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Design, and facile synthesis of 1,3 diaryl-3-(arylamino)propan-1-one derivatives as the potential alpha-amylase inhibitors and antioxidants

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ABSTRACT

Diabetes is the most prevalent metabolic disorder causing a high rate of mortality and morbidity. Recently alpha-amylase is reported to be good drug design target for the treatment of diabetes mellitus. We have designed 116 molecules based on aza-Michael adduct of *trans*-chalcone as 1,3 diaryl-3-(arylamino)propan-1-ones which were studied by molecular docking and among them best six derivatives were synthesized easily via aza-Michael addition on *trans*-chalcone using KOH as a catalyst and evaluated for alpha-amylase inhibition along with antioxidant activity. It was observed that all compounds have alpha-amylase inhibitory activity but at different extents. The molecule **3e** is the most potent alpha-amylase inhibitor of this series. **3a** is the second most potent compound, whereas only one molecule **3d** has shown antioxidant activity.

1. Introduction

Diabetes mellitus is the most prevalent non-infective metabolic disorder among the millions of people around the world [1]. Recent studies have shown that the prevalence of the disease is growing, from a worldwide prevalence of 422 million persons in 2016 to 844 million persons in 2030 [2]. Diabetes mellitus is classified into two types: type 1 and type 2 [1]. Type 2 diabetes is responsible for 90–95 percent of diabetes cases around the world. In type 2 diabetes, insulin is available but the body cannot use it which is referred to as insulin resistance. On the progression of type 2 diabetes, it gets worse and leading to produce less insulin. There are several factors cause diabetes, among them, the main risk factor for type 2 diabetes is obesity and overweight [3]. Looking at the main drawback with insulin and its preparations, various oral hypoglycemic agents were discovered as antidiabetic agents including alpha-amylase inhibitors (Fig. 1) [4]. Pancreatic and salivary glands in human are the main regions for production and secretion of alpha-amylase enzyme [5]. The enzyme can be obtained from three main sources *viz* plants, animals, and microorganisms have a number of industrial and biotechnological applications. Alpha-amylases play a major role in the metabolism of starch and glycogen to control the blood glucose level. In human salivary and pancreatic enzymes are alpha-amylase enzymes [6]. Both enzymes are studied well due to their importance in a number of disorders like diabetes, pancreatitis, and parotitis. Inhibitors of alpha-amylase enzyme may reduce the glucose levels that can occur after a meal by slowing the speed of conversion of starch to monosaccharides [7]. Recently various newer alpha amylase inhibitors were identified as small molecules like indole [8], benzofuran hydrazine [9], thiadiazole [10], and chromone [11].

We designed our target molecules employing aza-Michael addition reaction due to its versatility in constructions of C—N bond via nucleophilic addition of aniline/amine to alpha, beta-unsaturated compounds in a straightforward manner.

There are several catalyst used for aza-Michael reaction like Lewis acidic catalysts $PtCl_4{\cdot}5H_2O$ [12], $Cu(OTf)_2$ [13], $InCl_3$ [14], $Yb(OTf_3)$ [15], $LiClO_4$ [16], $Bi(NO_3)$ [17], $FeCl_3{\cdot}6H_2O$ [18], $CeCl_3{\cdot}7H_2O$ [19], ZnO [20], MgO [21], silica supported perchloric acid [22,23], ionic liquid [24], sulfated zirconia [25], organocatalytic [26], and bases like 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [27].

2. Results & discussion

Since *trans*-chalcone is the scaffold which can inhibit the alpha-amylase enzyme [28,29] and reduces the absorption of smaller absorbable carbohydrates like glucose thus, we have designed 116 molecules based on *trans*-chalcone via aza-Michael addition (Fig. 2) to investigate potentiation in alpha-amylase inhibition. The designed molecules were screened by molecular docking study.

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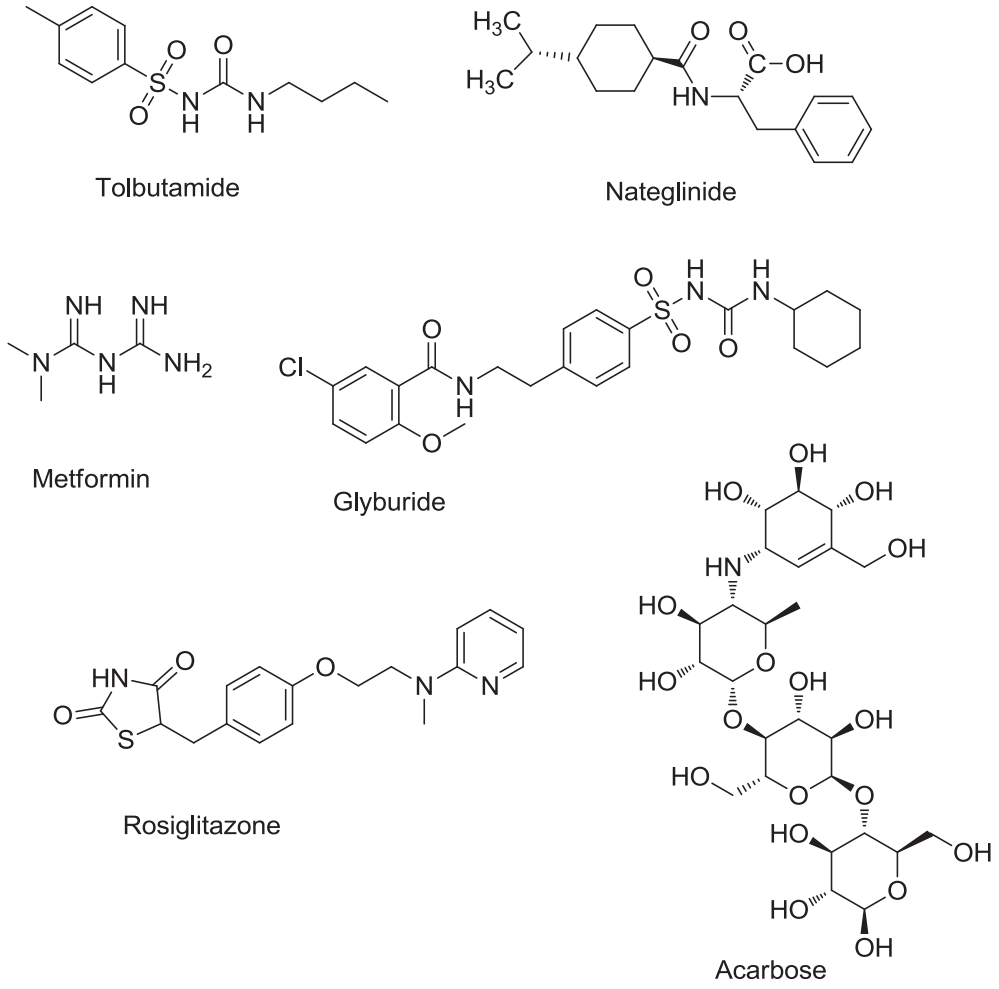


Fig. 1. Representative oral antidiabetic drugs.

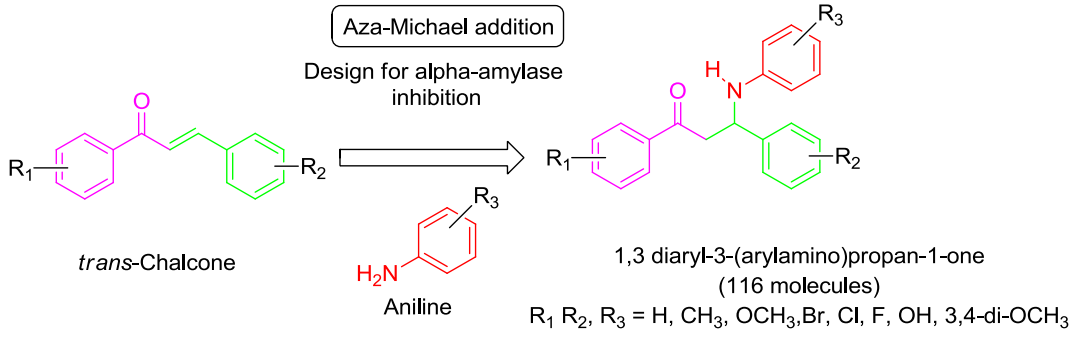
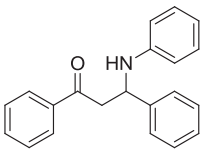
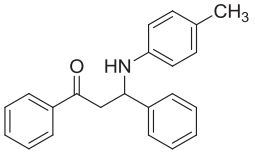
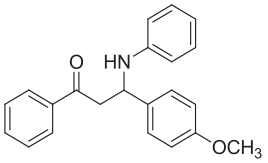
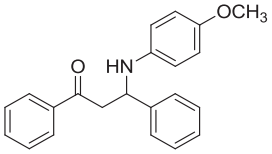
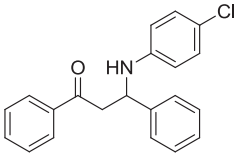
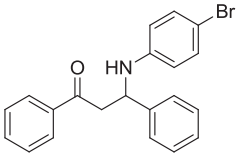


Fig. 2. Design of alpha-amylase inhibitor based on *trans*-chalcone.

For the identification of potentially active ligands, 116 designed molecules as 1,3 diaryl-3-(arylamino)propan-1-ones were analyzed by molecular docking using Auto dock Vina 1.5.6, a molecular docking software [30]. Alpha-amylase protein 4gqr was downloaded from pdb data bank [31] and validated from the internal ligand (MYC504) using Autodock Vina molecular docking software. For the preparation of protein, it was reloaded and various problems were fixed such as missing bonds or atoms and removed extraneous structures like water molecules. Polar hydrogens were added along with the Kollman charges. After saving the macromolecule (as pdbqt file) the 4gqr.pdbqt was loaded and set it as map type by choosing ligand and grid box was generated. The protein was validated by the extraction of ligand and docking it in the same manner as an actual ligand. All the designed molecules were drawn by prepared ChemDraw program and their geometry was optimized using the MM2 method [32]. All the optimized ligands were saved in pdb format and the validated protein was used for docking study to find out binding affinities for 116 designed molecules which revealed that these molecules were found good compared to MYC504, *trans*-chalcone, and acarbose (Refer Table S1 in supplementary information). The most active compounds which were having a high binding affinity towards protein 4gqr were identified and taking in account of binding affinity (kcal/mol), availability, cost, and feasibility of reaction, some of the *trans*-chalcone derivatives i.e. **3a-f** were selected as shown in Table 1.

For in-depth study, an analysis at the binding site of 4gqr protein was done with **3a** molecule to understand the binding interactions. As

Table 1
Selected *trans*-chalcone derivatives based on binding affinity to 4gqr.

S. No.	Code	Structure	Binding affinity on 4gqr (kcal/mol)
1	**3a**		−8.0
2	**3b**		−7.4
3	**3c**		−7.4
4	**3d**		−8.3
5	**3e**		−8.2
6	**3f**		−7.5
7	Internal ligand	MYC504	−7.8
8	Basic scaffold	*trans*-Chalcone	−7.5
9	Standard	Acarbose	−6.9

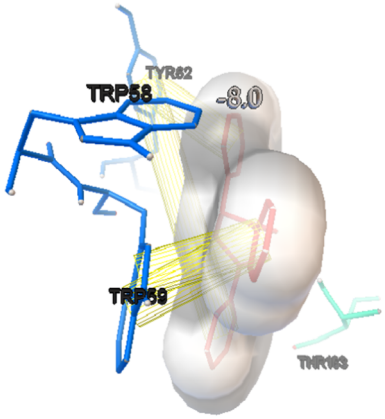


Fig. 3. The interaction of 3a with human pancreatic alpha-amylase (4gqr).

in the case of compound **3a** which is having aniline substituent at β-carbon was found to interact with indole ring of TRP59 through pi-pi interaction. Aryl ring of TYR62 was found to be linked to both aryl rings of **3a** through the pi-pi bond, along with it amino acid, THR163 was also attached leading to high binding affinity i.e., −8.0 kcal/mol (Fig. 3).

Introductions of other substituents such as CH_3, OH, Br, Cl, F, and OCH_3 at different *meta* and *para* positions of three aromatic rings will be leading to the formation of a number of derivatives with higher affinities than acarbose (Refer Table S1 in supplementary information). According to above assumptions, we can conclude that aniline and the other aromatic rings at β carbon are the lipophilic ones which can make stronger interactions with the active site, α carbon is the linker and aromatic keto is the phenyl part.

Synthesis of the synthetically feasible most potent molecule (**3a-3f**) was carried out using the scheme depicted below (Scheme 1). In step 1 the *trans*-chalcones (**1a-1b**) were prepared by the aldol condensation reaction using acetophenone with benzaldehyde (**a**) or 4-methoxy benzaldehyde (**b**) and sodium hydroxide as a base in ethanol with 98% and 57% yield respectively. Further *trans*-chalcone were reacted with aniline (**2a-2e**) in presence of potassium hydroxide as a catalyst in ethanol to afford 1,3 diaryl-3-(arylamino)propan-1-one (**3a-3f**) in 68–97% yield via aza-Michael addition (Table 2). Optimization of reaction condition for the aza-Michael addition was done with prototype reaction using *trans*-chalcone and aniline (Table 3) which suggested that ethanol and potassium hydroxide to be best to afford 1,3 diaryl-3-(arylamino)propan-1-one in 97% yield.

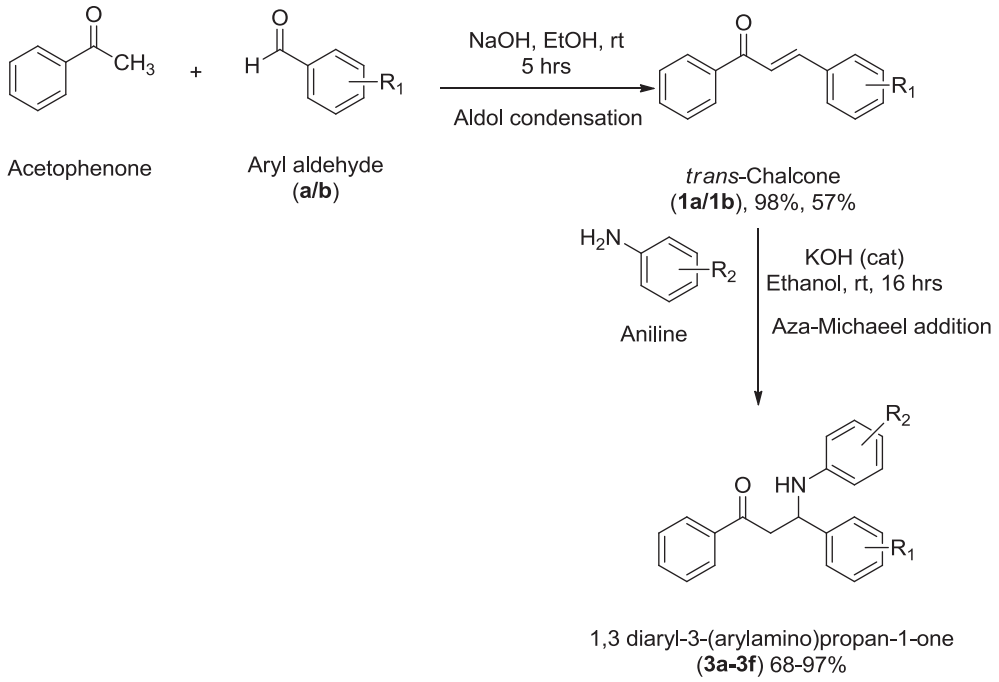
All six synthesized 1,3 diaryl-3-(arylamino)propan-1-ones (**3a-3f**) were tested for their alpha-amylase inhibitory activity with little modification described by Sudha et al. [33] and others [34–36]. It was observed that all of them found active inhibitors of alpha-amylase enzyme at different extents but better than acarbose (Table 4 and Fig. 4). Log concentrations versus % inhibition curves were plotted and IC_{50} values were calculated for all molecules (Fig. 5). Among these **3e** has shown highest activity against alpha-amylase enzyme with IC_{50} 23.17 μg/mL whereas **3a** and **3f** were the second and third most potent alpha-amylase inhibitors with IC_{50} 25.70 μg/mL and 48.17 μg/mL respectively. All these were found better tha acarbose which was having IC_{50} 891.25 μg/mL.

Further, all 1,3 diaryl-3-(arylamino)propan-1-ones **(3a-3f)** were evaluated for their antioxidant activity which was compared with a standard reducing agent i.e. ascorbic acid [37]. Among all molecules, the only **3d** has reduced DPPH with higher potency (IC_{50} = 79.43 μg/mL) whereas other found less potent compared to ascorbic acid. These results suggested that substitution on all three phenyl rings have variant effect particularly un-substituted or electron withdrawing groups tend to increase the potency for alpha amylase whereas this is reverse in case of antioxidant activity.

3. Materials and methods

3.1. Molecular docking

For the identification of potentially active ligands, 116 designed molecules as 1,3 diaryl-3-(arylamino)propan-1-ones were analyzed by molecular docking using Auto dock Vina 1.5.6 software, a molecular docking software [30]. For the extraction and preparation of ligands, 4gqr (alpha-amylase) protein was downloaded from the protein data bank (PDB 2017) [31]. For the preparation of protein, it was reloaded and various problems were fixed such as missing bonds or atoms and removed extraneous structures like water molecules. Polar hydrogens were added along with the Kollman charges. After saving the macromolecule (as pdbqt file) the 4gqr.pdbqt was loaded and set it as map type by choosing ligand and grid box was generated. The protein was validated by the extraction of ligand and docking it in the same manner as an actual ligand. The designed molecules were drawn by ChemDraw Ultra and converted to 3D structures. The geometry of all compounds was optimized by semi-empirical MM2 method (ChemBioDraw 2012) [32]. Molecular docking was performed on the optimized structure of the protein.



Scheme 1. Synthesis of 1,3 diaryl-3-(arylamino)propan-1-ones via aza-Michael addition.

3.2. Chemistry

The ^{1}H NMR spectra were recorded at 400 MHz on a Bruker Avance 400 (400 MHz) spectrometer in DMSO using TMS as an internal standard. The chemical shifts (δ) for ^{1}H are given in ppm. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; m, multiplet. The reactions were monitored by TLC (Merck). Evaporation of solvents was performed under reduced pressure using rotator evaporator commercial grade reagents and solvents were used without further purification. The open capillary method was employed for the determination of melting points and was uncorrected. Fourier Transformed Infrared (FT-IR) spectroscopic data was recorded on a spectrophotometer (Perkin Elmer 783, Pyrogen 1000 Spectrophotometer, USA) employing the KBr disc method. Mass spectra of all the synthesized compounds were recorded on a JEOL GC Mass spectrometer (Japan).

3.2.1. The general synthetic procedure of trans-chalcone

trans-Chalcones (**1a/1b**) were prepared by dissolving acetophenone (1 eq, 8.3 mmol) in 10 mL of ethanol in a single-necked RBF fitted on a magnetic stirrer. Then aryl aldehyde (1 eq, 8.3 mmol) and sodium hydroxide (0.1 eq, 0.83 mmol) were added separately while stirring. The mixture was stirred for 5 h at room temperature and reaction progress was monitored by TLC. The white precipitate was formed which was collected by vacuum filtration. Thereafter the crude *trans*-chalcones were recrystallized from ethanol and characterized by melting point and FTIR.

*(E)-1,3-diphenyl-prop-2-en-1-one **(1a)***

Yield: 98%; white solid; m.p. 55 °C; FTIR (KBr, υ cm^{-1}): 1659(C═O); 1603(C═C); Rf (EtOAc:hexane; 05:95) = 0.69.

*(E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one **(1b)***

Yield: 57%; yellow solid; m.p. 62 °C; FTIR (KBr, υ cm^{-1}): 1700(C═O); 1655(C═C); 1262(C—O). Rf (EtOAc:hexane; 10:90) = 0.73.

3.2.2. General synthetic procedure of aza-Michael addition to prepare 1,3 diaryl-3-(arylamino)propan-1-ones

1,3-diphenyl-3-(arylamino)propan-1-ones (**3a-3e**) were prepared by dissolving *trans*-chalcone (**1a** or **1b**) [1 eq, 4.80 mmol] in ethanol in a single-necked RBF fitted on a magnetic stirrer. Then aniline (**2a-2c**) [1 eq, 4.80 mmol] and potassium hydroxide [0.5 eq, 2.4 mmol] were added separately and stirred well. The mixture was stirred for 16 h at room temperature and reaction progress was monitored by TLC. The white colored precipitate which was formed collected by vacuum filtration. Thereafter the crude product was recrystallized from ethanol and was obtained in good yield.

*1,3 diphenyl-3-(phenylamino)propan-1-one **(3a)***

Yield: 97%; white crystalline solid; m.p. 165 °C; IR (KBr, υ cm^{-1}): 3384 (NH), 1668 (C═O); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 3.2 (dd, $J_{1,2}$ = 4.6 Hz, $J_{1,3}$ = 16.9 Hz; 1H), 3.6 (dd, $J_{1,2}$ = 8.8 Hz, $J_{1,3}$ = 16.9 Hz, 1H), 4.98–5.03 (m, 1H), 6.1 (d, $J_{1,2}$ = 7.4 Hz, 1H, NH), 6.5 (t, $J_{1,2}$ = 7.3 Hz, 1H), 6.5 (d, $J_{1,2}$ = 7.7 Hz, 2H), 6.96 (t, $J_{1,2}$ = 0.9 Hz, 2H), 7.1 (t, $J_{1,2}$ = 1 Hz, 1H), 7.4 (t, $J_{1,2}$ = 12.5 Hz, 2H), 7.42–7.60 (m, 5H), 7.9 (d, $J_{1,2}$ = 1.3 Hz, $J_{1,3}$ = 5.9 Hz, 2H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 46.42, 52.81, 112.79, 115.77, 126.61, 126.68, 128.62, 128.28, 128.67, 133.15, 136.76, 144.01, 147.79, 197.30 (C═O); MS-TOF (m/z) = 302 (M + H); anal $C_{21}H_{19}NO$ calcd. C, 83.69; H, 6.35; N, 4.65. Found C, 83.71; H, 6.42; N, 4.68.

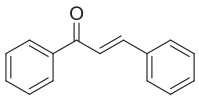
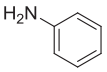
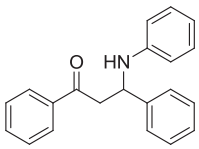
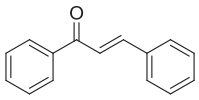
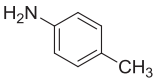
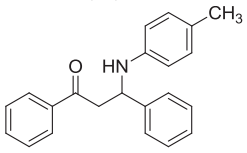
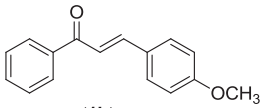
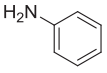
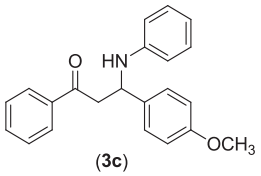
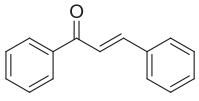
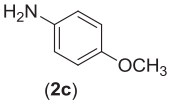
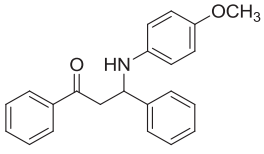
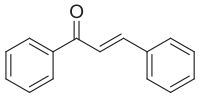
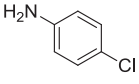
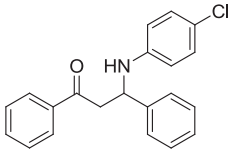
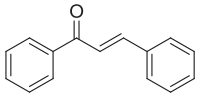
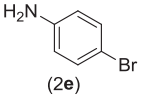
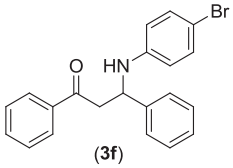
*1,3-diphenyl-3-(p-tolylamino)propan-1-one **(3b)***

Yield: 66%; white solid m.p. 170 °C; IR (KBr, υ cm^{-1}): 3401(NH), 1660(C═O); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 2.07 (s, 3H, CH_3), 3.3 (dd, $J_{1,2}$ = 4.6 Hz, $J_{1,3}$ = 4.8 Hz, 1H), 3.6 (dd, $J_{1,2}$ = 8.6 Hz, $J_{1,3}$ = 16.7 Hz, 1H), 4.94–4.97 (m, 1H), 5.98 (d, $J_{1,2}$ = 7.9 Hz, 1H, NH), 6.4 (d, $J_{1,2}$ = 8.3 Hz, 2H), 6.8 (d, $J_{1,2}$ = 8.2 Hz, 2H), 7.2 (t, $J_{1,2}$ = 7.3 Hz, 1H), 7.3 (t, $J_{1,2}$ = 7.7 Hz, 2H), 7.4 (d, $J_{1,2}$ = 7.3 Hz, 2H), 7.5 (t, $J_{1,2}$ = 7.5 Hz, 2H), 7.63 (t, $J_{1,2}$ = 7.4 Hz, 1H, Ar H), 7.9 (d, $J_{1,2}$ = 7.3 Hz, 2H, Ar H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 19.98, 46.44, 53.16, 113.03, 124.18, 126.63, 128.01, 128.22, 128.66, 129.11, 133.12, 136.79, 144.11, 145.49, 197.44(C═O); MS-TOF (m/z) = 316 (M + H); anal $C_{22}H_{21}NO$ calcd. C, 83.78; H, 6.71; N, 4.44. Found C, 83.84; H, 6.79; N, 4.61.

*3-(4-methoxyphenyl)-1-phenyl-3-(phenylamino)propan-1-one **(3c)***

Yield: 71%; light yellow crystalline solid; m.p.155 °C; IR (KBr, υ cm^{-1}): 3376(NH), 1666(C═O), 1286(C—O); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 3.3 (dd, $J_{1,2}$ = 4.8 Hz, $J_{1,3}$ = 11.9 Hz; 1H), 3.6 (dd, $J_{1,2}$ = 8.6 Hz, $J_{1,3}$ = 8.2 Hz, 1H), 3.7 (s, 3H, OCH_3), 4.92–4.97 (m, 1H), 6.2 (d, $J_{1,2}$ = 7.76, Hz, 1H, NH), 6.46 (t, $J_{1,2}$ = 7.3 Hz, 1H), 6.5 (d, $J_{1,2}$ = 7.8 Hz, 2H), 6.85 (d, $J_{1,2}$ = 8.7 Hz, 2H), 6.97 (t, $J_{1,2}$ = 7.5 Hz, 2H), 7.4 (d, $J_{1,2}$ = 8.7, Hz, 2H), 7.5 (t, $J_{1,2}$ = 7.4 Hz, 2H), 7.62 (t, J

Table 2
Synthesis of 1,3 diaryl-3-(arylamino)propan-1-one derivatives.*

S. No.	*trans*-Chalcone	Aniline	Product	Yield (%)
1	 (**1a**)	 (**2a**)	 (**3a**)	97
2	 (**1a**)	 (**2b**)	 (**3b**)	66
3	 (**1b**)	 (**2a**)	 (**3c**)	71
4	 (**1a**)	 (**2c**)	 (**3d**)	70
5	 (**1a**)	 (**2d**)	 (**3e**)	82
6	 (**1a**)	 (2**e**)	 (**3f**)	68

* *trans*-chalcone (1 eq, 4.8 mmol), dissolved in 10 mL ethanol and added aniline (1 eq, 4.8 mmol) and potassium hydroxide (0.5 eq, 2.4 mmol) were added and stirred at rt (room temperature) for 16 h.

$_{1,2}$ = 6.3, 1H) 7.95 (d, $J_{1,2}$ = 1.3 Hz, $J_{1,3}$ = 8.5 Hz, 2H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 46.51, 52.28, 54.92, 112.87, 113.64, 115.72, 127.68, 128.00, 128.66, 133.12, 135.76, 136.80, 147.81, 158.00, 197.46(C═O); MS-TOF (m/z) = 332 (M + H); anal $C_{22}H_{21}NO_2$ calcd. C, 79.73; H, 6.39; N, 4.23. Found C, 79.82; H, 6.45; N, 4.29.

3-((4-methoxyphenyl)amino)-1,3-diphenylpropan-1-one ***(3d)***

Yield: 70% light green solid; m.p. 145 °C; IR (KBr, υ cm^{-1}): 3371(NH), 1666 (C═O), 1276(C−O); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 3.3(dd, $J_{1,2}$ = 4.8 Hz, $J_{1,3}$ = 16.6 Hz;1H), 3.5 (s, 3H, OCH_3), 3.6(dd, $J_{1,2}$ = 7.2 Hz, $J_{1,3}$ = 16.6 Hz, 1H), 4.89–4.94 (m, 1H), 5.8 (d, J = 8.1 Hz, 1H, NH), 6.5(d, $J_{1,2}$ = 10.3 Hz, 2H), 6.6 (d, $J_{1,2}$ = 10.3 Hz, 2H), 7.15 (t, $J_{1,2}$ = 0.9 Hz, 1H), 7.3 (t, $J_{1,2}$ = 7.7 Hz, 2H), 7.4 (d, $J_{1,2}$ = 7.1 Hz, 2H), 7.51(t, $J_{1,2}$ = 5.9 Hz, 2H), 7.6 (t, $J_{1,2}$ = 7.4, 1H), 7.9 (d, $J_{1,2}$ = 4.2 Hz, $J_{1,3}$ = 12.7 Hz, 2H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 46.52, 53.78, 55.17, 113.99, 114.38, 126.62, 126.66, 128.02, 128.22, 128.66, 133.11, 136.82, 141.93, 144.16, 150.69, 197.53(C═O); MS-TOF (m/z) = 332 (M + H); anal $C_{22}H_{21}NO_2$ calcd. C, 79.73; H, 6.39; N, 4.23. Found C, 79.81; H, 6.42; N, 4.27.

3-((4-chlorophenyl)amino)-1,3-diphenylpropan-1-one ***(3e)***

Yield: 82%; white crystalline solid; m.p. 160 °C; IR (KBr, υ cm^{-1}): 3370(NH), 1664(C═O), 681(C-Cl); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 3.31 (dd, $J_{1,2}$ = 4.5 Hz, $J_{1,3}$ = 17 Hz; 1H), 3.6 (dd, $J_{1,2}$ = 8.9 Hz, $J_{1,3}$ = 17 Hz, 1H), 4.94–4.99 (m, 1H), 6.4 (d, $J_{1,2}$ = 7.6 Hz, 1H, NH), 6.5 (d, $J_{1,2}$ = 2 Hz, 2H), 7.0 (d, $J_{1,2}$ = 3.3 Hz, 2H), 7.2 (t, $J_{1,2}$ = 1.1 Hz, 1H), 7.3(t, $J_{1,2}$ = 7.8 Hz, 2H), 7.4 (d, $J_{1,2}$ = 7.1 Hz, 2H), 7.5 (t, $J_{1,2}$ = 17.1 Hz, 2H), 7.6 (t, $J_{1,2}$ = 1 Hz, 1H), 7.95(d, $J_{1,2}$ = 1.4 Hz, $J_{1,3}$ = 8.5 Hz, 2H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 46.33, 52.84, 114.11, 119.04, 126.58, 126.80, 128.01, 128.34, 128.39, 128.67, 133.18, 136.69, 143.55, 146.73, 197.10(C═O); MS-TOF (m/z) = 336 (M + H); anal $C_{21}H_{18}ClNO$ calcd. C, 75.11; H, 5.40; Cl, 10.56; N, 4.17. Found C, 75.21; H, 5.51; Cl, 10.61; N, 4.21.

3-((4-bromophenyl)amino)-1,3-diphenylpropan-1-one ***(3f)***

Yield: 68%; greenish-white crystalline solid; m.p. 175 °CIR (KBr, υ cm^{-1}): 3369(NH), 1665(C═O), 565(C−Br); ^{1}H NMR (400 MHz), DMSO-d_6, δ (ppm): 3.3 (dd, $J_{1,2}$ = 4.6 Hz, $J_{1,3}$ = 17.08 Hz; 1H), 3.6 (dd, $J_{1,2}$ = 7.8 Hz, $J_{1,3}$ = 17.0 Hz, 1H), 4.93–4.98 (m, 1H), 6.4(d, $J_{1,2}$ = 1.96 Hz, 1H, NH), 6.5 (d, $J_{1,2}$ = 2.4 Hz, 2H), 7.1 (d, $J_{1,2}$ = 1.8 Hz, 2H), 7.2 (t, $J_{1,2}$ = 7.3 Hz, 1H), 7.3 (t, $J_{1,2}$ = 7.8 Hz, 2H), 7.4 (d, $J_{1,2}$ = 7.2 Hz, 2H), 7.5 (t, $J_{1,2}$ = 1.6 Hz, 2H), 7.6 (t,

Table 3
Effects of reagents and solvents on the aza-Michael addition of *trans*-chalcone and aniline.*

S. No.	Reagent (0.5 equiv)	Solvent	Yield (%)	Time (h)
1	Triethylamine	Ethanol	69	24
2	Triethylamine	Methanol	17	20
3	Triethylamine	Water	NA	16
4	Triethylamine	Acetonitrile	4	12
5	Triethylamine	Benzyl alcohol	23	12
6	Triethylamine	Hexane	NA	12
7	Triethylamine	Toluene	NA	12
8	Triethylamine	DMF	NA	12
9	Triethylamine	Chloroform	48	12
10	NaOH	Ethanol	83	16
11	$LiOH{\cdot}H_2O$	Ethanol	69	20
12	Pyridine	Ethanol	NA	12
13	KOH	Ethanol	97	16
14	Triethylamine	Ethanol	69	24
15	$AlCl_3$	Ethanol	NA	12
16	Silica gel 100–200	Ethanol	17	12
17	Lithium perchlorate	Ethanol	NA	24

* *trans*-chalcone (1 eq, 4.8 mmol), dissolved in 5 mL ethanol and added aniline (1 eq, 4.8 mmol) and reagent (0.5 eq, 2.4 mmol) were added and stirred at rt (room temperature); NA = No reaction.

Table 4
IC_{50} values obtained by alpha-amylase inhibition and antioxidant activity.

S. No.	1,3 diaryl-3-(arylamino) propan-1-one	IC_{50} (μg/mL) Alpha-amylase inhibition	IC_{50} (μg/mL) Antioxidant (DPPH assay)
1	**3a**	25.70	N
2	**3b**	225.89	N
3	**3c**	177.82	N
4	**3d**	251.18	79.43
5	**3e**	23.17	N
6	**3f**	48.17	N
7	Acarbose	891.25	–
8	Ascorbic acid	–	831.76

N = Low activity.

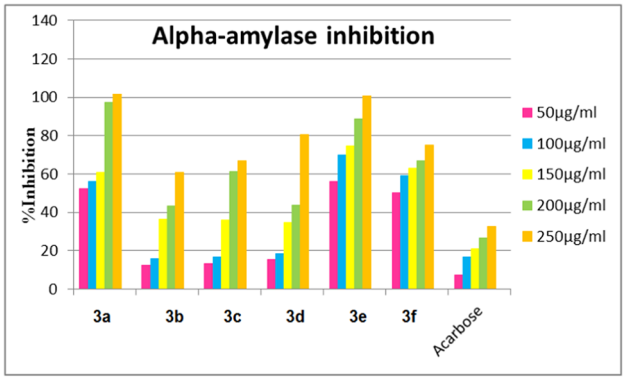


Fig. 4. % inhibition of alpha-amylase enzyme by 1,3 diaryl-3-(arylamino) propan-1-one and acarbose at different concentrations.

$J_{1,2}$ = 1.1 Hz, 1H), 7.95 (d, $J_{1,2}$ = 1.3 Hz, $J_{1,3}$ = 8.5 Hz, 2H); ^{13}C NMR (400 MHz), DMSO-d_6, δ (ppm): 46.31, 52.74, 106.43, 114.69, 126.57, 126.80, 128.01, 128.35, 128.67, 131.20, 133.18, 136.68, 143.49, 147.08, 197.08(C=O); MS-TOF (m/z) = 382 (M + 2); anal $C_{21}H_{18}BrNO$ calcd. C, 66.33; H, 4.77; Br, 21.01; N, 3.68. Found C, 66.41; H, 4.81; Br, 21.09; N, 3.74.

3.3. In-vitro alpha-amylase inhibitory activity assay

Alpha-amylase inhibitory activity assay was done by iodine-starch method [33–36]. Acarbose was used as a standard. The test is based on the formation of a complex between iodine and starch which is blue in color and has a maximum absorbance at 580 nm. In positive control solution, we added both alpha-amylase enzyme and starch but not any inhibitor so that there should be 100% enzymatic activity and minimum absorbance (since maximum starch is degraded by an alpha-amylase enzyme). On the other hand, negative control solution did not contain alpha-amylase enzyme and inhibitor but an only starch solution which upon addition of I_2 solution a dark green colored complex was formed which has shown maximum absorbance and no enzymatic activity. Overall, the absorbance of test solutions should be in between the absorbance of positive and negative control solutions and color intensity of them as well [33].

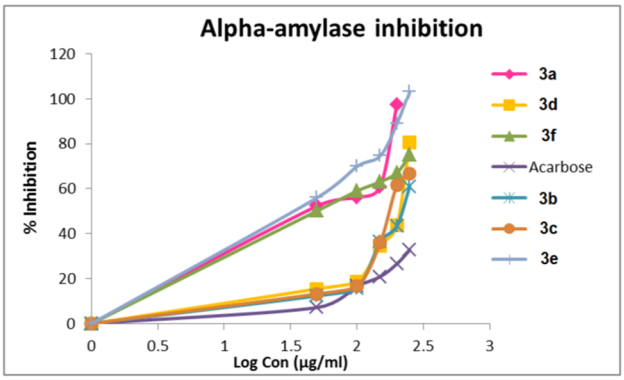


Fig. 5. Comparison of alpha-amylase inhibitory activity of 1,3 diaryl-3-(arylamino)propan-1-ones with acarbose.

3.3.1. Preparation of phosphate buffer (0.02 M, pH = 6.9)

Solutions of 0.02 M of $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}H_2O$ were prepared separately and their pH was detected by pH meter, thereafter the solution with lower pH ($NaH_2PO_4{\cdot}H_2O$) was poured into solution with higher pH ($Na_2HPO_4{\cdot}12H_2O$) and rechecked pH of the solution again, and adjusted by drop-wise addition of 0.006 M NaCl or 1% NaOH solution to 6.9.

3.3.2. Preparation of (0.5 mg/mL) alpha-amylase solution

12.5 mg of aspergillus alpha-amylase was dissolved in minimum quantity of prepared phosphate buffer (pH 6.9) in a 25 mL volumetric flask, thereafter complete solubility; volume was made up to 25 mL by the same solution.

3.3.3. Preparation of 1% starch solution

1 g of potato starch was dissolved in 100 mL of distilled water and boiled for 30 s on a hot plate. The solution was cooled at room and filtered through simple filter paper. Filtrates were used for the alpha-amylase inhibitory assay.

3.3.4. Preparation of 1% iodine solution

1 g of iodine and 2 g of KI were dissolved in 50 mL of distilled water in a baker and stirred on a magnetic stirrer overnight for complete solubility of I_2, thereafter solution was transferred to a 100 mL volumetric flask and volume was made up to 100 mL by the same solvent and used for the assay.

3.3.5. Preparation of 1 M HCl solution

8.6 mL of concentrated HCl solution was diluted to 100 mL with distilled water.

3.3.6. Preparation of stock sample solution

10 mg of each compound was dissolved in 10 mL methanol in a volumetric flask and was labeled as a stock sample solution 1000 ppm.

3.3.7. Preparation of working sample solution

From a solution of stock (1000 ppm), 0.5 mL, 1 mL, 1.5 mL, 2 mL, and 2.5 mL were transferred to separate 10 mL volumetric flasks and volumes were made up by methanol, thereafter labeled as working sample solutions 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm.

Enzyme activity and %inhibition were calculated using the formula below:

$$\text{Enzyme activity} = \frac{(\text{Abs. of negative control} - \text{Abs. of positive control}) - \text{Abs. of test sample}}{(\text{Abs. of negative control} - \text{Abs. of positive control})} \times 100$$

Inhibition (%) = 100-enzyme activity.

Where, the absorbance of the sample (test sample + alpha-amylase), the absorbance of negative control (no alpha-amylase, 0% enzymatic activity, and 100% absorbance), and the absorbance of positive control (no inhibitor, 100 enzymatic activity, and 0% absorbance).

Inhibition concentration (IC_{50}): It is the concentration of the drugs at which 50% inhibition takes place.

3.4. In-vitro antioxidant activity assay

The assay has been carried out according to Murti et al. [37]. Ascorbic acid was used as a standard. The test is based on the reduction of DPPH which is a free radical with dark purple color and the maximum absorbance at 516 nm. As the DPPH is getting reduced with an antioxidant it will turn its color to yellow, means with an increase in the concentration of antioxidant the absorbance should be decreased.

3.4.1. Preparation of DPPH solution

A solution of DPPH (1.3 mg/mL) in methanol was prepared and was kept in dark for 2 h, and used freshly in the same day.

3.4.2. Preparation of stock sample solution

10 mg of each compound was dissolved in 10 mL methanol in a volumetric flask and was labeled as a stock sample solution 1000 ppm.

3.4.3. Preparation of working sample solution

From a solution of stock (1000 ppm), 0.5 mL, 1 mL, 1.5 mL, 2 mL, and 2.5 mL were transferred to separate 10 mL volumetric flasks and volumes were made up of methanol, thereafter labeled as working sample solutions 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm.

The DPPH free radical scavenging activity was calculated using the following formula:

$$\%\ \text{scavenging} = \frac{\text{Abs. of positive control} - \text{Abs. of test sample}}{\text{Abs. of positive control}} \times 100$$

Where; positive control is absorbance of a DPPH solution without compound; the sample is the absorbance of the test molecules with DPPH.

4. Conclusion

In conclusion we have explored the aza-Michael adducts of *trans*-chalcone by taking help of Autodock Vina a molecular docking software to identify the best molecules. The identified best molecules 1,3 diaryl-3-(arylamino)propan-1-one derivatives (**3a-f**) were synthesized in a simpler manner using KOH as a catalyst via aza-Michael addition. Further biological evaluation revealed that these were found active as alpha-amylase inhibitors and antioxidants. The molecule **3e** was found to be the most potent alpha-amylase inhibitor whereas **3a** was to be second most potent. Wheras DPPH antioxidant assay showed that only **3d** has potent antioxidant. Thus these 1,3 diaryl-3-(arylamino)propan-1-ones derivative would be considered as potential antidiabetic agents and further study could lead to the development of the better antidiabetic drug in future.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

Acknowledgment

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.010.

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