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Evaluation of synthesized methoxy chalcones for therapeutic potential through *in vitro* and *in silico* methods

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ABSTRACT: Chalcones are the precursors of flavonoids and have a wide range of biological activities. A series of methoxy chalcones (**1-12**) were synthesized using the Claisen-Schmidt method and identified by NMR analyses. Enzyme inhibition, antimicrobial, and antioxidant activities of all compounds were investigated. The enzyme kinetics and ADMET profile of the compounds were evaluated by *in silico* methods. The highest inhibition activities for lipase, AChE, BChE, tyrosinase, *α*-amylase, and *α*-glucosidase were observed at the following IC_{50} values (µg/mL): **7** (39.83±1.1216), **2** (60.39±1.24), **1** (39.79±1.29), **2** (40.40±1.01), **1** (98.61±3.17), and **2** (55.91±1.78), respectively. Compounds **1** and **4** exhibited the highest antioxidant activity against FRAP and CUPRAC tests, while **1** and **3** were the most effective in the DPPH method. All compounds showed the best activity against gram (-) bacteria. The top docking scores were compound **1** against *α*-amylase and BChE, **2** against *α*-glucosidase, AChE, and tyrosinase, and **7** against lipase. All compounds met the drug-likeness criteria using the SwissADME. All compounds have high bioavailability with lower toxicity profiles using SwissADME and pkCSM. According to the AMES test, compounds **3**, **6**, **9**, and **10** were predicted to be mutagenic. ProTox (v.3.0) predicts that all compounds have an oral LD_{50} value of 2100 mg/kg bw and are classified as GHS Category V, indicating relatively low acute toxicity. Overall, the study results indicate that compounds **1** and **2** show promise for animal studies targeting Alzheimer's disease and diabetes, while **7** appears promising for obesity.

KEYWORDS: Chalcones; Molecular docking; ADMET; Enzyme inhibition; Antimicrobial; Antioxidant.

1. INTRODUCTION

Heterocyclic compounds such as chalcone and its derivatives have become very important and interesting in medicinal chemistry in the 21st century due to their therapeutic potential and broad spectrum of pharmacological properties [1]. These compounds are key to the development of many vital therapeutic agents. It has received extraordinary attention due to a wide range of physicochemical and pharmacological activities on the cerebrovascular, cardiovascular, and neurovascular systems and its contributions to life-sustaining processes [2]. Many synthetic analogues obtained from chalcones have therapeutic properties. It has been highlighted that these compounds may be potential therapeutic agents through their structural modifications [3]. Chalcones are compounds that serve as precursors to flavonoids and are classified as flavonoids themselves. They primarily consist of polyhydroxylated aromatic rings. They have radical quenching activity due to their phenolic groups, which is of interest to researchers in finding therapeutically useful compounds [4]. Chalcone-derived compounds (their structural analogs, either synthetic or natural) have been reported to exhibit a wide range of biological activities such as; anticancer [5-7], neuroprotective

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[1, 8], antibacterial [1, 3, 9], antimalarial [3, 5, 10], antileishmanial [5, 11, 12], anti-diabetic [13, 14], anti-inflammatory [5, 15], antioxidant [1, 3, 16], antituberculosis [3, 5], antiviral [1, 17], and antifungal [3, 5].

Today, diseases like Alzheimer's, Parkinson's, diabetes, and obesity are among the major health challenges in modern society. These conditions are increasingly important due to their rising prevalence worldwide, the substantial burden they place on healthcare systems, and their impact on individuals' quality of life. Alzheimer's disease is the most common form of dementia, primarily affecting older adults. Parkinson's disease is a neurological disorder that impacts motor functions. Despite decades of research, there are still no curative treatments for either of these neurodegenerative conditions [18]. Diabetes and obesity are major contributors to global health problems, leading to serious complications such as cardiovascular diseases, kidney failure, and metabolic syndrome. In 2022, the World Health Organization (WHO) reported that approximately 890 million adults were classified as obese [19]. Until recently, the two most effective anti-obesity medications available were orlistat and sibutramine. In 2010, the U.S. Food and Drug Administration (FDA) withdrew sibutramine due to its serious cardiovascular side effects [20]. Consequently, the search for safer anti-obesity drugs remains a priority. There is an association between obesity and diabetes mellitus. Elevated postprandial glucose levels and increased free fatty acids can lead to β-cell failure and suppress insulin production, resulting in high blood glucose levels [21]. Enzymes are known to influence the pathology of various diseases, including inflammation, microbial infections, neoplasms, HIV, diabetes, and obesity. Enzyme inhibition is used as a universal strategy to treat these and similar diseases or to elucidate the mechanism of action. In drug development and research, designing effective enzyme inhibitors is crucial. Inhibiting AChE and BChE enzymes in Alzheimer's disease patients is important in terms of increasing the activity of cognitive functions [22]. Studies have reported that AChE inhibitors do not completely cure the disease but reduce symptoms and slow down the progression of the disease [23]. The tyrosinase enzyme has been associated with melanin pigmentation, which provides hair and skin color, and neurotoxicity. It has been stated that inhibition of tyrosinase enzyme may guide treatment research in pigmentation disorders and Parkinson's disease [24]. *α*-Amylase and *α*-glucosidase enzymes are the main enzymes of glucose metabolism. Blood glucose levels increase with the activity of these enzymes. In this context, inhibition of *α*-amylase and *α*-glucosidase enzymes constitutes an important mechanism in controlling blood glucose levels in diabetes [22]. Nowadays, pancreatic lipase inhibitors are of great importance in obesity treatment. However, it has been shown that pancreatic lipase inhibitors such as orlistat can reduce obesity caused by a high-fat diet while also leading to fatty stools [25]. Moreover, oxidative stress is seen when the body's antioxidant defense mechanism is weakened, and it cannot remove free radicals. Studies show that oxidative stress is involved in the etiology of many diseases such as cardiovascular diseases, cancer, and diabetes, and that phenolic compounds with antioxidant effects may be useful in protecting against oxidative stress-related disorders [26, 27].

The conventional approach to pharmacokinetics involves separating the various factors that influence target access into individual parameters, including absorption, distribution, metabolism, and excretion (ADME). These parameters are assessed individually using specialized methods. In drug design and discovery, early estimation of ADME and toxicity (ADMET) properties has been shown to mitigate potential drawbacks in clinical and phase stages to a great extent. For ADMET prediction, computer models are recommended as powerful alternatives to experimental procedures, especially in the early stages when numerous chemical structures are being explored, but compound availability is limited [28, 29].

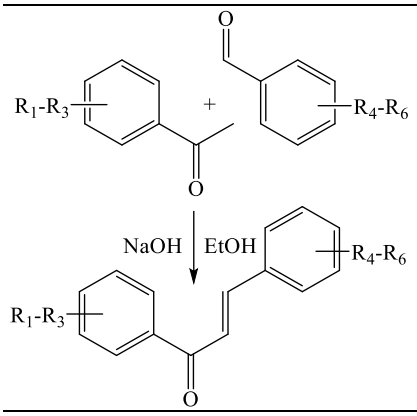
Based on the above considerations, methoxy chalcones (1-12) were synthesized in the first stage. In the second stage, enzyme-inhibiting, antimicrobial, and antioxidant effects of the compounds were investigated. *In silico* analyses, including molecular docking and ADMET prediction, were performed using SwissADME, pKCSM, and ProTox (v.3.0). The literature search indicated that compound **8** is novel while others have been synthesized previously.

2. RESULTS

2.1. Chemistry

The synthesis of methoxy chalcones (**1-12**) was shown in Scheme 1. Equivalent amounts of methoxy-substituted acetophenone and methoxy-substituted benzaldehyde were reacted at room temperature by dissolving them with water and alcohol solvent in a basic environment. The reactions were terminated by controlling the thin-layer chromatography (TLC). The synthesized compounds were further purified by crystallization. After the purity of the synthesized compounds was checked by TLC, their structures were

determined using NMR (^{1}H and ^{13}C-APT) spectroscopy. The original spectra are included as figures in the Supplementary Material.



No	-R_1	-R_2	-R_3	-R_4	-R_5	-R_6
1	3-OCH_3	4-OCH_3	5-OCH_3	3-OCH_3	4-OCH_3	5-OCH_3
2	3-OCH_3	4-OCH_3	5-OCH_3	2-OCH_3	3-OCH_3	4-OCH_3
3	3-OCH_3	4-OCH_3	5-OCH_3	3-OCH_3	4-OCH_3	-H
4	2-OCH_3	3-OCH_3	4-OCH_3	3-OCH_3	4-OCH_3	5-OCH_3
5	2-OCH_3	3-OCH_3	4-OCH_3	2-OCH_3	3-OCH_3	4-OCH_3
6	2-OCH_3	3-OCH_3	4-OCH_3	3-OCH_3	4-OCH_3	-H
7	2-OCH_3	4-OCH_3	5-OCH_3	3-OCH_3	4-OCH_3	5-OCH_3
8	2-OCH_3	4-OCH_3	5-OCH_3	2-OCH_3	3-OCH_3	4-OCH_3
9	2-OCH_3	4-OCH_3	5-OCH_3	3-OCH_3	4-OCH_3	-H
10	3-OCH_3	4-OCH_3	-H	3-OCH_3	4-OCH_3	5-OCH_3
11	3-OCH_3	4-OCH_3	-H	2-OCH_3	3-OCH_3	4-OCH_3
12	3-OCH_3	4-OCH_3	-H	3-OCH_3	4-OCH_3	-H

Scheme 1. Synthesis scheme for the methoxy chalcones (**1-12**).

2.2. Evaluation of enzyme inhibitions

Methoxy chalcones (**1-12**) were tested for their *in vitro* lipase [30], *a*-glucosidase [31], *a*-amylase [32], tyrosinase [33], AChE [34], and BChE [34] inhibition. Their calculated IC_{50} (µg/mL) values are shown in Table 1.

Table 1. Enzyme inhibitions (IC_{50} values) of methoxy chalcones **1-12**.

No	Lipase	AChE	BChE	Tyrosinase	*a*-Amylase	*a*-Glucosidase
1	100.34±2.05	78.15±0.58	39.79±1.29	119.41±2.13	98.61±3.17	60.93±0.93
2	46.67±1.61	60.39±1.24	61.53±1.07	40.40±1.01	146.54±1.65	55.91±1.78
3	43.86±2.18	110.25±2.47	124.39±2.24	76.11±3.17	235.81±1.08	617.49±3.72
4	123.03±4.35	163.01±2.19	175.84±2.43	96.10±2.19	192.98±3.09	>1000
5	161.07±3.53	552.77±3.28	444.13±3.78	>1000	>1000	>1000
6	169.82±5.74	>1000	>1000	>1000	>1000	>1000
7	39.83±1.12	>1000	>1000	>1000	406.98±2.08	>1000
8	42.43±1.75	142.84±3.71	54.34±0.34	202.63±3.03	337.40±2.42	161.57±2.23
9	288.84±10.23	81.13±1.24	201.26±1.12	320.88±2.91	159.84±1.25	341.76±1.24
10	94.84±4.65	>1000	>1000	>1000	>1000	>1000
11	55.26±3.41	251.71±1.09	82.51±1.04	650.68±1.28	532.94±11.59	>1000
12	65.16±3.12	>1000	>1000	>1000	>1000	274.65±1.48
Orlistat	13.49±1.23	N/A	N/A	N/A	N/A	N/A
Galantamine	N/A	6.13±0.47	26.59±0.71	N/A	N/A	N/A
Kojic acid	N/A	N/A	N/A	18.83±1.09	N/A	N/A
Acarbose	N/A	N/A	N/A	N/A	79.55±3.12	37.31±3.12

*IC_{50} values (µg/mL) are the mean±SD (*n*=3); AchE: Acetylcholinesterse; BChE: Butyrylcholinesterase; N/A: Not available.

2.3. Evaluation of antimicrobial activities

The *in vitro* antimicrobial activities of methoxy chalcones (**1-12**) were examined against eight bacteria, one yeast, and one fungus. The minimum inhibitory concentration (MIC) values (in µg/mL) were determined after measuring the inhibition diamers (in mm) [35, 36]. The results are presented in Table 2.

Table 2. Screening for the antimicrobial activity of methoxy chalcones **1-12**.

No	Stock Sol. (µg/mL)	MIC (µg/mL)									
		Gram (-) Bac.			Gram (+) Bac.				No Gr.	Fungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
1	10,300	643.8	643.8	643.8	N/A	N/A	N/A	N/A	321.9	643.8	643.8

2	10,000	625	1250	1250	N/A	N/A	N/A	N/A	625	1250	1250
3	10,400	627.5	1255	1255	N/A	N/A	N/A	N/A	627.5	1255	1255
4	10,100	631.3	1262.5	1262.5	N/A	N/A	N/A	N/A	1262.5	1262.5	1262.5
5	55,500	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6	12,800	800	1600	800	N/A	N/A	N/A	N/A	400	800	800
7	12,200	381.3	762.5	762.5	N/A	N/A	N/A	N/A	381.3	762.5	762.5
8	10,500	164.1	328.3	328.3	N/A	N/A	N/A	N/A	164.1	328.3	328.3
9	10,200	637.5	637.5	637.5	N/A	N/A	N/A	N/A	318.8	637.5	637.5
10	10,000	625	1250	1250	N/A	N/A	N/A	N/A	625	1250	1250
11	10,400	1255	2510	1255	N/A	N/A	N/A	N/A	627.5	1255	1255
12	40,000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Amp.	10	10	18	128	35	10	15	15	N/A	N/A	N/A
Strep.	10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4	N/A	N/A
Flu	5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8	8

MIC: Minimal inhibitory concentration (µg/mL); Ec: *Escherichia coli*; Yp: *Yersinia pseudotuberculosis*; Pa: *Pseudomonas aeruginosa*; Sa: *Staphylococcus aureus*; Ef: *Enterococcus faecalis*; Lm: *Listeria monocytogenes*; Bc: *Bacillus cereus* 702 Roma; Ms: *Mycobacterium smegmatis*; Ca: *Candida albicans*; Sc: *Saccharomyces cerevisiae*; Amp.: Ampicillin; Strep.: Streptomycin; Flu.: Fluconazole; (-): No activity; N/A: Not available.

2.4. Evaluation of antioxidant activities

Antioxidant activities of **1-12** were tested against FRAP [37, 38], CUPRAC [38], and DPPH [37] methods according to the literature and experimental procedure described in our previous work [39, 40] (Table 3). The standards used were butylated hydroxytoluene for DPPH, Trolox for CUPRAC, and FRAP.

Table 3. Antioxidant activities of methoxy chalcones **1-12**.

No	FRAP[a]	CUPRAC[b]	DPPH[c]
1	1633.70±2.52	2093.33±3.53	0.320±0.002
2	861.80±3.16	2732.00±7.42	0.663±0.001
3	531.13±3.12	1360.00±1.25	0.542±0.003
4	1048.70±4.32	1651.40±3.91	2.311±0.006
5	95.70±3.25	81.40±4.36	8.135±0.004
6	66.37±1.59	294.00±1.92	5.865±0.004
7	102.03±3.06	216.00±3.25	5.038±0.002
8	249.70±2.09	870.00±7.18	4.379±0.001
9	881.70±4.15	682.40±1.36	3.346±0.004
10	49.03±3.09	155.33±2.57	14.165±0.006
11	516.03±8.03	670.00±8.06	7.349±0.008
12	457.03±1.87	104.67±2.13	11.524±0.001
BHT	N/A	N/A	0.032±0.001

Data are the mean±SD (n=3); [a]FRAP: µM Trolox equivalent/g; [b]CUPRAC: µM Trolox equivalent/g; [c]DPPH: mg/mL; BHT: Butylated hydroxy toluene; N/A: Not available.

2.5. *In silico* studies

2.5.1. Molecular docking analysis

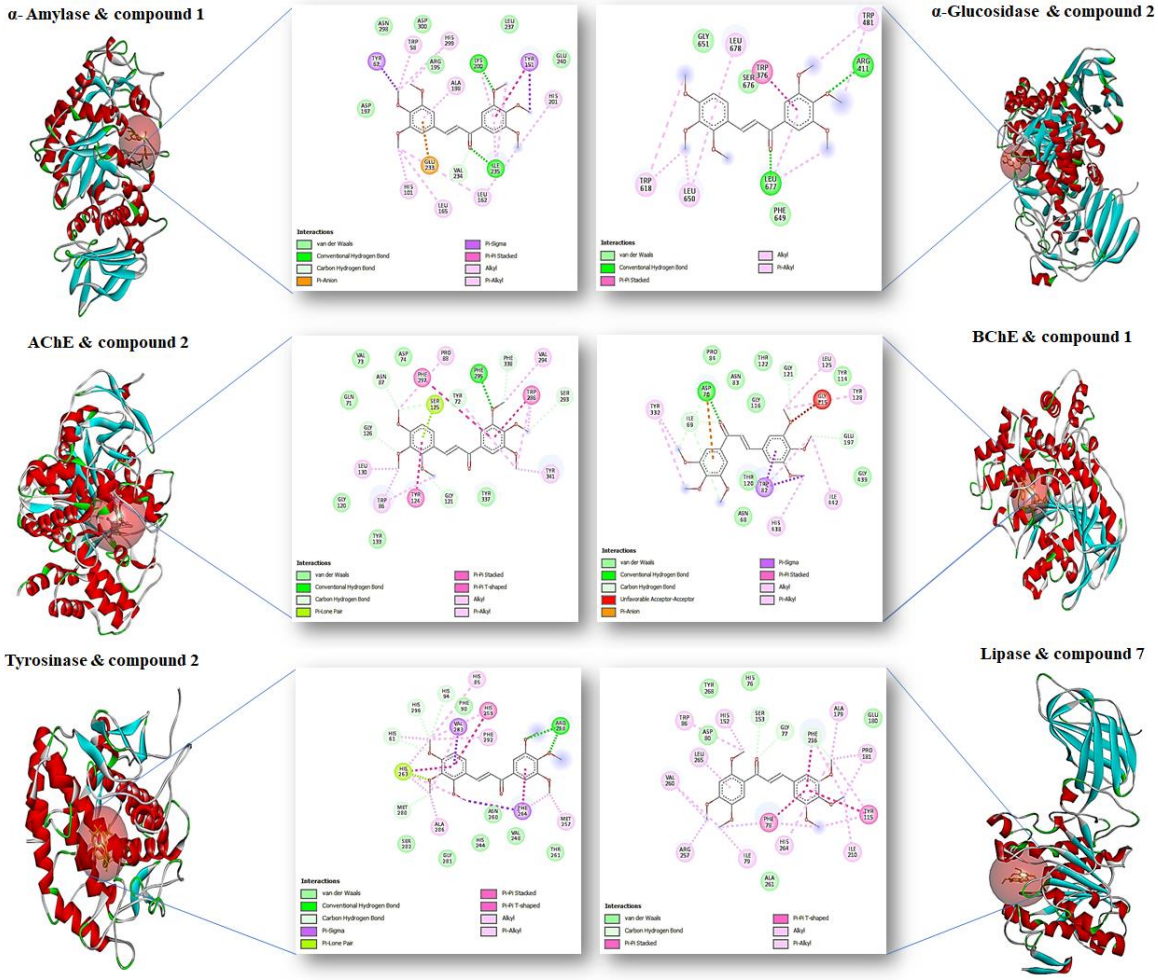
Molecular docking was used to study enzyme-ligand interactions between methoxy chalcones **(1-12)** and *α*-amylase, *α*-glucosidase, tyrosinase, AChE, BChE, and lipase enzymes. Compared to the reference substance, acarbose, the tested compounds showed better binding affinity for *α*-amylase and *α*-glucosidase enzymes (Table 4). The 2D and 3D analysis of methoxy chalcones with the best docking score against *α*-amylase, *α*-glucosidase, AChE, BChE, tyrosinase, and lipase are given in Scheme 2.

Table 4. The lowest binding energy values (kcal/mol) and inhibition constant (Ki) of the methoxy chalcones (**1-12**) and positive controls from each docking analysis in the active site of *α*-amylase, *α*-glucosidase, AChE, BChE, tyrosinase, and lipase.

No	*α*-Amylase	*α*-Glucosidase	AChE	BChE	Tyrosinase	Lipase
1	-6.83	-6.15	-8.35	-6.49	-6.34	-7.35
2	-6.66	-5.88	-8.46	-6.59	-6.38	-7.60
3	-7.01	-6.18	-8.21	-6.97	-6.45	-7.81
4	-6.38	-5.45	-8.39	-6.60	-6.65	-7.66

5	-6.31	-5.77	-8.50	-6.46	-6.34	-7.80
6	-6.54	-6.08	-8.63	-6.47	-6.37	-7.69
7	-6.79	-5.90	-8.28	-7.37	-6.64	-7.74
8	-6.62	-5.48	-8.68	-6.84	-6.71	-7.71
9	-6.35	-6.49	-9.01	-7.37	-6.43	-8.07
10	-7.00	-5.96	-8.68	-6.67	-6.88	-7.75
11	-6.79	-6.09	-8.58	-7.14	-6.81	-7.84
12	-7.02	-6.33	-8.72	-6.87	-6.98	-8.24
Acarbose	-6.30	-4.66	N/A	N/A	N/A	N/A
Galantamine	N/A	N/A	-9.13	-7.61	N/A	N/A
Kojic acid	N/A	N/A	N/A	N/A	-3.96	N/A
Orlistat	N/A	N/A	N/A	N/A	N/A	-6.52

AchE: Acetylcholinesterse; BChE: Butyrylcholinesterase; N/A: Not available.



Scheme 2. The 2D and 3D analysis of methoxy chalcones with the best docking score against *a*-amylase (**1**), *a*-glucosidase (**2**), AChE (**2**), BChE (**1**), tyrosinase (**2**), and lipase (**7**).

2.5.2. Toxicity predictions

The structural features of the methoxy chalcones were input into the web server tools SwissADME, pkCSM, and ProTox using canonical SMILES from the PubChem database. SwissADME's output panels were used to assess the overall characteristics, physicochemical properties (given in Table 5), lipophilicity and water solubility (Tables 6 and 7), drug-likeness rule and bioavailability score (Table 8), medicinal chemistry friendliness (Table 9), and bioavailability radar (Supplementary Material). Pharmacokinetic parameters provided by the SwissADME and pkCSM and the acute oral toxicity (LD_{50}) values obtained with ProTox-3.0 web tools are used to evaluate the ADMET profiles of methoxy chalcones. The predictions are presented in Table 10.

Table 5. Physicochemical properties of methoxy chalcones **1-12**.

No	Fraction Csp3	Num. H-bond acceptors	Num. H-bond donors	Num. RB	MR	TPSA (0Å2)	MW (g/mol)
1	0.29	7	0	9	105.20	72.45	388.41
2	0.29	7	0	9	105.20	72.45	388.41
3	0.25	6	0	8	98.71	63.22	358.39
4	0.29	7	0	9	105.20	72.45	388.41
5	0.29	7	0	9	105.20	72.45	388.41
6	0.25	6	0	8	98.71	63.22	358.39
7	0.29	7	0	9	105.20	72.45	388.41
8	0.29	7	0	9	105.20	72.45	388.41
9	0.25	6	0	8	98.71	63.22	358.39
10	0.25	6	0	8	98.71	63.22	358.39
11	0.25	6	0	8	98.71	63.22	358.39
12	0.21	5	0	7	92.22	53.99	328.36

Fraction Csp3: Ratio of sp3 hybridized carbons over the total carbon count of the molecule; H-bond: Hydrogen bond; RB: Rotable bond; MR: Molecular refractivity; TPSA: Topological polar surface area; MW: Molecular weight; Optimal range: Csp3≤0.25, HBA≤10, HBD≤5, RB≤9, MR≤130, TPSA 20 to ≤130 Å^2.

Table 6. Lipophilicity characteristics of methoxy chalcones **1-12**.

No	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT Log P	Consensus P[a]
1	3.87	3.52	3.53	1.36	4.33	3.32
2	4.09	3.52	3.53	1.36	4.33	3.36
3	3.64	3.55	3.52	1.67	4.24	3.32
4	4.00	5.52	3.53	1.36	4.33	3.35
5	4.06	3.52	3.53	1.36	4.33	3.36
6	3.81	3.55	3.52	1.67	4.24	3.36
7	3.76	3.52	3.53	1.36	4.33	3.30
8	3.80	3.52	3.53	1.36	4.33	3.31
9	3.54	3.55	3.52	1.67	4.24	3.30
10	3.65	3.55	3.52	1.67	4.24	3.32
11	3.79	3.55	3.52	1.67	4.24	3.35
12	3.48	3.58	3.51	1.99	4.15	3.34

[a]Optimal range: log P≤5.

Table 7. Water solubility characteristics of methoxy chalcones **1-12**.

No	ESOL Solubility				Ali Solubility				SLICOS-IT Solubility			
	Log S	mg/mL	mol/L	Class	Log S	mg/mL	mol/L	Class	Log S	mg/mL	mol/L	Class
1	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
2	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
3	-4.11	2.77e-02	7.73e-05	MS	-4.56	9.81e-03	2.74e-05	MS	-5.55	1.02e-03	2.84e-06	MS
4	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
5	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
6	-4.11	2.77e-02	7.73e-05	MS	-4.56	9.81e-03	2.74e-05	MS	-5.55	1.02e-03	2.84e-06	MS
7	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
8	-4.19	2.51e-02	6.47e-05	MS	-4.73	7.31e-03	1.88e-05	MS	-5.65	8.68e-04	2.23e-06	MS
9	-4.11	2.77e-02	7.73e-05	MS	-4.56	9.81e-03	2.74e-05	MS	-5.55	1.02e-03	2.84e-06	MS
10	-4.11	2.77e-02	7.73e-05	MS	-4.56	9.81e-03	2.74e-05	MS	-5.55	1.02e-03	2.84e-06	MS
11	-4.11	2.77e-02	7.73e-05	MS	-4.56	9.81e-03	2.74e-05	MS	-5.55	1.02e-03	2.84e-06	MS
12	-4.04	3.00e-02	9.14e-05	MS	-4.40	1.31e-02	3.98e-05	MS	-5.44	1.20e-03	6.65e-06	MS

PS: Poorly soluble; S: Soluble; MS: Moderately soluble; and VS: Very soluble; ESOL Log S: Insoluble ≤-10, poorly soluble ≤-6, moderately soluble ≤-4, soluble ≤-2, very soluble ≤0; Ali Log S: Insoluble ≤-10, poorly soluble ≤-6, moderately soluble ≤-4, soluble ≤-2, very soluble ≤0; SILICOS-IT Log S: Insoluble ≤-10, poorly soluble ≤-6, moderately soluble ≤-4, soluble ≤-2, very soluble ≤0.

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Table 8. Drug-likeness rule and bioavailability scores of methoxy chalcones **1-12**.

No	Lipinski[a]	Ghose[b]	Veber[c]	Egan[d]	Muegge[e]	Bioavailability score[f]
1	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
2	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
3	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
4	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
5	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
6	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
7	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
8	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
9	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
10	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
11	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
12	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55

[a]Lipinski rules (Pfizer): MW ≤500 g/mol, MLOGP ≤4.15, N or O ≤10, NH or OH ≤5; [b]Ghose rules: MW 160 to 480 g/mol, WlogP -0.4 to 5.6, MR 40 to 130, total no. of atom 20-70; [c]Verber rules: No. of rotatable bonds ≤10, TPSA ≤140Å2, HBD and HBA ≤12; [d]Egan rules: WLOGP ≤5.88, TPSA ≤131.6; [e]Muegge rules: MW 200 to 600 g/mol, XLOGP –2 to 5, TPSA ≤150, no. of rings ≤7, no. of carbon atoms >4, number of heteroatoms >1, no. of rotatable bonds ≤15, HBA ≤10, HBD ≤5; [f]Bioavailability score, at least 0.10.

Table 9. Medicinal chemistry properties of methoxy chalchones **1-12**.

No	PAINS	Brenk[a]	Lead-likeness[b]	Synthetic accessibility[c]
1	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.39
2	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.52
3	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.24
4	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.46
5	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.59
6	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.31
7	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.47
8	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.60
9	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.33
10	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.24
11	0 alert	1 alert: michael_acceptor_1	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.37
12	0 alert	1 alert: michael_acceptor_1	No; 1 violation: XLOGP3>3.5	3.04

[a]Brenk model: ClogP/ClogD 0 to 4, HBD and HBA <4 to 7, no. of heavy atoms 10 to 27, no. of rotatable bonds <8, ring system <5, no ring systems with fused rings >2; [b]Lead-likeness: MW 100 to 350 g/mol, ClogP 1 to 3.0; [c]Synthetic acceptibility: Range from 1 (very easy to synthesize) to 10 (difficult to synthesize).

Table 10. Pharmocokinetic profile and toxicity prediction of mexhoxy chalchones (**1-12**) using pkCSM, SwissADME, and ProTox-3.0.

	Parameter	1	2	3	4	5	6	7	8	9	10	11	12
Absorption (A)	Water solubility (log mol/L)	-4.687	-4.729	-4.752	-4.936	-5.464	-5.383	-4.478	-5.026	-5.008	-4.724	-5.311	-4.785
	Caco-2 permeability (log Papp, cm/s)	1.37	1.381	1.328	1.281	1.341	1.288	1.393	1.359	1.295	1.292	1.258	1.597
	Intestinal absorption (human) %	99.147	99.125	98.122	98.475	98.876	97.742	99.443	99.877	98.567	99	99.302	98.14
	Skin permeability (log Kp)	-2.741	-2.741	-2.737	-2.746	-2.752	-2.741	-2.739	-2.748	-2.739	-2.738	-2.745	-2.707
	P-gp substrate (from SwissADME)	No	No	No	No	No	No	No	No	No	No	No	No
Distribut ion (D)	VDss (human) (log L/kg)	-0.425	-0.384	-0.444	-0.549	-0.358	-0.417	-0.601	-0.456	-0.367	-0.531	-0.381	-0.25
	BBB permeability (log BB)	-0.984	-0.974	0.321	-0.965	-0.968	0.188	-0.981	-0.985	0.22	-0.068	-0.206	-0.009

	BBB perm. (SwissADME)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Metabolism (M)	CYP1A2 inhibitor	No	No	Yes	No	Yes	Yes	No	No	No	No	Yes	Yes
	CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Excretion (E)	Total clearance (log ml/min/kg)	0.341	0.873	0.329	0.278	0.839	0.256	0.378	0.87	0.346	0.316	0.794	0.303
	Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity (T)	Ames test	No	No	Yes	No	No	Yes	No	No	Yes	Yes	No	No
	Hepatotoxicity	No	No	No	No	No	No	No	No	No	No	No	No
	Oral rat acute toxicity (ProTox-3.0) (LD_{50}, in mg/kg)	2100	2100	2100	2100	2100	2100	2100	2100	2100	2100	2100	2100

3. DISCUSSION

The geometry of the double bond in the C-2 and C-3 positions of **1-12** was determined to be in the *trans* position by measuring the coupling constant as 15.5-16.2 Hz from the ^{1}H-NMR spectra. ^{1}H-NMR spectra of **1-12** resulted in the doublet peaks at 7.37 ppm and 7.72 ppm with a proton integral ratio of 1 each are attributed to olefinic *trans* protons of H-2 and H-3. ^{1}H NMR of **1-12** was also given peaks at 7.27 (2H) ppm and 6.87 (2H) ppm, belonging to the H-2′, 6′ and H-2″, 6″ aromatic methine protons in the rings A and B, respectively. ^{1}H-NMR spectra of **1-12** showed peaks at 3.90-3.95 ppm with an integral ratio of 12, 15, or 18 protons confirming that there are 4, 5, or 6-OCH_3 groups. The ^{13}C-NMR spectrum revealed the carbonyl carbon peaks in the range of 188.6-191.4 ppm (C-1); trans olefinic carbons (C-2 and C-3) at 121.3 ppm and 145.0 ppm, respectively; Aromatic CH carbons in the rings A and B were detected at 106.2 ppm and 105.7 ppm, respectively. The carbon signals for -OCH_3 peaks were observed at 61.0 ppm belonging to 4′ and 4″ methoxy and the carbon signals at 56.3 ppm and 56.5 ppm were attributed to 3′,5′ and 3″,5″ methoxy, respectively. Some of the carbon peaks of -OCH_3 were overlapped (Supplementary Material).

Clinical studies have confirmed that orlistat, as a pancreatic lipase inhibitor, may reduce obesity caused by a high-fat diet and that taking orlistat may cause fatty stools [25]. It causes gastrointestinal side effects such as bloating, diarrhea, and fecal incontinence, and decreased absorption of fat-soluble vitamins is observed in people taking orlistat. The current situation regarding obesity treatment emphasizes the need for new research. In our study, when the lipase enzyme activity results of methoxy chalcones (**1-12**) were evaluated against orlistat (IC_{50}:13.49±1.23), the highest lipase enzyme inhibition activity was detected on 1-(2,4,5-trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-on (**7**) and its IC_{50} value was found to be 39.83±1.1216 µg/mL. Afterward, the best lipase enzyme activity was observed in compound **8**, **3,** and **2,** and the best IC_{50} values were 42.43±1.7501 µg/mL, 43.86±2.1826 µg/mL, and 46.67±1.6145 µg/mL, respectively. The lowest activity was detected in compound **9** (IC_{50}: 288.84±10.2278 µg/mL). It is thought that compound number 7 is promising for the treatment of obesity and may shed light on further detailed studies. There are many studies in the literature using orlistat as a positive control [41]. The activities of isolated benzoic acid derivatives and four synthesized analogs against orlistat (IC_{50}: 0.73±0.02 µM) were evaluated and IC_{50} values were found to be between 28.32 and 55.8 µM [42]. In another study, several pyrazole-fused benzimidazole derivatives were designed as potential pancreatic lipase inhibitors. Orlistat used as standard showed 91±1.68% enzyme inhibition, with only -4.5 kcal/mol binding affinity with pancreatic lipase. (E)-N2-((naphthalen-1-yl)methylene)-N5-(1H-pyrazol-3-yl)-3H-benzo[d]imidazole-2,5-diamine was found to be the most effective pancreatic lipase inhibitor for the treatment of obesity and can be further optimized using more *in vivo* and *in vitro* models [43].

Inhibiting AChE and BChE enzymes in Alzheimer's disease patients is important in terms of increasing the activity of cognitive functions [22]. Studies have reported that AChE inhibitors do not completely cure the disease but reduce symptoms and slow down its progression [23]. AChE and BChE enzyme inhibition activities of methoxy chalcones (**1-12**) were evaluated according to the galantamine

standard (IC_{50}: 6.13±0.47 µg/mL, IC_{50}: 26.59±0.71 µg/mL, respectively). The highest AChE inhibitory activity was detected in compounds **2** (IC_{50}: 60.39±1.24 µg/mL), **1** (IC_{50}: 78.15±0.58 µg/mL), and **9** (IC_{50}: 81.13±1.24 µg/mL), respectively. The highest BChE inhibitory activity was detected in compounds **1** (IC_{50}: 39.79 ± 1.29 µg/mL), **8** (IC_{50}: 54.34±0.34 µg/mL) and **2** (IC_{50}: 61.53±1.07 µg/mL), respectively. These results show that the best activity is in compounds **1** and **2**, which contain methoxy groups at positions C-3, C-4, and C- 5 in the ring A. In addition, the lowest activity was determined in compounds **6**, **7**, and **12** in both enzyme activities. It is thought that the decrease in AChE and BChE enzyme inhibition activities may be caused by methoxy binding to positions C-3 and C-4 of ring B. As can be seen, the different binding order of methoxy groups to the chalcone molecule caused the inhibitory effects of these molecules at different concentrations. There are studies in the literature that inhibitory effects change when functional groups such as fluoro, hydroxy, and methoxy are attached differently to the chalcone molecule [44]. In a study conducted by Aslan et al [44], AChE activities were found in the order of 4-hydroxychalcone, 4-methoxychalcone, 2-hydroxy-4′-methoxychalcone, 4-fluoro-4′-methoxychalcone, 4-fluorochalcone, and 4,4′-difluorochalcone [44]. It has been observed that some hydroxy chalcone derivatives show activity against AChE with IC_{50} values of 28.2-134.5 µM [24]. In another study, heterocyclic compounds containing benzothiophene skeleton were synthesized, and their inhibitory activities against cholinesterases were evaluated. Benzothiophenes and benzothiophene-chalcone hybrids were tested against both AChE and BChE, and interesting structure-activity relationships were revealed. In general, benzothiophene-chalcone hybrids showed better inhibitory activity of both enzymes; they were the best inhibitors of AChE (IC_{50}: 62.10 µM) and BChE (IC_{50}: 24.35 µM), with IC_{50} values similar to those of the reference compound galantamine (IC_{50}: 28.08 µM) [45]. AChE is an important target for the prevention of Alzheimer's disease progression. To develop potential acetylcholinesterase inhibitors, a series of novel chalcone derivatives of ursolic acid were synthesized and AChE inhibitory abilities were tested *in vitro*. Three compounds were showed high AChE inhibitory potential with IC_{50} values of 1.82, 4.65, and 5.36 µM, respectively. Methoxymethyl 3-oxo-2-(3,5-bis(trifluoromethyl)-benzylidene)-urs-12-ene-28-oate was the most potent derivative compared to the standard inhibitor (galantamine, IC_{50}: 1.96 µM) [46]. It has been reported that some synthetic chalcones show strong inhibition against cathepsin B and μ-calpain, which may be useful in the treatment of Alzheimer's disease-related disorders, and another study reported that a coumarin chalcone hybrid, as a strong AChE inhibitor, may be useful in the treatment of neurodegenerative disorder [1]. It has been reported that some nitro-substituted chalcones show catechol-O-methyltransferase enzyme inhibitory activities and may be useful in controlling neurodegenerative disorders such as Parkinson's [47].

The tyrosinase enzyme activity of methoxy chalcones (**1-12**) was evaluated against α-kojic acid (IC_{50}: 18.83±1.09 µg/mL). The highest tyrosinase enzyme inhibition activity was detected in compounds **2** (IC_{50}: 40.40±1.01 µg/mL), **3** (IC_{50}: 76.11±3.17 µg/mL), and **4** (IC_{50}: 96.10±2.19 µg/mL), respectively. These three compounds share 4-methoxy substituents in ring A and 3,4-dimethoxy substituents in ring B. Additionally, when the 3,4,5-trimethoxy configuration was positioned in ring A (**2**), the activity increased compared to positioning in B ring (**4**). As can be seen, the binding of methoxy groups to the chalcone molecule in different orders changed the activities of these molecules. There are similar studies in the literature that a series of chalcones were synthesized and examined for their tyrosinase inhibitory activities. It has been reported that synthesized different derivative chalcones are potential tyrosinase inhibitors with IC_{50} values at 14.20-14.38 µM [48]. In another study, chalcone compound bearing 3'-ethoxy and 4'-hydroxy showed higher tyrosinase inhibitory activity than kojic acid (IC_{50}: 9.28 µM, the positive control) with an IC_{50} value of 6.19±1.05 µM [49]. Moreover, a new chalcone was designed to be incorporated into topical formulations and is effective against the hyperpigmentation process. The chalcone with the best efficacy inhibited tyrosinase by 56%, 22%, and 16% in cell extracts from treated melanocytes, respectively. The positive control compounds hydroquinone and kojic acid inhibited tyrosinase by 16% and 23%, respectively [50]. Chalcones may be effective in preventing or treating neurodegenerative diseases [1, 47, 51].

a-Amylase and *a*-glucosidase enzyme inhibition activities of methoxy chalcones (**1-12**) were evaluated against acarbose. The highest *a*-amylase enzyme inhibition activity was detected in compounds **1** (IC_{50}, 98.61±3.17 µg/mL), **2** (IC_{50}, 146.54±1.65 µg/mL), and **9** (IC_{50}, 159.84±1.25 µg/mL), respectively. When *a*-glucosidase enzyme inhibition activity was evaluated, the highest activity was detected in compounds **2** (IC_{50}, 55.91±1.78 µg/mL), **1** (IC_{50}, 60.93±0.93 µg/mL), and **8** (IC_{50}, 161.57±2.23 µg/mL), respectively. In *a*-amylase enzyme inhibition activity, while the A ring configuration of compounds **1** and **2** was the same, the difference in the C-5' and C-2' methoxy positioning in the B ring changed the activity, and the highest activity was detected at the C-5' position. In *a*-glucosidase enzyme inhibition activity, the opposite

positioning changed the effect, and the highest activity was detected at the C-2″ methoxy position in the B ring. In the literature, it has been reported that the presence of hydroxy groups on C-2 and C-4 of ring A and C-3 and C-4 of ring B supports *α*-glucosidase activity [52]. It has been stated that different azochalcone derivatives show good inhibitory activities against *α*-amylase and *α*-glucosidase enzymes in the concentration range of 23.08-26.94 µM (IC_{50}) and 26.08-27.99 µM (IC_{50}), respectively [53]. In another study, the *in vitro α*-amylase inhibition activity of sixteen synthetic chalcones was investigated, and they exhibited good inhibitory activities in the range of 1.25±1.05 to 2.40±0.09 µM (IC_{50}) compared to standard acarbose (IC_{50}, 34±0.3 µM) (50). Moreover, in the search for new antidiabetic agents, a series of novel chalcones with benzimidazole skeleton were synthesized and their *α*-glucosidase and *α*-amylase inhibition studies were evaluated. The novel benzimidazole-equipped chalcones emerged as a potent antidiabetic agent with IC_{50} of 22.45±0.36 µg/mL and 20.47±0.60 µg/mL against *α*-glucosidase and *α*-amylase enzymes, respectively [54]. It has been reported that chalcones showed good *α*-glucosidase and *α*-amylase inhibitory activities [55-57].

When the antimicrobial activity of the compounds was evaluated, it was determined that none of the compounds showed activity against gram (+) bacteria. The best activity was detected against *E. coli* in compounds **8** (MIC: 164.1 µg/mL) and **7** (MIC: 381.3 µg/mL), respectively. Compound **8** (MIC: 328.3 µg/mL) also showed the highest activity against *Y. pseudotuberculosis* and *P. aeruginosa*. The highest antibacterial activity against *M. smegmatis* was detected in compounds **8** (MIC, 164.1 µg/mL), **9 (**MIC, 318.8 µg/mL), and **1** (MIC, 321 µg/mL), respectively. The highest antifungal effect against yeast fungi was determined in compound **8** (MIC: 328.3 µg/mL). Generally, the strongest broad-spectrum was detected in compound **8** against gram (-), non-gram (*M. smegmatis*), and yeast fungi (*C. albicans*, *S. cerevisiae*) within the range of 164.1-328.3 µg/mL MIC values. No antibacterial activity was detected in compounds **5** and **12**. In the literature, acebogenin, which is a dihydrochalcone, is reported to be effective against *S. aureus*. Isobavachalcone and bavachalcone were isolated from *P. corylifolia* are reported as natural antibacterial agents, and the 3-(carboxyalkyl) rhodanine is reported to be effective against human pathogens at low concentrations. They have been reported to exhibit strong inhibition as an antibacterial as well as an antifungal agent [1]. It has been reported that lycochalcone A and lycochalcone C show strong antibacterial activity, especially against *B. subtilis*, *S. aureus*, and *M. luteus* [5]. Several studies in the literature showing that chalcone compounds exhibit antimicrobial activities [1, 3, 5].

The antioxidant capacities of the compounds were calculated as Trolox equivalent using FRAP and CUPRAC methods. According to the FRAP method, the highest antioxidant capacity was observed in compounds **1** (1633.70±2.52 µM Trolox equivalent/g), **4** (1048.70±4.32 µM Trolox equivalent/g), and **9** (881.70±4.15 µM Trolox equivalent/g), respectively. The lowest activity was determined in compound **10** (49.03±3.09 µM Trolox equivalent/g). According to the CUPRAC method, the highest antioxidant capacity was observed in compounds **2** (2732.00±7.42 µM Trolox equivalent/g), **1** (2093.33±3.53 µM Trolox equivalent/g), and **4** (1651.40±3.91 µM Trolox equivalent/g), respectively. The lowest activity was determined in compound **5** (81.40±4.36 µM Trolox equivalent/g). The DPPH radical scavenging capacity of methoxy chalcones (**1-12**) was measured against the BHT standard, expressed as SC_{50} (50%scavenging capacity). The highest radical scavenging activity was detected in compounds **1** (SC_{50}: 0.320±0.002 mg/mL), **3** (SC_{50}: 0.542±0.003 mg/mL), and **2** (SC_{50}: 0.663±0.001 mg/mL), respectively. The lowest activity was detected in compound **10** (SC_{50}: 14.165±0.006 mg/mL). According to the FRAP and DDPH method, the best activity was determined in compound **1**. As can be seen, the binding of methoxy groups to the chalcone molecule in different orders changed the activities of these molecules. It has been stated that the structure-antioxidant activities of chalcones are associated with several different mechanisms such as free radical neutralization, hydrogen donation, singlet oxygen quenching, and metal chelation [58]. In the literature, many hydroxychalcones with isoprenyl substituents in their phenyl rings have antioxidant potential [5]. 3,4-Dihydroxy chalcones such as butein, sappanchalcone, and okanin showed effective antioxidant activity, and the broussochalcone A compound isolated from *Broussonetia papyrifera* Vent is as strong an antioxidant as BHT [5, 59]. In another study, 3-hydroxyxanthoangelol showed stronger DPPH radical scavenging activity than α-tocopherol, and xanthoangelol B and xanthokeismins A were found to be stronger superoxide scavengers than the positive control resveratrol [60]. Antioxidant activity studies of chalcone compounds are common in the literature [1, 5].

Compound**s 1, 2, 3, 10,** and **1** had the lowest binding energies for *α*-amylase (-7.02, -7.01, -7.00, and -6.83 kcal/mol, respectively). Moreover, compound **1** showed significant inhibition activity in *in vitro* studies (IC_{50}: 98.61±3.17 µg/mL). This compound formed hydrogen bonds with Lys200 and Ile235, pi-sigma interactions with Tyr62 and Tyr151, and hydrophobic interactions with Asn298, Asp300, Asp197, Arg195,

Leu237, and Glu240 in *a*-amylase. Compound **2** was also the most active in *silico* and *in vitro* studies with inhibition activity (IC_{50}: 55.91±1.78 µg/mL) and binding energy of -6.15 kcal/mol for *a*-glucosidase. This compound formed hydrogen bonds with Leu677 and Arg411, pi-pi stacked interaction with Trp376, and hydrophobic interactions with Gly651, Phe649, and Ser676 in *a*-glucosidase. The amino acids identified in these interactions through molecular docking were consistent with known interaction sites in the literature [61, 62]. Furthermore, compounds **2** and **1** showed significant inhibition effects on AChE and BChE with IC_{50} values of 60.39±1.24 µg/mL and 39.79±1.29 µg/mL, respectively. According to molecular docking studies, compounds **2** and **1** have strong binding energies towards AChE (-8.46 kcal/mol) and BChE (-6.49 kcal/mol), respectively. These compounds formed hydrogen bonding interactions with residues Asn87, Ser125, Gly121, Ser293, and Phe338 in AChE and Ile69, Asp70, Gly121, and Glu197 in BChE. They also exhibited hydrophobic interactions with residues Gln71, Val73, Asp74, Gly120, Tyr133, and Tyr337 in AChE and Pro84, Asn83, Gly116, Thr122, Tyr114, Gly439, Asn68, and Thr120 in BChE. These amino acids have important roles in the catalytic activity of these enzymes [63, 64]. The binding energies of methoxy chalcones (**1-12**) for tyrosinase were assessed using molecular docking. All compounds exhibited better binding affinity (binding energy values ranging from -6.98 to -6.34 kcal/mol) compared to kojic acid, the reference standard with a binding energy of -3.96 kcal/mol. Furthermore, compound **2** showed good inhibitory activity in *in vitro* studies with an IC_{50} value of 40.40±1.01 µg/mL. This compound formed hydrogen bonds with residues Arg268, His296, His94, His61, and Met280, pi-sigma interactions with residues Phe264 and Val283, and hydrophobic interactions with Ser282, Gly281, His244, Val248, Thr261, and Asn260 in tyrosinase enzyme. Compound **7** was the most active lipase inhibitor with an IC_{50} value of 39.83±1.12 µg/mL. *In silico* molecular docking, results revealed that compound **7** had a higher binding affinity (binding energy: -7.74 kcal/mol) than orlistat and formed hydrogen bonding interactions with Ser153 and Gly77, which are important for catalytic activity. In addition, this compound exhibited pi-alkyl interactions with Ile79, Val260, Ile210, Ala179, and Pro181. Molecular docking results showed that the compounds bound to the active sites of *a*-amylase, *a*-glucosidase, tyrosinase, AChE, BChE, and lipase interacted with amino acid residues required for catalytic activity [61-66].

The optimal ranges for intestinal permeability or oral absorption are represented as a fraction of carbons in the sp3 hybridization not less than 0.25, no more than nine rotatable bonds, and TPSA between 20 and 130 Å2 [67]. All methoxy chalcones were predicted to meet these criteria, except **12,** fraction Csp3 was to be 0.21. None of the methoxy chalcones were found to be H-bond donors and H-bond acceptors were found to be less than ten for all compounds. The molecular refractivity of all methoxy chalcones (**1-12**) was in the optimal range, equal to or less than 130. The TPSA for compounds **1**, **2**, **4**, **5**, **7**, and **8** was 72.45 Å2, while for **3**, **6**, **9**, **10**, and **11** was 63.22 Å2. Compound **12** has the lowest TPSA of 53.99 Å2. Compounds **1**, **2**, **4**, **5**, **7**, and **8** share the same molecular weight of 388.41 g/mol while **3**, **6**, **9**, **10**, and **11** are 358.39 g/mol. Compound **12** differs from the others and has the lowest molecular weight of 328.36 g/mol. The molecular weights of methoxy chalcones (**1-12**) were within the acceptable range specified by Lipinski's rule of five [68], indicating potential for oral bioavailability. Methoxy chalcones had a Consensus LogP value from 3.30 to 3.36. The optimal range for lipophilicity should be equal to or less than 5. All the tested methoxy chalcones (**1-12**) were determined to be lipophilic. The Log S values of the Estimated SOLubility (ESOL), Ali, and SILICOS-IT are not within the recommended range, indicating that the compounds are moderately soluble. For assessing drug-likeness of methoxy chalcones, Lipinski, Ghose, Veber, Egan, Muegge Filters, and the Bioactivity Score were used. All compounds (**1-12**) passed the Lipinski, Ghose, Veber, Egan, and Muegge criteria. A Bioactivity Score of 0.55 was obtained for each compound. All methoxy chalcones passed the Panassay Interference compounds (PAINS) with 0 violations, and Brenk filters with 1 alert of Michael acceptor_1. Compounds **1-11** had 3 violations for lead-likeness and compound **12** had 1 violation. All methoxy chalcones (**1-12**) passed the Lipinski's rule of five (MW≤500, LOGP≤5, HBDs≤5, and HBAs≤10.6) but **1-11** violated the 3 criteria for lead-likeness (MW>350, Rotors>7, XLOGP3>3.5) and **12** violated one (XLOGP3>3.5). Bioavailability radar gives an insight into a compound's drug-likeness, which is estimated based on 6 physicochemical features: lipophilicity (LIPO), size (SIZE), polarity (POLAR), insolubility (INSOLU), insaturation (INSATU), and flexibility (FLEX). All methoxy chalcones had physicochemical features in the appropriate range (pink region), except for the saturation property of compound **12**, as indicated by the out-of-range red dot of its skewed hexagons for less than 0.25 of the carbons in the Sp3 hybridization (Supplementary Material).

Despite the simpler molecular structure and potentially promising effects of chalcones, a limited number of studies have reported the *in vivo* toxicity and ADME properties. Those studies primarily

evaluated biological activity in various pathological models [69-75]. Methoxy chalcones (**1-12**) were predicted to have higher permeability with a Caco-2 Papp value of more than 8×10^{-6} cm/s (predicted values >0.9). Compound **12** has the highest intestinal permeability with a log Papp of 1.597 cm/s. Based on the results of the human intestinal absorption model, methoxy chalcones exhibited good efficiency, with an average absorption rate (%) of 98.82±0.62 (97.74-99.86). The log Kp values of methoxy chalcones (**1-12**) indicating all the compounds have good skin permeability. P-glycoprotein (P-gp) is an ATP-binding cassette (ABC) transporter that behaves like a biological barrier in cells. All the tested methoxy chalcones (**1-12**) were not predicted as a substrate of P-gp. The distribution volume (VD) indicates the extent to which a compound is distributed in tissue rather than plasma, with higher values indicating greater distribution in tissue. The logarithm of VDss is considered low if below <-0.15 and high if above >0.45. All methoxy chalcones were predicted lower than -0.15 indicating VDss is poor, compound **7** has the lowest distribution to the tissue (-0.60) and **12** is the highest (-0.25). The blood-brain barrier (BBB) protects the central nervous system (CNS) from exogenous compounds. The ability of a molecule to cross into brain tissue is an important parameter to consider, as it may exert its pharmacological or toxicological activity on the central nervous system. A compound with a log BBB of >0.3 is considered to readily cross the blood-brain barrier, while <-1 is poorly distributed into the brain. The pkCSM predicted all methoxy chalcones in the mid zone (>-1 but <0.3), with compound **3** being the boundary (0.321). The SwissADME implies that all the tested methoxy chalcones can able to cross the BBB. The CYP450 enzyme family is mainly found in the liver. It is important for the detoxification and metabolism of many drugs in the body. CYP-mediated metabolic properties of methoxy chalcones showed that all compounds (**1-12**) are inhibitors for CYP219 and CYP2C9, but non-inhibitors for CYP2D6. Compounds **3**, **5**, **6**, **11**, and **12** are predicted to be inhibitors of CYP1A2 while others are not. Compounds **1-8** and **12** are predicted to be CYP3A4 inhibitors while **9**, **10**, and **11** are not. Excretion features of the methoxy chalcones were predicted from renal OCT2 substrate affinity and total clearance. OCT2 is a transporter found in the kidneys that is responsible for the uptake and clearance of drugs and other substances in the body. OCT2 substrates have the potential to interact with OCT2 inhibitors, which can lead to adverse effects [76]. There were no renal OCT2 substrates for any of the compounds. The total clearances of compounds **2**, **5**, **8**, and **11** were predicted similarly with an average of 0.84±0.04 (represented as log mL/min/kg), corresponding to ~6.9 mL/min/kg. While the other methoxy chalcones had a 2.8-fold higher renal clearance (0.30±0.04 log mL/min/kg) corresponding to ~2.0 mL/min/kg. The results suggest that methoxy chalcones (**1-12**) were non-hepatotoxic. However, according to the AMES test compounds **3**, **6**, **9**, and **10** were predicted to be mutagenic. The oral rat acute toxicity of methoxy chalcones (**1-12**) was all predicted with the same LD_{50} value of 2100 mg/kg bw. These LD_{50} values correspond to the Globally Harmonized System (GHS) [77] of class V (acute oral toxicity), which may be harmful if swallowed ($2000<LD_{50}\leq5000$).

4. CONCLUSION

This study revealed that compound **1** exhibits the highest *α*-amylase and BChE inhibition activity. Compound **2** shows the highest *α*-glucosidase, AChE, and tyrosinase inhibition activity, and Compound **7** displays the highest lipase inhibition activity. Compounds **1** and **4** exhibited the most effective antioxidant activity according to FRAP and CUPRAC, while **1** and **3** were the most effective compounds using the DPPH method. The methoxy chalcones (**1-12**) showed activity against gram (-) bacteria. Our molecular docking results indicate that all compounds (**1-12**) bind to the active sites of *α*-amylase, *α*-glucosidase, AChE, BChE, and lipase and interact with amino acid residues crucial for catalytic activity. Moreover, the methoxy chalcones with the highest docking scores were identified as compound **1** against *α*-amylase and BChE, compound **2** against *α*-glucosidase, AChE, and tyrosinase, and compound **7** against lipase. Both physicochemical and drug-likeliness analysis using SwissADME favored methoxy chalcones (**1-12**). The ADMET evaluation indicates that all compounds may have a low toxicity profile. However, compounds **3**, **6**, **9**, and **10** were predicted to be mutagenic according to the AMES assay. Overall, the results of the study revealed that compounds **1** and **2** have the potential to be good candidates for animal studies for Alzheimer's disease and diabetes and compound **7** for obesity. Toxicity estimates are constrained by computational tools that lack experimental evidence; thus, future studies, including animal studies, are needed for experimental confirmation.

5. MATERIALS AND METHODS

5.1. Materials and equipment

Solvents (ethanol, chloroform, ethyl acetate and diethyl ether), acetophenone compounds (3,4,5-trimethoxy acetophenone, 2,3,4-trimethoxy acetophenone, 2,4,5-trimethoxy acetophenone, and 3,4-dimethoxyacetophenone), aldehyde compounds (3,4,5-trimethoxybenzaldehyde, 2,3,4-trimethoxy-benzaldehyde, and 3,4-dimethoxybenzaldehyde), and any used reagent were purchased from by Sigma-Aldrich, BLDpharm or Merck unless otherwise stated. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C), using TMS as an internal standard. $CDCl_3$ was used as an NMR solvent. Chemical shifts were expressed in δ (ppm) and coupling constants (J) were reported in hertz (Hz). ACD NMR program was also used for the interpretation of NMR spectra. FT-IR spectra were taken using the Perkin-Elmer 1600 (ATR) (4000-400 cm^{-1}) spectrophotometer. 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).

5.2. General synthesis of methoxy chalcones (1-12)

NaOH (15 mmol) was dissolved in 2 mL of distilled water in a beaker with a magnetic stirrer, and 4 mL of 95% ethanol was added. The beaker was placed in an ice water bath and the reaction medium was cooled. Methoxy acetophenone (5 mmol) was completely dissolved in 5 mL of ethanol, then slowly added to the NaOH solution, and stirring continued. After 30 minutes, methoxy benzaldehyde (5 mmol) was dissolved in 5 mL of ethanol and added dropwise to the mixture. After 2 hours, the mixture was removed from the ice bath and allowed to stir at room temperature for 12 hours to give a crude mixture then compounds (**1-12**) were purified with crystallization using, diethyl ethers. Complete consumption of reactions was monitored through TLC. Compound **8** is novel. Experimental methods used for the syntheses of methoxy chalcones (**1-12**) were presented in Scheme 1. The structures of the methoxy chalcones (**1-12)** were confirmed through NMR, FT-IR, UV, LC-QTOF-MS analysis, and ACD NMR program's help. Their spectral data are given below.

5.2.1. ((2E)-1,3-bis (3,4,5-trimethoxyphenyl)prop-2-en-1-one), (**1**)

Yield: 86.4 %; R_f = 0.57 (chloroform-ethyl acetate: 8:1); light yellow; $C_{21}H_{24}O_7$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.72 (d, 1H, J=15.6 Hz, H-3); 7.37 (d, 1H, J=15.6 Hz, H-2); 7.27 (s, 2H, H-2′, 6′); 6.87 (s, 2H, H-2″, 6″); 3.90-3.95 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 189.5 (C=O), 153.5 (C-3″, 5″), 153.1 (C-3′, 5′), 145.0 (C-3), 142.5 (C-4′), 140.4 (C-4″), 133.6 (C-1′), 130.4 (C-1″), 121.3 (C-2), 106.2 (C-2′, 6′), 105.7 (C-2″, 6″), 61.0, 56.5 (x2), 56.3 (x3) (6x-OCH_3).

5.2.2. ((2E)-3-(2,3,4-trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one), (**2**)

Yield: 92.1 %; R_f = 0.56 (chloroform-ethyl acetate: 8:1); light yellow; $C_{21}H_{24}O_7$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 8.00 (d, 1H, J=15.8 Hz, H-3); 7.52 (d, 1H, J=15.8 Hz, H-2); 7.41 (d, 1H, J=8.8 Hz, H-6″); 7.28 (s, 2H, H-2′, 6′); 6.75 (d, 1H, J=8.8 Hz, H-5″); 3.91-3.97 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 189.8 (C=O), 155.8 (C-4″), 153.8 (C-2″), 153.1 (C-3′, 5′), 142.5 (C-4′), 142.1 (C-3″), 140.2 (C-3), 133.9 (C-1′), 124.3 (C-6″), 122.0 (C-1″), 121.2 (C-2), 107.6 (C-5″), 106.0 (C-2′, 6′), 61.4, 61.0, 56.4 (x3), 56.1 (6x-OCH_3).

5.2.3. ((2E)-3-(3,4-dimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one), (**3**)

Yield: 91.4 %; R_f = 0.50 (chloroform-ethyl acetate: 8:1); light yellow; $C_{20}H_{22}O_6$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.76 (d, 1H, J=15.6 Hz, H-3); 7.34 (d, 1H, J=15.6 Hz, H-2); 7.25 (d, 1H, J=8.4 Hz, H-6″); 7.26 (s, 2H, H-2′, 6′); 6.91 (d, 1H, J=8.4 Hz, H-5″); 3.93-3.95 (s, 15H, 5x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 189.4 (C=O), 153.1 (C-3′, 5′), 151.4 (C-4″), 149.2 (C-3″), 145.0 (C-3), 142.5 (C-4′), 133.8 (C-1′), 127.8 (C-1″), 122.9 (C-2), 119.8 (C-6″), 111.1 (C-2″), 110.4 (C-5″), 106.1 (C-2′, 6′), 61.0, 56.4 (x2), 56.0 (x2) (5x-OCH_3).

5.2.4. ((2E)-3-(3,4,5-trimethoxyphenyl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one), (**4**)

Yield: 80.8 %; R_f = 0.50 (chloroform-ethyl acetate: 8:1); light yellow; $C_{21}H_{24}O_7$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.59 (d, 1H, J=16.2 Hz, H-3); 7.47 (d, 1H, J=8.8 Hz, H-6′); 7.37 (d, 1H, J=16.2 Hz, H-2); 6.84 (s, 2H, H-2″, 6″); 6.76 (d, 1H, J=8.8 Hz, H-5′); 3.89-3.93 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 190.9 (C=O), 157.0 (C-4′), 153.6 (C-2′),153.4 (C-3″,5″), 143.3 (C-3), 142.1 (C-3′), 140.1 (C-4″), 130.7 (C-1″), 126.8 (C-1′), 125.9 (C-6′), 125.7 (C-2), 107.4 (C-5′),105.5 (C-2″,6″), 62.2, 61.1 (x2), 56.2 (x2), 56.1 (6x-OCH_3).

5.2.5. ((2E)-3-(2,3,4-trimetoksifenil)-1-(2,3,4-trimetoksifenil)prop-2-en-1-on), (**5**)

Yield: 87.5 %; R_f = 0.63 (chloroform-ethyl acetate: 8:1); light yellow; $C_{21}H_{24}O_7$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.90 (d, 1H, *J*=16.0 Hz, H-3); 7.45 (d, 1H, *J*=8.8 Hz, H-6′); 7.46 (d, 1H, *J*=16.0 Hz, H-2); 7.39 (d, 1H, *J*=8.8 Hz, H-6″); 6.76 (d, 1H, *J*=8.8 Hz, H-5″); 6.72 (d, 1H, *J*=8.8 Hz, H-5′); 3.89-3.94 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 191.4 (C=O), 156.7 (C-4′), 155.6 (C-4″), 153.7 (C-2′), 153.6 (C-2″), 142.4 (C-3′), 142.1 (C-3″), 138.5 (C-3), 127.2 (C-1″), 125.7 (C-6′,6″), 123.4 (C-2), 122.2 (C-1′), 107.6 (C-5″), 107.3 (C-5′), 62.1, 61.9, 61.5, 61.1, 56.1 (x2) (6x-OCH_3).

5.2.6. ((2E)-1-(2,3,4-trimetoksifenil)-3-(3,4-dimetoksifenil)prop-2-en-1-on), (**6**)

Yield: 95.8 %; R_f = 0.56 (chloroform-ethyl acetate: 8:1); light yellow; $C_{20}H_{22}O_6$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.63 (d, 1H, J=15.8Hz, H-3); 7.46 (d, 1H, J=8.8 Hz, H-6′); 7.35 (d, 1H, J=15.8 Hz, H-2); 7.21 (d, 1H, J=8.3 Hz, H-6″); 7.14 (s, 1H, H-2″); 6.89 (d, 1H, J=8.3 Hz, H-5″); 6.77 (d, 1H, J=8.8 Hz, H-5′); 3.89-3.94 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 191.1 (C=O), 156.8 (C-4′), 153.5 (C-2′), 151.2 (C-4″), 149.2 (C-3″), 143.5 (C-3), 142.4 (C-3′), 128.1 (C-1″), 127.0 (C-1′), 125.7 (C-6′), 124.6 (C-6″), 123.0 (C-2), 111.1 (C-5″), 110.0 (C-2″), 107.3 (C-5′), 62.2, 61.1, 56.1, 56.0 (x2) (5x-OCH_3).

5.2.7. ((2E)-1-(2,4,5-trimethoxyphenyl), 3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one), (**7**)

Yield: 85.8 %; R_f = 0.60 (chloroform-ethyl acetate: 8:1); light yellow; $C_{21}H_{24}O_7$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.62 (d, 1H, J=15.6 Hz, H-3); 7.52 (d, 1H, J=15.6 Hz, H-2); 7.37 (s, 1H, H-6′); 6.84 (s, 2H, H-2″,6″); 6.55 (s, 1H, H-3′); 3.88-3.97 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 189.8 (C=O), 154.7 (C-2′), 153.5 (C-4′), 153.4 (C-3″,5″), 143.4 (C-5′), 142.1 (C-3), 139.9 (C-4″), 131.1 (C-1″), 126.6 (C-2), 120.4 (C-1′), 113.1 (C-6′), 105.4 (C-2″,6″), 97.0 (C-3′), 61.0, 56.8, 56.3, 56.2, 56.1 (x2) (6x-OCH_3).

5.2.8. ((2E)-3-(2,3,4-trimethoxyphenyl)-1-(2,4,5-trimethoxyphenyl)prop-2-en-1-one), (**8**)

Yield: 86.5 %; R_f = 0.60 (chloroform-ethyl acetate: 8:1); melting point (°C): 152-154; light yellow; $C_{21}H_{24}O_7$; UV (EtOAc) λ max nm (logε): 230 (4.43); FT-IR (cm^{-1}): 2360, 2328, 1699, 1541, 1512; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.92 (d, 1H, *J*=15.9 Hz, H-3); 7.62 (d, 1H, *J*=15.9 Hz, H-2); 7.38 (s, 1H, H-6′); 7.37 (d, 1H, *J*=8.8 Hz, H-6″); 6.72 (d, 1H, *J*=8.8 Hz, H-5″); 6.55 (s, 1H, H-3′); 3.87-3.97 (s, 18H, 6x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 190.3 (C=O), 155.3 (C-2′), 154.6 (C-4″), 153.7 (C-4′), 153.3 (C-2″), 143.3 (C-5′), 137.3 (C-3,3″), 126.3 (C-6″), 123.6 (C-2), 122.6 (C-1″), 120.8 (C-1′), 113.2 (C-6′), 107.6 (C-5″), 97.1 (C-3′), 61.5, 60.9, 56.8, 56.3, 56.1 (x2) (6x-OCH_3); Positive LC-QTOF-MS m/z (%): ($C_{21}H_{24}O_7$) $[M+Na]^+$: 411.3629 (100), calc. 411.3659.

5.2.9. ((2E)-3-(3,4-dimethoxyphenyl)-1-(2,4,5-trimethoxyphenyl)prop-2-en-1-one), (**9**)

Yield: 77.5 %; R_f = 0.55 (chloroform-ethyl acetate: 8:1); light yellow; $C_{20}H_{22}O_6$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.67 (d, 1H, *J*=15.6 Hz, H-3); 7.50 (d, 1H, *J*=15.6 Hz, H-2); 7.37 (s, 1H, H-6′); 7.21 (d, 1H, *J*=8.3 Hz, H-6″); 7.13 (s, 1H, H-2″); 6.89 (d, 1H, *J*=8.3 Hz, H-5″); 6.55 (s, 1H, H-3′); 3.90-3.97 (s, 15H, 5x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 190.0 (C=O), 154.6 (C-2′), 153.4 (C-4′), 150.9 (C-4″), 149.1 (C-3″), 143.3 (C-5′), 142.2 (C-3), 128.5 (C-1″), 125.2 (C-2,6″), 120.6 (C-1′), 113.1 (C-5″), 111.1 (C-2″), 110.2 (C-6′), 97.1 (C-3′), 56.9, 56.3, 56.1, 56.0, 55.9 (5x-OCH_3).

5.2.10. ((2E)-1-(3,4-dimethoxyphenyl),3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one), (**10**)

Yield: 86.4 %; R_f = 0.50 (chloroform-ethyl acetate: 8:1); light yellow; $C_{20}H_{22}O_6$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.73 (d, 1H, *J*=15.8 Hz, H-3); 7.69 (d, 1H, *J*=8.4 Hz, H-6′); 7.63 (s, 1H, H-2′); 7.45 (d, 1H, *J*=15.8 Hz, H-2); 6.92 (d, 1H, *J*=8.4 Hz, H-5′); 6.88 (s, 2H, H-2″, 6″); 3.91-3.98 (s, 15H, 5x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 188.6 (C=O), 153.6 (C-3″,5″), 153.3 (C-4′), 149.3 (C-3′), 144.2 (C-3), 140.2 (C-4″), 131.3 (C-1′), 130.6 (C-1″), 123.0 (C-2), 121.0 (C-6′), 110.8 (C-2′), 109.9 (C-5′), 105.6 (C-2″,6″), 61.0, 56.2 (x2), 56.1 (x2) (5x-OCH_3).

5.2.11. ((2E)-3-(2,3,4-trimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one), (**11**)

Yield: 80.3 %; R_f = 0.68 (chloroform-ethyl acetate: 8:1); light yellow; $C_{20}H_{22}O_6$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.99 (d, 1H, *J*=15.8 Hz, H-3); 7.69 (d, 1H, *J*=8.4 Hz, H-6′); 7.64 (s, 1H, H-2′); 7.60 (d, 1H, *J*=15.8 Hz, H-2); 7.40 (d, 1H, *J*=8.8 Hz, H-6″); 6.94 (d, 1H, *J*=8.4 Hz, H-5′); 6.74 (d, 1H, *J*=8.8 Hz, H-5″); 3.90-3.98 (s, 15H, 5x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 189.1 (C=O), 155.6 (C-4′), 153.7 (C-4″), 153.0 (C-2″), 149.1 (C-3′),139.4 (C-3′,3″), 131.6 (C-1′), 123.9 (C-6″), 122.9 (C-2), 122.2 (C-1″), 121.1 (C-6′), 110.8 (C-2′), 109.9 (C-5′), 107.6 (C-5″), 61.4, 61.0, 56.1 (x2), 56.0 (5x-OCH_3).

5.2.12. ((2E)-1,3 bis-(3,4-dimethoxyphenyl)prop-2-en-1-one), (**12**)

Yield: 89.5 %; R_f = 0.50 (chloroform-ethyl acetate: 8:1); light yellow; $C_{19}H_{20}O_5$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.78 (d, 1H, *J*=15.5 Hz, H-3); 7.70 (d, 1H, *J*=8.3 Hz, H-6′); 7.63 (s, 1H, H-2′); 7.43 (d, 1H, *J*=15.5 Hz, H-2); 7.25 (d, 1H, *J*=8.2 Hz, H-6″); 7.17 (s, 1H, H-2″); 6.94 (d, 1H, *J*=8.3 Hz, H-5′); 6.91 (d, 1H, *J*=8.2 Hz, H-5″); 3.94-3.98 (s, 12H, 4x-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 188.7 (C=O), 153.1 (C-4′), 151.3 (C-4″), 149.2 (C-3′,3″), 144.2 (C-3), 131.5 (C-1′), 128.0 (C-1″), 123.0 (C-2), 122.9 (C-6″), 119.6 (C-6′), 111.1 (C-2″), 110.8 (C-5″), 110.0 (C-2′), 109.9 (C-5′), 56.1 (x2), 56.0 (x2) (4x-OCH_3).

5.3. Antioxidant activity

Antioxidant activities of **1-12** were tested against FRAP [37, 38], CUPRAC [38], and DPPH [37] methods according to the literature and experimental procedure described in our previous work [39, 40] (Table 3). Butylated hydroxytoluene for DPPH and Trolox for CUPRAC and FRAP was used as standard.

5.4. 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 tuberculosis; *Mycobacterium smegmatis* ATCC607, and fungi; *Candida albicans* ATCC 60193. Inhibition diameters were measured by the agar well diffusion method [35, 36], and the MIC value was determined as microgram-milliliter (µg /mL) to the microdilution techniques. The antimicrobial screening test using the agar-well diffusion method as adapted was used earlier [35, 36] and the experimental procedure described in our previous work [40, 78]. The antimicrobial properties of **1-12** were investigated quantitatively in respective broth media by using the microdilution method, and the minimal inhibition concentration (MIC) values (µg/mL) were examined [79]. Experimental procedure described in our previous work [40].

5.5. Enzyme inhibition activity

Lipase, *a*-Amylase, *a*-glucosidase, tyrosinase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) inhibition experimental procedure described in our previous work [30, 39, 80].

5.6. *In silico* evaluations

5.6.1. Molecular docking analysis

The AutoDock 4.2 was used to investigate the enzyme-ligand interaction mechanisms between methoxy chalcones (1-12) and the enzymes *a*-amylase, *a*-glucosidase, AChE, BChE, and tyrosinase [81]. The three-dimensional (3D) structures of each target enzyme and ligand required for this analysis were obtained from the Protein Data Bank website (http://www.rcsb.org/pdb) (PDB IDs: 4W93, 5NN4, 4EY6, 6QAA, 2Y9X, and 1ETH respectively). The APBS-PDB2PQR software package was utilized to remove the crystallized ligands, water molecules, and non-interacting ions from the protein structures, and add the appropriate hydrogen atoms [82]. After the protein structures were prepared, the grid box was adjusted according to the position of the binding site of the crystallized ligand. Docking was performed using a rigid protein and flexible ligand with the Lamarckian Genetic Algorithm, conducting 100 independent runs per ligand. The conformation of the protein and ligand with the lowest binding energy was visualized with the BIOVIA Discovery Studio Client [83].

5.6.2. Toxicological predictions

The ChemInfo (https://cheminfo.org/) was used to generate the simplified molecular-input line-entry system (SMILES) code of methoxy chalcones. The generated SMILES codes were then subjected to *in silico* web servers, including SwissADME (https://swissadme.ch), pkCSMpharmacokinetics (http://biosig.unimelb.edu.au/pkcsm/), and ProTox-3.0 (http://tox.charite.de/protoxII/). These well-validated tools share the goal of estimating absorption, distribution, metabolism, excretion, and toxicity (ADMET) features from a chemical structure with some differences in particular computational approaches. SwissADME® was used for the assessment of parameters such as physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry of methoxy chalcones [67]. pkCSM (Predicting

Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures) is utilized for the computation of pharmacokinetics and toxicity properties [76]. ProTox-3.0 (http://tox.charite.de/protoxII/) was used to predict acute oral toxicity values (LD_{50}) of the methoxy chalcones [84].

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