 (d, J = 8.0 Hz, 1H), 6.72 (ddd, J = 8.9, 8.3, 3.9 Hz, 5H), 5.77 (br, 1H), 5.53 (br, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 2.96–2.79 (m, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 146.4, 146.3, 143.7, 143.6, 134.4, 127.8, 122.5, 121.1, 119.3, 114.2, 111.3, 108.6, 56.0, 55.9, 35.7, 32.3. LC/MS (ESI): 273.0 [M-H]⁻. HPLC purity: 95.0%.

(3,5-dimethoxyphenethyl)-2-methoxyphenol (14d).  Compound 14d was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 14d (0.10 g, 90%) as a blue oil. ¹H NMR (400 MHz, CDCl₃) δ 6.83 (d, J = 8.0 Hz, 1H), 6.69 (ddd, J = 8.0, 1.7 Hz, 1H), 6.63 (d, J = 1.6 Hz,
1H), 6.32 (dd, J = 7.4, 2.0 Hz, 3H), 5.54 (br, 1H), 3.83 (s, 3H), 3.76 (s, 6H), 2.83 (s, 4H). 13C NMR (101 MHz, CDCl3) δ 160.7, 146.3, 144.2, 143.8, 133.7, 121.0, 114.3, 111.2, 106.6, 98.0, 55.9, 55.3, 38.6, 37.4. LC/MS (ESI): 289.1 [M+H]+. HPLC purity: 96.9%.

4-chloro-2-(4-hydroxy-3-methoxyphenethyl)phenol (14e). Compound 14e was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 1:1) to yield 14e (0.05 g, 46%) as a colorless oil. 1H NMR (400 MHz, CDCl3) δ 7.06 (d, J = 1.6 Hz, 1H), 7.03 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.68 (t, J = 8.3 Hz, 2H), 6.61 (s, 1H), 5.52 (br, 1H), 4.82 (br, 1H), 3.82 (s, 3H), 2.83 (s, 4H). 13C NMR (101 MHz, CDCl3) δ 152.3, 146.4, 143.9, 133.4, 130.1, 129.8, 127.0, 125.4, 121.0, 116.7, 114.4, 111.2, 55.9, 35.7, 32.5. LC/MS (ESI): 277.0 [M-H]-. HRMS calcd for C15H15O3Cl [M-H]-: 277.0637, found: 277.0644. HPLC purity: 99.9%.

4-[2-(3-hydroxy-5-methoxyphenyl)ethyl]-2-methoxyphenol (14f, gigantol). Compound 14f was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 5:1) to yield gigantol (0.50 g, 68%) as a white solid. 1H NMR (400 MHz, CDCl3) δ 6.83 (d, J = 8.0 Hz, 1H), 6.68 (dd, J = 8.0, 1.8 Hz, 1H), 6.62 (d, J = 1.8 Hz, 1H), 6.31 (s, 1H), 6.25 (d, J = 2.0 Hz, 2H), 5.47 (s, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 2.80 (dt, J = 11.8, 5.9 Hz, 4H). 13C NMR (101 MHz, CDCl3) δ 160.9, 156.6, 146.3, 144.6, 143.8, 133.6, 121.0, 114.2, 111.2, 108.1, 106.9, 99.1, 55.9, 55.3, 38.3, 37.3. HPLC purity: 98.5%.

3-methoxy-5-((4-methoxyphenylamino)methyl)phenol (17a). Compound 17a was synthesized according to the general procedure 4. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 3:1) to yield 17a (0.19 g, 74%) as a yellow oil. 1H NMR (400 MHz, CDCl3) δ 6.77 (d, J = 8.9 Hz, 2H), 6.59 (d, J = 8.9 Hz, 2H), 6.49 (s, 1H), 6.42 (s, 1H), 6.29 (s, 1H), 4.18 (s, 2H), 3.75 (s, 3H), 3.73 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 161.1, 157.3, 152.4, 142.3, 142.2, 115.0, 114.8, 107.0, 105.5, 100.3, 55.9, 55.3, 49.4. LC/MS (ESI): 260.1 [M+H]+. HRMS calcd for C15H17NO3 [M+H]+: 260.1281, found: 260.1287. HPLC purity: 95.3%.

4-((3,4-dimethoxyphenethylamino)methyl)phenol (17b). Compound 17b was synthesized according to the general procedure 4. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 5:1) to yield 17b (0.24 g, 82%) as a white solid. mp: 116.7–118.6°C. 1H NMR (400 MHz, CDCl3) δ 7.05 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.1 Hz, 1H), 6.76–6.72 (m, 2H), 6.62 (d, J = 8.5 Hz, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.71 (s, 2H), 2.93 (t, J = 7.0 Hz, 2H), 2.81 (t, J = 6.9 Hz, 2H). 13C NMR (101 MHz, CDCl3) δ 156.3, 149.0, 147.5, 131.9, 129.7, 129.6, 120.6, 115.9, 112.0, 111.5, 55.9, 55.8, 53.2, 50.2, 35.1. LC/MS (ESI): 288.1 [M+H]+. HPLC purity: 98.0%.

2-((benzo[d]thiazol-2-ylamino)methyl)-5-methoxyphenol (17c). Compound 17c was synthesized according to the general procedure 4. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 4:1) to yield 17c (0.19 g, 65%) as a white solid. mp: 184.3–185.7°C. 1H NMR (400 MHz, MeOD) δ 7.57 (dd, J = 7.9, 0.7 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.26 (td, J = 7.8, 1.2 Hz, 1H), 7.16 (d, J = 8.8 Hz, 1H), 7.06 (td, J = 7.8, 1.1 Hz, 1H), 6.48–6.36 (m, 2H), 4.49 (s, 2H), 3.74 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 167.8, 160.8, 156.8, 150.4, 131.5, 130.1, 129.7, 126.0, 121.9, 120.8, 118.1, 117.8, 106.2, 103.2, 55.2, 54.2, 35.1. LC/MS (ESI): 287.0 [M+H]+. HRMS calcd for C15H14N2O2S [M+H]+: 287.0849, found: 287.0863. HPLC purity: 97.6%.

(E)-3-(3,5-dimethoxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one (19). A mixture of 1-(4-fluorophenyl)ethanone (2 mmol) and 3,5-dimethoxybenzaldehyde (2.2 mmol) in ethanol was added to K2CO3 (20 mmol) and stirred at room temperature overnight. After thorough extraction with EtOAc, the combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude
compound 19 was purified using silica gel column chromatography with EtOAc/petroleum ether (1/20) as eluent to produce 0.51 g yellow oil, yield: 89%. \( ^1 \text{H NMR (400 MHz, CDCl}_3 \) δ 8.05 (t, \( J = 6.0 \) Hz, 2H), 7.72 (d, \( J = 15.7 \) Hz, 1H), 7.44 (d, \( J = 15.7 \) Hz, 1H), 7.18 (t, \( J = 7.9 \) Hz, 2H), 6.77 (s, 2H), 6.54 (s, 1H), 3.84 (s, 6H).

1-(3-(4-fluorophenyl)propyl)-3,5-dimethoxybenzene (20). Compound 20 was synthesized according to the general procedure 2. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 10:1) to yield 20 (0.23 g, 48%) as a colorless oil. \( ^1 \text{H NMR (400 MHz, CDCl}_3 \) δ 7.18–7.01 (m, 2H), 7.00–6.85 (m, 2H), 6.43–6.17 (m, 3H), 3.74 (s, 6H), 2.69–2.42 (m, 2H), 2.71–2.38 (m, 2H), 2.13–1.65 (m, 1H), 2.05–1.79 (m, 1H).

5-(3-(4-fluorophenyl)propyl)benzene-1,3-diol (21). Compound 21 was synthesized according to the general procedure 3. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 4:1) to yield 21 (0.19 g, 92%) as a yellow oil. \( ^1 \text{H NMR (400 MHz, CDCl}_3 \) δ 7.06 (s, 2H), 6.92 (t, \( J = 8.4 \) Hz, 2H), 6.22 (s, 2H), 6.18 (s, 1H), 2.55–2.51 (m, 2H), 2.45–2.41 (s, 2H), 1.81 (d, \( J = 4.7 \) Hz, 2H). \( ^{13} \text{C NMR (101 MHz, CDCl}_3 \) δ 162.4, 160.0, 156.4, 145.7, 137.7, 137.7, 129.7, 127.7, 115.1, 114.9, 108.3, 100.4, 35.1, 34.4, 32.4. LC/MS (ESI): 247.1 [M+H]⁺. HRMS calcd for C₁₅H₁₅O₂F [M-H]-: 245.0983, found: 245.0987. HPLC purity: 98.8%.

N-(4-hydroxyphenyl)-4-methylbenzenesulfonamide (23). Compound 23 was synthesized according to the general procedure 3. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 5:1) to yield 23 (0.17 g, 63%) as a yellow oil. \( ^1 \text{H NMR (400 MHz, MeOD) \) δ 7.31 (d, \( J = 8.2 \) Hz, 2H), 7.04 (d, \( J = 7.9 \) Hz, 2H), 6.61 (d, \( J = 8.8 \) Hz, 2H), 6.38 (d, \( J = 8.8 \) Hz, 2H), 3.15–3.03 (m, 3H). \( ^{13} \text{C NMR (101 MHz, CDCl}_3 \) δ 159.2, 147.3, 140.4, 133.0, 132.7, 130.9, 128.9, 119.1, 24.0. LC/MS (ESI): 262.0 [M-H]-. HPLC purity: 99.8%.

N-(3,4-dimethoxyphenethyl)-2-(4-hydroxyphenyl)acetamide (25). 2-(4-hydroxyphenyl)acetic acid (1.0 mmol) and 2-(3,4-dimethoxyphenyl)ethanamine (1.0 mmol) were mixed and stirred at 180°C for 4 hours under N₂. Crude product 25 was purified by recrystallization using acetonitrile to produce 0.25 g gray solid. Yield: 80%, mp: 151.7–153.2°C. \( ^1 \text{H NMR (400 MHz, MeOD) \) δ 7.02 (d, \( J = 8.4 \) Hz, 2H), 6.80 (dd, \( J = 15.8, 4.9 \) Hz, 2H), 6.71 (d, \( J = 8.5 \) Hz, 2H), 6.68–6.62 (m, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.39 (t, \( J = 7.1 \) Hz, 2H), 3.35 (s, 2H), 2.71 (t, \( J = 7.0 \) Hz, 2H). \( ^{13} \text{C NMR (101 MHz, CDCl}_3 \) δ 174.6, 157.5, 150.4, 149.1, 133.3, 131.1, 127.6, 122.3, 116.4, 113.8, 113.2, 56.6, 55.4, 43.2, 42.0, 35.9. LC/MS (ESI): 316.1 [M+H]⁺. HPLC purity: 98.1%.

Biological assay

AR inhibition assays. AR was purchased from Prospec-TanyTechnogene Ltd. (Israel). AR activity was evaluated as previously described [48, 49]. Briefly, the reaction mixture was composed of enzyme solution (20 mmol/L, 20 μL), NADPH (0.104 mmol/L, 50 μL; Italian Roth, Italy), DL-glyceraldehyde (10 mmol/L, 50 μL; Sigma, U.S.), buffer phosphate (0.1 mol/L), and selected concentrations of test compounds. The reaction was initiated by the addition of DL-glyceraldehyde as the substrate, and PBS was used as the blank control. The use of NAPDH, in parallel to AR activity, was monitored at room temperature for 10 min at 40 s intervals at 340 nm in an EnSpire™ Multimode Plate Reader. AR inhibition was expressed as inhibition rate (%) = \[ 1 - (A_2 - A_0)/(A_1 - A_0) \] ×100% [50], where \( A_0 \) represents the decrease in NADPH absorbance without AR, substrate, or compounds; \( A_1 \) represents the decrease in NADPH absorbance prior to the test compounds addition; \( A_2 \) represents decrease in NADPH absorbance following the test compounds addition. All assays were performed in triplicate.

iNOS inhibition assays. iNOS produces NO by catalyzing a reaction involving L-Arg and oxygen. Its activity was assessed by monitoring NO production using a Nitric Oxide Synthase Assay Kit (Nanjing Jiancheng Bioengineering Institute, China), which was developed based on
the method of Fröhlich et al. [51]. The magnitude of iNOS inhibition is expressed as inhibition rate which was calculated using the following equation: 

\[
\% = \frac{(B_1 - B_2)}{(B_1 - B_0)} \times 100\% 
\]

where \(B_0\) and \(B_1\) represented the absorbance values obtained for the blank (control solution) and standard, respectively, and \(B_2\) represented the absorbance of the test compounds. All assays were performed in triplicate.

**Cell viability assays.** Human lens epithelial cells (HLECs; SRA 01/04) were obtained from Dr. Fu Shang, USDA HNRCA at Tufts University, Boston, MA, U.S. and Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, P. R. China [52, 53] and cultured in minimal essential medium (MEM) supplemented with 20% fetal bovine serum (FBS) and a cocktail of Penicillin-Streptomycin at 37°C in a humid environment containing 5% CO₂ [52]. Cells were harvested at 80% confluency by trypsinization, and then fresh culture medium was added to generate single-cell suspensions for use in cell viability assays. HLECs were then seeded in 96-well cell culture grade microplates at a density of \(1 \times 10^5\) cells/mL. After 24 h of incubation, the cells were treated for 72 h with 250 mmol/L D-galactose with and without test compounds (0.1, 0.5, or 1.0 μg/mL) [45].

Cell viability was assessed using an MTT assay. After the treatments, 20 μL of 5 mg·mL⁻¹ MTT solution was added to each well, followed by incubation for 4 h at 37°C. The resulting formazan crystals were then dissolved in 150 μL DMSO and absorbance measured at 570 nm using an EnSpire™ Multimode Plate Reader. The results are presented as percentages of cell survival as compared to the untreated control group, and all assays were performed in triplicate.

**Statistical methods.** Statistical analyses and data processing were performed using the SPSS v.16.0 statistical software. Cell viability data are presented as mean ± SD. One-way ANOVA was performed to assess statistical significance between the test compounds. \(P < 0.05\) was considered statistically significant.

**Supporting Information**

S1 File. Contents: ^1H and ^13C NMR spectra of target compounds.

(DOCX)

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (81274157, 81102674), Guangzhou Science & Technology Planning Project (2014J1400082), Guangdong Natural Science Foundation (S2011010005661), Guangdong Science & Technology Planning Project (2011B031700076, 2009B093003335) and the U.S. Department of Agriculture (USDA)/Agricultural Research Service (Cooperative Agreement No. 1950-5100-087).

**Author Contributions**

Conceived and designed the experiments: XW. Performed the experiments: CL JW XL. Analyzed the data: CL JW. Contributed reagents/materials/analysis tools: HF. Wrote the paper: XW JW CL XL HF WW QY XS MW XH CYOC.

**References**

1. Yang L, Wang Z, Xu L (2006) Simultaneous determination of phenols (bibenzyl, phenanthrene, and fluorenone) in Dendrobium species by high-performance liquid chromatography with diode array detection. Journal of Chromatography A 1104:230–237. PMID:16378617

2. Miyazawa M, Shimamura H, Nakamura S, Kameoka H (1997) Antimutagenic activity of Gigantol from Dendrobium nobile. Journal of Agricultural and Food Chemistry 45: 2849–2853
3. Hart JH (1981) Role of phytostilbenes in decay and disease resistance. Annual Review of Phytopathology. 19: 437–458.

4. Chanvorachote P, Kowitdamrong A, Ruanghirun T, Sritularak B, Likhitwitayawuid (2013) Natural Product Communications 8:115–118.

5. Song JX, Shaw PC, Sze CW, Tong Y, Yao XS, Ng TB (2010) Chrysotoxine, a novel bibenzyl compound, inhibits 6-hydroxydopamine induced apoptosis in SH-SY5Y cells via mitochondria protection and NF-κB modulation. Neurochemistry International, 57:676–689.

6. Simmler C, Antheaume C, Lobstein A (2010) Antioxidant biomarkers from Vanda coerulea stems reduce irradiated HaCaT PGE-2 production as a result of COX-2 inhibition. PLoS One 5(10): e13713. doi: 10.1371/journal.pone.0013713 PMID: 21060890

7. Tezuka Y, Hirano H, Kikuchi T, Xu GJ (1991) Constituents of Ephemerantha lonchophylla, isolation and structure elucidation of new phenolic compounds, Ephemeranthol-A, Ephemeranthol-B, and Ephemeranthoquinone, and of a new diterpene glucoside, Ephemeranthoside. Chemical & Pharmaceutical Bulletin 39:593–598.

8. Fan C, Wang W, Wang Y, Qin G, Zhao W (2001) Chemical constituents from Dendrobium densiflorum. Phytochemistry 57:1255–1258. PMID:11454354

9. Estrada-Soto S, López-Guerrero JJ, Villalobos-Molina R, Mata R (2006) Endothelium-independent relaxation of aorta rings by two stilbenoids from the orchids Scaphyglottis livida. Fitoterapia 77:236–239. PMID:16533577

10. Hernández-Romero Y, Acevedo L, Sánchez Mde L, Shier WT, Abbas HK (2005) Phytotoxic activity of bibenzyl derivatives from the orchid Epidendrum rigidum. Journal of Agricultural and Food Chemistry 53: 6276–6280. PMID: 16076106

11. Gutierrez RM, Solis RV (2009) Relaxant and antispasmodic effects of extracts of the orchid Encyclia michuacana on isolated guinea pig ileum. Journal of Natural Medicines 63:65–68.

12. Estrada S, Rojas A, Mathison Y, Israel A, Mata R (1999) Nitric oxide/cGMP mediates the spasmolytic action of 3,4′-dihydroxy-5,5′-dimethoxybibenzyl from Scaphyglottis livida. Planta Medica 65:109–114. PMID: 10193199

13. Won JH, Kim JY, Yun KJ, Lee JH, Back NJ, Chung HG, et al. (2006) Gigantol isolated from the whole plants of Cymbidium goeringii inhibits the LPS-induced iNOS and COX-2 expression via NF-kappaB inactivation in RAW 264.7 macrophages cells. Planta Medica 72: 1181–1187. PMID: 16924582

14. Sangshetti JN, Chouthe RS, Sakle NS, Gonjari I, Shinde DB (2014) Aldose reductase: a multi-disease target. Curr Enzym Inhib. 10: 2–12.

15. Oguntibeju OO. Diabetes Mellitus—Insights and Perspectives. InTech Press; 2013.

16. Alexiou P, Demopoulos VJ (2010) A diverse series of substituted benzenesulfonamides as aldose reductase inhibitors with antioxidant activity: design, synthesis, and in vitro activity. J Med Chem, 53: 7756–7766. doi:10.1021/jm101008m PMID: 20936791

17. Fang H, Hu X, Wang M, Wan W, Yang Q, Sun X, et al. (2015) Anti-osmotic and antioxidant activities of gigantol from Dendrobium aurantiacum var. denneanum against cataractogenesis in galactosemic rats. Journal of Ethnopharmacology 172: 238–46 doi: 10.1016/j.jep.2015.06.034 PMID: 26119284

18. Harding JJ, van Heyningen R (1998) Is month of birth associated with cataract in later life? Journal of Ophthalmology 82:526–527.

19. Pathania S, Randhawa V, Bagler G (2013) Prospecting for novel plant-derived molecules of Rauvolfia serpentina as inhibitors of Aldose Reductase, a potent drug target for diabetes and its complications. PLoS One 8:e61327. doi:10.1371/journal.pone.0061327 PMID: 23613832

20. Hofseth LJ, Robles AI, Espey MG, Harris CC (2005) Nitric oxide is a signaling molecule that regulates gene expression. Methods in Enzymology 396:326–340. PMID: 16291242

21. Olofsson EM, Marklund SL, Behndig A (2007) Glucose-induced cataract in CuZn-SOD null lenses: an effect of nitric oxide? Free Radical Biology and Medicine 42:1098–1105. PMID: 17349936

22. Kim J, Kim CS, Sohn E, Kim H, Jeong IH (2010) Lens epithelial cell apoptosis initiates diabetic cataractogenesis in the Zucker diabetic fatty rat. Clinical and Experimental Ophthalmology 248:811–818.

23. Costantino L, Rastelli G, Vianello P, Cignarella G, Barlocco D (1999) Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. Medicinal Research Reviews 19:3–23. PMID: 9918192

24. Suzen S, Buyukbingol E (2003) Recent studies of aldose reductase enzyme inhibition for diabetic complications. Current Medicinal Chemistry 10:1329–1352. PMID: 12871133

25. Kinoshita JH (1974) Mechanisms initiating cataract formation. Proctor Lecture. Investigative Ophthalmology & Visual Science 13:713–724.
26. Lechner M, Lirk P, Rieder J (2005) Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Seminars in Cancer Biology 15:277–289. PMID: 15914026
27. Pollreisz A, Schmidt-Erfurth U (2010) Diabetic cataract-pathogenesis, epidemiology and treatment. Journal of Ophthalmology 2010:608751. doi: 10.1155/2010/608751 PMID: 20634936
28. Stanga PE, Boyd SR, Hamilton AM (1999) Ocular manifestations of diabetes mellitus. Current Opinion in Ophthalmology 10:483–489. PMID: 10662255
29. Tabin G, Chen M, Espandar L (2008) Cataract surgery for the developing world. Current Opinion in Ophthalmology 19:55–59. PMID: 18090899
30. Reyes-Ramírez A, Leyte-Lugo M, Figueroa M, Serrano-Alba T, González-Andrade M, Mata R (2011) Synthesis, biological evaluation, and docking studies of gigantol analogs as calmodulin inhibitors. European Journal of Medicinal Chemistry 46:2699–2708. doi: 10.1016/j.ejmech.2011.03.057 PMID: 21514702
31. Lechner M, Lirk P, Rieder J (2005) Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Seminars in Cancer Biology 15:277–289. PMID: 15914026
32. Aß(1–42) aggregation inhibitors, antioxidants, and neuroprotective agents. Bioorganic & Medicinal Chemistry letters 22:7683–7687.
33. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820. PMID: 11742414
34. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
35. Gacche RN, Dhole NA (2011) Synthesis and in vitro evaluation of botryllazine B analogues as a new class of inhibitor against human aldose reductase. Tetrahedron 65:3019–3026.
36. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820. PMID: 11742414
37. Matsuda H, Morikawa T, Toguchi I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
38. Morikawa T, Ninomiya K, Imamura M, Akaki J, Fujikura S (2014) Acylated phenylethanoid glycosides, echinacoside and acteoside from Cistanche tubulosa, improve glucose tolerance in mice. Journal of Natural Medicines 68:561–566. doi: 10.1007/s11418-014-0837-9 PMID: 24748124
39. Matsumoto H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
40. Aß(1–42) aggregation inhibitors, antioxidants, and neuroprotective agents. Bioorganic & Medicinal Chemistry letters 22:7683–7687.
41. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
42. Gacche RN, Dhole NA (2011) Synthesis and in vitro evaluation of botryllazine B analogues as a new class of inhibitor against human aldose reductase. Tetrahedron 65:3019–3026.
43. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820. PMID: 11742414
44. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
45. Matsumoto H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
46. Aß(1–42) aggregation inhibitors, antioxidants, and neuroprotective agents. Bioorganic & Medicinal Chemistry letters 22:7683–7687.
47. Matsuda H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
48. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
49. Matsumoto H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
50. Aß(1–42) aggregation inhibitors, antioxidants, and neuroprotective agents. Bioorganic & Medicinal Chemistry letters 22:7683–7687.
51. Matsuda H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
52. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
53. Matsumoto H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
54. Rao VR, Muthenna P, Shankaraiah G, Akileswari C, Babu KH (2012) Synthesis and biological evaluation of new pipiramine analogues as potent aldose reductase inhibitors (ARIs). European Journal of Medicinal Chemistry 57:344–361. doi: 10.1016/j.ejmech.2012.09.014 PMID: 23124161
55. Matsumoto H, Morikawa T, Toguchida I, Yoshikawa M (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. chemical & pharmaceutical bulletin (Tokyo) 50:788–795.
47. Ines B, Luis C, Jose MS, Julio AS, Rafael S, Gabriel T (1985) 4,5-O-Substituted phenanthrenes from cyclophanes. The total synthesis of cannithrene II. Journal of Organic Chemistry 50:2236–2240.

48. Terashima H, Hama K, Yamamoto R, Tsuboshima M, Kikawa R, Hatanaka I (1984) Effects of a new aldose reductase inhibitor on various tissues in vitro. Journal of Pharmacology and Experimental Therapeutics 229:226–230. PMID: 6423811

49. La Motta C, Sartini S, Mugnaini L, Simorini F, Taliani S, Salerno S (2007) Pyrido[1,2-a]pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. Journal of Medicinal Chemistry 50:4917–4927. PMID: 17845019

50. Halder N, Joshi S, Gupta SK (2003) Lens aldose reductase inhibiting potential of some indigenous plants. Journal of Ethnopharmacology 86:113–116. PMID: 12686449

51. Fröhlich LG, Kotsonis P, Traub H, Taghavi-Moghadam S, Al-Masoudi N (1999) Inhibition of neuronal nitric oxide synthase by 4-amino pteridine derivatives: structure-activity relationship of antagonists of (6R)-5,6,7,8-tetrahydrobiopterin cofactor. Journal of Medicinal Chemistry 42:4108–4121. PMID: 10514281

52. Bian Q, Fernandes AF, Taylor A, Wu M, Pereira P, Shang F (2008) Expression of K6W-ubiquitin in lens epithelial cells leads to upregulation of a broad spectrum of molecular chaperones. Mol Vis 14:403–412. PMID: 18334961

53. Ibaraki N, Chen SC, Lin LR, Okamoto H, Pipas JM, Reddy VN (1998) Human lens epithelial cell line. Exp Eye Res 67:577–585. PMID: 9878220