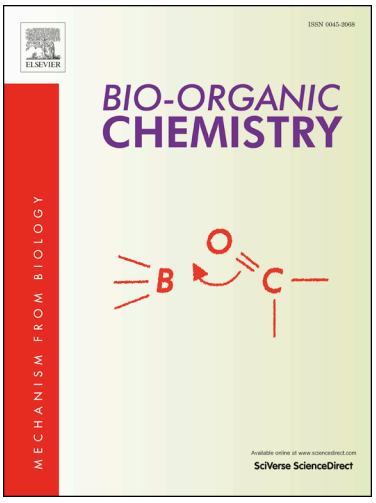

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Chalcones and *Bis*-Chalcones: As Potential *α*-Amylase Inhibitors; Synthesis, *In Vitro* Screening, and Molecular Modelling Studies

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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 ^{1}H-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 IC_{50} = 1.25 ± 1.05 - 2.40 ± 0.09 μM as compared to the standard acarbose (IC_{50} = 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.

Keywords: Chalcones; *bis*-chalcones; *in vitro* *α*-amylase inhibitory activity; structure-activity relationship; *in silico* studies

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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 irresponsive 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 got the considerable attention of 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] (Figure-1).

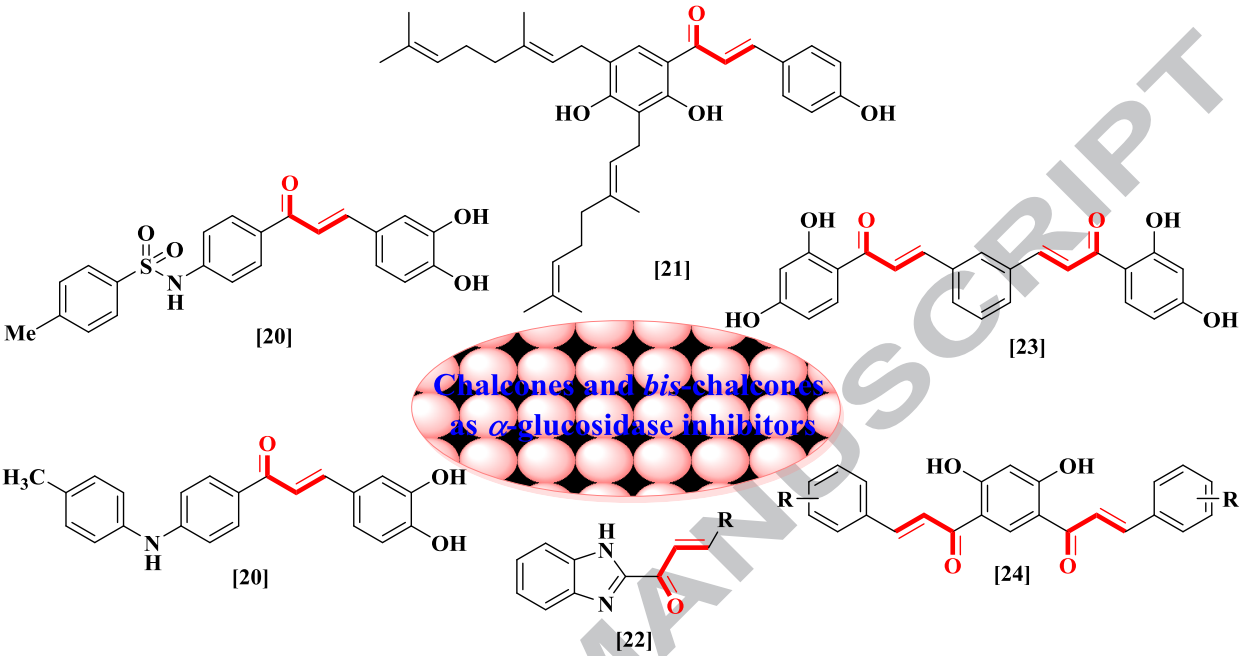


Figure-1: Identified α-glucosidase inhibitors based on chalcones and *bis*-chalcones scaffold

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 afore-mentioned activity (Figure-2).

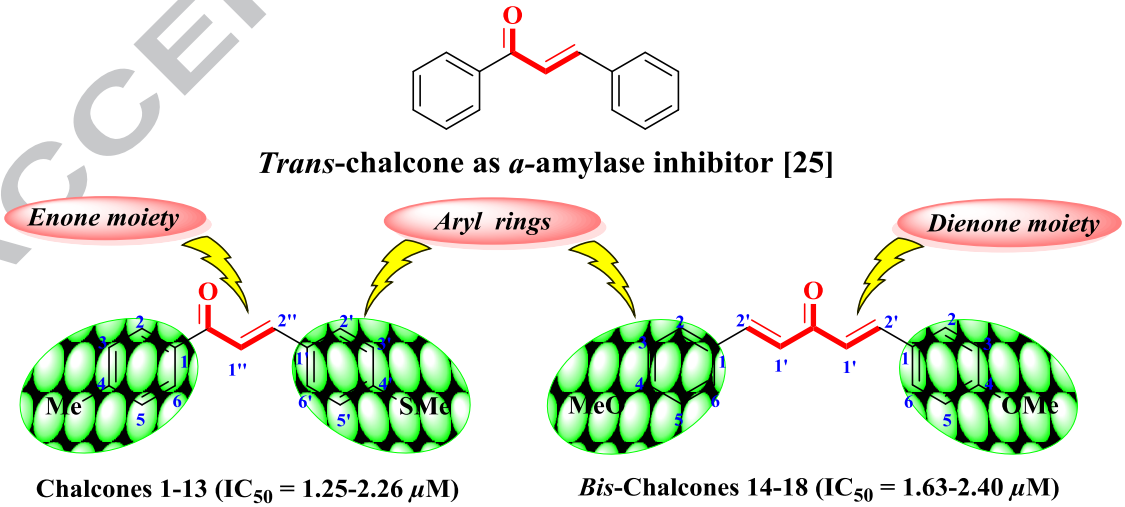
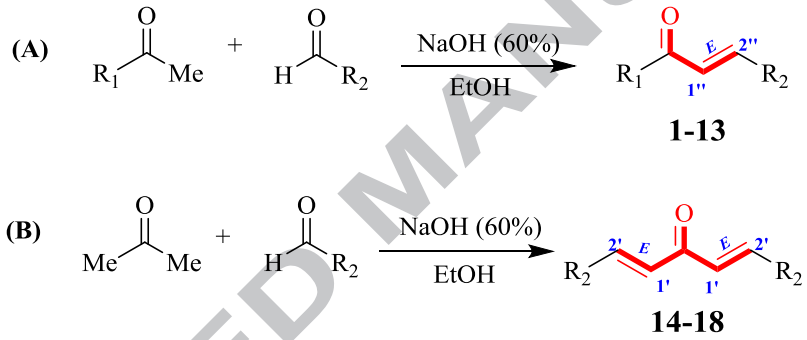


Figure-2: Overview of the structural features and α-amylase inhibitory activity of chalcones **1-13** and *bis*-chalcones **14-18**

Results and Discussion

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 ^{1}H-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.



Scheme-1: Synthesis of (**A**) chalcones **1-13** and (**B**) *bis*-chalcones **14-18**

^{1}H- and ^{13}C-NMR spectra of compound **18** were recorded in deuterated DMSO-d_6 at 400 MHz machine. Being symmetrical in structure, ^{1}H- and ^{13}C-NMR signals are representing the each half of the molecule. In ^{1}H-NMR spectrum, two characteristic signals for H-1′ and -2′ were appeared at δ_H 7.88 and δ_H 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_H 5.85-6.78. Two signals for methylene δ_H (3.89) and methylδ_H (1.28) groups of the ethoxy moiety were appeared as quartet and triplet, respectively (Figure-3A).

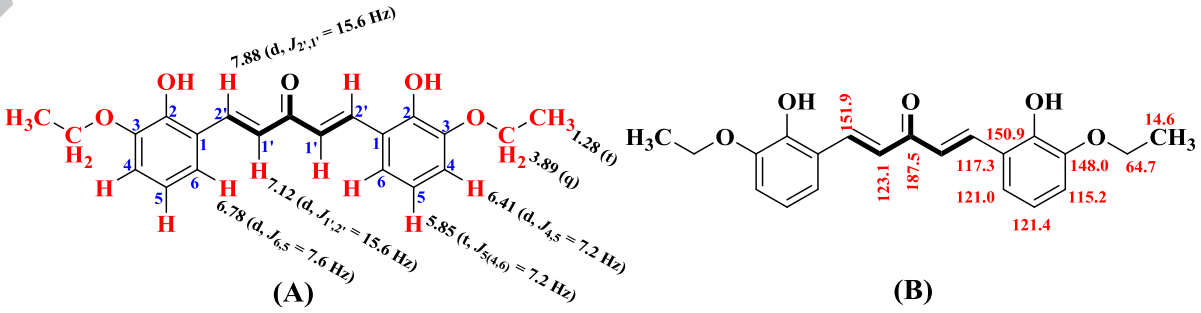


Figure-3: ^{1}H- (**A**) and ^{13}C-NMR (**B**) chemical shifts of new compound **18**

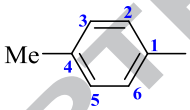
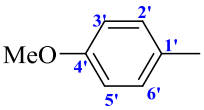
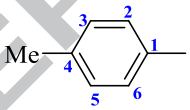
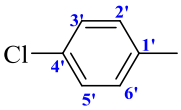
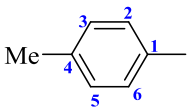
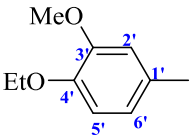
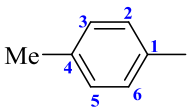
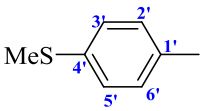
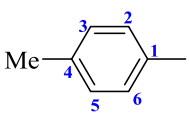
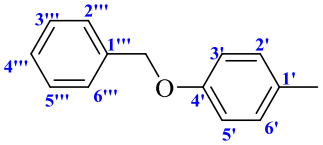
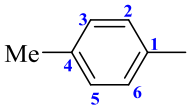
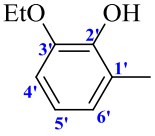
^{13}C-NMR broad-band decoupled spectrum (DMSO-d_6) showed total 11 carbon signals including two methyl, two methylene, ten methine, and seven quarternary carbons (Figure-3B).

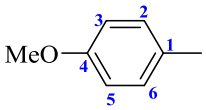
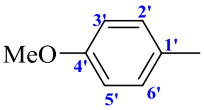
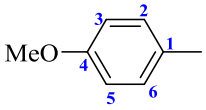
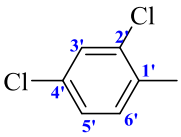
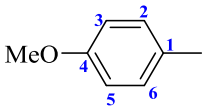
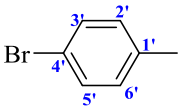
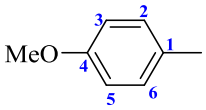
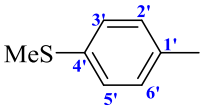
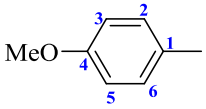
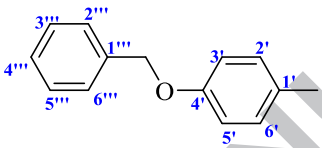
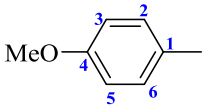
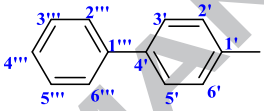
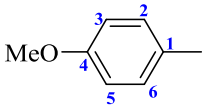
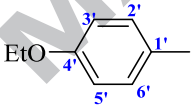
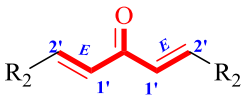
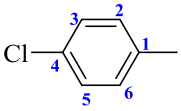
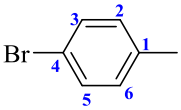
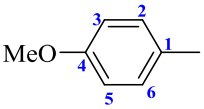
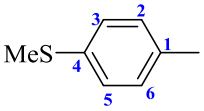
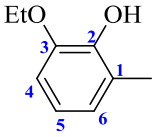
EI-MS of compound **18** displayed the molecular ion peak $[M]^+$ at *m/z* 354 representing the formation of the desired molecule.

***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 IC_{50} = 1.25 ± 1.05-2.40 ± 0.09 μM as compared to the standard acarbose (IC_{50} = 1.04 ± 0.3 μM) (Table-1).

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

Compounds	**R_1**	**R_2**	**α-Amylase inhibitory activity IC_{50} ± SEM[a]**
 **Chalcones**			
1			2.06 ± 0.04
2			1.85 ± 0.09
3			2.07 ± 0.08
4			1.27 ± 0.7
5			1.99 ± 0.05
6			2.26 ± 0.07

7			1.92 ± 0.12
8			1.97 ± 0.08
9			1.98 ± 0.07
10			1.25 ± 1.05
11			2.15 ± 0.07
12			1.99 ± 0.09
13			2.00 ± 0.08
Compounds	**R_2**		**α-Amylase inhibitory activity IC_{50} ± SEM[a]**
 ***Bis*-Chalcones**			
14			1.72 ± 0.1
15			1.80 ± 0.07
16			1.63 ± 0.18
17			2.40 ± 0.09
18			2.12 ± 0.1

Standards = Acarbose[b]	1.04 ± 0.3

SEM[a] (Standard error mean); Acarbose[b] (Standard inhibitor for α-amylase enzyme)

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**, dienone moiety in *bis*-chalcones **14**-**18**, and aryl rings in all compounds (Figure-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 IC_{50} = 1.27 ± 0.7-2.26 ± 0.07 μM as compared to the standard acarbose (IC_{50} = 1.04 ± 0.3 μM). The difference in the inhibitory activity of these compounds is the result of different substituents on aryl ring (R_2). Amongst them, compound **4** (IC_{50} = 1.27 ± 0.7 μM) with 4′-SCH_3 group was found to be the most active and showed inhibitory activity comparable to standard (IC_{50} = 1.04 ± 0.3 μM). Comparison of its activity with derivative **1** (IC_{50} = 2.06 ± 0.04 μM) having OCH_3 instead of SCH_3, showed less activity than compound **4**, revealed that the sulfur atom of SCH_3 is taking part in the interaction with the active site of enzyme. Similarly, replacing SCH_3 to Cl and OBz in compounds **2** (IC_{50} = 1.85 ± 0.09 μM) and **5** (IC_{50} = 1.99 ± 0.05 μM), respectively, brought decline in the inhibitory activity. Furthermore, compounds **3** (IC_{50} = 2.07 ± 0.08 μM) having 3′-OMe and 4′-OEt groups and compound **6** (IC_{50} = 2.26 ± 0.07 μM) having 2′-OMe and 3′-OEt groups on aryl ring (R_2) showed moderate inhibitory activity (Figure-4).

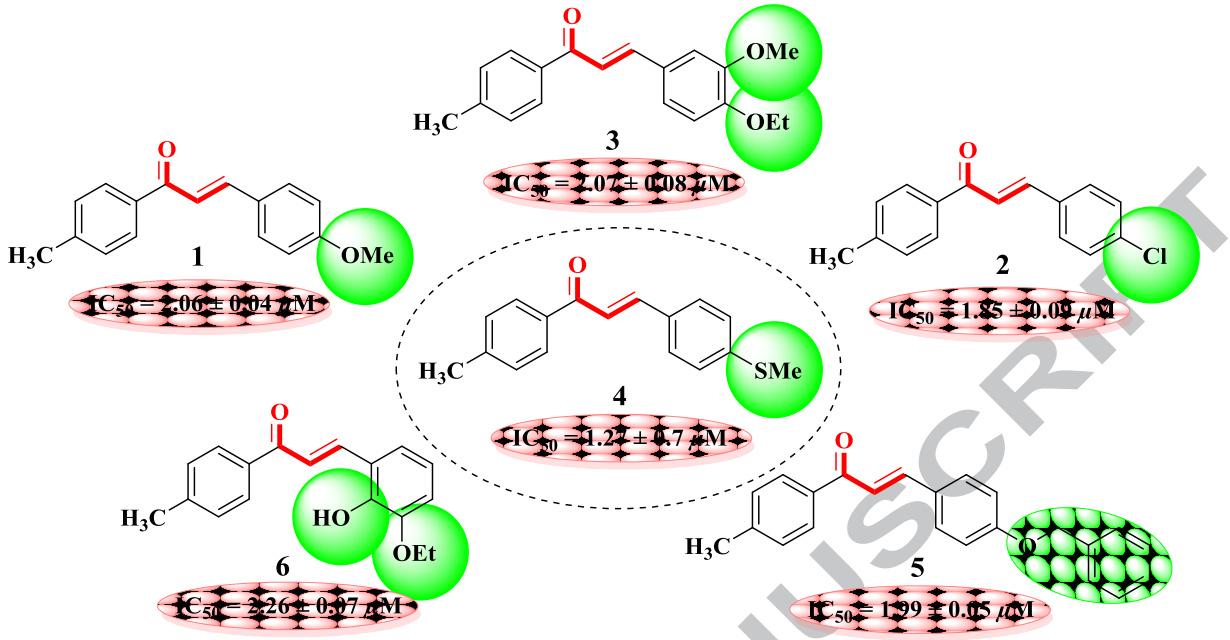


Figure-4: Comparison of α-amylase inhibitory activity of compounds **1-6**

Chalcones **7-13**, derived from 1-(4-methoxyphenyl)ethan-1-one demonstrated α-amylase inhibitory activity ranging from IC_{50} = 1.25 ± 1.05-2.15 ± 0.07 μM. Different groups attached to the aryl ring (R_2) are responsible for varying inhibitory activity. Again in this group of compounds, the 4′-SCH_3 substituted derivative **10** (IC_{50} = 1.25 ± 1.05 μM) was found to be the most active analog. Its activity may compared to distinctively similar compound **4** (IC_{50} = 1.27 ± 0.7 μM) having 4-CH_3 instead of 4-OCH_3 group on aryl ring (R_1), showed almost same activity which further confirmed the important role of SCH_3 group in the inhibitory potential. However, replacement of 4′-SCH_3 with 4′-OMe, 4′-OEt, 4′-Ph, 4′-OBz, 4′-Br, and 2′,4′-di-Cl in compounds **7** (IC_{50} = 1.92 ± 0.12 μM), **13** (IC_{50} = 2.00 ± 0.08 μM), **12** (IC_{50} = 1.99 ± 0.09 μM), **11** (IC_{50} = 2.15 ± 0.07 μM), **9** (IC_{50} = 1.98 ± 0.07 μM), and **8** (IC_{50} = 1.97 ± 0.08 μM), respectively, showed decreased activity (Figure-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.

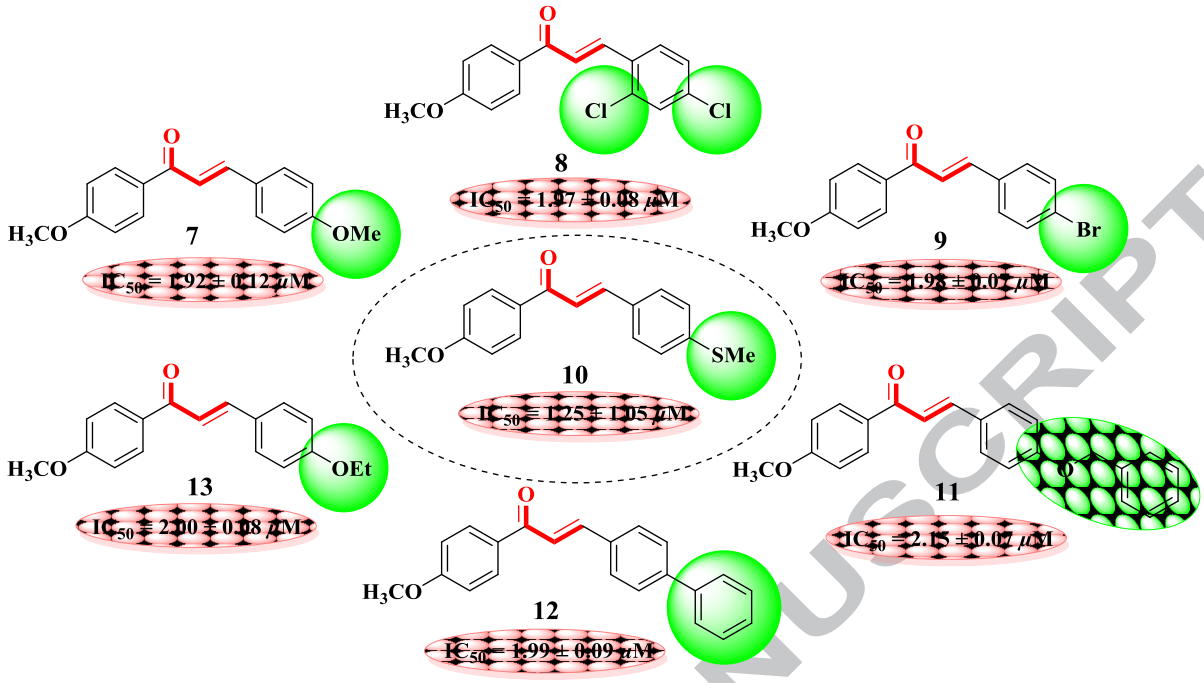


Figure-5: Comparison of α-amylase inhibitory activity of compounds **7-13**

Bis-chalcones **14-18** derived from acetone and demonstrated α-amylase inhibitory potential in the range of IC_{50} = 1.63 ± 0.18 - 2.40 ± 0.09 μM as compared to the standard acarbose (IC_{50} = 1.04 ± 0.3 μM). Amongst these compounds, compound **16** (IC_{50} = 1.63 ± 0.18 μM) with 4/4′-OMe substitution on aryl rings R_1/R_2 was found to be the most active. Replacement of 4/4′-OMe with 4/4′-SMe as in compound **17** (IC_{50} = 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** (IC_{50} = 1.72 ± 0.1 μM) and **15** (IC_{50} = 1.8 ± 0.07 μM) having 4/4′-Cl and 4/4′-Br, respectively, a comparable inhibitory activity was observed. Another compound **18** (IC_{50} = 2.12 ± 0.1 μM) having 2/2′-OH and 3/3′-OEt showed moderate inhibitory activity (Figure-6).

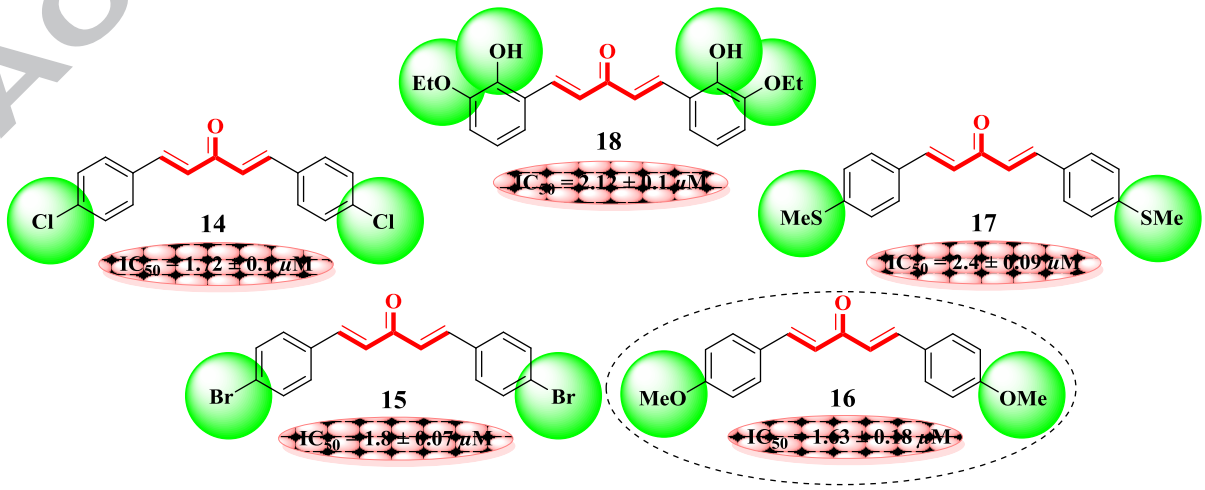


Figure-6: Comparison of α-amylase inhibitory activity of compounds **14-18**

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 compounds with different substituents on aryl ring R_2 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.

***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 Figure-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 electronegative 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

Figure-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 (Figure-7C). Whereas, compound **14** formed five polar and one arene-cation interactions with the active site residues of the *α*-amylase enzyme (Figure-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 showed good docking scores and demonstrated sound *in silico* inhibition of the *α*-amylase enzyme. Overall a good correlation between the docking study and biological evaluation of active chalcones and *bis*-chalcones was perceived. The correlation graph and the correlation coefficient values are given in Figure-8.

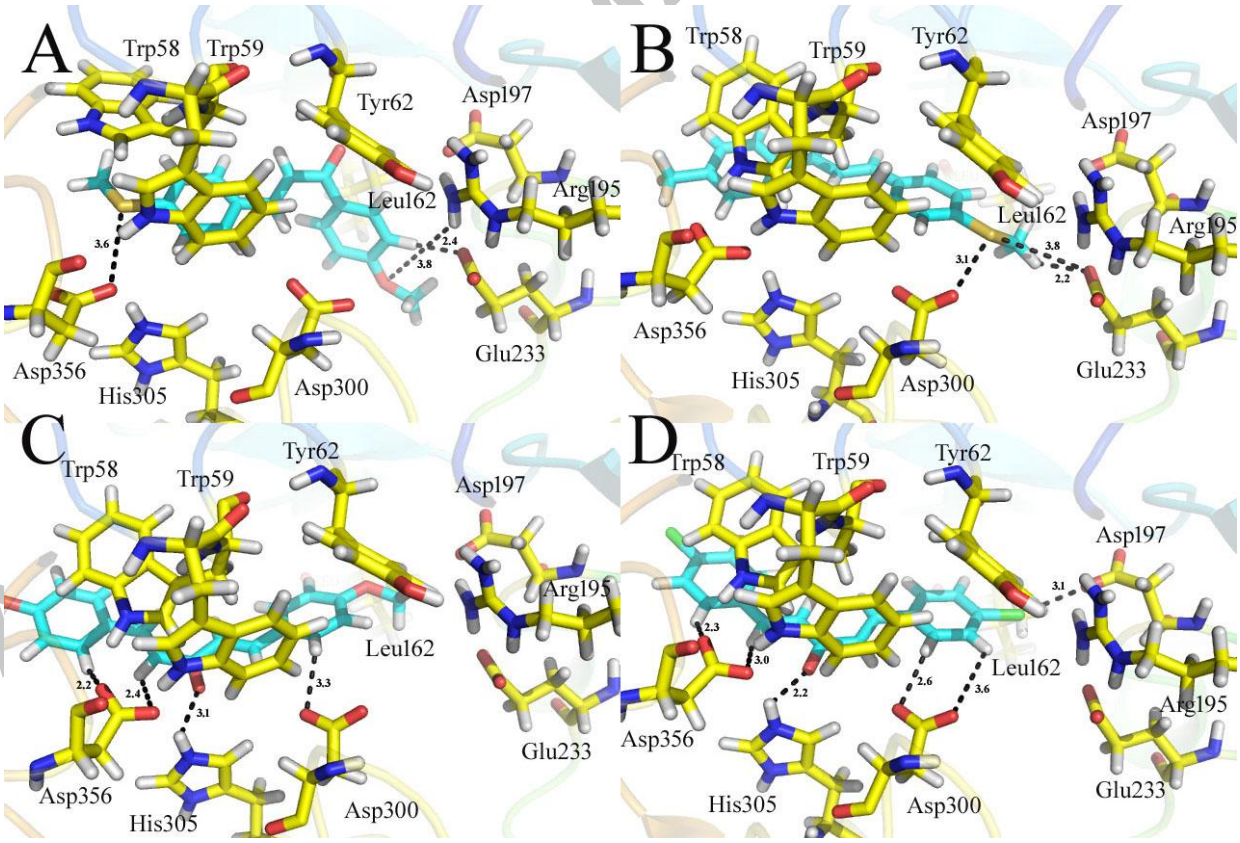


Figure-7: Docking conformations of compounds on *α*-amylase enzyme. (**A**) 3D binding mode of compound **10** as inhibitor of *α*-amylase enzyme. (**B**) 3D binding mode of compound **4**. (**C**) 3D binding mode of compound **16**. (**D**) 3D binding mode of compound **14** in binding cavity of *α*-amylase enzyme. Ligands are shown in cyan color.

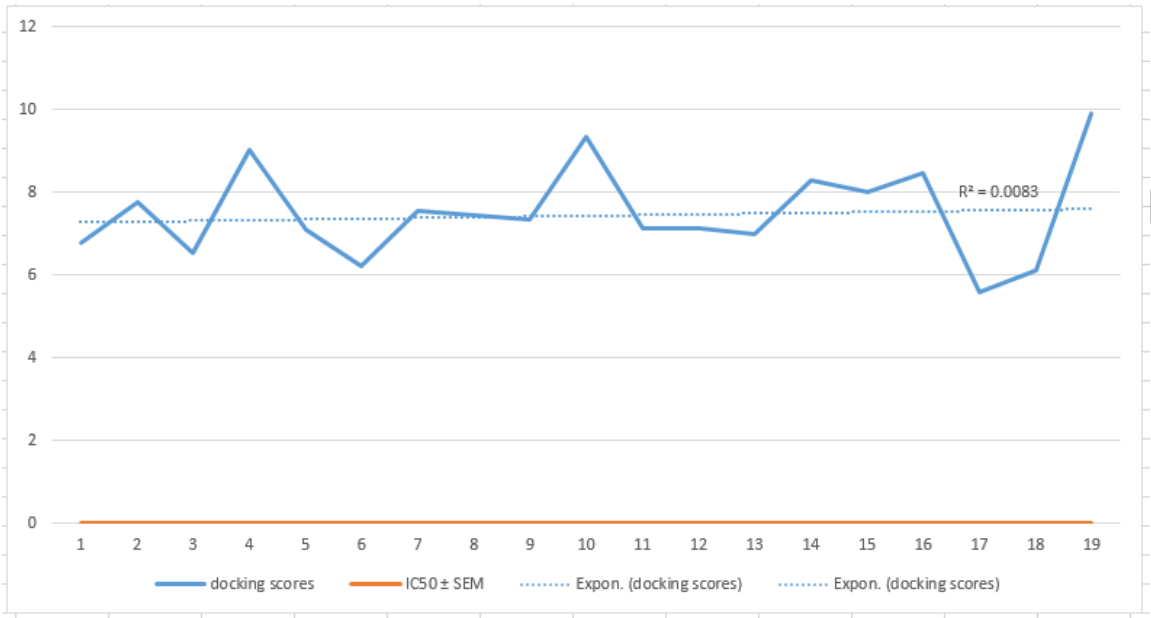


Figure-8: A correlation graph for predicted docking score and IC_{50} values

Table-2: Docking scores and report of predicted interactions of docked conformations

Interaction Report						
Comp.	**Docking Score**	**Ligand**	**Receptor**	**Interaction**	**Distance**	**E (kcal/mol)**
1	-6.7845	C1 1 C19 32	OD2 ASP 300 (A) 6-ring TRP 59 (A)	H-donor H-π	3.36 3.61	-0.2 -0.3
2	-7.7641	CL18 31 6-ring 6-ring 6-ring	OD2 ASP 356 (A) CB TRP 59 (A) CD1 LEU 162 (A) CD2 LEU 162 (A)	H-donor π-H π-H π-H	3.24 3.56 4.57 3.79	-0.0 -0.3 -0.2 -0.9
3	-6.5211	6-ring	CB TRP 59 (A)	π-H	3.72	-0.2
4	-9.0231	S18 31 S18 31 C19 32 6-ring 6-ring	OE1 GLU 233 (A) OD2 ASP 300 (A) OE1 GLU 233 (A) 6-ring TRP 59 (A) 5-ring TRP 59 (A)	H-donor H-donor H-donor π-π π-π	3.82 3.13 2.20 3.76 3.86	-0.1 -1.0 -0.5 -0.0 -0.0
5	-7.1093	C4 6 C19 32 6-ring 6-ring	OD2 ASP 356 (A) OD1 ASP 197 (A) 5-ring TRP 59 (A) 6-ring TRP 59 (A)	H-donor H-donor π-π π-π	3.52 3.50 3.56 3.95	-0.3 -0.2 -0.0 -0.0
6	-6.2198	O18 30 C21 37 6-ring	OD1 ASP 300 (A) OE1 GLU 233 (A) CB TYR 62 (A)	H-donor H-donor π-H	2.99 3.59 4.10	-2.7 -0.3 -0.2
7	-7.5432	C18 28 C18 28 C20 33 6-ring	OD1 ASP 300 (A) 6-ring TYR 62 (A) 5-ring TRP 59 (A) 6-ring TRP 59 (A)	H-donor H-π H-π π-π	3.45 3.88 4.66 3.45	-0.3 -0.8 -0.4 -0.0
8	-7.4389	C13 19 CL17 26 6-ring 6-ring	OD1 ASP 300 (A) OE1 GLU 233 (A) 5-ring TRP 59 (A) 6-ring TRP 59 (A)	H-donor H-donor π-π π-π	3.55 3.21 3.67 3.70	-0.3 -0.6 -0.0 -0.0
9	-7.3421	C4 6 BR17 27 BR17 27	OD2 ASP 356 (A) OD1 ASP 197 (A) OD2 ASP 197 (A)	H-donor H-donor H-donor	3.45 3.47 3.88	-0.5 -0.5 -0.5

		C10 15	6-ring TRP 58 (A)	H-π	4.78	-0.3
		C19 29	6-ring TRP 59 (A)	H-π	4.70	-0.2
		6-ring	5-ring TRP 59 (A)	π-π	3.46	-0.0
		6-ring	6-ring TRP 59 (A)	π-π	3.88	-0.0
10	-9.3467	C3 4	OE1 GLU 233 (A)	H-donor	2.39	-0.5
		O	NH ARG 195 (A)	H-donor	3.84	-0.0
		S19 32	OD2 ASP 356 (A)	H-donor	3.60	-0.5
		C20 33	6-ring TRP 59 (A)	H-π	3.67	-0.8
		6-ring	CB TRP 59 (A)	π-H	3.85	-0.3
		6-ring	CD2 LEU 162 (A)	π-H	3.83	-0.7
		6-ring	CB ALA 198 (A)	π-H	4.71	-0.2
11	-7.1239	C23 38	OD1 ASP 356 (A)	H-donor	3.53	-0.2
		C18 28	6-ring TRP 59 (A)	H-π	3.61	-0.6
12	-7.1192	O8 12	CB TYR 62 (A)	H-acceptor	3.58	-0.2
		6-ring	CB TRP 59 (A)	π-H	3.73	-0.5
		6-ring	CD1 LEU 162 (A)	π-H	4.11	-0.2
		6-ring	CD2 LEU 162 (A)	π-H	3.78	-0.5
		6-ring	5-ring TRP 59 (A)	π-π	3.54	-0.0
13	-7.0027	C13 20	OD1 ASP 300 (A)	H-donor	3.57	-0.3
		6-ring	5-ring TRP 59 (A)	π-π	3.65	-0.0
		6-ring	6-ring TRP 59 (A)	π-π	3.67	-0.0
14	-8.2893	C4 6	OD1 ASP 300 (A)	H-donor	2.62	-0.3
		C8 13	OD1 ASP 300 (A)	H-donor	3.62	-1.1
		C12 19	OD1 ASP 356 (A)	H-donor	2.30	-0.3
		C18 29	OD1 ASP 356 (A)	H-donor	3.00	-0.5
		CL19 31	OD1 ASP 197 (A)	H-donor	3.12	-0.4
		6-ring	6-ring TRP 59 (A)	π-π	3.78	-0.0
15	-7.9887	C12 19	OD2 ASP 356 (A)	H-donor	3.61	-0.3
		C18 29	OD1 ASP 356 (A)	H-donor	3.57	-0.4
		BR19 31	OD1 ASP 197 (A)	H-donor	3.46	-0.4
		BR19 31	OD2 ASP 197 (A)	H-donor	3.83	-0.8
		C7 11	6-ring TRP 58 (A)	H-π	4.78	-0.2
		6-ring	CG PRO 54 (A)	π-H	4.54	-0.4
		6-ring	CB TRP 357 (A)	π-H	4.58	-0.2
16	-8.4512	C4 6	OD1 ASP 356 (A)	H-donor	2.21	-0.3
		C8 13	OD1 ASP 356 (A)	H-donor	2.42	-0.3
		C12 19	OD1 ASP 300 (A)	H-donor	3.33	-0.3
		O	NH HIS 305 (A)	H-donor	3.15	-0.3
17	-5.5673	C12 19	OD2 ASP 356 (A)	H-donor	3.50	-0.5
		S21 36	OD1 ASP 197 (A)	H-donor	3.27	-0.3
		S21 36	OD2 ASP 197 (A)	H-donor	3.64	-1.2
18	-6.1107	C16 22	OD1 ASP 300 (A)	H-donor	3.37	-0.3
		C23 35	OD1 ASP 300 (A)	H-donor	3.66	-0.3
		O11 15	CB THR 163 (A)	H-acceptor	3.29	-0.2
		C23 35	6-ring TYR 62 (A)	H-π	3.90	-0.2
Standard	-9.8935	C 19	OD1 ASP 300 (A)	H-donor	3.42	-0.7
		C 36	OD1 ASP 300 (A)	H-donor	3.09	-1.2
		C 36	OD2 ASP 300 (A)	H-donor	2.96	-0.8
		O 61	OD1 ASP 197 (A)	H-donor	2.67	-4.2
		O 65	OD1 ASP 356 (A)	H-donor	2.83	-3.3
		O 69	OD1 ASP 197 (A)	H-donor	2.60	-1.6
		O 79	OG1 ARG 195 (A)	H-donor	2.81	-1.8
		C 5	6-ring TRP 59 (A)	H-π	3.64	-0.6

Conclusion

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

Experimental

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 (δ) 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.

General procedure for the synthesis of chalcones 1-13

To a stirred solution of 4-methyl/4-methoxy acetophenone (0.5 mmol) in EtOH (10 mL), solution of NaOH (60%) (3 mL) was added drop-wise by maintaining the temperature at 0 $^\circ$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 ^{1}H-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; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.05 (d, $J_{2,3/6,5}$ = 8.4 Hz, 2H, H-2, H-6), 7.84 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.80 (d, $J_{2'',1''}$ = 14.4 Hz, 1H, H-2″ Vinylic), 7.70 (d, $J_{1'',2''}$ = 15.2 Hz, 1H, H-1″ Vinylic), 7.37 (d, $J_{3,2/5,6}$ = 8.0 Hz, 2H, H-3, H-5), 7.01 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 3.81 (s, 3H, OCH_3), 2.39 (s, 3H, CH_3); EI-MS: *m/z* (rel. abund. %), 252 (M^+, 100), 237 (43), 161 (29), 108 (16), 90 (19), 64 (7); Anal. Calcd for $C_{17}H_{16}O_2$: 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; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 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, $J_{2',3'/6',5'}$ = 7.2 Hz, 2H, H-2′, H-6′), 7.70 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″ Vinylic), 7.50 (d, $J_{3',2'/5',6'}$ = 8.1 Hz, 2H, H-3′, H-5′), 7.36 (d, $J_{3,2/5,6}$ = 8.1 Hz, 2H, H-3, H-5), 2.37 (s, 3H, CH_3); 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 $C_{16}H_{13}ClO$: 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; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.06 (d, $J_{2,3/6,5}$ = 8.4 Hz, 2H, H-2, H-6), 7.81 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.68 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″ Vinylic), 7.52 (d, $J_{2',6'}$ = 1.6 Hz, 1H, H-2′), 7.37 (ovp, 3H, H-3, H-5, H-6′), 7.00 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5′), 4.09 (q, 2H, O-$\underline{CH_2}CH_3$), 3.85 (s, 3H, O$\underline{CH_3}$), 2.39 (s, 3H, -CH_3), 1.35 (t, 3H, O-$CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 188.9, 149.5, 149.3, 145.3, 144.0, 134.7, 129.6, 129.6, 129.3, 129.3, 126.4, 122.0, 121.2, 118.0, 111.2, 64.7, 56.2, 21.5, 14.6; EI-MS: *m/z* (rel. abund. %), 296 (M^+, 100), 281 (6), 267 (76), 119 (20), 91 (18); Anal. Calcd for $C_{19}H_{20}O_3$: 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 (4)

Yellow solid; Yield: 69%; M.p.: 96-98 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.06 (d, $J_{2,3/6,5}$ = 8.0 Hz, 2H, H-2, H-6), 7.89 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.82 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2′, H-6′), 7.70 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″ Vinylic), 7.37 (d, $J_{3,2/5,6}$ = 8.0 Hz, 2H, H-3, H-5), 7.31 (d, $J_{3',2'/5',6'}$ = 8.4 Hz, 2H, H-3′, H-5′), 2.51 (s, 3H, -S$\underline{CH_3}$) 2.39 (s, 3H, -CH_3); EI-MS: *m/z* (rel. abund. %), 268 (M^+, 100), 253 (77), 221 (76), 177 (31), 119 (38), 91 (40); Anal. Calcd for $C_{17}H_{16}OS$: C, 76.08; H, 6.01; Found: C, 76.10; H, 6.04.

(*E*)-3-(4-(Benzyloxy)phenyl)-1-(*p*-tolyl)prop-2-en-1-one (5)

Yellow solid; Yield: 72%; M.p.: 110-112 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.05 (d, $J_{2,3/6,5}$ = 8.0 Hz, 2H, H-2, H-6), 7.84 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.80 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.70 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″, Vinylic), 7.47 (d, $J_{3,2/5,6}$ = 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, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 5.17 (s, 2H, -O$\underline{CH_2}C_6H_5$), 2.39 (s, 3H, -CH_3); EI-MS: *m/z* (rel. abund. %), 328 (M^+, 75), 237 (15), 119 (22), 91 (100), 65 (16); Anal. Calcd for $C_{23}H_{20}O_2$: 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; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.02 (d, $J_{2'',1''}$ = 14.8 Hz, 1H, H-2″ Vinylic), 7.86 (ovp, 3H, H-2, H-6, H-1″ Vinylic), 7.29 (d, $J_{3,2/5,6}$ = 7.6 Hz, 2H, H-3, H-5), 6.92 (d, $J_{6',5'}$ = 7.6 Hz, 2H, H-6′), 6.49 (d, $J_{4',5'}$ = 6.8 Hz, 2H, H-4′), 5.95 (bd.t, 1H, H-5′), 3.91 (q, 2H, -O-$\underline{CH_2}CH_3$), 2.36 (s, 3H, -CH_3), 1.28 (s, 3H, -O-$CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 188.8, 151.3, 147.7, 144.0, 141.2, 134.7, 129.7, 129.7, 129.3, 129.3, 121.5, 121.1, 120.4, 117.0, 115.2, 64.7, 21.5, 14.6; EI-MS: *m/z* (rel. abund. %), 282 (M^+, 73), 265 (55), 119 (100), 91 (38); Anal. Calcd for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43; Found: C, 76.59; H, 6.45.

(*E*)-1,3-*Bis*(4-methoxyphenyl)prop-2-en-1-one (7)

Yellow solid; Yield: 76%; M.p.:101-103 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.15 (d, $J_{2,3/6,5}$ = 8.8 Hz, 2H, H-2, H-6), 7.84 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.81 (d, $J_{2'',1''}$ = 15.2 Hz, 1H, H-2″ Vinylic), 7.68 (d, $J_{1'',2''}$ = 15.2 Hz, 1H, H-1″ Vinylic), 7.08 (d, $J_{3,2/5,6}$ = 8.8 Hz, 2H, H-3, H-5), 7.01 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 3.85 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3); EI-MS: *m/z* (rel. abund. %), 268 (M^+, 100), 253 (48), 237 (28), 225 (33), 161 (33), 135 (50), 77 (17); Anal. Calcd for $C_{17}H_{16}O_3$: 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; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.26 (d, $J_{6',5'}$ = 8.4 Hz, 1H, H-6′), 8.18 (d, $J_{2,3/6,5}$ = 5.2 Hz, 2H, H-2, H-6), 8.04 (d, $J_{2'',1''}$ = 15.2 Hz, 1H, H-2″ Vinylic), 7.94 (d, $J_{1'',2''}$ = 15.2 Hz, 1H, H-1″ Vinylic), 7.74 (d, $J_{3',5'}$ = 2.0 Hz, 1H, H-3′), 7.55 (dd, $J_{5',3'}$ = 2.0 Hz, $J_{5',6'}$ = 10.0 Hz, 1H, H-5′), 7.09 (d, $J_{3,2/5,6}$ = 8.8 Hz, 2H, H-

3, H-5), 3.86 (s, 3H, -OCH_3); EI-MS: *m/z* (rel. abund. %), 306 (M^+, 54), 308 (M+2, 30), 310 (M+4, 7), 291 (6), 271 (100), 135 (14); Anal. Calcd for $C_{16}H_{12}Cl_2O_2$: C, 62.56; H, 3.94; Found: C, 62.58; H, 3.96.

(*E*)-3-(4-Bromophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (9)

Intense yellow solid; Yield: 71%; M.p.:152-154 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.02 (d, $J_{2,3/6,5}$ = 8.8 Hz, 2H, H-2, H-6), 7.73 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.54 (ovp, 5H, H-3, H-5, H-2′, H-6′, H-1″ Vinylic), 6.98 (d, $J_{3',2'/5',6'}$ = 9.2 Hz, 2H, H-3′, H-5′), 3.87 (s, 3H, -O$\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 316 (M^+, 97), 318 (M+2, 100), 301 (19), 288 (17), 237 (29), 135 (82); Anal. Calcd for $C_{16}H_{13Br}O_2$: C, 60.59; H, 4.13; Found: C, 60.61; H, 4.15.

(*E*)-1-(4-Methoxyphenyl)-3-(4-(methylthio)phenyl)prop-2-en-1-one (10)

Intense yellow solid; Yield: 67%; M.p.: 97-101 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.02 (d, $J_{2,3/6,5}$ = 8.8 Hz, 2H, H-2, H-6), 7.76 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.54 (d, $J_{2',3'/6',5'}$ = = 8.4 Hz, 2H, H-2′, H-6′), 7.50 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″ Vinylic), 7.24 (d, $J_{3,2/5,6}$ = 8.4 Hz, 2H, H-3, H-5), 6.97 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 3.87 (s, 3H, -O$\underline{CH_3}$), 2.50 (s, 3H, -S$\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 284 (M^+, 100), 269 (43), 237 (40), 135 (53), 77 (18); Anal. Calcd for $C_{17}H_{16}O_2S$: C, 71.80; H, 5.67; Found: C, 71.83; H, 5.65.

(*E*)-3-(4-(Benzyloxy)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (11)

Yellow solid; Yield: 74%; M.p.:135-137 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.74 (d, $J_{2,3/6,5}$ = 8.4 Hz, 2H, H-2, H-6), 7.72 (d, $J_{2'',1''}$ = 15.6 Hz, 1H, H-2″ Vinylic), 7.46-7.33 (ovp, 9H, H-3, H-5, H-2′, H-6′, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴), 7.19 (d, $J_{1'',2''}$ = 15.6 Hz, 1H, H-1″ Vinylic), 7.10 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 5.17 (s, 2H, -$\underline{CH_2}C_6H_5$), EI-MS: m/z (rel. abund. %), 344 (M^+, 7), 327 (3), 300 (10), 91 (100); Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85; Found: C, 80.23; H, 5.88.

(*E*)-3-([1,1′-Biphenyl]-4-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (12)

Yellow solid; Yield: 76%; M.p.: 203-206 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.90 (d, $J_{2,3/6,5}$ = 8.4 Hz, 2H, H-2, H-6), 7.97 (d, $J_{2'',1''}$ = 16.0 Hz, 1H, H-2″ Vinylic), 7.79 (d, $J_{2',3'/6',5'}$ = = 8.0 Hz, 2H, H-2′, H-6′), 7.75 (d, $J_{2''',3'''/6''',5'''}$ = = 7.2 Hz, 2H, H-2‴, H-6‴), 7.51 (ovp, 8H, H-3, H-5, H-3′, H-5′, H-1″ Vinylic, H-3‴, H-5‴), 3.30 (s, 3H, -O$\underline{CH_3}$); EI-MS: *m/z* (rel. abund.

%), 314 (M^+, 43), 178 (81), 386 (100); Anal. Calcd for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77; Found: C, 84.03; H, 5.75.

(*E*)-3-(4-Ethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (13)

Yellow solid; Yield: 78%; M.p.:105-107 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.15 (d, $J_{2,3/6,5}$ = 8.8 Hz, 2H, H-2, H-6), 7.84 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.81 (d, $J_{2'',1''}$ = 15.2 Hz, 1H, H-2″ Vinylic), 7.68 (d, $J_{1'',2''}$ = 15.2 Hz, 1H, H-1″ Vinylic), 7.08 (d, $J_{3,2/5,6}$ = 8.8 Hz, 2H, H-3, H-5), 7.01 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 3.91 (q, 2H, -O<u>CH$_2$</u>-CH$_3$), 3.85 (s, 3H, -OCH$_3$), 1.28 (s, 3H, -O-CH$_2$<u>CH$_3$</u>); EI-MS: *m/z* (rel. abund. %), 282 (M^+, 23), 265 (24), 253 (19), 237 (17), 200 (45); Anal. Calcd for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43; Found: C, 76.57; H, 6.43.

General procedure for the synthesis of *bis*-chalcones 14-18

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 maintaining the temperature at 0 °C. Reaction mixture was 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. Structures of compounds **14-18** were deduced by EI-MS and ^{1}H-NMR spectroscopic techniques. CHN analysis was also carried out.

(1*E*,4*E*)-1,5-*Bis*(4-chlorophenyl)penta-1,4-dien-3-one (14)

Brown solid; Yield: 69%; M.p.:192-194 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.82 (d, $J_{3,2/5,6}$ = 6.4 Hz, 2H, H-3, H-5), 7.80 (d, $J_{2',1'}$ = 16.0 Hz, 1H, H-2′ Vinylic), 7.54 (d, $J_{2,3/6,5}$ = 8.4 Hz, 2H, H-2, H-6), 7.37 (d, $J_{1',2'}$ = 16.0 Hz, 1H, H-1′ Vinylic); EI-MS: *m/z* (rel. abund. %), 302 (M^+, 100), 304 (M+2, 70), 306 (M+4, 15), 267 (62), 239 (23), 204 (55), 165 (61), 137 (38), 101 (7), 75 (18); Anal. Calcd for $C_{17}H_{12}Cl_2O$: C, 67.35; H, 3.99; Found: C, 67.33; H, 3.97.

(1*E*,4*E*)-1,5-*Bis*(4-bromophenyl)penta-1,4-dien-3-one (15)

Brown solid; Yield: 66%; M.p.:148-150 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.78 (ovp, 3H, H-2, H-6, H-2′ Vinylic), 7.67 (d, $J_{3,2/5,6}$ = 8.4 Hz, 2H, H-3, H-5), 7.38 (d, $J_{1',2'}$ = 16.0 Hz, 1H, H-1′ Vinylic); EI-MS: *m/z* (rel. abund. %), 390 (M^+, 70), 392 (M+2, 100), 394 (M+4, 67), 311 (72), 209 (27), 204 (75), 102 (35); Anal. Calcd for $C_{17}H_{12}Br_2O$: C, 52.08; H, 3.09; Found: C, 52.06; H, 3.12.

(1*E*,4*E*)-1,5-*Bis*(4-methoxyphenyl)penta-1,4-dien-3-one (16)

Yellow solid; Yield: 74%; M.p.: 130-132 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.74 (d, $J_{2,3/6,5}$ = 8.8 Hz, 2H, H-2, H-6), 7.73 (d, $J_{2',1'}$ = 15.6 Hz, 1H, H-2′ Vinylic), 7.19 (d, $J_{1',2'}$ = 15.6 Hz, 1H, H-1′ Vinylic), 7.02 (d, $J_{3,2/5,6}$ = 8.8 Hz, 2H, H-3, H-5), 3.80 (s, 3H, OCH_3); EI-MS: *m/z* (rel. abund. %), 294 (M^+, 100), 279 (28), 186 (47), 161 (39), 133 (25), 121 (20); Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16; Found: C, 77.55; H, 6.18.

(1*E*,4*E*)-1,5-*Bis*(4-(methylthio)phenyl)penta-1,4-dien-3-one (17)

Yellow solid; Yield: 76%; M.p.: 143-145 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.74 (ovp, 3H, H-2, H-6, H-2′ Vinylic), 7.32 (ovp, 3H, H-3, H-5, H-1′ Vinylic), 2.51 (s, 3H, -SCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 187.3, 141.8, 141.8, 138.4, 138.4, 131.8, 131.8, 128.7, 128.7, 128.7, 128.7, 127.0, 127.0, 127.0, 127.0, 123.0, 123.0, 14.5, 14.5; EI-MS: *m/z* (rel. abund. %), 326 (M^+, 100), 311 (6), 279 (34), 204 (38), 177 (10), 134 (19), 102 (11); Anal. Calcd for $C_{19}H_{18}OS_2$: C, 69.90; H, 5.56; Found: C, 69.93; H, 5.58.

(1*E*,4*E*)-1,5-*Bis*(3-ethoxy-2-hydroxyphenyl)penta-1,4-dien-3-one (18)

Reddish brown solid; Yield: 79%; M.p.: >300 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.88 (d, $J_{2',1'}$ = 15.6 Hz, 1H, H-2′ Vinylic), 7.12 (d, $J_{1',2'}$ = 15.6 Hz, 1H, H-1′ Vinylic), 6.78 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6), 6.41 (d, $J_{4,5}$ = 7.2 Hz, 1H, H-4), 5.85 (t, $J_{5(4,6)}$ = 7.2 Hz, 1H, H-5), 3.89 (q, 4H, -O-$\underline{CH_2}CH_3$), 1.28 (t, 3H, -O-$CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 187.5, 151.9, 151.9, 150.9, 150.9, 148.0, 148.0, 123.1, 123.1, 121.4, 121.4, 121.0, 121.0, 117.3, 117.3, 115.2, 115.2, 64.7, 64.7, 14.6, 14.6; EI-MS: *m/z* (rel. abund. %), 354 (M^+, 16), 336 (73), 321 (100), 279 (56), 163 (52), 134 (23), 43 (52); Anal. Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26; Found: C, 71.19; H, 6.28.

***In vitro* α-amylase inhibitory assay**

The α-amylase inhibitory activity was determined as method reported by Uzma *et al*. [38]. A volume of 500 μL of test sample (100, 200, 400, 800, 1000 μg/mL) and 500 μL of α-amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. After pre-incubation, 500 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25 °C for 10 minutes. The reaction was arrested with 1 mL of dinitrosalicylic acid colour reagent. The tubes were then incubated in

boiling water for 5 min and cooled to room temperature. The solutions were diluted after adding 10 mL distilled water and the absorbance was measured at 540 nm [39-40].

The percentage of inhibition was calculated as illustrated:

$$\% \text{ Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}} \times 100$$

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

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Highlights:

- Substituted chalcones **1-13** and *bis*-chalcones **14-18** were synthesized and structurally characterized.
- Compounds **1-18** were screened for *in vitro* α-amylase inhibitory activity.
- Limited structure-activity relationship was established.
- *In silico* study was conducted to predict the binding interactions of compounds with the active site of α-amylase enzyme.

Chalcones and *Bis*-Chalcones: As Potential α-Amylase Inhibitors; Synthesis, *In Vitro* Screening, and Molecular Modelling Studies

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