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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Oxindole-based chalcones: synthesis and their activity against glycation of proteins

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Abstract

Diabetes mellitus, a metabolic disorder, is characterized by a substantial hyperglycaemia. Prevalence of hyperglycaemia for longer period of time can cause nonenzymatic condensation of sugar in blood with amino group of protein and give rise to advanced glycation end products (AGEs). AGEs play a major role in the onset of late diabetic complications including diabetic retinopathy, nephropathy, neuropathy and cardiovascular diseases. There is a need to establish potential therapeutic regimens that can effectively inhibit the formation of AGEs. To this end a series of novel oxindole-based chalcones have been investigated for their antiglycation potential. Analogues **1** ($IC_{50} = 155.22 \pm 2.98$ μM), **3** ($IC_{50} = 195.95 \pm 0.43$ μM), **4** ($IC_{50} = 289.47 \pm 2.47$ μM), **5** ($IC_{50} = 222.44 \pm 4.03$ μM), **7** ($IC_{50} = 251.27 \pm 2.80$ μM), and **20** (224.23 ± 1.93 μM) showed potent inhibitory activity against glycation compared to the reference Rutin ($IC_{50} = 294.5 \pm 1.5$ μM). These results reveal that multiple hydroxyl substituents and their position on the aromatic ring play a key role in inhibitory effect due to their hydrogen bonding potential. The study also reveals the influence of substituents on the binding capabilities and in turn inhibitory potential of different analogues.

Keywords Diabetes mellitus · Antiglycation · Oxindole-based chalcones · Rutin

Introduction

Diabetes mellitus (DM) is a very common metabolic disorder which is linked with a range of acute and chronic complications (Danaei et al. 2011; Shaw et al. 2010). Diabetes is caused by hyperglycaemia (increased blood sugar level), which plays a major role in diabetic complications (Miyata 1996). Prevalence of hyperglycaemia for longer period of time stimulates the synthesis of advanced glycation end products (AGEs) (Schmidt et al. 1995). The nonenzymatic condensation of carbonyl part of sugar and the reactive amino group of protein (preferentially on lysine

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and arginine) (Raj et al. 2000) occurs, resulting in the formation of AGEs and this process is termed as "glycation" (Thorpe and Baynes 2003). A French food chemist Louis Camille Millard reported the process of glycation for the first time called "Millard reactions", also referred as Browning or nonenzymatic glycation. It was identified that upon heating a mixture of amino acids and reducing sugars, a yellowish-brown product is formed (Maillard 1912). Millard reaction is not a single reaction but comprises a series of nonenzymatic reactions; at first carbonyl part of reducing sugar and active amino group form Schiff bases, subsequent rearrangement leads to the formation of a fairly stable Amadori adduct. Further series of reactions involving oxidation, rearrangement, dehydration and condensation reactions lead to irreversible protein-bound compounds termed as AGEs (Méndez et al. 2010). Glucose acts as a long-term fuel for diabetic complications (Peppa et al. 2003). At early stages formation of AGEs through glycation is dependent on the blood glucose levels; the higher concentration of glucose causes greater chances of AGEs formation (Furth 1997). Hyperglycaemia increases the chance of inducing the glycation of various structural and functional proteins like collagen or plasma proteins giving rise to AGEs and leading to the stimulation of several detrimental effects like free-radical species generation, alteration in drug binding affinity in plasma, impaired fibrinolysis etc (Singh et al. 2014). The process of glycation is complex, and a series of reactions are involved, that can give rise to structurally diverse heterocycles and cross-linked structures. However, the synthesis of monolysyl AGEs and their incorporation into the collagen proteins has been reported to facilitate the investigations into AGEs role in the pathogenesis of diabetes and other pertinent diseases (Woods et al. 2012). Another study shows the synthesis of advance peptides with site-specific AGEs (Kaur et al. 2016). Notwithstanding the detrimental effect of AGEs, the molecular mechanism of action of AGEs towards the progression of diseases still remains unclear.

In several pathophysiological processes, AGEs play a central role, leading to development of proteopathy, an array of macrovascular and microvascular complications, mainly diabetic retinopathy, nephropathy, neuropathy and cardiovascular diseases (Cooper 2004). The pathogenesis of late diabetic complications is highly complex; thus, potential therapeutic regimens are needed urgently to effectively prevent the onset of late diabetic complications through inhibition of glycation of proteins under hyperglycaemic conditions. For this purpose, antiglycation potential of different oxindole-based chalcone analogues have been investigated, as it was envisioned that they may offer therapeutic potential in inhibiting AGEs formation or delaying the process of glycation.

Several oxindoles and indole derivatives are found in mammalian cells and body fluids that exhibit diverse chemical and biological properties. Oxindole derivatives have gained medicinal and pharmaceutical importance due to their specific selectivity in biological space. They have shown several biological activities including anaesthetic (Kornet and Thio 1976), anti-inflammatory (Kadin 1985), anticancer (Peddibhotla 2009), antifungal (Strigacova et al. 2001), and antiglycation activities (Khan et al. 2013) in several reports.

To target a specific pathological process, chemists are developing promising chemotherapeutic leads. A series of oxindole-based chalcones were synthesized (Taha et al. 2015) and were then evaluated for their in vitro antiglycation activity. Each analogue comprises multiple functionalities, including oxindole, Schiff base and benzohydrazide, which were envisioned to effectively enhance the therapeutic potential of a series of chalcones.

Results and discussion

Antiglycating activity of oxindole-based chalcones and their structure−activity relationship

The NH_2 group of nitrogen-containing compounds acts like aminoguanidine to form Schiff base adducts with carbonyl group of sugar moiety, which inhibited the production of AGEs through inhibition of protein glycation. The preliminary structure−activity relationship revealed that the activity of a compound to inhibit AGEs formation mainly depend on the number and the position of hydroxyl substituents on the aromatic ring. Analogues **1**, **2**, and **5** are trihydroxy substituted; out of which analogues **1** and **5** exhibited excellent antiglycation activities with IC_{50} value of 155.22 ± 2.98 and 222.44 ± 4.03 μM, respectively, lower than the reference compound "rutin" ($IC_{50} \pm SEM = 294.5 \pm 1.5$) (Fig. 4). The activity of these analogues can be attributed to their ability to inhibit glycation. However, analogue **1** exhibited better activity than analogue **5** which might be due to the position of hydroxyl groups. Analogue **1** has ortho and para–OH substitutions while analogue **5** has ortho, meta, and para-substituted hydroxyl groups at the aromatic ring (Fig. 1). The formation of hemiacetal by interaction of lone pair of hydroxyl group or other hetero atom with the carbonyl carbon participates in hemiacetal or ketal formation (Fig. 2) and results in the inhibition glycation by inhibiting the final step towards the formation of AGEs.

Moreover, analogue **2** possesses lower antiglycation activity than analogues **1** and **5** with IC_{50} value of 336.96 ± 3.42 μM, which might be due to the presence of methyl

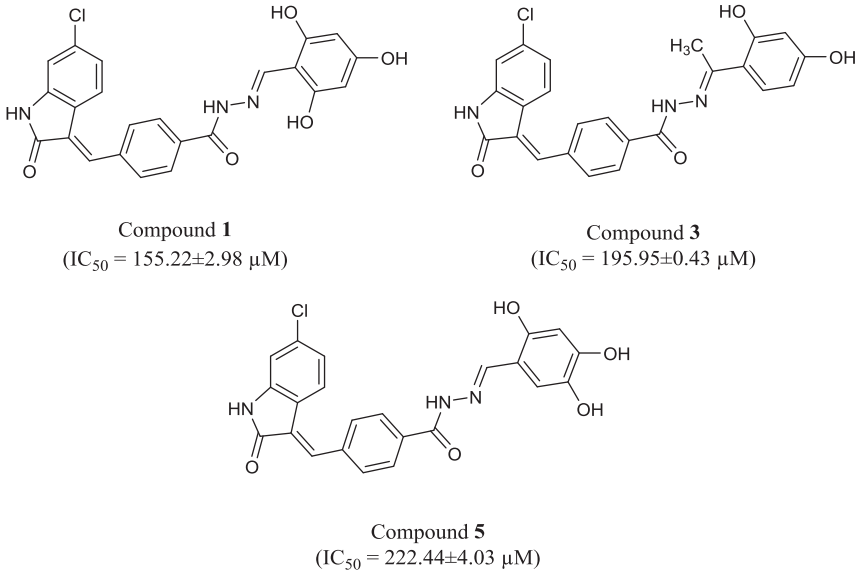


Fig. 1 Structure and antiglycation inhibition potential of oxindole-based chalcone **1**, **3**, and **5**

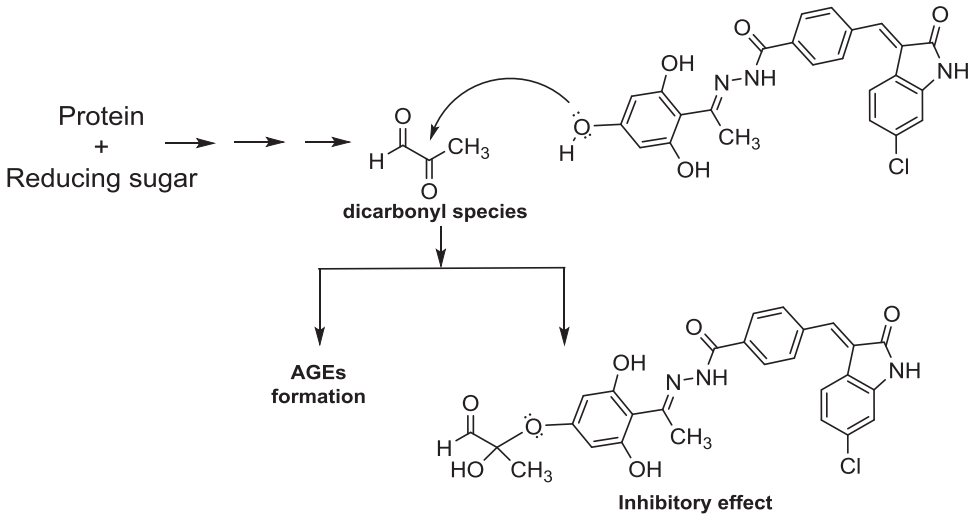


Fig. 2 Inhibitory effect due to hemiketal/acetal formation

group that sterically hinders the binding interaction and slightly lowers the activity (Fig. 4). Five analogues with dihydroxy substituents (i.e. analogues **3**, **4**, **6**, **7**, and **8**) depicted excellent to moderate activity. Analogue **3** (IC_{50} = 195.95 ± 0.43 μM), **4** (IC_{50} = 289.47 ± 2.47 μM), **7** (IC_{50} = 251.27 ± 2.80 μM) possess potent antiglycation profile than the reference rutin as shown in Fig. 4. As discussed earlier, the position of a substituent affects the binding capabilities; therefore, analogue **3** (with ortho- and para-OH) is found to be more active than **7** (with two meta-OH), which in turn showed better inhibitory potential than **4** (with ortho- and meta-OH) (Table 1). Analogue **6** has meta and para, while **8** has ortho and meta dihydroxyl substitutions; both these analogues involve intramolecular hydrogen bonding interactions (Fig. 3); thus, their activity is lowered with IC_{50} values of 527.54 ± 4.23 and 423.83 ± 4.81 μM, respectively.

Analogues **9**, **10**, and **11** have mono –OH substitution at meta, para, and ortho positions of aromatic ring, respectively (Table 1). Due to the least number of –OH substituents, these compounds exhibited moderate antiglycation activities. However, analogues **12** and **13** that also have mono –OH at the aromatic ring with a substituted $–CH_3$ group at R^2, lower the activity due to steric hindrance or the electron-donating effect of the methyl group. Analogue **12** was found to be inactive while **13** possessed slight activity with IC_{50} value of 843.99 ± 3.23 μM.

Analogue **20** (with para $–CH_3$ substitution at the aromatic ring) exhibited better antiglycation activity as

Table 1 In vitro antiglycation activity of oxindole-based chalcone derivatives

Analogue#	R_1	R_2	$IC_{50} \pm S.E.M^a$ (μM)
1	H		155.22 ± 2.98
2	CH_3		336.96 ± 3.42
3	H		195.95 ± 0.43
4	H		289.47 ± 2.47
5	H		222.44 ± 4.03
6	H		527.54 ± 4.23
7	H		251.27 ± 2.80
8	H		423.83 ± 4.81
9	H		559.37 ± 2.12
10	H		466.54 ± 3.88
11	H		368.36 ± 2.02
12	CH_3		NA[b]
13	CH_3		843.99 ± 3.23
14	H		NA[b]
15	H		NA[b]
16	H		NA[b]
17	H		382.36 ± 4.66
18	H		NA[b]
19	H		NA[b]
20	H		224.23 ± 1.93

Standard drug = Rutin ($IC_{50} \pm SEM = 294.5 \pm 1.5$ μM)

SEM standard error of the mean, *NA* not active

compared to the reference rutin (IC_{50}: 224.23 ± 1.93 μM). This potent antiglycation enhanced activity could be due to the binding interactions with methyl glyoxal (Table 1). However, compounds **14**–**19** failed to interact effectively in order to inhibit AGEs formation and found to be inactive (with percent inhibition < 50%); thus their IC_{50} were not evaluated eventually.

Overall, five analogues of oxindole-based chalcones showed better potential than the reference standard rutin (Fig. 4). The potential activity can be attributed to the presence of several hydroxyl substituents. These substituents possessing lone-pairs of electrons can interact with the dicarbonyl compounds synthesized at the intermediate stage of glycation and hinder the completion of final step of glycation which results in AGEs formation (Fig. 2). Likewise, not only the number but the position also influences the activity of candidates which is envisioned to be due to the binding interactions between the dicarbonyl species and the approaching candidate. Moreover, the availability of electron pairs is highly dependent on the position of the substituents, as the intramolecular hydrogen bonding interactions can lead to the unavailability of electron pairs (Fig. 3), thus immensely affecting the ability of inhibition potential of a candidate.

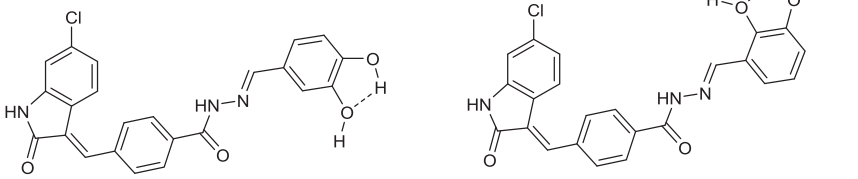
Conclusion

In the current study, a wide range of the depiction of antiglycation potential of a series of oxindole-based chalcones was scrutinized, due to the position of the substituents on the aromatic ring. Among the tested compounds, **1**, **3**–**5** and **20** depicted excellent antiglycation potential as compared to the standard reference rutin (Fig. 4). The analogues containing multiple –OH groups on the aromatic ring were more potent and possessed excellent potential to inhibit glycation. Involvement of –OH groups in intramolecular hydrogen bonding significantly lowers the inhibition potential of the analogues. Moreover, sterically hindered analogues showed lower inhibitory potential of glycation due to insignificant binding with the dicarbonyl species. To this end, these results can significantly help in designing more potent and novel drugs against diabetes, by targeting glycation process that leads to the formation of AGEs and in turn to diverse late diabetic conditions.

Materials and methods

Dimethyl sulfoxide (DMSO), sodium azide (NaN_3), disodium hydrogen phosphate (Na_2HPO_4), methyl glyoxal (MG), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma-Aldrich. Bovine serum albumin

Fig. 3 Intramolecular hydrogen bonding interactions of analogues **6** and **8**



Compound **6**
IC_{50} = 527.54 ± 4.23 µM
Intramolecular Hydrogen Bonding Interactions

Compound **8**
IC_{50} = 423.83 ± 4.81
Intramolecular Hydrogen Bonding Interactions

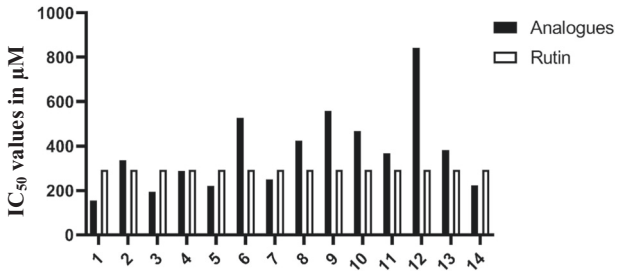


Fig. 4 Comparison of in vitro antiglycation activity of oxindole-based chalcones with reference (rutin)

(BSA) and fetal bovine serum were purchased from Research Organics, Cleveland (USA).

Sodium phosphate buffer of pH 7.4 and a concentration of 67 mM was prepared by mixing disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) in deionized water. Sodium azide (3 mM) was also added to maintain aseptic conditions. BSA and MG solutions were prepared in buffer with concentrations of 10 and 50 mg/ml, respectively, while test samples (concentration 1 mM/ml) were prepared in DMSO.

Synthesis of oxindole-based chalcones

6-Chlorooxindole (**I**) and 4-formylbenzoic acid (**II**) were used as starting material to synthesize (*E*)-4-((5-chloro-2-oxoindolin-3-ylidene) methyl) benzoic acid (**III**) via condensation using piperidene. Crude mixture was purified using silica gel column chromatography to obtain pure isomer, which was then further reacted to give (*E*)-4-((6-chloro-2-oxoindolin-3-ylidene)methyl) benzohydrazide (**IV**) (Scheme 1) by using a known methodology (Zhang et al. 2002). The product obtained was then reacted with different acetophenone and aryl aldehydes using acetic acid to synthesize range of different analogues (**1–20**) (Table 1). The detailed spectroscopic studies of these compounds (**1–20**) were reported in our previous study (Taha et al. 2015).

In vitro antiglycation assay

In vitro antiglycation activity was performed according to the modified protocol followed by Ahmed (2005). Triplicate samples, in a 96-well plate assay in each well, had a reaction mixture of 200 µl, a glycated control containing 20 µl of test compound solution, 50 µl of BSA, 50 µl of MG and 80 µl of phosphate buffer, while a blank control containing 20 µl of DMSO, was incubated for 9 days (at 37 °C temperature). After incubation, fluorescence intensity for the change was evaluated using the microplate ELISA reader, SpectraMax M2 (Molecular Devices, CA, USA) at 37 °C. The percentage inhibition of each compound was calculated by using the following formula:

$$\%\text{Inhibition} = \left(1 - \text{Fluorescence}_{\text{test compound}}/\text{Fluorescence}_{\text{control}}\right) \times 100.$$

Rutin, a natural flavonoid inhibiting protein glycation in vitro (Cervantes-Laurean et al. 2006), was used as a positive control with an IC_{50} value of 294.5 ± 1.5 µM.

Statistical analysis

SEM = IC_{50} value was presented as mean ± SEM (standard error of the mean) calculated by using the formula

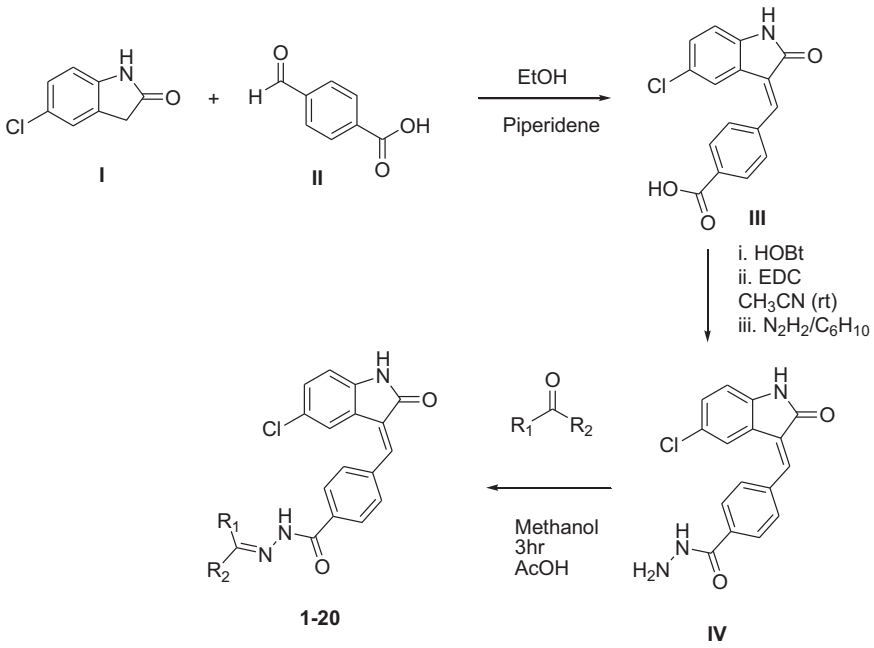
$$\text{SEM} = \frac{s}{\sqrt{N}},$$

where s = sample standard deviation

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2},$$

x_i–x_n = sample data set, $\bar{x}$ = mean value of sample data, N = size of data.

Scheme 1 Synthesis of oxindole-based chalcone derivatives



Compliance with ethical standards

Conflicts of Interest The authors declare no conflict of interest.

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