ORIGINAL ARTICLE

ACG publications

Org. Commun. 15:2 (2022) 129-147

organic communications

Molecular docking, synthesis and biological evaluation (enzyme inhibition, antimicrobial and antioxidant) of methoxy benzoin/benzil/stilbenoid derivatives

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(Received March 30, 2022; Revised April 25, 2022; Accepted April 29, 2022)

Abstract: In this study, methoxy benzoin compounds (**1-10**) were synthesized from the corresponding aromatic aldehydes based on a screening of biological activity. Oxidation and reduction of benzoins (**1-10**) yielded the corresponding benzils (**11-20**) and stilbenoids (**21-29**), respectively. The enzyme inhibition, antimicrobial, and antioxidant activities of **1-29** were evaluated. **1, 14, 19,** and **28** against α-amylase, **15** and **19** against α-glucosidase, **2**, **4, 14**, **18**, **25** and **26** against tyrosinase, **2, 7,** and **23** against AChE, and **7**, and **13** against BChE showed similar activity to the standard used. Among the methoxy benzoin derivatives, **4** proved to be the most active compound against *E.coli*, *Y.pseudotuberculosis*, *M. smegmatis*, and *C.albicans* in the range of 41-82 µg/mL MIC values. All benzil derivatives displayed bioactivity against *M.smegmatis* and *C. albicans*. Compounds **18** and **11** were found to be most effective against *M.smegmatis*, and compounds **11** and **17** were found to be the most effective against *C.albicans*. All stilbenoid type compounds showed selective activity against *B.cereus*. Compounds **21** and **22** were the most effective stilbenoid compounds against *M. smegmatis*. Benzoins (**1-10)** were the most effective antioxidants among all three groups compared to the tested methods, which can be attributed to the free hydroxyl at the benzylic position. As a result, the change of carbon skeleton and substitution at different positions of synthesized organic compounds also caused the variation of biological activity.

Keywords: Methoxy benzoin/benzil/stilbenoid; molecular docking; enzyme inhibition; antimicrobial; antioxidant.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/organic-communications © April-June 2022 EISSN:1307-6175
DOI: http://doi.org/10.25135/acg.oc.125.2203.2407
Available online: May 15, 2022

1. Introduction

Benzoin, benzil, and stilbenoid are important natural compounds due to their broad biological activities.[1-4] Many natural compounds related to the above mentioned structure had been reported as licoagrodione,[5] narceinone,[6] loddigesiinol D,[7] derrisdione A,[5] coriaceol,[8] sophodibenzoside A,[9] 1-(4-hydroxyphenyl)-2-(3,5-dihydroxyphenyl)-2-hydroxyethone,[10] and 1,2-(3,5-dimetoxy-4-hyroxyphenyl)-ethane-1,2-dione.[11]

Benzoin is a leading intermediate for the synthesis of new chemicals, which has gained importance in medicinal chemistry in recent years. Mixed benzoin synthesis is very talented and reliable with the substituent on the phenyl ring.[2, 12-14]

Enzyme inhibition is one of the approaches used in metabolic and neurodegenerative diseases. One way to control hyperglycemia is to inhibit enzymes that prevent the digestion of macromolecules as a result of suppression of hyperglycemia.[15-17] In the literature, many studies have demonstrated the enzyme inhibitory effect of benzoin/benzil compounds.[2, 18-29] The enzyme inhibition of 1,2-diphenylethanone has also been reported.[30] On the other hand, the antimicrobial and antioxidant activities of benzoin/benzil compounds have also been an important topic reported in the literature.[2, 18, 31-34]

The synthesis of compounds **1**,[35] **2**,[36] **3**,[37] **4**,[38] **5**,[39] **6**,[40] **10**,[41] **11**,[42] **12**,[43] **13**,[44] **14**,[45] **15**,[46] **16** and **17**,[47] **18**,[48] **20**,[49] **21-23**,[50] **24**,[51] **25**,[52] **27**,[53] **28**,[54-55] and **29**[56] were reported. Our literature search revealed resulted that compounds **6a+b**, **7**, **9**, **19**, and **26a+b** have not been previously mentioned.

In our continuous research, the present study aimed to synthesize methoxy benzoin/benzil/stilbenoid for therapeutic potential. In this work, methoxy benzoin compounds (**1-10**) were first synthesized from the condensation of methoxy-substituted benzaldehydes, oxidation of benzoins yielded methoxy benzils (**11-20**), and reductions yielded methoxy 1,2-diphenylethanones (**21-29**). Due to the biological activities of **1-29**, their potential to inhibit enzyme, their antimicrobial and antioxidant properties were investigated and their molecular docking studies were also studied.

2. Experimental

2.1. Chemical Material and Apparatus

Aldehydes (3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 3,4-dimethoxy benzaldehyde, and 3,5-dimethoxybenzaldehyde), solvents (chloroform, *n*-hexane, ethyl acetate, methanol, acetone and dimethyl sulfoxide), and other used reagent were purchased from by Fluka, Merck or Sigma-Aldrich unless otherwise stated. Ultrasonic bath was used for the benzoin synthesis (340 W, VUC-A06H, WiseClean). Perkin-Elmer 1600 (ATR) (4000-400 cm^{-1}) spectrophotometer was used to take FT-IR spectra. The mass spectral analyses were carried out on an Agilent 6230 LC-QTOF-MS. A Bruker 400 MHz NMR spectrometer was also used to obtain ^{1}H and ^{13}C NMR spectra (400 MHz for ^{1}H, 100 MHz for ^{13}C), using TMS as an internal standard. $CDCl_3$ was used as NMR solvent. ^{13}C and APT spectra were adjusted according to deutero solvent peaks. Chemical shifts were expressed in δ (ppm), and coupling constants (*J*) were reported in hertz (Hz). ACD NMR program was used for the interpretation of spectra. Melting points were determined using the Thermo-var apparatus fitted with a microscope. TLC was carried out on Silica gel 60 F254, and the spots were visualized by UV lamp (254 nm and 366 nm). Normal phase silica gel (230-400 mesh) was used in vacuum column chromatography (VLC).

2.2. Chemistry

*2.2.1. General Synthesis of Hydroxy Methoxy Benzoins **1-10***

Methoxy benzaldehydes (0.001 mol) in EtOH:H_2O (10:2 mL) were reacted with potassium cyanide (0.001 mol) using ultrasonic bath (320 W, 120 min) at 70-85ºC in a close vessel. Complete consumption of reactant was monitored through TLC. Water (30 mL) was added to the mixture, then extracted with ethyl acetate (3x30 mL) to give crude mixture and compounds **1-10** were purified by repeated vacuum liquid chromatography (VLC, Silica gel 230-400 mesh) by increasing the solvent polarity of *n*-hexane, chloroform, ethyl acetate, and methanol mixtures and the fractions were checked by TLC. Of ten benzoin compounds, **6a+b**, **7,** and **9** are new compounds. Experimental methods used for the syntheses of methoxy benzoins (**1-10**) were presented in Table 1.

Table 1. Experimental method for the synthesis of methoxy benzoins (**1-10**)[a]

Reagents (0.01mol each)	Possible benzoin products ($R_1PhCOCH(OH)PhR_2$)	No	Yields (%)
Benzaldehyde	R_1, R_2= -H		-
3-Methoxybenzaldehyde	R_1, R_2= 3-OCH_3		24
KCN	R_1= 3-OCH_3, R_2= -H / R_1= -H, R_2= 3-OCH_3	**1a+b**	52
Benzaldehyde	R_1, R_2= -H		-
4-Methoxybenzaldehyde	R_1, R_2= 4-OCH_3		16
KCN	R_1= 4-OCH_3, R_2= -H	**2**	58
Benzaldehyde	R_1, R_2= -H		-
3,4-Dimethoxybenzaldehyde	R_1, R_2= 3,4-diOCH_3		20
KCN	R_1= 3,4-diOCH_3, R_2= -H	**3**	56
3-Methoxybenzaldehyde	R_1, R_2= 3-OCH_3	**4**	78
KCN			
3-Methoxybenzaldehyde	R_1, R_2= 3-OCH_3		16
4-Methoxybenzaldehyde	R_1, R_2= 4-OCH_3		18
KCN	R_1= 4-OCH_3, R_2= 3-OCH_3	**5**	58
3-Methoxybenzaldehyde	R_1, R_2= 3-OCH_3		18
3,5-Dimethoxybenzaldehyde	R_1, R_2= 3,5-diOCH_3		37
KCN	R_1= 3-OCH_3, R_2= 3,5-diOCH_3 / R_1= 3,5-diOCH_3, R_2= 3-OCH_3	**6a+b**	42
3-Methoxybenzaldehyde	R_1, R_2= 3-OCH_3		22
3,4-Dimethoxybenzaldehyde	R_1, R_2= 3,4-diOCH_3		16
KCN	R_1= 3,4-diOCH_3, R_2= 3-OCH_3	**7**	49
3,4-Dimethoxybenzaldehyde	R_1, R_2= 3,4-diOCH_3	**8**	52
KCN			
3,4-Dimethoxybenzaldehyde	R_1, R_2= 3,4-diOCH_3		21
3,5-Dimethoxybenzaldehyde	R_1, R_2= 3,5-diOCH_3		42
KCN	R_1=3,4-diOCH_3, R_2=3,5diOCH_3	**9**	26
3,5-Dimethoxybenzaldehyde	R_1, R_2= 3,5-diOCH_3	**10**	72
KCN			

[a] US, 340 Watt, EtOH-H_2O (10:2 mL) in closed flask, 70-85 °C, 120 min.

The structures of the benzoins (**1-10**) were confirmed through NMR, FT-IR, UV, LC-QTOF-MS analysis, and ACD NMR program's help. Their spectral data are given below.

*2-(3,5-dimethoxyphenyl)-2-hydroxy-1-(3-methoxyphenyl)ethanone(**6a**) and 1-(3,5-dimethoxyphenyl)-2-hydroxy-2-(3-methoxyphenyl)ethanone(**6b**):* Yield: 42%; R_f = 0.48 (*n*-hexane-EtOAc: 1:1); light yellow oil; UV (EtOAc) λ max nm (logε): 230(4,43); FT-IR (cm^{-1}): 3456, 3025, 2939, 2838, 1682, 1594, 1457, 1428, 1296, 1208,1156, 845, 683; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 5.89 (s, 1H, H-2); 5.84 (s, 1H, H-2); 7.49-7.02 (m, 11H, Ar-H); 6.48 (s, 2H, H-2",6"); 6.35 (s, 1H, H-4"); 4.77 (s, 2x -OH); 3.71-3.65 (s, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 198.63, 198.51(C=O), 161.21, 160.70, 160.03, 159.65, 141.21, 141.18, 140.55, 135.29 and 134.83 (Ar-C), 130.12, 129.63, 121.66, 120.27, 120.07, 114.02, 113.35, 113.11, 106.86, 106.85, 105.74, 105.70, 100.33, and 100.28, (Ar-CH), 76.27 and 76.29 (CH, C-2), 55.42, 55.27, 55.23, 55.10 (6x-OCH_3); Positive LC-QTOF-MS *m/z* (%): ($C_{17}H_{18}O_5$) $[M+Na]^+$: 325.2351(100), calcd. 325.2351.

*1-(3,4-dimethoxyphenyl)-2-hydroxy-2-(3-methoxyphenyl)ethanone (**7**)*: Yied: 49%; R_f = 0.57 (*n*-hexane-EtOAc: 1:1); light orange oil; UV (EtOAc) λ max nm (logε): 247(3,82); FT-IR (cm^{-1}): 3455, 3020, 2937, 2837, 1668, 1598, 1583, 1513, 1462, 1260, 1146, 1076,1019, 874, 751, 706; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 5.83 (s, 1H, H-2); 7.49-7.39 (m, 2H, H-2'/6'); 6.89 (d, *J*=8.0 Hz, 1H, H-5'); 6.84 (s, 1H, H-2"); 6.77-6.70 (m, 2H, H-4"/6"); 7.18-7.14 (m, 1H, H-5"); 4.72 (bs, 1H, -OH); 3.77 (s, 3'/4'-OCH_3); 3.65 (s, 3"-OCH_3); ^{13}C-NMR

(100 MHz, $CDCl_3$, δ, ppm): 197.08 (C-1), 75.64 (C-2), 126.27 (C-1'), 113.77 (C-2'), 148.90 (C-3'), 153.80 (C-4'), 110.05 (C-5'), 124.20 (C-6'), 141.22 (C-1"), 110.97 (C-2"), 159.97 (C-3"), 113.12 (C-4"), 130.05 (C-5"), 119.95 (C-6"), 55.79 (3'-OCH_3), 55.93 (4'-OCH_3), 55.09 (3"-OCH_3); Positive LC-QTOF-MS *m/z* (%): ($C_{17}H_{18}O_5$) $[M+Na]^+$: 325.2574(100), calcd. 325.2572.

*1-(3,4-dimethoxyphenyl)-2-(3,5-dimethoxyphenyl)-2-hydroxyethanone(**9**)*: Yield: 26%; R_f = 0.5 (*n*-hexane-EtOAc: 1:1); melting point (°C): 94-95; FT-IR (cm^{-1}): 3448, 3027, 2938, 2839, 1670, 1595, 1515, 1463,1268, 1205, 1156, 1022, 802; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 5.69 (s, 1H, H-2); 7.32 (s, 1H, H-2'); 6.51 (bd, 1H, H-5'); 7.33 (bd, 1H, H-6'); 6.36 (s, 2H, H-2",6"); 6.10 (s, 1H, H-4"); 4.75 (bs, 1H, -OH); 3.49 (s, 3'-OCH_3); 3.54 (s, 4'-OCH_3); 3.41 (s, 3"/4"-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 196.94 (C-1), 75.52 (C-2), 126.21 (C-1'), 110.75 (C-2'), 148.70 (C-3'), 153.63 (C-4'), 109.77 (C-5'), 123.88 (C-6'), 141.93 (C-1"), 105.50 (C-2"), 160.98 (C-3"), 99.67 (C-4"), 160.98 (C-5"), 105.50 (C-6"), 55.44 (3'-OCH_3), 55.56 (4'-OCH_3); 54.90 (3"/5"-OCH_3). Positive LC-QTOF-MS *m/z* (%): ($C_{18}H_{20}O_6$) $[M+K-H]^+$: 371.0913(75), calcd. 371.0901.

*2.2.2. General Synthesis of Methoxy Benzils **11-20***

Methoxy benzoins (100-300 mg) were dissolved in HPLC grade acetone (3-5 mL) and Fehling reagent (Fehling A: 1 mL + Fehling B: 0.5 mL)[57] was added. Reaction mixtures were stirred at 0-5 °C for 20-30 minutes and were terminated after the TLC control. Water (10 mL) was added to the flask after solvent evaporation and reaction content was extracted with ethyl acetate (3x20 mL) to give crude mixture. The compounds **11-20** were purified by repeated vacuum liquid chromatography (VLC, Silica gel 230-400 mesh) by increasing the solvent polarity of *n*-hexane, chloroform, ethyl acetate, and methanol mixtures and the fractions were checked by TLC. Compound **19** was a new compound. The structures of benzil (**11-20)** derivatives were verified through NMR, UV, FT-IR, LC-QTOF-MS analysis, and ACD NMR program's help.

*1-(3,4-Dimethoxyphenyl)-2-(3,5-dimethoxyphenyl)ethane-1,2-dione(**19**):* Yield: 65% ; R_f = 0.52 (*n*-hexane-EtOAc: 2:1); melting point (°C): 169-170; UV (EtOAc) λ max nm (logε): 330(4,19); FT-IR (cm^{-1}): 3027, 2938, 1773, 1661, 1593, 1510, 1465, 1424, 1275, 1159, 1063; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.58 (s, 1H, H-2'); 6.88 (d, *J*=8.0 Hz, 1H, H-5'); 7.44 (d, *J*=8.0 Hz, 1H, H-6'); 7.08 (s, 2H, H-2", 6"); 6.71 (s, 1H, H-4"); 3.94 (s, 6H, 3'/4'-OCH_3); 3.80 (s, 6H, 3"/5"-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 194.53 (C-1), 193.10 (C-2), 126.14 (C-1'), 110.35 (C-2'), 149.57 (C-3'), 154.96 (C-4'), 110.15 (C-5'), 126.37 (C-6'), 134.95 (C-1"), 107.37 (C-2"), 161.08 (C-3"), 107.37 (C-4"), 161.08 (C-5"), 107.37 (C-6"), 56.07 (3'-OCH_3), 56.23 (4'-OCH_3), 55.65 (3"/5"-OCH_3). Positive LC-QTOF-MS *m/z* (%): ($C_{18}H_{18}O_6$) $[M+Na]^+$: 353.1080(100), calcd. 353.1067.

*2.2.3. General Synthesis of Methoxy Stilbenoids **21-29***

Zn(Hg) amalgam (2 equivalents)[58] was added to ethanolic solution (3-5 mL) of methoxy benzoins (100-300 mg) and mixtures were stirred at ice bath for 10 minutes. Then, concentrated HCl-H_2O mixture (1: 1,2 mL) was added. The reactions were terminated after the TLC control (1-6 hours). Water (10 mL) was added to the flask after solvent evaporation and reaction content was extracted with chloroform (3x20 mL) to give crude mixture. The compounds **21-29** were purified by repeated vacuum liquid chromatography (VLC, Silica gel 230-400 mesh) by increasing the solvent polarity of n-hexane, chloroform, ethyl acetate, and methanol mixtures and the fractions were checked by TLC. Compound **26** was a new compound. The structures of the stilbenoid (methoxy diphenyl ethanone) (**21-29)** were verified through NMR, UV, FT-IR, LC-QTOF-MS analysis, and ACD NMR program's help.

*2-(3,5-Dimethoxyphenyl)-1-(3-methoxyphenyl)ethanone(**26a**) and 1-(3,5-dimethoxyphenyl)-2-(3-methoxyphenyl)ethanone(**26b**)*: Yield: 16%; R_f = 0.68 (*n*-hexane-ethyl acetate: 4:2); light yellow oil; FT-IR (cm^{-1}): 3027, 3002, 2937, 2837, 1692, 1596, 1460, 1427, 1261, 1209, 1157, 1052; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): (**26a**): 7.63 (d, *J*=7.8 Hz, 1H, H-6'); 7.36 (bs, 1H, H-2'); 7.26 (t, *J*= 7.6 Hz, 1H, H-5'), 6.92 (d, *J*=7.8 Hz, H-4'), 6.48 (s, 2H, H-2", 6"); 6.37 (s, 1H, H-4"); 4.22 (m, H-2, -CH_2); 3.85, 3.78 (s, 3x -OCH_3). (**26b**): 7.38 (t, *J*=7.8 Hz, 1H, H-5'); 7.18 (s, 2H, H-2",6"); 6.68 (s, 1H, H-4"); 6.88 (d, *J*= 7.8 Hz, 1H, H-6'); 6.88-6.82 (m, 1H, H-2',4'); 4.22 (m, H-2, -CH_2); 3.83, 3.80 (s, 3x -OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 197.27/197.21 (C-1), 45.92/45.68 (C-2), 160.97, 160.88, 159.86, 138.49, 137.91, 136.77, and 136.08 (Ar-C),

129.68, 129.64, 121.81, 121.33, 119.66, 115.13, 112.86, 112.42, 107.52, 106.50, 105.40, and 98.96 (Ar-CH), 55.55, 55.40, 55.28, 55.16 (-OCH_3). Positive LC-QTOF-MS *m/z* (%): ($C_{17}H_{18}O_4$) $[M+K]^+$: 325.2351(100), calcd. 325.2356.

2.3. Biological Assays

2.3.1. Enzyme Inhibitions

α-Amylase, α-glucosidase, tyrosinase, AChE, and BChE enzyme inhibition experimental procedure was described in our previous work. [18]

2.3.2. Antimicrobial Activities

2.3.2.1. Microorganisms Used for Antimicrobial Activity

The test microorganisms used in the study were obtained from Refik Saydam Hıfzısıhha Institute (Ankara) and are as follows. Three Gram (-); *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288; five Gram(+); *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans*, *Paenobascillus* sp., *Bacillus cereus* 709 ROMA, *Bacillus subtilis* ATCC 10774, one tuberculosise; *Mycobacterium smegmatis* ATCC607, and fungi; *Candida albicans* ATCC 60193. Inhibition diameters were measured by the agar well diffusion method,[59-64] and the MIC value was determined as microgram-milliliter (µg /mL) to the microdilution technics.

2.3.2.2. Antimicrobial Activity Assessment (Agar-well Diffusion Method)

The antimicrobial screening test using the agar-well diffusion method as adapted was used earlier[59-64] and procedure was described in our previous work. [18]

2.3.2.3. Minimal Inhibition Concentration (MIC) Assay

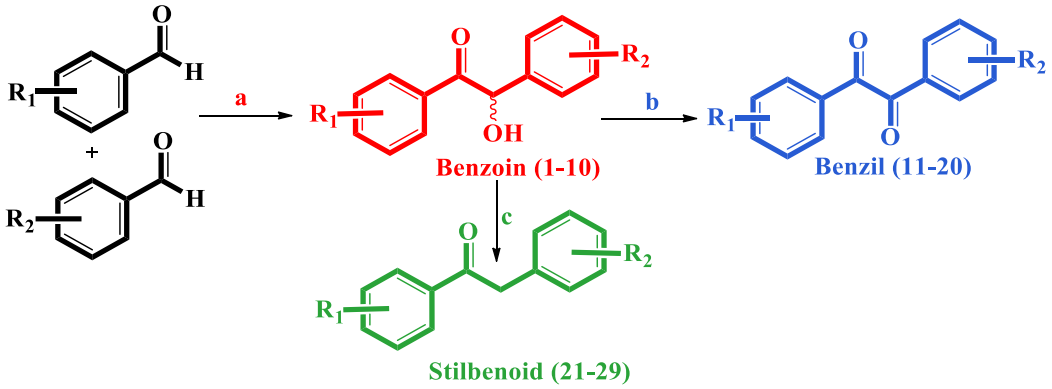
The antimicrobial properties of **1-29** were investigated quantitatively in respective broth media by using the microdilution method, and the minimal inhibition concentration (MIC) values (µg/mL) were examined.[60] Experimental procedure was described in our previous work.[18]

2.2.4. Antioxidant Activities

Antioxidant activities of **1-29** were tested against FRAP,[65-67] CUPRAC,[66] and DPPH[68-73] methods according to the literature[65-70] and experimental procedure describe in our previous work.[18] Trolox for CUPRAC and FRAP and butylated hydroxytoluene for DPPH were used as standards.

2.4. Molecular Docking Analysis Procedure

Molecular docking analysis was performed using the AutoDock 4.2 program[74] to elucidate the enzyme-ligand interaction mechanisms between compounds **1-29** and the enxzymes α-glucosidase, α-amylase, AChE, BChE and tyrosinase. This method requires three-dimensional (3D) structural information of the target enzyme and ligand to investigate the enzyme-ligand interaction mechanisms. First, the 3D crystal structures of target enzymes α-amylase, α-glucosidase, AChE, BchE, and tyrosinase were obtained from the protein data bank website (http://www.rcsb.org/pdb) (PDB ID: 5NN4, 4W93, 4EY6, 6QAA and 2Y9X, respectively). Crystallized ligands, water molecules, and noninteracting ions were then removed from these protein structures, and appropriate hydrogen atoms were added using the APBS-PDB2PQR software package. [75] After the preparation of the protein structures was completed, the grid box was adjusted according to the location of the binding site of the crystallized ligands and the amino acids responsible for the activity of the enzymes were selected and the binding site was determined with the AGFR1.2 program.[76] Docking was performed with a rigid protein and a flexible ligand using the Lamarckian Genetic algorithm and 100 independent runs per ligand. After this analysis, the protein and ligand conformation with the lowest binding energy was generated and visualized using BIOVIA Discovery Studio Client.[77]



a: KCN, EtOH-H_2O (10:2), 340 Watt-US, 70-85°C, in closed balloon; **b:** Fehling reagent, 0-5°C; **c:** Zn(Hg)

No	R_1	R_2
1a+b	3-OCH_3/ -H	-H/ 3-OCH_3
2	4-OCH_3	-H
3	3,4-diOCH_3	-H
4	3-OCH_3	3-OCH_3
5	4-OCH_3	3-OCH_3
6a+b	3-OCH_3 / 3,5-diOCH_3	3,5-diOCH_3 / 3-OCH_3
7	3,4-diOCH_3	3-OCH_3
8	3,4-diOCH_3	3,4-diOCH_3
9	3,4-diOCH_3	3,5-diOCH_3
10	3,5-diOCH_3	3,5-diOCH_3
11	3-OCH_3	-H
12	4-OCH_3	-H
13	3,4-di-OCH_3	-H
14	3-OCH_3	3-OCH_3
15	4-OCH_3	3-OCH_3
16	3-OCH_3	3,5-diOCH_3
17	3,4-diOCH_3	3-OCH_3
18	3,4-diOCH_3	3,4-diOCH_3
19	3,4-diOCH_3	3,5-diOCH_3
20	3,5-diOCH_3	3,5-diOCH_3
21a+b	3-OCH_3/ -H	-H/ 3-OCH_3
22	4-OCH_3	-H
23	3,4-diOCH_3	-H
24	3-OCH_3	3-OCH_3
25	4-OCH_3	3-OCH_3
26a+b	3-OCH_3 / 3,5-diOCH_3	3,5-diOCH_3 / 3-OCH_3
27	3,4-diOCH_3	3-OCH_3
28	3,4-diOCH_3	3,4-diOCH_3
29	3,5-diOCH_3	3,5-diOCH_3

Scheme 1. Synthesis scheme for the methoxy benzoin, benzil, and stilbenoid derivatives

3. Results and Discussion

3.1. Chemistry

The synthetic method for all compounds (**1**-**29**) is shown in Scheme 1. Benzoins (**1**-**10**)[2,18] were obtained from commercially substituted various benzaldehydes using KCN/US (340 W) which were then were oxidized with Fehling reagent to give the corresponding benzil compounds (**11**-**20**).[57] Benzoins were also reduced with Zn(Hg) amalgam to afford methoxy-substituted stilbenoids (**21**-**29**).[58] A total of 29 methoxy-substituted benzoin/benzil/stilbenoid were synthesized and characterized by 1D NMR (^{1}H, APT/^{13}C), LC-QTOF-MS, FT-IR, UV, and by the help of ACD 1D NMR program.

Our previous works reported the biological evaluation (enzyme inhibition, antioxidant and antimicrobial activity) of hydroxy and hydroxymethoxy benzoin/benzyl, which showed promising activities selectively due to their different substitution and position.[2, 18] Therefore, we aimed to synthesize a series of new/known methoxy benzoin/benzil/stilbenoid compounds to evaluate their biological activity, which were then screened for their enzyme inhibition, antimicrobial and antioxidant activity.

The synthesis of benzoin from two different benzaldehydes can give four different products, and the product yield depends on the substituent groups.[2, 18] Compounds **2**-**5** and **7**-**10** were obtained as single products and compounds **1** and **6** were obtained as mutual isomeric mixtures, which were purified by a vacuum liquid column chromatography (Table 1). The synthesized benzoin compounds are racemic, and the ^{1}H NMR spectra revealed the benzylic -CH(OH) peaks at δ 5.69-5.89 ppm and the ^{13}C NMR spectra indicated peaks at δ 197-198 ppm for C-1 (C=O) and δ 75-77 ppm for C-2. The disappearance of the benzylic proton and carbon peaks for the benzoin and the appearance of two asymmetric carbonyl peaks at δ 193-195 ppm for C-1 (C=O) and C-2 confirmed the benzil skeletons. Fot the stilbenoid compounds, the presence of the benzylic -CH_2 peak at δ 4.22 (m) ppm in the ^{1}H NMR spectra and the peaks at δ 197-198 ppm for C=O and δ 45-46 ppm for -CH_2 in the ^{13}C NMR spectra indicated the stilbenoid structure.

3.2. Biological Assay

3.2.1. Enzyme Inhibition Assays

Methoxy benzoin/benzil/stilbenoids (**1**-**29**) were tested *in vitro* for their inhibition of α-glucosidase[73,78] α-amylase,[78] tyrosinase,[79-80] AChE,[81-82] and BChE[81] inhibition. Their measured IC_{50} (µg/mL) values are shown in Table 2.

Among the compounds obtained (**1**-**29**), the most effective structures were **1** (118.28 µg/mL), **14** (103.32 µg/mL), **19** (101.83 µg/mL), and **28** (114.78 µg/mL) according standard (acarbose 93.12 µg/mL) used for the α-amylase. Compounds **19** and **14** were as active as the standard (acarbose) and the highest activities were seen with benzils for α-amylase. Only compounds **16** (59.30 µg/mL), **15** (64.17 µg/mL), and **19** (69.44 µg/mL) were effective in inhibiting α-glucosidase compared with the standard (acarbose, 36.65 µg/mL). Many of the benzoin/benzil/stilbenoid compounds were not active against α-glucosidase. Compounds **2** (66.22 µg/mL), **7** (114.47 µg/mL), and **23** (109.39 µg/mL) were found to be most effective compounds against AChE compared with the standard used (galantamine, 9.26 µg/mL). Compared to the galantamine standard used (33.72 µg/mL) against BChE, compounds **7** (93.51 µg/mL) and **13** (70.94 µg/mL) were found to be the most effective. The most effective compounds were **2** (33.29 µg/mL), **4** (54.13 µg/mL), **14** (62.89 µg/mL), **16** (31.89 µg/mL), **18** (32.15 µg/mL), **25** (48.11 µg/mL), and **26** (32.44 µg/mL) according to the standard used for the tyrosinase (kojic acid, 12.78 µg/mL). Compounds **2** (33.29 µg/mL), **16** (31.89 µg/mL), and **26a+b** (32.44 µg/mL) wereproved to be the most effective for the each group of compounds, respectively. Methoxy benzoins were seen as the most effective compounds in the range of 33.29-83.55 µg/mL in all three groups. Evaluation of the inhibition of five enzymes showed that each class of methoxy benzoin/benzil/stilbenoids had different activities against the tested enzymes. Compounds 1**9** against α-amylase, **16** against α-glucosidase, and tyrosinase, **2** against AChE, and **13** against BChE were found to be the most effective among each group compared to the standards used.

When the structure-activity relationship (SAR) of the compounds was considered, the following results were obtained. Benzoin/benzil/stilbenoid (**1-29)** has mono/di-OCH_3 groups on ring A and B as R_1 and R_2, respectively. Based on the structural properties of **1-29,** a limited structure-activity relationship (SAR) was developed (Scheme 1). Among them, compounds **1** (IC_{50} = 118.28 µg/mL), **14** (IC_{50} = 103.32 µg/mL), and **19** (IC_{50} = 101.83 µg/mL) with –OCH_3/di–OCH_3 at 3-/3,4-as R_1 and –OCH_3/di–OCH_3 at 3- and 3,5- as R_2 to each other exhibited similar inhibitory activity against *α*-amylase compared with the standard acarbose (IC_{50} = 93.12 µg/mL). The activity increased when the position of one of the –OCH_3 groups in the compounds was shifted to the 3- position as R_1 and to the 3- and 3,5- positions as R_2. When α-amylase inhibition was evaluated, it was seen that the most active compound in all three groups was found to be **19** (IC_{50} = 101.83 µg/mL) with

the 3,4-diOCH_3 as R_1, and with the 3,5-diOCH_3 as R_2 compared to the standard (acarbose). Thus, -OCH_3 benzils (**11-20**) are significant in interaction with the enzyme and the decrease in activity for oxidized derivatives may be due to low interaction of hydrogen bonding. Compounds **15** (IC_{50} = 64.17 µg/mL), and **19** (IC_{50} = 69.44 µg/mL) with methoxy at 4- and 3,4- as R_1 and methoxy/dimethoxy at 3-, and 3,5- positions as R_2 to each other resulted closer inhibition as the standard (acarbose, IC_{50} = 36.65 µg/mL) against *α*-glucosidase. Enzyme inhibition had shown that methoxy benzil analogues were usually more active for *α*-glucosidase and gave higher interaction with the enzyme.

Table 2 . Enzyme inhibition of **1-29** (IC_{50}, µg/mL)

No	α-Amylase	α-Glucosidase	AChE	BChE	Tyrosinase
Benzoins					
1a+b	**118.28**	372.85	>1000	147.24	83.55
2	155.89	>1000	**66.22**	>1000	**33.29**
3	>1000	417.04	>1000	342.12	76.99
4	456.25	>1000	384.81	335.51	**54.13**
5	137.47	>1000	166.31	241.00	>1000
6a+b	271.73	>1000	185.91	106.04	>1000
7	>1000	>1000	**114.47**	**93.51**	71.75
8	468.49	>1000	>1000	>1000	61.75
9	431.74	>1000	>1000	772.12	>1000
10	>1000	>1000	354.54	>1000	>1000
Benzils					
11	>1000	>1000	>1000	>1000	>1000
12	428.32	>1000	163.90	208.53	80.73
13	121.41	>1000	>1000	**70.94**	>1000
14	**103.32**	>1000	372.85	126.43	**62.89**
15	433.34	**64.17**	394.34	153.55	>1000
16	>1000	**59.30**	>1000	>1000	31.89
17	>1000	>1000	274.44	228.15	>1000
18	>1000	>1000	>1000	317.76	**32.15**
19	**101.83**	**69.44**	>1000	242.44	>1000
20	>1000	203.10	>1000	>1000	>1000
Stilbenoids					
21a+b	253.43	>1000	139.87	>1000	87.65
22	>1000	>1000	>1000	303.55	>1000
23	>1000	>1000	**109.39**	156.03	>1000
24	788.82	>1000	>1000	>1000	>1000
25	391.48	>1000	509.43	182.53	**48.11**
26a+b	177.03	325.88	>1000	>1000	**32.44**
27	476.01	>1000	>1000	400.68	258.52
28	**114.78**	>1000	210.86	>1000	158.74
29	>1000	>1000	>1000	>1000	>1000
Acarbose	93.12	36.65	-	-	-
Galantamine	-	-	9.26	33.72	-
Kojic Acid	-	-	-	-	12.78

Compounds **2** (IC_{50} = 66.22 µg/mL), **7** (IC_{50} = 114.47 µg/mL), and **23** (IC_{50} = 109.39 µg/mL) with methoxy/dimethoxy at 4- and 3,4- as R_1 and methoxy group at 3- as R_2 to each other showed moderate inhibition effect against AChE in comparison with the standard galantamine (IC_{50} = 9.26 µg/mL). IC_{50} values of most of the benzoin/benzil/stilbenoid compounds gave the low inhibition (IC_{50} = >1000 µg/mL) against *α*-

glucosidase enzyme. The IC_{50} value of **7** and **13** were found to be 93.51 µg/mL and 70.94 µg/mL with dimethoxy at 3,4- position as R_1 and methoxy at 3- position as R_2 (galantamine, IC_{50} = 33.72 µg/mL), respectively. Other compounds gave the low activity with IC_{50} values of >1000 µg/mL against BChE. These results showed that -OCH_3 substituted benzoin/benzil/stilbenoid compounds were usually not active on AChE and BChE enzyme inhibitions. Compounds **2** (IC_{50} = 33.29 µg/mL), **4** (IC_{50} = 54.13 µg/mL), **14** (IC_{50} = 62.89 µg/mL), **16** (IC_{50} = 31.89 µg/mL), **18** (IC_{50} = 32.15 µg/mL), **25** (IC_{50} = 48.11 µg/mL), and **26** (IC_{50} = 32.44 µg/mL) with -OCH_3 /di-OCH_3 at 4-, 3-, 3,4-, and 3,5- positions as R_1 and -OCH_3 /-OH at 3-, 3,4-, and 3,5-positions as R_2 to each other gave the parallel inhibition against tyrosinase in comparison with the kojic acid (IC_{50} = 12.78 µg/mL).

Compounds **5, 6, 9, 10, 11, 15, 17-19, 22-24,** an**d 29** resulted the low activity (IC_{50}, >1000 µg/mL). The numbers of more active compounds among the all three group were observed against tyrosinase comparison with other tested enzymes (Table 2). Compound **19** (IC_{50} = 101.83 µg/mL) having 3,4-diOCH_3 as R_1 and 3,5-di-OCH_3 as R_2, compound **15** (IC_{50} = 64.17 µg/mL) having 4-OCH_3 as R_1 and 3-OCH_3 as R_2, compound **2** (IC_{50} = 66.22 µg/mL) having 4-OCH_3 as R_1, compound **13** (IC_{50} = 70.94 µg/mL) having 3,4-di-OCH_3 as R_1, compound **16** (IC_{50} = 31.89 µg/mL) having 3--OCH_3as R_1 and 3,5-di-OCH_3 as R_2 displayed the best inhibitory activity against *α*-glucosidase, *α*-amylase, BChE, AChE, and tyrosinase enzymes, respectively. The results have shown that some of the methoxy substituted benzoin/benzil/stilbenoid (**1-29**) were active against the tested enzymes comparison with used standard. Nevertheless, highly differences in the activity was observed due to the number and positions of substitution on the benzoin/benzil/stilbenoid structures (**1-29**).

In the literature, many enzymatic studies were reported for the benzoin/benzil/stilbenoid compounds. In our previous work, hydroxy and hydroxy methoxy benzoin/benzil compounds showed enzyme inhibition as much as used standards against α-glucosidase, α-amylase, tyrosinase, AChE, and BChE.[2,18] Enzyme inhibition of hydroxy, hydroxymethoxy and methoxy benzoin and benzil compounds are compared, hydroxybenzoin were the most active against α-amylase and α-glucosidase. Whereas, among the all three benzoin/benzil, the best activity were observed from hydroxymethoxy benzoin/benzil compounds against tyrosinase, AChE, and BChE. In another work, synthesis of benzoin and their phosphatidyl inositol 3-kinase activities (PI3Kα) were reported.[19] 1,2-Diphenyliminoethanols were the more closely resemble the fingerprint of PI3K enzyme inhibitors were mentioned.[20] In a work, benzil compounds were reported to be inhibitor of carboxylesterases (CEs).[21] In another work, studies of α-hydroxyketones and α-diketones on Jack bean urease have been reported.[22] The imidazole derivatives of benzils were evaluated for their α-glucosidase inhibitory effect and the results revealed that most of the compounds investigated good activity at low micro-molar concentration.[23] Benzils were reported as mammalian CEs inhibitor[24] and inhibitory force of 1,2-dione depends on the hydrophobicity of the R group and electrophilicity effect of the C=O group.[24] A work had shown the CEs activity of long alkyl chain benzil inhibitors in situ.[25] Recently, a class of CEs inhibitors based on benzil was identified and these compounds showed inhibition effect on CEs in the low nanomolar range. Results suggested that carboxylesterase activity with benzil or its analogs applied to minimize the toxicity of normal cells to CPT-11.[26] Benzil was also reported as a selective inhibitor of CEs, and the inhibitory activity of related 1,2-diones was evaluated toward enzymes.[83] Another paper described the structural basis for the inhibition of hCE1 by the nanomolar affinity of benzil. It was suggested that the efficacy of clinical drugs could be modulated by targeted hCE1 inhibitors.[27] It was mentioned in the literature that cytosolic epoxide hydrolase activity was supressed by benzil and the enzyme appeared to be inhibited by benzyl.[28] Compounds affecting rodent microsomal epoxide hydrolase activity on human adrenal enzyme were also reported in the literature. 1,1,1-Trichloropropene-2,3-oxide and cyclohexene oxide were reported to inhibit the activity, whereas benzil had a stimulatory effect.[29] Amino-carboxylic based pyrazoles were mentioned as inhibitors of protein tyrosine phosphatase 1B (PTP1B), and compounds with different hydrophobic tails, such as 1,2-diphenylethanone and dibenzil amines, were evaluated in the PTP1B enzymatic assay.[30] Thus, the enzymatic studies showed that benzoin/benzil/stilbenoid compounds could be potentially drug-active compounds and require further studies.

3.2.2. Antimicrobial Activity Assays

The antimicrobial activities of **1-29** against eight bacteria, one yeast, and one fungus were investigated. MIC values (µg/mL) were calculated[59-60] after inhibition diameters (mm) were observed and the data are shown in Table 3.

Table 3. Antimicrobial minimum inhibition concentration of **1-29**

No	Stock sol. µg/mL	Microorganism and minimum inhibition concentration (MIC, µg/mL)									
		Gram (-)			Gram (+)				Tuberculosis	Fungi	
		Ec	*Yp*	*Pa*	*Sa*	*Sm*	*Psp*	*Bc*	*Bs*	*Ms*	*Ca*
Benzoins											
1a+b	70500	440	440	440	440	-	-	220	-	110	110
2	101900	-	-	-	-	-	-	-	-	-	-
3	65300	1632	1632	204	408	-	-	408	-	102	408
4	3300	41	82	-	165	-	-	-	-	41	41
5	82300	4115	4115	-	-	-	-	514	-	64	514
6a+b	88800	2220	2220	-	-	-	-	1110	-	69	1110
7	17700	885	885	-	-	-	-	442	-	221	221
8	1400	-	-	-	-	-	-	-	-	-	-
9	17300	-	-	216	-	-	-	-	-	-	-
10	16800	-	-	-	-	-	-	-	-	-	-
Benzils											
11	3500	88	-	-	-	-	-	22	-	22	44
12	2000	-	-	-	-	-	-	-	-	50	100
13	9900	123	247	495	123	-	-	-	-	61	247
14	6100	76	8152	152	305	305	-	-	-	38	305
15	7600	95	190	380	190	-	-	-	-	190	380
16	20700		-	517	129	517		129		517	1035
17	3000		150	-	75	-		75		75	75
18	3200	80	-	160	-	-	-	160	-	20	160
19	4600	230	-	-	-	-	-	-	-	230	230
20	3700	185	-	-	185	-	-	-	-	185	185
Stilbenoids											
21a+b	3000	150	150	-	-	-	-	-	18.8	33	-
22	5400	270	270	-	-	-	-	-	16.9	34	-
23	6500	325	-	-	-	-	-	-	395	-	-
24	24400	-	-	-	-	-	-	-	610	-	-
25	19700	-	-	-	-	-	-	-	493	-	-
26a+b	10900	-	-	-	-	-	-	-	504	-	-
27	6000	-	-	-	-	-	-	-	150	-	-
28	3200	160	-	160	160	160	160	-	160	160	-
29	8600	-	-	-	-	-	-	-	860	-	-
Amp.	10	10	10	NT	35	10	NT	NT	15		
Strep.										35	
Flu.	5										25

Ec: Escherichia coli. *Kp*: *Klepsiella pneumonia*. *Yp*: *Yersinia pseudotuberculosis*. *Pa*: *Pseudomonas aeruginosa*. *Ns*: *Neisseria* sp.. *Mca*: *Moraxella catarrhalis*. *Sa*: *Staphylococcus aureus*. *Ef*: *Enterococcus faecalis*. *Spy*: *Streptococcus pyogenes*. *Spn*: *Streptococcus pneumonia*. *Sm*: *Streptococcus mutans*. *Lm*: *Listeria monocytogenes*. *Psp*: *Paenibacillus* sp. *Bc*: *Bacillus cereus*. *Bs*: *Bacillus suptilis*. *Ms*: *Mycobacterium smegmatis*. *Ca*: *Candida albicans*. *Sc*: *Saccharomyces cerevisiae*. Amp.: Ampisilin. Strep.: Streptomisin. Flu.: Flukonazol. (-): No activity. NT: Not tested.

Compound **4** was the most active benzoin with MIC values in the range of 41-165 µg/mL against *E. coli*, *Y. pseudotuberculosis*, *Staphylococcus aureus*, *M. smegmatis*, and *C. albicans*, respectively.

Of the benzoins tested, **1-7,** and **9** showed MIC in the range of 41-2220 µg/mL against *E. coli*, *Pseudomonas aeruginosa*, *Y. pseudotuberculosis*, *B. cereus*, *S. aureus*, *M. smegmatis*, and *C. albicans* only. Compounds **8** and **10** showed no activity against any of the microorganisms tested. None of the benzoin compounds were effective against *Streptococcus mutans*, *Paenobascillus* sp., and *Bacillus subtilis*. Of the benzil compounds (**11-20**), **11** against *E. coli*, *B. cereus*, *M. smegmatis*, and *C. albicans* and **14** and **18** against *E. coli* and *M. smegmatis* were the most active with the MIC values within the range of 22-88 µg/mL, and 20-80 µg/mL, respectively. All of the benzil compounds tested were active against *M. smegmatis* and *C. albicans* in the range of 20-1035 µg/mL MIC values, respectively. None of the benzil compounds were active against *Paenobascillus* sp. and *B. subtilis*. Compounds **11**, **13**, **14**, **15**, **17** and **18** within the benzil group were most effective against various microorganisms with MIC values in the range of 20-247 µg/mL. In case of stilbenoid (**21-29**), compounds **21** and **22** were the most active against the *M. smegmatis* within the range of 33-34 µg/mL MIC values. None of the stilbenoids showed activity against *B. cereus*, and *C. albicans*, but all stilbenoid compounds (**21-29**) were active against *B. subtilis* with MIC values of 18.8-860 µg/mL, respectively. As a result of the antimicrobial activities in all three groups, the benzil compounds (**11-20**) were found to be most active antimicrobial agents. The antimicrobial activity showed that the compounds with methoxy (-OCH_3) as -R_1 group at *meta*/*para* position on ring A and at 3- position as -R_2 group on ring B were more active.

Antimicrobial activities of hydroxy and hydroxy methoxy benzoin/benzil were reported in the literature, which showed promising activities for some of the microorganisms tested.[2, 18] Addanki et al. mentioned the anti-bacterial activity of dinitrobenzil against Gram (+) bacteria such as *S. aureus*, *B. subtilis*, *Staphylococcus epidermidis* and Gram (-) bacteria such as *P. aeruginosa* and *E. coli*. The compounds had the highest inhibitory activity against *Staphylococcus epidermidis* in the case of Gram (+) bacteria and *E. coli* in the case of Gram (-) bacterium compared to standard streptomycin.[31] Nithya et al. reported the antimicrobial activity of 4,4'-dimethoxy benzoin/benzil. And the cytotoxic, and antibacterial activity *in vitro*,which inhibits cell growth, was also demonstratedat different concentration. The results show that the antibacterial activity of benzoin/benzil is higher than the antimicrobial activity.[32] Nithya et al. also synthesized many benzil compounds and reported excellent antimicrobial and cytotoxic activity. The antimicrobial activity results showed that some of the tested compounds exhibited the most promising antibacterial activities. The antioxidant activity of benzil and its analogs was also reported.[33-34]

3.2.3. Antioxidant Activity Assays

The antioxidant activities of **1-29** were studied by the FRAP, CUPRAC and DPPH methods and the data can be seen in Table 4.

Among all three groups of compounds, the benzoins (**1-10**), were found to be the most effective by all three methods used. It is clear that the benzylic hydroxyl (-OH) in the benzoin structures affected the activity and thus the compounds were more active. The highest CUPRAC and FRAP values of **7** were 1508.17±6.5 (µg/mL) and 2100±42.7 (µg/mL) respectively. The lowest DPPH value of **11** and **1a+b** was found to be 6.72 ± 0.7 mg/mL and 7.20 ± 0.5 mg/mL, respectively. Considering the substitution positions, they are generally more active when -OCH_3 is substituted at the 3,4-position on ring A and at the 3-position on ring B in the case of the CUPRAC and FRAP methods. However, in the DPPH method, the benzoin compound with -OCH_3 at the 3- position of the ring A/B was more effective. The antioxidant activities of benzoin/benzil compounds containing different substituents have been reported in the literature, and the antioxidant activities of some of the reported benzoin/benzil compounds were found to be more effective than the standards used.[2, 18, 32]

3.3. Molecular Docking

The enzyme-ligand interactions between α-amylase, α-glucosidase, tyrosinase, AChE, BChE, enzymes with methoxy benzoin (**1-10**), benzil (**11-20**) and stilbenoid (**21-29**) were studied by molecular docking. The lowest binding energy of the compounds indicated better binding affinity to the protein.

Table 4. Antioxidant activities of **1-29**

No	FRAP[a]	CUPRAC[b]	DPPH[c]
Benzoins			
1a+b	1759 ± 89.6	1468.33 ± 21.6	7.20 ± 0.5

2	1625 ± 112.7	1875.00 ± 31.5	10.07 ± 1.5
3	1917 ± 57.1	46.67 ± 5.1	23.20 ± 1.8
4	1741 ± 12.9	965.01 ± 26.4	35.54 ± 0.6
5	1475 ± 37.4	1428.33 ± 32.4	36.26 ± 1.5
6a+b	1875 ± 78.9	1407.83 ± 21.8	43.93 ± 0.4
7	2100 ± 42.7	1508.17 ± 6.5	13.39 ± 1.2
8	1427 ± 69.3	591.67 ± 16.2	71.73 ± 0.2
9	1867 ± 87.4	287.50 ± 17.5	13.69 ± 0.6
10	2001 ± 37.3	580.00 ± 36.1	35.56 ± 1.5
Benzils			
11	1477 ± 12.6	1633.33 ± 21.6	6.72 ± 0.7
12	1515 ± 69.4	168.34 ± 8.2	23.68 ± 2.3
13	1779 ± 51.7	40.00 ± 3.8	65.83 ± 0.9
14	1476 ± 48.0	2966.6 ± 53.2	83.75 ± 2.1
15	1585 ± 46.3	18.33 ± 1.4	25.42 ± 0.6
16	1969 ± 67.2	1501.50 ± 5.8	14.25 ± 1.5
17	1784 ± 69.5	20.00 ± 1.5	34.98 ± 1.1
18	1204 ± 38.0	236.68 ± 6.8	96.60 ± 1.2
19	1370 ± 59.5	98.33 ± 11.4	42.54 ± 0.9
20	1370 ± 68.2	212.67 ± 3.1	14.36 ± 0.7
Stilbenoids			
21a+b	1364 ± 58.3	315.00 ± 1.3	12.54 ± 1.4
22	1407 ± 34.8	610.00 ± 5.3	73.51 ± 0.8
23	1400 ± 90.7	33.34 ± 1.3	82.86 ± 0.2
24	1421 ± 94.8	808.32 ± 14.2	60.51 ± 2.0
25	1338 ± 57.3	931.67 ± 2.6	15.28 ± 0.2
26a+b	1178 ± 86.4	580.00 ± 11.7	36.78 ± 0.5
27	1005 ± 37.6	16.68 ± 1.8	26.79 ± 0.9
28	1124 ± 37.9	17.83 ± 1.4	81.69 ± 0.8
29	1008 ± 14.6	640.00 ± 3.7	54.52 ± 0.3
BHT	-	-	6.44 ± 0.1

[a]FRAP. the iron reducing antioxidant power (µg/mL trolox/gram DW). [b]CUPRAC. Copper reducing antioxidant power (µg/mL trolox/gram DW). [c]DPPH. 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (mg/mL). BHT: di-t-butyl hydroxy toluene.

In general, the tested compounds showed better binding affinity for α-amylase and α-glucosidase enzymes than the reference compound (acarbose) (Table 5). In particular, compounds **6a** and **9** have the lowest binding energy (-7.19 and -6.93 kcal/mol) for the enzyme α-amylase and α-glucosidase, respectively, and formed hydrogen bond interactions with residues Lys200, Ile235, Leu237, Asp236 and Glu233 in α-amylase and residues Arg600, Leu677, Asp616 and Asp518 in α-glucosidas. Accordingly, the amino acids that play a role in the interaction according to the molecular docking process were found to be compatible with the interaction sites known in the literature.[84-85]

In addition, for AChE and BChE enzymes, it was found that the binding energy (galantamine, reference compound) was -9.13 and -7.61 kcal/mol, respectively. According to the molecular docking results, all tested compounds showed lower binding affinity towards AChE and BChE enzymes than the reference

compound. This situation was also observed in experimental studies. At the same time, compounds **9** and **14** showed the lowest binding energy (-8.41 and 8.09 kcal/mol) against AChE and BChE, respectively. These compounds exhibited hydrogen bond interactions with the Gly121, Gly122, Ser203 and Glu202 residues of the AChE enzyme, and the Gly116, Gly117, Ser198, Leu286, Gly439 and Pro285 residues of the BChE enzyme, as shown in Figure 1 and Table 5. These amino acid residues are important for the catalytic activity of these enzymes.[86-87]

Finally, the binding energies of **1**-**29** compounds for the enzyme tyrosinase were analyzed by the molecular docking method. According to this analysis, all compounds showed better binding affinity (binding energy value from -6.69 to -6.15 kcal/mol) than the reference compound (kojic acid, binding energy: 3.96 kcal/mol) and compound **6a** had the best binding affinity. These active compounds interacted with residues Met280, Ser282, Gly281 and Asn260 located in the tyrosine enzyme.

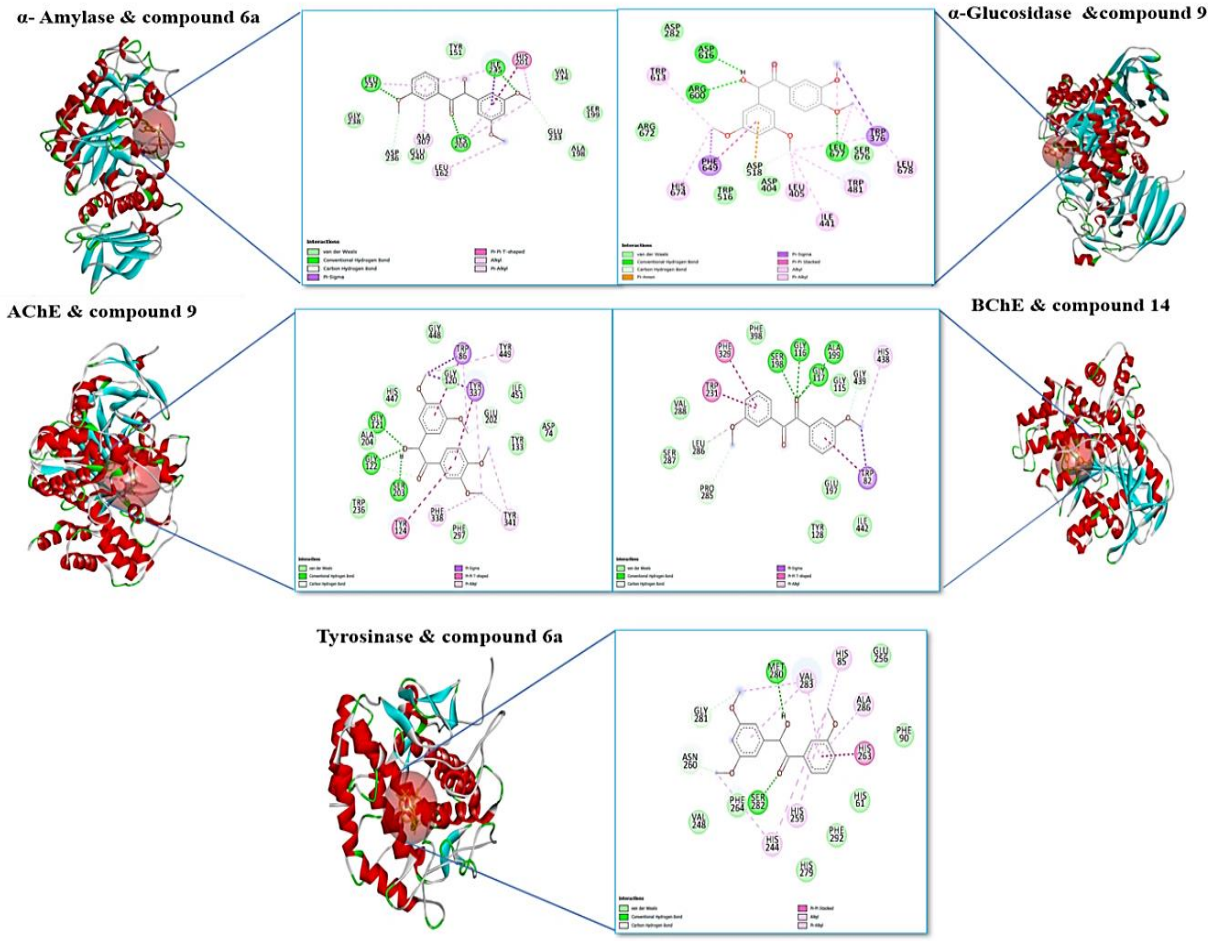


Figure 1. The 2D analysis of compounds with the best docking score against α-amylase, α-glucosidase, AChE, BChE and tyrosinase enzymes

In summary, the molecular docking results show that the compounds bind to the active sites of AChE, BChE and tyrosinase and interact with amino acid residues that are important for catalytic activity. Also, after *in vitro* evaluation of enzyme inhibition analysis, compounds **1**, **14**, **19**, and **28** against α-amylase, **15** and **19** against α-glucosidase, **2**, **4**, **14**, **18**, **25** and **26** against tyrosinase, **2**, **7**, and **23** against AChE, and **7**, and **13** against BChE showed similar activity to the standard used. In this study, these compounds showed similar and higher binding affinity compared to the standard compounds (Table 5).

Table 5. The lowest binding energy values and inhibition constant (Ki) of the compounds **1**-**29** and positive control compound from each docking analysis in the active site of α-amylase, α-glucosidase, AChE, BChE, and tyrosinase

No	Binding Energy (kcal/mol)				
	α-Amylase	α-Glucosidase	AChE	BChE	Tyrosinase
Benzoins					
1a	-6.40	-5.81	-7.61	-7.39	-6.69
1b	-6.83	-6.08	-7.76	-7.35	-6.64
2	-6.74	-6.10	-7.83	-7.12	-6.23
3	-6.39	-6.15	-7.86	-7.12	-6.42
4	-7.17	-6.11	-8.03	-7.69	-6.69
5	-6.66	-6.50	-7.94	-7.15	-6.63
6a	**-7.19**	-6.78	-8.28	-7.41	**-6.71**
6b	-7.15	-6.23	-8.05	-7.49	-6.58
7	-7.17	-6.58	-8.29	-7.23	-6.63
8	-6.79	-6.24	-8.10	-7.11	-6.15
9	-6.72	**-6.93**	**-8.41**	-7.36	-6.50
10	-7.08	-6.54	-8.21	-7.77	-6.65
Benzils					
11	-6.57	-6.04	-7.51	-7.82	-6.60
12	-6.51	-5.88	-7.56	-7.57	-6.49
13	-6.57	-5.98	-7.67	-7.55	-6.41
14	-7.10	-6.17	-7.87	**-8.09**	-6.66
15	-6.83	-6.43	-7.83	-7.58	-6.66
16	-7.10	-6.54	-8.03	-7.90	-6.66
17	-7.03	-6.52	-8.01	-7.54	-6.58
18	-6.82	-6.12	-8.31	-7.14	-6.43
19	-6.92	-6.79	-8.15	-6.99	-6.62
20	-7.09	-6.58	-8.04	-7.59	-6.57
Stilbenoids					
21a	-6.48	-5.67	-7.18	-7.77	-6.45
21b	-6.26	-5.79	-7.51	-7.67	-6.34
22	-6.14	-5.79	-7.32	-7.52	-6.20
23	-6.46	-5.90	-7.41	-7.47	-6.18
24	-6.90	-6.30	-7.78	-8.00	-6.43
25	-6.33	-6.26	-7.66	-7.20	-6.37
26a	-6.76	-6.47	-8.16	-7.65	-6.50
26b	-6.89	-6.61	-7.76	-7.45	-6.46
27	-6.72	-6.11	-7.72	-7.03	-6.41
28	-6.74	-6.26	-7.85	-6.99	-6.17
29	-6.81	-6.72	-8.10	-7.42	-6.36
Acarbose	-6.30	-4.66	-	-	-
Galantamine	-	-	-9.13	-7.61	-
Kojic acid	-	-	-	-	-3.96

4. Conclusion

This work presented the synthesis of new and known methoxy benzoin/benzil/stilbenoid compounds with their biological activities (enzyme inhibition, antimicrobial, and antioxidant). Many benzoins/benzils have been described reported for their potential therapeutic use. The molecular docking study of **1-29** against the five different enzymes revealed that compounds **6a** and **9** generally had the best binding affinity against α-amylase, α-glucosidase, tyrosinase, AChE, and BChE. The highest FRAP and CUPRAC values were observed as 2100±42.7 (µg/mL) and 1508.17±6.5 (µg/mL) in compound **7**, respectively. The lowest DPPH values for **11** and **1a+b** were 6.72 ± 0.7 mg/mL and 7.20 ± 0.5 mg/mL, respectively. Compound **4** was the most effective benzoin against *E. coli*, *M. smegmatis*, and *C. albicans* (MIC, IC_{50} 41 µg/mL). All benzil compounds (**11-20**) were active against *M. smegmatis*, and *C. albicans* within the range of 20-1035 µg/mL MIC values, respectively. In contrast, all stilbenoid compounds (**21-29**) tested were active only against *B. subtili*, with MIC

values ranging from 18.8-860 µg/mL. Benzil compounds (**11**-**20**) were found to be the most effective antimicrobial agents. Compound **19** (IC_{50} = 101.83 µg/mL), **15** (IC_{50} = 64.17 µg/mL), **16** (IC_{50} = 31.89 µg/mL), **2** (IC_{50} = 66.22 µg/mL) and **13** (IC_{50} = 70.94 µg/mL) showed the best inhibitions against *α*-amylase, *α*-glucosidase, tyrosinase, AChE, and BChE, respectively. Indeed, according to the enzyme inhibition results, no significant correlation was observed in the SAR of compounds **1**-**29** compared to R_1 and R_2. Nevertheless, further pharmacological studies could be performed for **1**-**29** to be used use as potential drug agents.

Acknowledgements

The authors are very thankful to the Turkey Academy of Sciences (TÜBİTAK 107R048) and KTÜ-BAP (TDK-2020-8881) for providing financial support.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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