Chalcones and bis-chalcones: As potential α-amylase inhibitors; synthesis, in vitro screening, and molecular modelling studies

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ABSTRACT

Despite of a diverse range of biological activities associated with chalcones and bis-chalcones, they are still neglected by the medicinal chemist for their possible α-amylase inhibitory activity. So, the current study is based on the evaluation of this class for the identification of new leads as α-amylase inhibitors. For that purpose, a library of substituted chalcones 1–13 and bis-chalcones 14–18 were synthesized and characterized by spectroscopic techniques EI-MS and 1H NMR. CHN analysis was carried out and found in agreement with the calculated values. All compounds were evaluated for in vitro α-amylase inhibitory activity and demonstrated good activities in the range of IC50 = 1.25 ± 1.05–2.40 ± 0.09 µM as compared to the standard acarbose (IC50 = 1.04 ± 0.3 µM). Limited structure–activity relationship (SAR) was established by considering the effect of different groups attached to aryl rings on varying inhibitory activity. SMe group in chalcones and OMe group in bis-chalcones were found more influential on the activity than other groups. However, in order to predict the involvement of different groups in the binding interactions with the active site of α-amylase enzyme, in silico studies were also conducted.

1. Introduction

Diabetes mellitus is a chronic syndrome associated with the endocrine system. It affects the metabolism of many molecules including carbohydrates, proteins, fat, electrolytes, and water. Diabetes is mainly characterized by hyperglycemia, caused by either the deficiency of insulin secretion or cells becomes unresponsive towards the production of insulin resulted in the elevated blood glucose level. Amongst the different therapeutic approaches to treat diabetes, one is to decrease postprandial hyperglycemia by the inhibition of hydrolyzing enzymes such as α-amylase and α-glucosidase. α-Amylase is involved in the digestion of carbohydrates. It is a metalloenzyme that breakdowns long chain carbohydrates into smaller molecules e.g., (conversion of starch into glucose and maltose) by hydrolyzing the α-1,4-glycosidic linkage. It serves as the major digestive enzymes and facilitates intestinal absorption. The high blood glucose level is associated with many pathological conditions such as diabetes, obesity, and oral diseases. Diabetes in turn give rise to other pathological conditions including neuropathy, retinopathy, microangiopathy, nephropathy, and cardiovascular diseases. Therefore, the inhibitors for α-amylase is among the potential targets in the development of lead compounds for the treatment of diabetes [1–10].

Chalcones, are abundantly found in edible plants and serves as a precursors for the synthesis of flavonoids and isoflavonoids. The chalcones are α,β-unsaturated ketones having an enone system between two aromatic rings. The keto-ethylenic group (−COCH−CH−) serves as the reactive site and acts as a chromophore giving chalcones their characteristic color. The α,β-unsaturation is responsible for the pharmacological properties of chalcones. These molecules display interesting biological activities including antioxidant, antiinflammatory, cytotoxic,
anticancer, analgesic, antipyretic, antianginal, antihepatotoxic, antimicrobial, antimalarial, and antiallergic activities. A common synthetic approach toward the synthesis of chalcones is via the Knoevenagel condensation, many of which utilize microwave techniques [11–17].

**Bis-chalcones** have also received considerable attention from biologists and synthetic chemists due to their wide variety of biological activities. Their analogues are found to exhibit potential antioxidant activities and are the potent NO production inhibitors. These compounds are also reported for their anticancer potential against A549, DU145, KB, and KB-VIN human cancer cell lines. Biphenyl based bis-chalcones have anticancer activity against MCF-7 and MDA-MB-231 human breast cancer, HeLa cell lines, and human embryonic kidney (HEK-293) cells. Some bis-chalcones have appreciable antibacterial and antifungal activities [18–19]. Chalcones and bis-chalcones have been studied in past years for α-glucosidase inhibitory activity [20–24] (Fig. 1).

Unfortunately, chalcones and bis-chalcones are rarely studied for α-amylase inhibitory activity. Najafian et al., reported one unsubstituted trans-chalcone as α-amylase competitive inhibitor with in silico study [25]. By keeping in mind that there is utter need to explore this class for α-amylase inhibitory potential, we decided to synthesize a library of substituted chalcones and bis-chalcones to subject them for the aforementioned activity (Fig. 2).

**2. Results and discussion**

**2.1. Chemistry**

Substituted acetophenones were reacted with a variety of benzaldehydes to afford chalcone (Scheme 1A) derivatives 1–13, however, bis-chalcones 14–18 were synthesized by treating acetone with a variety of benzaldehydes (Scheme-1B). Both reactions were carried out at room temperature under basic conditions (60% NaOH). The extent of reaction progress was checked by TLC monitoring. Synthetic compound 1–18 were characterized by spectroscopic techniques EI-MS and 1H NMR. CHN analysis was also carried out. To the best of our knowledge, structures of compounds 1, 2, 4, 5, and 7–16 [26–36] are known while remaining derivatives are found to be new.

1H- and 13C NMR spectra of compound 18 were recorded in deuterated DMSO-<d>6</d> at 400 MHz machine. Being symmetrical in structure, 1H- and 13C NMR signals are representing the each half of the molecule. In 1H NMR spectrum, two characteristic signals for H-1’ and -2’ were appeared at δ<sub>H</sub> 7.88 and δ<sub>H</sub> 7.12, respectively, showed trans coupling with each other with a coupling value 15.6 Hz. H-4, H-5, and H-6 were resonated in a usual aromatic region δ<sub>H</sub> 5.85–6.78. Two signals for methylene δ<sub>H</sub> (3.89) and methyl δ<sub>H</sub> (1.28) groups of the ethoxy moiety were appeared as quartet and triplet, respectively (Fig. 3A).

![Fig. 1. Identified α-glucosidase inhibitors based on chalcones and bis-chalcones scaffold.](image1)

![Fig. 2. Overview of the structural features and α-amylase inhibitory activity of chalcones 1–13 and bis-chalcones 14–18.](image2)
2.2. In vitro α-amylase inhibitory activity

Synthetic chalcones 1–13 and bis-chalcones 14–18 were screened for in vitro α-amylase inhibitory activity. All compounds demonstrated a good α-amylase inhibitory activity in the range of IC50 = 1.25 ± 1.05–2.40 ± 0.09 µM as compared to the standard acarbose (IC50 = 1.04 ± 0.3 µM) (Table 1).

2.3. Structure-activity relationship (SAR)

Limited structure-activity relationship (SAR) was established by assuming that all structural features such as enone moiety in chalcones 1–13, diene moiety in bis-chalcones 14–18, and aryl rings in all compounds (Fig. 2), are responsible for exhibiting the α-amylase inhibitory activity. However, the variation in the inhibitory activity is attributed by different groups attached to aryl rings.

Chalcones 1–6, derived from 1-(p-tolyl)ethan-1-one showed inhibition potential in the range of IC50 = 1.27 ± 0.7–2.26 ± 0.07 µM as compared to the standard acarbose (IC50 = 1.04 ± 0.3 µM). The difference in the inhibitory activity of these compounds is the result of different substituents on aryl ring (R2). Amongst them, compound 4 (IC50 = 1.27 ± 0.7 µM) with 4′-SCH3 group was found to be the most active and showed inhibitory activity comparable to standard (IC50 = 1.04 ± 0.3 µM). Comparison of its activity with derivative 1 (IC50 = 2.06 ± 0.04 µM) having OCH3 instead of SCH3, showed less activity than compound 4, revealed that the sulfur atom of SCH3 is taking part in the interaction with the active site of enzyme. Similarly, replacing SCH3 to Cl and OBz in compounds 2 (IC50 = 1.85 ± 0.09 µM) and 5 (IC50 = 1.99 ± 0.05 µM), respectively, brought decline in the inhibitory activity. Furthermore, compounds 3 (IC50 = 2.07 ± 0.08 µM) having 3′-OMe and 4′-OEt groups and compound 6 (IC50 = 2.26 ± 0.07 µM) having 2′-OMe and 3′-OEt groups on aryl ring (R2) showed moderate inhibitory activity (Fig. 4).

Chalcones 7–13, derived from 1-(4-methoxyphenyl)ethan-1-one demonstrated α-amylase inhibitory activity ranging from IC50 = 1.25 ± 0.05–2.15 ± 0.07 µM. Different groups attached to the aryl ring (R2) are responsible for varying inhibitory activity. Again in this group of compounds, the 4′-SCH3 substituted derivative 10 (IC50 = 1.25 ± 0.05 µM) was found to be the most active analog. Its activity may compared to distinctively similar compound 4 (IC50 = 1.27 ± 0.7 µM) having 4-CH3 instead of 4-OCH3 group on aryl ring (R2), showed almost same activity which further confirmed the important role of SCH3 group in the inhibitory potential. However, replacement of 4′-SCH3 with 4′-OMe, 4′-OEt, 4′-Ph, 4′-OBz, 4′-Br, and 2′,4′-di-Cl in compounds 7 (IC50 = 1.92 ± 0.12 µM), 13 (IC50 = 2.00 ± 0.08 µM), 12 (IC50 = 1.99 ± 0.09 µM), 11 (IC50 = 2.15 ± 0.07 µM), 9 (IC50 = 1.98 ± 0.07 µM), and 8 (IC50 = 1.97 ± 0.08 µM), respectively, showed decreased activity (Fig. 5). It is important to note that derivatives 7–9, and 11–13 showed much closed inhibitory potential which revealed that these compounds have almost similar extent of interactions with the active site of α-amylase enzyme.

Bis-chalcones 14–18 derived from acetone and demonstrated α-amylase inhibitory potential in the range of IC50 = 1.63 ± 0.18–2.40 ± 0.09 µM as compared to the standard acarbose (IC50 = 1.04 ± 0.3 µM). Amongst these compounds, compound 16 (IC50 = 1.63 ± 0.18 µM) with 4′/4′-OMe substitution on aryl rings R1/R2 was found to be the most active. Replacement of 4′/4′-OMe with 4′/4′-SMe as in compound 17 (IC50 = 2.4 ± 0.09 µM), resulted in slight decreased in the inhibitory activity which might be due to slight low polarity of compounds 17 than 16. Activity of compound 16 may also compared with halogenated derivatives 14 (IC50 = 1.72 ± 0.2 µM) and 15 (IC50 = 1.8 ± 0.07 µM) having 4′/4′-Cl and 4′/4′-Br, respectively, a comparable inhibitory activity was observed. Another compound 18 (IC50 = 2.12 ± 0.1 µM) having 2′/2′-OEt and 3′/3′-OEt showed moderate inhibitory activity (Fig. 6).

Precisely, it may extracted out from the limited SAR that in case of chalcones, SMe group is playing a crucial role in the activity while other
Table 1

In vitro α-amylase inhibitory, DPPH and ABTS radical scavenging activities of chalcones 1–13 and bis-chalcones 14–18.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>R2</th>
<th>α-Amylase inhibitory activity IC50 ± SEMa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalcones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>2.06 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>1.85 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>2.07 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>1.27 ± 0.7</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>1.99 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>2.26 ± 0.07</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>1.92 ± 0.12</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>1.97 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>1.98 ± 0.07</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>1.25 ± 1.05</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>2.15 ± 0.07</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>1.99 ± 0.09</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>2.00 ± 0.08</td>
</tr>
<tr>
<td>Bis-Chalcones</td>
<td>R2</td>
<td></td>
<td>α-Amylase inhibitory activity IC50 ± SEMa</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>1.72 ± 0.1</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>1.80 ± 0.07</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>1.63 ± 0.18</td>
</tr>
</tbody>
</table>

(continued on next page)
compounds with different substituents on aryl ring R2 have almost same extent of inhibitory potential. However, in case of bis-chalcones, OMe group has big influence on the activity than other groups as well as extended aromatic part also showed the moderate inhibitory activity. Furthermore, by keeping in mind that solely SAR is insufficient to have clear picture of the participation of different moieties of compound in the inhibitory activity. So, in silico study was conducted to decipher the ligands (synthetic compounds) interactions with active site of α-amylase enzyme which is as follows.

### 2.4. In silico studies

In order to predict the binding conformations of the synthetic chalcones 1–13 and bis-chalcones 14–18 into the active site of α-amylase enzyme, MOE-Dock module implemented in MOE program was used. The default parameters of MOE-Dock program were used in the docking protocol. Best conformations of chalcones and bis-chalcones for hydrogen bonding/arene-arene/arene-cation interactions were analyzed on the basis of docking score. It was perceived from the docking calculation study that the top ranked conformations of almost all chalcones and bis-chalcones were well fitted inside the active site of α-amylase enzyme and were involved in several types of interactions with the active site residues of α-amylase enzyme. i.e., Trp58, Trp59, Tyr62, Leu162, Arg195, Asp197, Glu233, Asp300, His305, and Asp356. Table 2 represents the details of interactions including docking scores for all chalcones and bis-chalcones. It is observed that the presence of electronegative groups and electron rich species like -Cl, -Br, -S, and -O are the main structural features for the active nature of compounds while bulky groups such as methyl and ethyl slightly lowered the activity of some of the compounds. From the conformation of compound 10 (docking score = −9.3467, Table 2), it was observed that this compound formed three hydrogen bonds and four π-H linkages with the active site residues of α-amylase. Arg195 was observed in making H-donor interaction with the oxygen atom of the methoxy moiety of the compound while Glu233 and Asp356 established interactions with carbon and sulphur atoms of the compound, respectively, as shown in Fig. 7A. Trp59, Leu162, and Ala198 of active site residues formed π-H interactions with the compound. The high potent inhibitory activity may be due to the presence of the electron accepting group (carbonyl oxygen) and electron donative group (MeO) which create an electron flow making the compound more active, polarizable, and potent. Compound 4 (docking score = −9.0231) was observed to make three polar interactions with the Glu233 and Asp300 residues of the binding pocket of the enzyme as shown in Fig. 7B. Trp59 formed arene-arene contact with benzene moiety of the same compound. The inhibition of this compound might be due to the availability of the electron accepting group (carbonyl oxygen) and electron donating groups (methyl group). Compounds 16 and 14 has docking scores of -8.4512 and -8.2893, respectively, against α-amylase enzyme. Compound 16 is bound to the α-amylase enzyme in an adequate manner through four hydrogen bonds and several hydrophobic interactions (Fig. 7C). Whereas, compound 14 formed five polar and one arene-cation interactions with the active site residues of the α-amylase enzyme (Fig. 7D). The structural features observed in this group for the active nature of compounds (16 and 14) are the presence of electronegative groups like halogen (-Cl) and Methoxy (MeO) groups. The confirmations obtained after docking

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>R2</th>
<th>α-Amylase inhibitory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>2.40 ± 0.09</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>2.12 ± 0.1</td>
</tr>
</tbody>
</table>

Standards = Acarbose

SEM*: Standard error mean; Acarbose (Standard inhibitor for α-amylase enzyme)
showed good docking scores and demonstrated sound \textit{in silico} inhibition of the \(\alpha\)-amylase enzyme. Overall a good correlation between the docking study and biological evaluation of active chalcones and \textit{bis}-chalcones was perceived. The correlation graph and the correlation coefficient values are given in Fig. 8.

3. Conclusion

Synthetic chalcones 1–13 and \textit{bis}-chalcones 14–18 showed significant \textit{in vitro} \(\alpha\)-amylase inhibitory activity as compared to the standard acarbose. Limited structure–activity relationship (SAR) suggested that SM and OM groups are playing crucial role in the inhibitory activity in the case of chalcones and \textit{bis}-chalcones, respectively. However, \textit{in silico} study predicted a number of interacting sites of the ligands (synthetic compounds) with the active site of \(\alpha\)-amylase enzyme. This study has identified a number of lead compounds which can further explore in order to get a powerful inhibitor for \(\alpha\)-amylase enzyme.

4. Experimental

4.1. Materials and methods

All chemicals were of analytical grade and purchased from Sigma-Aldrich, USA. Silica gel coated aluminium plates (Kieselgel 60, 254, E. Merck, Germany) were used for thin layer chromatography (TLC). Spots were visualized with a dual wavelength (254 and 365 nm) UV light. Electron impact mass experiment were recorded on MAT 312 and MAT 113D mass spectrometer. The \(^1\)H NMR spectra were recorded on Bruker AM machines, operating at 300 and 400 MHz. The chemical shift are presented in ppm (\(\delta\)) relative to tetramethylsilane (TMS) as an internal standard and the coupling constant (\(J\)) are in Hz. Melting points of the compounds were determined on Stuart* SMP10 melting point apparatus. CHN Analyses were carried out on a Carlo Erba Strumentazione-Mod-1106, Italy.

4.2. General procedure for the synthesis of chalcones 1–13

To a stirred solution of 4-methyl/4-methoxy acetophenone
## Table 2
Docking scores and report of predicted interactions of docked conformations.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Docking score</th>
<th>Ligand</th>
<th>Receptor</th>
<th>Interaction</th>
<th>Distance</th>
<th>E (kcal/mol)</th>
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<tbody>
<tr>
<td>1</td>
<td>−6.7845</td>
<td>C1 1</td>
<td>OD2 ASP 300 (A)</td>
<td>H-donor</td>
<td>3.36</td>
<td>−0.2</td>
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<tr>
<td></td>
<td>−6.7614</td>
<td>C19 32</td>
<td>6-ring TRP 59 (A)</td>
<td>H-π</td>
<td>3.61</td>
<td>−0.3</td>
</tr>
<tr>
<td>2</td>
<td>−6.5211</td>
<td>C18 31</td>
<td>OD2 ASP 356 (A)</td>
<td>H-donor</td>
<td>3.24</td>
<td>−0.0</td>
</tr>
<tr>
<td></td>
<td>−6.5342</td>
<td>C19 32</td>
<td>OD2 ASP 300 (A)</td>
<td>H-donor</td>
<td>3.13</td>
<td>−1.0</td>
</tr>
<tr>
<td>3</td>
<td>−7.1093</td>
<td>C4 6</td>
<td>OD2 ASP 356 (A)</td>
<td>H-donor</td>
<td>3.52</td>
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<tr>
<td></td>
<td>−6.2198</td>
<td>C19 32</td>
<td>OD1 ASP 197 (A)</td>
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<td>4</td>
<td>−7.5432</td>
<td>C18 28</td>
<td>OD1 ASP 300 (A)</td>
<td>H-donor</td>
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<td>−7.4389</td>
<td>C17 26</td>
<td>OD1 ASP 233 (A)</td>
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<td>5</td>
<td>−7.3421</td>
<td>C4 6</td>
<td>OD2 ASP 356 (A)</td>
<td>H-donor</td>
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<td></td>
<td>−9.3467</td>
<td>C3 4</td>
<td>OD1 ASP 197 (A)</td>
<td>H-donor</td>
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<td>6</td>
<td>−6.1349</td>
<td>C20 33</td>
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<td>H-π</td>
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<td>7</td>
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<td>O8 12</td>
<td>OD1 ASP 300 (A)</td>
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<td>8</td>
<td>−7.1239</td>
<td>C23 38</td>
<td>OD2 ASP 356 (A)</td>
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<tr>
<td></td>
<td>−7.1192</td>
<td>C18 28</td>
<td>OD1 ASP 300 (A)</td>
<td>H-donor</td>
<td>3.57</td>
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<td>9</td>
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<td>OD1 ASP 356 (A)</td>
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<td>−8.2893</td>
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<td>OD1 ASP 300 (A)</td>
<td>H-donor</td>
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<td>−8.4512</td>
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<td>11</td>
<td>−5.5673</td>
<td>C12 19</td>
<td>OD1 ASP 300 (A)</td>
<td>H-donor</td>
<td>3.33</td>
<td>−0.3</td>
</tr>
</tbody>
</table>

(continued on next page)
In EtOH (10 mL), solution of NaOH (60%) (3 mL) was added drop-wise by maintaining the temperature at 0 °C. Substituted benzaldehyde derivative (0.5 mmol) was added to the above-mentioned mixture and further stirred for 2–3 h. Completion of reaction was monitored by TLC analysis. After completion of reaction, it was kept in refrigerator for overnight and then diluted with ice cold water, the resulting precipitates were filtered and washed with excess of distilled water. The precipitates were crystallized from methanol.[37] Structures of compounds 1–13 were deduced by EI-MS and 1H NMR spectroscopic techniques. CHN analysis was also performed.

(E)-3-(4-Methoxyphenyl)-1-(p-tolyl)prop-2-en-1-one (1)
Yellow solid; Yield: 72%; M.p.: 94–96 °C; 1H NMR (400 MHz, DMSO-d6): \( \delta \) 8.05 (d, \( J_{2,3/6,5} = 8.4 \) Hz, 2H, H-2, H-6), 7.84 (d, \( J_{3,2/5,6} = 14.4 \) Hz, 1H, H-3), 7.52 (d, \( J_{3,2/5,6} = 8.1 \) Hz, 2H, H-3, H-5), 3.81 (s, 3H, OCH3), 2.39 (s, 3H, CH3); EI-MS: \( m/z \) (rel. abund.), 252 (M+, 100), 237 (43), 161 (29), 108 (16), 90 (19), 64 (7); Anal. Calcd for C17H16O2: C, 80.93; H, 6.39; Found: C, 80.95; H, 6.42.

(E)-3-(4-Chlorophenyl)-1-(p-tolyl)prop-2-en-1-one (2)
Yellow solid; Yield: 75%; M.p.: 122–124 °C; 1H NMR (300 MHz, DMSO-d6): \( \delta \) 8.05 (d, \( J_{2,3/6,5} = 7.8 \) Hz, 2H, H-2, H-6), 7.96 (d, \( J_{2,1''} = 15.0 \) Hz, 1H, H-1′′ Vinylic), 7.91 (d, \( J_{3,2/5,6} = 7.2 \) Hz, 2H, H-3, H-5), 7.70 (d, \( J_{1,2''} = 15.6 \) Hz, 1H, H-1′′ Vinylic), 7.50 (d, \( J_{3,2/5,6} = 15.6 \) Hz, 1H, H-1′′ Vinylic), 7.36 (d, \( J_{3,2/5,6} = 8.1 \) Hz, 2H, H-3, H-5), 2.37 (s, 3H, CH3); EI-MS: \( m/z \) (rel. abund.), 256 (M+, 100), 258 (M+2, 30), 241 (20), 221 (23), 165 (18), 119 (40), 91 (24); Anal. Calcd for C16H13ClO: C, 74.86; H, 5.10; Found: C, 74.88; H, 5.13.

(E)-3-(4-Ethoxy-3-methoxyphenyl)-1-(p-tolyl)prop-2-en-1-one (3)
Yellow solid; Yield: 73%; M.p.: 113–115 °C; 1H NMR (400 MHz, DMSO-d6): \( \delta \) 8.06 (d, \( J_{2,3/6,5} = 8.4 \) Hz, 2H, H-2, H-6), 7.81 (d, \( J_{3,2/5,6} = 15.6 \) Hz, 1H, H-2′′ Vinylic), 7.65 (d, \( J_{1,2''} = 15.6 \) Hz, 1H, H-1′′ Vinylic), 7.52 (d, \( J_{3,2/5,6} = 1.6 \) Hz, 1H, H-2′′), 7.37 (ovp, 3H, H-3, H-5, H-6), 3.81 (s, 3H, OCH3), 3.75 (s, 3H, OCH3), 3.75 (s, 3H, OCH3), 2.39 (s, 3H, CH3); EI-MS: \( m/z \) (rel. abund.), 252 (M+, 100), 237 (43), 161 (29), 108 (16), 90 (19), 64 (7); Anal. Calcd for C19H19ClO2: C, 73.79; H, 6.07; Found: C, 73.75; H, 6.04.

-(E)-3-(4-Chlorophenyl)-1-(p-tolyl)prop-2-en-1-one (2)
Yellow solid; Yield: 75%; M.p.: 122–124 °C; 1H NMR (300 MHz, DMSO-d6): \( \delta \) 8.05 (d, \( J_{2,3/6,5} = 7.8 \) Hz, 2H, H-2, H-6), 7.96 (d, \( J_{2,1''} = 15.0 \) Hz, 1H, H-2′′ Vinylic), 7.91 (d, \( J_{3,2/5,6} = 7.2 \) Hz, 2H, H-3, H-5), 7.70 (d, \( J_{1,2''} = 15.6 \) Hz, 1H, H-1′′ Vinylic), 7.50 (d, \( J_{3,2/5,6} = 15.6 \) Hz, 1H, H-1′′ Vinylic), 7.36 (d, \( J_{3,2/5,6} = 8.1 \) Hz, 2H, H-3, H-5), 2.37 (s, 3H, CH3); EI-MS: \( m/z \) (rel. abund.), 256 (M+, 100), 258 (M+2, 30), 241 (20), 221 (23), 165 (18), 119 (40), 91 (24); Anal. Calcd for C16H13ClO: C, 74.86; H, 5.10; Found: C, 74.88; H, 5.13.
Fig. 8. A correlation graph for predicted docking score and IC50 values.

(E)-3-(4-(Methylthio)phenyl)-1-(p-tolyl)prop-2-en-1-one (4)
Yellow solid; Yield: 69%; M.p.: 96–98 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.06 (d, J2,3/6,5 = 8.0 Hz, 2H, H-2, H-6), 7.84 (d, J2,3/6,5 = 8.4 Hz, 2H, H-2, H-6), 7.70 (d, J1-2-3 = 15.6 Hz, 1H, H-1′ Vinylic), 7.37 (d, J3,2/5,6 = 8.0 Hz, 2H, H-3, H-5), 7.31 (d, J2,3/5,6 = 8.4 Hz, 2H, H-2′, H-3′), 2.51 (s, 3H, OCH3); EI-MS: m/z (rel. abund.%), 268 (M+100), 253 (271), 221 (177), 119 (39), 41 (40); Anal. Calcld for C19H20O3: C, 77.00; H, 6.80; Found: C, 77.02; H, 6.83.

(E)-3-(4-(Methylthio)phenyl)-1-(p-tolyl)prop-2-en-1-one (5)
Yellow solid; Yield: 72%; M.p.: 110–112 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.05 (d, J2,3/6,5 = 8.0 Hz, 2H, H-2′, H-6′), 7.80 (d, J2,3/6,5 = 15.6 Hz, 1H, H-1′ Vinylic), 7.47 (d, J3,2/5,6 = 7.2 Hz, 2H, H-3, H-5), 7.41 (ovp, 5H, H-2″, H-3″, H-4″, H-5″, H-6″), 7.09 (d, J2,3/5,6 = 8.8 Hz, 2H, H-3′, H-5′), 4.71 (s, 2H, -OCH2CH3), 2.39 (s, 3H, -CH3); EI-MS: m/z (rel. abund.%), 328 (M+100), 237 (15), 119 (22), 91 (1 0), 65 (16); Anal. Calcld for C19H20O3: C, 84.12; H, 6.14; Found: C, 84.15; H, 6.16.

(E)-3-(3-Ethoxy-2-hydroxyphenyl)-1-(p-tolyl)prop-2-en-1-one (6)
Red solid; Yield: 70%; M.p.: 278–280 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.02 (d, J2,3/1 = 14.8 Hz, 1H, H-2′ Vinylic), 7.86 (ovp, 3H, H-2, H-6, H-1′ Vinylic), 7.29 (d, J2,3/6,5 = 7.6 Hz, 2H, H-2, H-6), 6.92 (d, J2,3/6,5 = 7.6 Hz, 2H, H-2, H-6), 6.49 (d, J3,2/5,6 = 6.8 Hz, 2H, H-4′), 5.95 (bd, 1H, H-5′), 3.91 (q, 2H, -OCH2CH3), 2.36 (s, 3H, -CH3), 1.28 (s, 3H, -OCH2CH3); 13C NMR (100 MHz, DMSO-d6): δ 138.8, 151.3, 147.7, 144.0, 141.2, 134.7, 129.7, 129.3, 123.9, 123.1, 121.5, 120.4, 117.0, 115.2, 21.5, 14.6; EI-MS: m/z (rel. abund. %), 282 (M+100), 265 (255), 119 (100), 91 (38); Anal. Calcld for C19H18O3: C, 76.57; H, 6.43; Found: C, 76.59; H, 6.45.

(E)-1,3-Bis(4-methoxycarbonyl)prop-2-en-1-one (7)
Yellow solid; Yield: 76%; M.p.: 101–103 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.15 (d, J2,3/6,5 = 8.8 Hz, 2H, H-2, H-6), 7.84 (d, J2,3/6,5 = 8.8 Hz, 2H, H-2′, H-6′), 7.81 (d, J2,3/6,5 = 15.2 Hz, 1H, H-2′′ Vinylic), 7.68 (d, J1-2-3 = 15.2 Hz, 1H, H-1′′ Vinylic), 7.08 (d, J3,2/5,6 = 8.8 Hz, 2H, H-3, H-5), 7.01 (d, J2,3/5,6 = 8.8 Hz, 2H, H-3′, H-5′), 3.85 (s, 3H, -OCH3), 3.81 (s, 3H, -OCH3); EI-MS: m/z (rel. abund.%), 268 (M+100), 253 (48), 237 (28), 225 (33), 161 (33), 135 (50), 77 (17); Anal. Calcld for C17H12O3: C, 76.10; H, 6.01; Found: C, 76.12; H, 6.04.

(E)-3-(2,4-Dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (8)
Brownish yellow solid; Yield: 73%; M.p.: 134–136 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.26 (d, J2,3/6,5 = 5.2 Hz, 2H, H-2, H-6), 8.04 (d, J2,3/6,5 = 15.2 Hz, 1H, H-2′ Vinylic), 7.94 (d, J2,3/6,5 = 15.2 Hz, 1H, H-1′′ Vinylic), 7.74 (d, J2,3/6,5 = 2.0 Hz, 1H, H-3′), 7.55 (dd, J2,3/6,5 = 10.0 Hz, 1H, H-5′), 7.09 (d, J3,2/5,6 = 8.8 Hz, 2H, H-3, H-5), 3.86 (s, 3H, -OCH3); EI-MS: m/z (rel. abund. %), 306 (M+100), 308 (M+2,30), 310 (M+4,7), 291 (6), 271 (100), 135 (14); Anal. Calcld for C19H12Cl2O2: C, 62.56; H, 3.94; Found: C, 62.58; H, 3.96.

(E)-3-(4-Bromophenyl)-1-(4-methoxycarbonyl)prop-2-en-1-one (9)
Intense yellow solid; Yield: 71%; M.p.: 152–154 °C; 1H NMR (400 MHz, CDC13): δ 8.02 (d, J2,3/6,5 = 8.8 Hz, 2H, H-2, H-6), 7.73 (d, J2,3/6,5 = 15.6 Hz, 1H, H-2′ Vinylic), 7.54 (ovp, 5H, H-3, H-5′, H-6′, H-1′′ Vinylic), 6.98 (d, J2,3/5,6 = 9.2 Hz, 2H, H-3′, H-5′), 3.87 (s, 3H, -OCH3); EI-MS: m/z (rel. abund.%), 316 (M+100), 318 (M+2,100), 301 (19), 288 (17), 273 (29), 135 (82); Anal. Calcld for C19H15O3Br: C, 60.59; H, 4.13; Found: C, 60.61; H, 4.15.

(E)-1-(4-Methoxyphenyl)-3-(3-methylphenyl)prop-2-en-1-one (10)
Intense yellow solid; Yield: 67%; M.p.: 97–101 °C; 1H NMR (400 MHz, CDCl3): δ 8.02 (d, J2,3/6,5 = 8.8 Hz, 2H, H-2, H-6), 7.76 (d, J2,3/6,5 = 15.6 Hz, 1H, H-2′ Vinylic), 7.54 (d, J2,3/5,6 = 8.4 Hz, 2H, H-2′, H-6′), 7.50 (d, J2,3/6,5 = 15.6 Hz, 1H, H-1′′ Vinylic), 7.24 (d, J3,2/5,6 = 8.4 Hz, 2H, H-3, H-5), 6.97 (d, J2,3/5,6 = 8.8 Hz, 2H, H-3′, H-5′), 3.87 (s, 3H, -OCH3), 2.50 (s, 3H, -SCH3); EI-MS: m/z (rel. abund.%),...
To a stirred solution of aldehyde (1.0 mmol) and acetone (0.5 mmol) in EtOH (10 mL), 3 mL solution of NaOH (60%) was added by the help of energy minimization algorithm implemented in MOE. All the built structures were elucidated by EI-MS and 1H NMR spectroscopic techniques. CHN analysis was also carried out using builder tool in MOE (www.chemcomp.com). All the built structures were synthesized using Builder tool in MOE (www.chemcomp.com).

The percentage of inhibition was calculated as illustrated:

\[
\% \text{Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100
\]

The IC_{50} values, concentration required to inhibit the α-amylase activity by 50% were calculated by non-linear regression graph plotted between percentage inhibition (x axis) versus concentrations (y axis), using a Graph Pad Prism Software (Version 5).

4.5. In silico methodology

To understand the binding modes of the chalcones and bis-chalcones derivatives in the active site of α-amylase enzyme, all compound were docked into the binding site of α-amylase enzyme. The 3D structure of α-amylase (PDB ID: 1HNY) was obtained from Protein Data Bank. Water molecules were removed and the 3D protonation of the protein molecule was carried out. Energy of the protein molecule was minimized with the help of energy minimization algorithm implemented in MOE (Molecular Operating Environment) software and the minimized structure was used for docking. The 3D structures of ligands were built using builder tool in MOE (www.chemcomp.com). All the built structures were 3D protonated and were energy minimized. The 3D structure were saved in pdb file format as input file for docking.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2018.05.003.

References