Phenothiazine-based Chalcones as Potential Dual-Target Inhibitors Towards Cholinesterases (AChE, BuChE) and Monoamine Oxidases (MAO–A, MAO–B)

Cem Yamali*[1,2], Feyza Sena Engin[1], Sinan Bilginer[1], Mehtap Tugrak[1], Dilan Ozmen Ozgun[3], Gulsen Ozli[4], Serkan Levent[5], Begum Nurpelin Saglik[5], Yusuf Ozkay[5], Halise Inci Gul*[1]

Abstract:

Chalcones targeting neurodegenerative diseases have been known as attractive structures in drug design and discovery. In this study, phenothiazine-based chalcones as ChEs and MAOs inhibitors were designed and synthesized *via* base-catalyzed Claisen-Schmidt condensation, and chemical structures of the compounds were elucidated by NMRs and HRMS. Compounds **3** and **9** showed promising inhibition potency against AChE enzyme with IC_{50} values of 0.221 µM and 0.053 µM while compound **9** displayed remarkable inhibition potency towards MAO-B enzyme with IC_{50} value of 0.048 µM. Compound **9**, as a dual-target inhibitor, selectively inhibited AChE and MAO-B enzymes. This promising behavior is an advantage for the compound since MAO-B and AChE inhibition have a role in Alzheimer's disease (AD). Fused tricyclic ring systems such as phenothiazine incorporated with chalcone moiety being multitargeting ligands may help scientists for the rational design of novel lead compounds targeting neurodegenerative illnesses.

[1]* Assist. Prof. Dr. Cem Yamali, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
E-mail: c.yamali@yahoo.com
[2] Assist. Prof. Dr. Cem Yamali, Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Cukurova University, Adana, Turkey
E-mail: c.yamali@yahoo.com
[1] Feyza Sena Engin, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
E-mail: feyzasenaengin@gmail.com
[1] Assit. Prof. Dr.Sinan Bilginer, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey. E-mail: sinanbilginer25@yahoo.com
[1] Dr. Mehtap Tugrak, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey.
E-mail: mehtaptugrak@hotmail.com
[3] Dilan Ozmen Ozgun, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ibrahim Cecen University, Agri, Turkey.
E-mail: eczdilan@yahoo.com
[4] Gulsen Ozli, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erzincan University, Erzincan, Turkey.
E-mail: gulsengunesacar@gmail.com
[5] Serkan Levent, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey.
E-mail: serkanlevent@anadolu.edu.tr
[5] Dr. Begum Nurpelin Saglik, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey. E-mail: bnsaglik@anadolu.edu.tr
[5] Prof. Dr. Yusuf Ozkay, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey.
E-mail: yozkay@anadolu.edu.tr
[1]* Prof. Dr. Halise Inci Gul, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey.
E-mail: incigul1967@yahoo.com

1. INTRODUCTION

The investigation of therapeutic compounds to modify the pathogenesis of neurodegenerative diseases has been a hot topic in drug development. The improvement in learning molecular mechanisms of Alzheimer's disease (AD) which is the most prevalent disorder in older persons with mental deterioration has been fascinating[1, 2].

The current pharmaceuticals used in the treatment of AD are cholinesterase inhibitors (ChEIs) (Donepezil, Rivastigmine, Galantamine) and *N*-methyl-D-aspartate (NMDA) receptor antagonists (Memantine)[3]. ChEIs have been used to prevent the degradation of the neurotransmitter acetylcholine (ACh) by inhibiting acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) enzymes that influence breakdown of acetylcholine in synapses. Therefore, to treat some of the symptoms of AD, inhibition of ChEs is one of the popular approaches[4]. The enzyme levels in AD have differences. So, AChE levels are low in AD, while BuChE activity is enhanced[5]. Functionally, both enzymes hydrolyze acetylcholine efficiently at different rates. However, AChE has higher hydrolytic activity than BChE's[6].

Unfortunately, the current ChEIs remain as palliative treatments of AD and are ineffective as a long-term treatment for AD. Tacrine, the first ChEI, was withdrawn due to hepatotoxicity. On the other hand, donepezil and rivastigmine show dose–depended adverse effects[7]. Therefore, it is very desirable to investigate safe and effective pharmaceuticals for AD.

Although the pathogenesis of AD is not fully clear, the clinical hallmarks such as cholinergic dysfunction, amyloid aggregation, tau hyperphosphorylation, and oxidative stress that lead to neurodegeneration and loss of memory and cognitive were reported[8, 9]. Current drug design strategies and targets have been developed to obtain the most potent and selective anti-AD drugs with less side effects due to the multi–factorial structure of AD.

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As a new target of AD, monoamine oxidases (MAOs) are attracting attention. MAOs play a crucial role in the process of deactivation of the monoamines in the body. Monoamines convert to ammonia, hydrogen peroxide and corresponding aldehyde[10]. Two types of MAOs in mammals are reported as MAO-A and MAO-B enzymes, which bound covalently to their co-factor flavin adenine dinucleotide (FAD). MAO-A inhibitors are considered for the treatment of anxiety and depression, while MAO-B inhibitors could be prefered as therapeutic agents in Parkinson's and Alzheimer's diseases[11].

In the last few years, MAO-B enzyme has been proved as an essential target for research of AD since MAO-B catalyzes formation of neurotoxic products[12]. This induces generation of free radicals that lead to oxidative stress, neuronal cell death, and β-amyloid plaques[13, 14]. As MAO-B inhibitors can regulate neurotransmitters and inhibit oxidative damage, novel selective MAO-B inhibitors can be developed as therapeutic agents targeting AD.

Phenothiazines (Figure 1), nitrogen- and sulfur-containing tricyclic compounds, have been used as dyes, biological stains, and pharmaceutical agents to treat various diseases[15]. Chlorpromazine (Figure 1) was the first drug successfully employed as a neuroleptic in the treatment of psychotic disorders such as schizophrenia as an antagonist of dopamine receptors[16]. Phenothiazine bearing methylene blue, FDA-approved drug, is a therapeutic agent for malaria, fear and claustrophobia, and psychiatric diseases (Figure 1). Since methylene blue can easily cross the blood-brain barrier, its analogues can be considered as a main structure to design new compounds for diseases affecting the central nervous system[17]. Besides, phenothiazine-based compounds are described as mostly well tolerated and have few side effects[18].

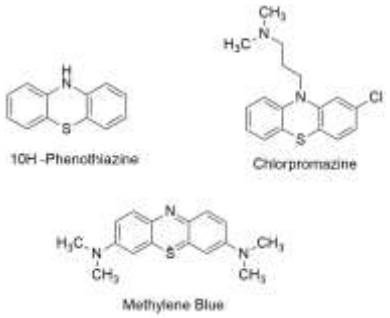


Figure 1. Chemical structures of phenothiazine and its derivatives

Methylene blue has been extended to phase 2 trials in humans with AD.[19]. Phenothiazine-derived compounds also show inhibitory potency against both cholinesterases, tau protein, and neuroprotective effects[15,19,20]. Besides, tacrine, the first ChEIs, is an example of a fused tricyclic drug against AD as a lead compound to be inspired.

Chalcones, as well as their synthetic analogues, display favorable biological activities against AD[21-26]. As they have flexible structures, chalcones can effectively bind to many kinds of enzymes or receptors targeting AD[27,28]. Designing phenothiazine-based chalcones to modulate specific enzymes may be a powerful strategy for the therapeutic aim of neurodegenerative disorders.

Due to the complexity of AD, the multitarget-directed ligands, which possess two or more complementary AD-related targets, have been considered as an effective way for the treatment of AD[29]. When compared with the clinical drugs which show their effects on a single biological target, the hybride structures that target multiple pathways can decrease toxicity and adverse effects. A study also reported synthesis of novel structures targeting AChE and MAO-B enzymes as dual inhibitors for the treatment of AD[30]. The obtained results encouraged many research groups to develop multitarget-directed ligands against AD.

By considering the points mentioned above, we designed phenothiazine-based chalcones, which include a fused tricyclic ring system similar to tacrine based on the hybrid approach to design multitarget-directed ligands. We aim to synthesize dual-targeting phenothiazine-based chalcones as ChEs and MAOs inhibitors as possible lead compounds for Alzheimer's disease as well as neurodegenerative diseases.

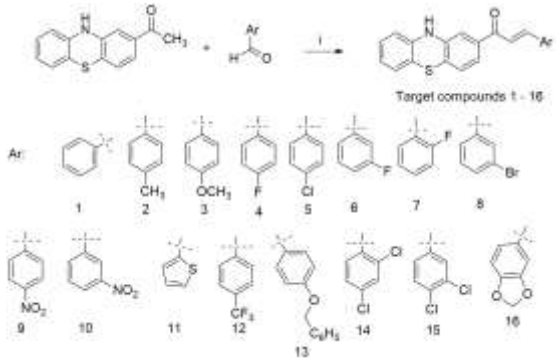
2. RESULTS AND DISCUSSION

2.1. Chemistry

In the present study, compounds were freshly synthesized by the reaction of 2-acetylphenothiazine with substituted-benzaldehydes *via* base-catalyzed Claisen-Schmidt condensation according to our previous studies[22,23,31] (Scheme 1). Chemical structures of compounds **1**–**16** were elucidated by spectral techniques such as ^{1}H NMR, ^{13}C NMR, and HRMS. Compounds **7**, **12**, and **15** were reported for the first time by this study.

Analysis of ^{1}H NMR spectra of compounds **1** – **16** showed that all synthesized were in *E* isomers with coupling constants (*J*) between 15.4 – 20.5 Hz for *α,β*-protons, observed in the

aromatic region of spectra. The proton of the NH group on the phenothiazine ring appeared in the range of 8.74–8.81 ppm as a singlet. According to the ^{13}C NMR spectra of the compounds, the carbonyl carbon of the compounds resonated at around 188 ppm, as expected. HRMS results also confirmed the chemical structures of the compounds. Spectrums of the compounds were given as a supplementary file.



: Methanol, aqueous solution of KOH (40 %), 70 °C.

Scheme 1. Synthesis of the phenothiazine-based chalcones

2.2. Cholinesterase (AChE, BuChE) Inhibitory Effects Of The Compounds

It is known that ChEs enzymes regulate cholinergic transmission. Furthermore, the hydrolytic activity of the AChE enzyme is higher than the BuChE's. Therefore, compounds targeting the AChE enzyme are favorable in the treatment of AD[32]. The compounds synthesized were evaluated against two ChEs to determine enzyme selectivity of the compounds. Donepezil and tacrine were used as reference drugs since the inhibitors differ in selectivity for different forms of ChEs [33]. Biological evaluation of compounds **1** – **16** as potential AChE and BuChE inhibitors was performed using *in vitro* modified-Ellman's assay [34]. The bioassay results are shown in Tables 1 and 2.

This study is the first report for the synthesized compounds in terms of AChE (except **1**, **2**, **3**, **5,** and **10**) and the BuChE enzyme inhibitory profile. Compounds **1**, **2**, **3**, **5**, and **10** were reported with AChE inhibition study in 2012. According to this study [35], the compounds showed an inhibitory potency with IC_{50} values between 1.0 to 6.4 µg/ml when compared to the neostigmine with IC_{50} value of 8.3 µg/ml.

Initially, the compounds' inhibitory potency was evaluated at 10^{-3} and 10^{-4} M (Table 1). It shows that the compounds were more potent against the AChE enzyme when compared with BuChE. Among the compounds, particularly, compounds **3** [(*E*)-3-(4-methoxyphenyl)-1-(10*H*-phenothiazin-2-yl)prop-2-en-1-one] and **9** [(*E*)-3-(4-nitrophenyl)-1-(10*H*-phenothiazin-2-yl)prop-2-en-1-one] attracted great attention since their inhibition percentages (% inhibition) were in the range of 87–93 % against AChE while Donepezil's % inhibition was 99 %. These two compounds were forwarded for further experiments to calculate their half-maximal inhibitory concentrations (IC_{50}) in the range of 10^{-3}–10^{-9} against AChE (Table 2). The compounds showed promising inhibitory potency when compared to donepezil. IC_{50} values of compounds **3**, **9**, and donepezil were as follows: 0.221, 0.053, and 0.02 µM, respectively. These results indicate that the AChE inhibitory potential of compound **9** is close to that of donepezil, and also, this compound can be modified to enhance enzyme inhibitory potency for having more potent lead compounds for future studies.

The compounds having different substituted-phenyl ring or bioisosteric ring were evaluated at different concentrations to see their enzyme inhibitory effects. The majority of the compounds were found less active as ChEs inhibitors, except for **3** and **9**. Some modifications or substituents made significant differences in bioactivity. Primary structure-bioactivity relationships were made according to the % inhibition of each compound obtained at 10^{-3} M to determine which modification is better and can be used for further designing of new enzyme inhibitors.

When compounds **1** (H, 55 %), **2** (4-CH_3, 60 %), and **3** (4-OCH_3, 93 %) were compared, the electron-releasing 4-OCH_3 group is favorable than 4-CH_3 since it increased activity. Among the halogenated compounds, fluorine substitution (73–77 %) led to increased activity compared to the parent compound **1** (H, 55 %), while the substitution pattern of the bromine or chlorine-bearing compounds did not increase the activity excessively. On the other hand, the substitution pattern of the electron-withdrawing NO_2 group has dramatically affected the activity. Compound **9** (*p*-NO_2, 91 %) was considered as one of the most potent in this study. *p*-NO_2 substitution increased inhibitory enzyme potency approximately 1.6 fold compared to compounds **10** (*m*-NO_2, 58 %) and **1** (H, 55 %). Based on these results, it can be stated that phenothiazine bearing chalcone-based compounds can be used for future studies to obtain more potent ChE inhibitors having increased pharmacological profile.

2.3. Monoamine Oxidase (MAO-A, MAO-B) inhibitory effects of synthesized compounds

MAOs enzyme inhibitory profiles of phenothiazine-based chalcones **1**–**16** were reported here firstly. The compounds were tested against both ChEs and MAOs to see whether they have dual inhibitory effects since MAO-B was reported as one of the hallmarks of AD[14].

If the compounds show their effects on both enzymes, they can be considered as potent dual inhibitors. From this point of view, the compounds were evaluated against MAO-A and MAO-B enzymes, and their inhibition results (% inhibition) was reported in Tables 3 and 4. Chlorgiline, a selective MAO-A inhibitor, and selegiline, a selective MAO-B inhibitor, were used in the bioassay as standard MAOs inhibitors. Firstly, compounds were tested at 10^{-3} and 10^{-4} M to see whether they have inhibitory potency against MAOs (Table 3). Then, the MAO-B enzyme was treated with different concentrations of the most potent compound in the range of 10^{-3}–10^{-9} for calculation IC_{50} values (Table 4).

Interestingly, compound **9**, which had significant AChE inhibitory potency, displayed inhibition potency towards MAO-B enzyme in the range of 88–92 % while reference drug selegiline's potency was in the range of 94–98 %. IC_{50} values of **9** and selegiline were 0.048 and 0.037 µM, respectively. Results showed that compound **9** could be considered as the most potent inhibitor of MAO-B enzyme, among others. Moreover, compound **9** was found more selective towards MAO-B when compared with MAO-A. This situation is an advantage for compound **9** since MAO-B inhibition has role in AD. It may show anti-AD effects by inhibiting AChE and MAO-B enzymes which need to be approved by further assays.

As a result of this study, the dual-target inhibitors showing enzyme inhibition properties selectively against both AChE and MAO-B enzymes were reported. For future concepts, further molecular modifications can be made to direct the lead compounds' physicochemical properties, enzyme selectivity, and pharmacological profile. (Figure 2).

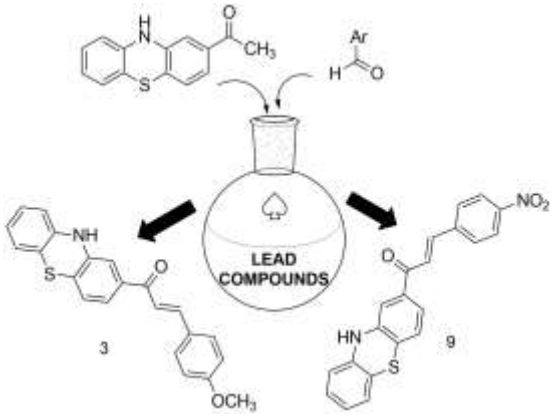


Figure 2. Lead compounds of the study: **3** (AChE) and **9** (AChE and MAO-B)

3. CONCLUSIONS

The chalcone-based compounds targeting neurodegenerative diseases have been reported as promising structures in the field of drug design and discovery. In this present study, phenothiazine-based chalcones as ChEs and MAOs inhibitors were freshly synthesized *via* base-catalyzed Claisen-Schmidt condensation. The lead compounds, preferably inhibited AD-related AChE and MAO-B enzymes. The compounds **3** and **9** showed remarkable inhibition potency against AChE enzyme with IC_{50} values of 0.221 µM and 0.053 µM while the compound **9** displayed significant inhibition potency towards MAO-B enzyme with IC_{50} values of 0.048 µM. The most potent and selective compound **9** can be declared as a promising dual-targeting lead inhibitor towards AChE and MAO-B enzymes. Hopefully, these results can help researchers for the rational design of novel phenothiazine-based lead compounds targeting neurodegenerative diseases.

Table 1. Cholinesterases (AChE, BuChE) inhibitory effects (% inhibition) of compounds **1–16**

Compound	% AChE		% BChE	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
1	55.128 ± 0.956	36.755 ± 0.529	38.165 ± 0.820	24.562 ± 0.651
2	60.965 ± 0.851	25.472 ± 0.637	36.521 ± 0.784	20.970 ± 0.425
3	**93.125 ± 1.185**	**87.256 ± 1.056**	41.066 ± 0.693	35.276 ± 0.451
4	77.255 ± 1.024	39.623 ± 0.419	39.527 ± 0.467	24.281 ± 0.748
5	55.129 ± 0.962	30.640 ± 0.511	27.394 ± 0.835	20.128 ± 0.881
6	76.255 ± 0.956	31.477 ± 0.492	34.288 ± 0.726	21.641 ± 0.547
7	73.955 ± 0.942	37.258 ± 0.365	33.018 ± 0.798	25.475 ± 0.756
8	69.529 ± 0.741	29.217 ± 0.660	28.750 ± 0.697	21.623 ± 0.841
9	**91.528 ± 1.118**	**87.642 ± 1.247**	25.623 ± 0.547	18.240 ± 0.462
10	58.955 ± 0.852	21.367 ± 0.407	27.985 ± 0.447	22.331 ± 0.632
11	41.488 ± 0.753	36.850 ± 0.510	29.748 ± 0.451	24.205 ± 0.787
12	68.264 ± 0.951	30.140 ± 0.628	31.488 ± 0.572	24.617 ± 0.510
13	59.621 ± 0.842	29.475 ± 0.453	34.170 ± 0.740	26.557 ± 0.529
14	60.250 ± 0.943	34.161 ± 0.467	31.629 ± 0.559	24.875 ± 0.421
15	47.206 ± 0.654	27.488 ± 0.617	29.118 ± 0.735	20.362 ± 0.460
16	54.859 ± 0.679	26.147 ± 0.477	26.578 ± 0.603	17.621 ± 0.435
Donepezil	**99.156 ± 1.302**	**97.395 ± 1.255**	-	-
Tacrine	-	-	**99.827 ± 1.378**	**98.651 ± 1.402**

Table 2. % Inhibition and IC_{50} (μM) values of the most potent compounds **3** and **9** against AChE

Compound	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	IC_{50} (μM)
3	93.125	87.256	70.148	58.117	40.205	35.622	22.148	**0.221**
	± 1.185	± 1.056	± 1.248	± 0.985	± 0.859	± 0.751	± 0.459	± 0.008
9	91.528	87.642	78.116	71.185	62.478	42.027	19.623	**0.053**
	± 1.118	± 1.247	± 1.063	± 0.974	± 0.851	± 0.632	± 0.575	± 0.001
Donepezil	99.156	97.395	93.583	91.277	76.982	35.459	18.410	**0.020**
	± 1.302	± 1.255	± 1.167	± 1.074	± 0.951	± 0.453	± 0.411	± 0.001

Table 3. Monoamine oxidases (MAO-A, MAO-B) inhibitory effects (% inhibition) of compounds **1–16**

Compound	% MAO-A		% MAO-B	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
1	33.015 ± 0.658	24.157 ± 0.514	45.258 ± 0.988	37.255 ± 0.716
2	28.114 ± 0.529	20.605 ± 0.411	41.597 ± 0.850	30.356 ± 0.621
3	39.250 ± 0.754	33.451 ± 0.588	46.753 ± 0.751	40.159 ± 0.549
4	40.158 ± 0.602	34.758 ± 0.684	37.264 ± 0.688	30.349 ± 0.533
5	27.529 ± 0.498	23.479 ± 0.477	39.314 ± 0.749	31.753 ± 0.451
6	40.744 ± 0.511	35.621 ± 0.618	50.116 ± 0.963	35.627 ± 0.481
7	34.708 ± 0.475	30.525 ± 0.455	49.276 ± 0.811	41.310 ± 0.507
8	29.265 ± 0.409	23.525 ± 0.575	51.741 ± 0.729	45.779 ± 0.562
9	37.529 ± 0.614	32.207 ± 0.416	**92.365 ± 1.158**	**88.126 ± 1.248**
10	34.525 ± 0.674	27.928 ± 0.507	44.358 ± 0.617	40.749 ± 0.477
11	28.955 ± 0.491	20.411 ± 0.531	36.719 ± 0.527	31.528 ± 0.469
12	36.754 ± 0.510	30.521 ± 0.619	38.955 ± 0.432	29.746 ± 0.488
13	39.852 ± 0.637	28.456 ± 0.488	48.260 ± 0.517	42.749 ± 0.591
14	32.369 ± 0.499	24.159 ± 0.579	42.637 ± 0.631	35.411 ± 0.317
15	27.753 ± 0.487	22.951 ± 0.412	40.957 ± 0.718	30.177 ± 0.467
16	29.753 ± 0.417	21.477 ± 0.497	49.855 ± 0.871	42.608 ± 0.475
Chlorgiline	**99.411 ± 2.955**	**98.257 ± 2.824**	-	-
Selegiline	-	-	**98.589 ± 2.055**	**94.850 ± 2.114**

Table 4. % Inhibition and IC_{50} (μM) value of the most potent compound **9** against MAO-B

Compound	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	IC_{50} (μM)
9	92.365 ± 1.158	88.126 ± 1.248	76.425 ± 1.051	70.628 ± 0.956	66.328 ± 0.854	41.259 ± 0.557	20.128 ± 0.632	**0.048** ± 0.001
Selegiline	98.589 ± 2.055	94.850 ± 2.114	87.412 ± 2.028	79.558 ± 1.057	66.248 ± 1.112	43.015 ± 1.014	15.107 ± 0.340	**0.037** ± 0.001

4. MATERIALS AND METHODS

4.1. Chemistry

NMR spectra of compounds **1**–**8** and **10** were recorded with a Bruker 300 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) while spectras of compounds **9** and **11**–**16** were recorded using a Varian Mercury Plus spectrometer 400 MHz (Varian Inc., Palo Alto, CA) in DMSO-*d6*. High Resolution Mass Spectra (HRMS) was taken using a liquid chromatography ion trap-time of flight tandem mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source, operating in both positive and negative ionization mode. Shimadzu's LCMS Solution software was used for data analysis. HRMS of compound **9** was recorded on an Agilent 6530 Accurate-Mass Q-TOF LCMS. Melting points were determined using an Electrothermal 9100 instrument (IA9100, Bibby Scientific Limited, Staffordshire, UK) and are uncorrected. Reactions were monitored by Thin Layer Chromatography (TLC) using silicagel HF_{254} (Merck Art 5715) plate under UV lamb (254 and 365 nm, Spectroline, Model ENF-240C/FE, Spectronics Corporation Westbury, New York U.S.A).

4.1.1. General synthesis method of 1-(10*H*-Phenothiazin-2-yl)-3-aryl-2-propen-1-ones, 1–16

Compounds **1**–**16** were synthesized according to the our previous study[36]. To the mixture of 2-acetylphenothiazine (4.14 mmol) and a suitable arylaldehyde in methanol (20 ml) in 1:1 mol ratios, an aqueous solution of KOH (40%, 4 mL) was added (Scheme 1). The mixture was refluxed at 70 ºC for 3 h. Reactions were followed by TLC. The mixture was cooled to room temperature and poured onto crushed ice containing few drops of concentrated HCl (37 %) solution and left overnight in a cooler. The precipitated solid product was collected by filtration and washed with cold methanol. The crude compounds were purified by crystallization from suitable amount of DMF:water mixtures.

1-(10H-Phenothiazine-2-yl)-3-phenyl-prop-2-en-1-one (1)

Yield 57 %. Mp: 225–226 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.79 (s, 1H, NH), 7.87–7.84 (m, 2H, arom. H), 7.79 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.71 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.62 (dd, 1H, arom. H, J_1 = 8.0 Hz, J_2 = 1.7 Hz), 7.47–7.45 (m, 3H, arom. H), 7.31 (d, 1H, arom. H, *J* =1.7 Hz), 7.08 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.5 Hz), 6.92 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.3 Hz), 6.77 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.1 Hz), 6.66 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.1 Hz). ^{13}C NMR (DMSO–d_6) δ (ppm) 188.5, 144.2, 142.6, 141.6, 137.3, 135.1, 131.1, 129.4, 129.3, 128.5, 126.7, 126.6, 124.1, 123.0, 122.6, 122.3, 115.7, 115.1, 113.4. HRMS (ESI–MS) m/z $C_{21}H_{15}NOS$ calculated $[M + H]^+$ 329.0869; measured 329.0872.

1-(10H-Phenothiazine-2-yl)-3-(4-methylphenyl)-prop-2-en-1-one (2)

Yield 48 %. Mp: 229–230 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.78 (s, 1H, NH), 7.76-7.69 (m, 4H, olefinic H, arom.H), 7.60 (dd, 1H, arom. H, J_1 = 8.0 Hz, J_2 = 1.8 Hz), 7.30 (d, 1H, arom. H, *J* = 1.7 Hz), 7.26 (d, 2H, arom. H, *J* = 8.0 Hz), 7.07 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.5 Hz), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.5 Hz), 6.75 (td, 1H, arom. H, J_1 = 7.4 Hz, J_2 = 1.2 Hz), 6.65 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.1 Hz), 2.34 (s, 3H, -CH_3). ^{13}C NMR (DMSO–d_6) δ (ppm) 188.4, 144.3, 142.6, 141.6, 141.2, 137.4, 132.4, 130.0, 129.3, 128.5, 126.7, 126.6, 123.9, 122.9, 122.6, 121.2, 115.7, 115.1, 113.4, 21.56. HRMS (ESI–MS) m/z $C_{22}H_{17}NOS$ calculated $[M + H]^+$ 343.1025; measured 343.1028.

1-(10H-Phenothiazine-2-yl)-3-(4-methoxyphenyl)-prop-2-en-1-one (3)

Yield 65 %. Mp: 226–227 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.79 (s, 1H, NH), 7.70 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.63 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.59 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2 = 1.7 Hz), 7.30 (d, 1H, arom. H, *J* = 1.7 Hz), 7.06 (d, 1H, arom. H, *J* = 8.0 Hz), 7.03–6.97 (m, 3H, arom. H), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.4 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.2 Hz), 6.66 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.1 Hz), 3.82 (s, 3H, -OCH_3).^{13}C NMR (DMSO–d_6) δ (ppm) 188.3, 161.8, 144.3, 142.6, 141.7, 137.6, 131.2, 128.4, 127.8, 126.7, 126.6, 123.7, 122.9, 122.5, 119.8, 115.7, 115.1, 114.9, 113.4, 55.9. HRMS (ESI–MS) m/z $C_{22}H_{17}NO_2S$ calculated $[M + H]^+$ 359.0975; measured 359.0982.

1-(10H-Phenothiazine-2-yl)-3-(4-fluorophenyl)-prop-2-en-1-one (4)

Yield 60 %. Mp: 224–225 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.78 (s, 1H, NH), 7.97–7.92 (m, 2H, arom. H), 7.82 (d, 1H, arom. H, *J* = 8.8 Hz), 7.77 (d, 1H, olefinic H, *J* = 15.8 Hz), 7.70 (d, 1H, olefinic H, *J* = 15.8 Hz), 7.66–7.61 (m, 1H, arom. H), 7.33–7.27 (m, 3H, arom. H), 7.09–7.05 (m, 1H, arom. H), 7.00 (td, 1H, arom. H, J_1 = 7.3 Hz, J_2 = 1.5 Hz), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.4 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.2 Hz), 6.66 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.0 Hz).^{13}C NMR (DMSO–d_6) δ (ppm) 188.3, 165.5, 162.0, 144.3, 142.8, 141.6, 137.5, 131.7, 131.2, 128.5, 126.6, 124.1, 123.0, 122.4, 119.8, 116.4, 115.7, 115.0, 113.4. HRMS (ESI–MS) m/z $C_{21}H_{14}FNOS$ calculated $[M + H]^+$ 347.0775; measured 347.0775.

1-(10H-Phenothiazine-2-yl)-3-(4-chlorophenyl)-prop-2-en-1-one (5)

Yield 64 %. Mp: 259–260 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.79 (s, 1H, NH), 7.90 (d, 2H, arom. H, *J*= 8.5 Hz), 7.82 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.69 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.63 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2 = 1.7 Hz), 7.52 (d, 2H, arom. H, *J* = 8.5 Hz), 7.29 (d, 1H, arom. H, *J* = 1.7 Hz), 7.08 (d, 1H, arom. H, *J* = 8.0 Hz), 7.03-6.98 (m, 1H, arom. H), 6.92 (dd, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.1 Hz), 6.79-6.74 (m, 1H, arom. H), 6.66 (dd, 1H, arom. H, J_1 = 7.4 Hz, J_2 = 1.0 Hz).^{13}C NMR (DMSO-d_6) δ (ppm) 188.4, 142.8, 142.6, 141.6, 137.2, 135.5, 134.1, 131.0, 129.4, 128.5, 126.7, 126.6, 124.3, 123.13, 123.07, 122.6, 115.6, 115.1, 113.3. HRMS (ESI–MS) m/z $C_{21}H_{14}ClNOS$ calculated $[M + H]^+$ 363.0479; measured 363.0477.

1-(10H-Phenothiazine-2-yl)-3-(3-fluorophenyl)-prop-2-en-1-one (6)

Yield 95 %. Mp: 230–232 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.79 (s, 1H, NH), 7.86 (d, 1H, olefinic H, *J* = 15.6 Hz), 7.82-7.79 (m, 1H, arom. H), 7.69 (d, 1H, olefinic H, *J* = 15.6 Hz), 7.68-7.63 (m, 2H, arom. H), 7.53-7.45 (m, 1H, arom. H), 7.30 (d, 1H, arom. H, *J* = 1.8 Hz), 7.28-7.24 (m, 1H, arom. H), 7.08 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.5 Hz), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.4 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.2 Hz), 6.66 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 188.4, 164.6, 161.3, 142.7, 141.6, 137.7, 137.1, 131.3, 128.5, 126.7, 126.6, 125.9, 124.4, 123.8, 123.2, 122.6, 117.8, 117.6, 115.6, 115.0, 113.3. HRMS (ESI–MS) m/z $C_{21}H_{14}FNOS$ calculated $[M + H]^+$ 347.0775; measured 347.0772.

1-(10H-Phenothiazine-2-yl)-3-(2-fluorophenyl)-prop-2-en-1-one (7)

Yield 38 %. Mp: 219–220 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.81 (s, 1H, NH), 8.06 (td, 1H, arom. H, J_1 = 7.8 Hz, J_2 = 1.5 Hz), 7.85 (d, 1H, olefinic H, *J* = 15.8 Hz), 7.78 (d, 1H, olefinic H, *J* = 15.8 Hz), 7.59 (dd, 1H, arom. H, J_1 = 8.0 Hz, J_2 = 1.8 Hz), 7.55-7.48 (m, 1H, arom. H), 7.35-7.28 (m, 3H, arom. H), 7.08 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.5 Hz), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.4 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.2 Hz), 6.66 (dd, 1H, arom. H, J_1 = 8.0 Hz, J_2 = 1.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm) 188.2, 142.7, 141.5, 137.0, 135.5, 133.2, 133.0, 129.8, 128.5, 126.7, 125.5, 124.4, 123.1, 122.8, 122.7, 122.6, 116.7, 116.4, 115.6, 115.1, 113.3. HRMS (ESI–MS) m/z $C_{21}H_{14}FNOS$ calculated $[M + H]^+$ 347.0775; measured 347.0766.

1-(10H-Phenothiazine-2-yl)-3-(3-bromophenyl)-prop-2-en-1-one (8)

Yield 69 %. Mp: 234–236 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.79 (s, 1H, NH), 8.16 (d, 1H, arom. H, *J* = 1.4 Hz), 7.87 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.84-7.79 (m, 1H, arom. H), 7.69-7.61 (m, 3H, arom. H), 7.40 (t, 1H, arom. H, *J* = 7.9 Hz), 7.30 (d, 1H, arom. H, *J* = 1.7 Hz), 7.07 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.4 Hz), 6.91 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.4 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.2 Hz), 6.66 (dd, 1H, arom. H, J_1 = 7.9 Hz, J_2 = 1.2 Hz).^{13}C NMR (DMSO-d_6) δ (ppm) 188.3, 142.6, 142.4, 141.6, 137.7, 137.1, 133.5, 131.4, 131.2, 128.6, 128.5, 126.7, 126.6, 124.4, 123.8, 123.3, 122.9, 122.6, 115.6, 115.1, 113.4. HRMS (ESI-MS) m/z $C_{21}H_{14}BrNOS$ calculated $[M + H]^+$ 408.0052; measured 408.0044.

1-(10H-Phenothiazine-2-yl)-3-(4-nitrophenyl)-prop-2-en-1-one (9)

Yield 81 %. Mp: 285–287 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.79 (s, 1H, NH), 8.25 (d, 2H, arom. H, *J* = 8.05 Hz), 8.11 (d, 2H, arom. H, *J* = 8.05 Hz), 7.96 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.76 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.64 (d, 1H, arom. H, *J* = 7.69 Hz), 7.27 (s, 1H, arom. H), 7.07 (d, 1H, arom. H, *J* = 7.7 Hz), 6.98 (t, 1H, arom. H, *J* = 7.3 Hz), 6.88 (d, 1H, arom. H, *J* = 7.3 Hz), 6.72 (t, 1H, arom. H, *J* = 7.3 Hz), 6.63 (d, 1H, arom. H, *J* = 7.7 Hz).^{13}C NMR (DMSO-d_6) δ (ppm) 188.5, 148.7, 142.9, 141.9, 141.7, 141.5, 137.1, 130.5, 130.4, 128.7, 127.0, 126.8, 126.6, 124.62, 124.61, 123.6, 122.8, 115.3, 113.5. HRMS (ESI-MS) m/z $C_{21}H_{14}N_2O_3S$ calculated $[M]^+$ 374.07251; measured 374.07134.

1-(10H-Phenothiazine-2-yl)-3-(3-nitrophenyl)-prop-2-en-1-one (10)

Yield 38 %. Mp: 250–252 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm) 8.80 (s, 1H, NH), 8.74 (s, 1H, arom. H), 8.32-8.24 (m, 2H, arom. H), 8.01 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.81 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.75 (d, 1H, arom. H, *J* = 8.0 Hz), 7.71-7.68 (m, 1H, arom. H), 7.30 (d, 1H, arom. H, *J* = 1.7 Hz), 7.08 (d, 1H, arom. H, *J* = 8.0 Hz), 7.00 (td, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.3 Hz), 6.90 (dd, 1H, arom. H, J_1 = 6.3 Hz, J_2 = 1.3 Hz), 6.76 (td, 1H, arom. H, J_1 = 7.6 Hz, J_2 = 1.3 Hz), 6.65 (dd, 1H, arom. H, J_1 = 8 Hz, J_2 = 1.1 Hz).^{13}C NMR (DMSO-d_6) δ (ppm) 188.3, 148.9, 142.6, 141.7, 141.5, 137.1, 137.0, 135.5, 130.8, 128.5, 126.7, 126.6, 125.1, 125.0, 124.6, 123.4, 122.6, 115.6, 115.1, 113.3. HRMS (ESI–MS) m/z $C_{21}H_{14}N_2O_3S$ calculated $[M + H]^+$ 375.0798; measured 375.0795.

1-(10H-Phenothiazine-2-yl)-3-(thiophene-2-yl)-prop-2-en-1-one (11)

Yield % 72. Mp: 244–245 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.79 (s, 1H, NH), 7.87 (d, 1H, olefinic H, *J* = 15.4 Hz), 7.77 (d, 1H, arom. H, *J* = 5.1 Hz), 7.65 (d, 1H, arom. H, *J* = 3.7 Hz), 7.49 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2= 1.5 Hz), 7.39 (d, 1H, olefinic H, *J* = 15.4 Hz), 7.26–7.17 (m, 1H, arom. H), 7.05 (d, 1H, arom. H, *J* = 7.7 Hz), 7.01–6.97 (m, 2H, arom. H), 6.90 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.1 Hz), 6.75 (td, 1H, arom. H, J_1 = 7.3 Hz, J_2 = 1.1 Hz), 6.64 (dd, 1H, arom. H, J_1 = 7.5 Hz, J_2 = 1.1 Hz).^{13}C NMR (DMSO–d_6) δ (ppm) 188.1, 142.8, 141.8, 140.4, 137.4, 137.3, 133.8, 131.1, 129.5, 128.7, 127.0, 126.9, 124.2, 123.0, 122.8, 120.6, 115.9, 115.3, 113.6. HRMS (ESI–MS) m/z $C_{19}H_{13}NOS_2$ calculated $[M + H]^+$ 336.0511; measured 336.0496.

1-(10H-Phenothiazine-2-yl)-3-(4-trifluoromethylphenyl)-prop-2-en-1-one (12)

Yield 85 %. Mp: 266–268 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.80 (s, 1H, NH), 8.07 (d, 2H, arom. H, *J* = 8.1 Hz), 7.92 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.79 (d, 1H, arom. H, *J* = 8.4 Hz), 7.74 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.64 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2 = 1.8 Hz), 7.28 (d, 1H, arom. H, *J* = 1.5 Hz), 7.08 (d, 1H, arom. H, *J* = 8.05 Hz), 7.00–6.92 (m, 1H, arom. H), 6.76–6.74 (m, 1H, arom. H), 6.90 (d, 1H, arom. H, *J* = 7.6 Hz), 6.64 (d, 2H, arom. H, *J* = 8.05 Hz).^{13}C NMR (DMSO–d_6) δ (ppm) 188.6, 142.9, 142.4, 141.7, 139.4, 137.2, 130.1, 128.7, 127.4, 127.0, 126.9, 126.4, 126.3, 125.2, 124.8, 123.5, 122.8, 115.8, 115.3, 113.5. HRMS (ESI–MS) m/z $C_{22}H_{14}F_3NOS$ calculated $[M + H]^+$ 398.0821; measured 398.0818.

1-(10H-Phenothiazine-2-yl)-3-(4-benzyloxyphenyl)-prop-2-en-1-one (13)

Yield 33 %. Mp: 217–219 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.76 (s, 1H, NH), 7.76 (d, 2H, arom. H, *J* = 8.8 Hz), 7.63 (d, 1H, olefinic H, *J* = 20.5 Hz), 7.57 (d, 1H, olefinic H, *J* = 20.5 Hz), 7.53 (d, 1H, arom. H, *J* = 8.1 Hz), 7.42 (d, 1H, arom. H, *J* = 7.3 Hz), 7.38–7.34 (m, 1H, arom. H), 7.32–7.30 (m, 1H, arom. H), 7.25 (d, 1H, arom. H, *J* = 0.7 Hz), 7.06–6.97 (m, 4H, arom. H), 6.88 (d, 2H, arom. H, *J* = 7.7 Hz), 6.75 (d, 1H, arom. H, *J* = 7.3 Hz), 6.64 (d, 2H, arom. H, *J* = 7.7 Hz), 5.13 (s, 2H, CH_2).^{13}C NMR (DMSO–d_6) δ (ppm) 188.7, 161.1, 144.5, 142.8, 141.8, 137.8, 137.3, 131.4, 129.2, 128.7, 128.44, 128.43, 128.1, 127.0, 126.9, 124.0, 123.1, 122.9, 120.0, 116.0, 115.9, 115.3, 113.5, 70.1. HRMS (ESI–MS) m/z $C_{28}H_{21}NO_2S$ calculated $[M + H]^+$ 436.1366; measured 436.1356.

1-(10H-Phenothiazine-2-yl)-3-(2,4-dichlorophenyl)-prop-2-en-1-one (14)

Yield 56 %. Mp: 228–230 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.78 (s, 1H, NH), 8.13 (d, 1H, arom. H, *J* = 8.5 Hz), 7.89 (d, 1H, olefinic H, *J* = 15.6 Hz), 7.80 (d, 1H, olefinic H, *J* = 15.6 Hz), 7.70 (d, 1H, arom. H, *J* = 2.0 Hz), 7.58 (dd, 1H, arom. H, J_1 = 8.1 Hz, J_2 = 1.8 Hz), 7.49 (dd, 1H, arom. H, J_1 = 8.5 Hz, J_2 = 2.0 Hz), 7.24 (s, 1H, arom. H), 7.05 (d, 1H, arom. H, *J* = 8.1 Hz), 7.0–6.95 (m, 1H, arom. H), 6.88 (d, 1H, arom. H, *J* = 7.7 Hz), 6.77–6.72 (m, 1H, arom. H), 6.62 (d, 1H, arom. H, *J* = 8.1 Hz). ^{13}C NMR (DMSO–d_6) δ (ppm) 184.4, 142.9, 141.6, 137.82, 137.81, 137.1, 136.33, 136.32, 135.8, 132.0, 130.3, 130.2, 128.8, 128.7, 127.0, 125.7, 125.0, 123.5, 123.0, 115.3, 113.4. HRMS (ESI–MS) m/z $C_{21}H_{13}Cl_2NOS$ calculated $[M + H]^+$ 398.0168; measured 398.0164.

1-(10H-Phenothiazine-2-yl)-3-(3,4-dichlorophenyl)-prop-2-en-1-one (15)

Yield 48 %. Mp: 236–238 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.77 (s, 1H, NH), 8.2 (d, 1H, arom. H, *J* = 1.8 Hz), 7.83 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.79 (dd, 1H, arom. H, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 7.67 (d, 1H, arom. H, *J* = 8.4 Hz), 7.66 (d, 1H, olefinic H, *J* = 15.7 Hz), 7.60 (dd, 1H, arom. H, J_1 = 7.7 Hz, J_2 = 1.8 Hz), 7.24 (d, 1H, arom. H, *J* = 1.8 Hz), 7.05 (d, 1H, arom. H, *J* = 8.1 Hz), 6.98–6.94 (m, 1H, arom. H), 6.89 (d, 1H, arom. H, *J* = 7.7 Hz), 6.76–6.72 (m, 1H, arom. H), 6.64 (d, 1H, arom. H, *J* = 7.3 Hz). ^{13}C NMR (DMSO–d6) δ (ppm) 184.6, 142.8, 141.67, 141.66, 137.2, 136.2, 133.4, 132.5, 131.7, 130.8, 129.7, 127.0, 126.9, 124.8, 124.5, 123.5, 122.9, 115.8, 115.3, 113.5. HRMS (ESI–MS) m/z $C_{21}H_{13}Cl_2NOS$ calculated [M + H]+ 398.0168; measured 398.0159.

1-(10H-Phenothiazine-2-yl)-3-(3,4methylenedioxyphenyl)-prop-2-en-1-one (16)

Yield 87 %. Mp: 220–222 ºC. ^{1}H NMR (DMSO–d_6) δ (ppm) 8.74 (s, 1H, NH), 7.59 (s, 1H, arom. H), 7.55 (dd, 1H, arom. H, J_1 = 8.05 Hz, J_2 = 1.8 Hz), 7.51 (d, 1H, arom. H, *J* = 1.1 Hz), 7.26 (d, 1H, arom. H, *J* = 1.8 Hz), 7.24 (d, 1H, arom. H, *J* = 1.8 Hz), 7.23 (s, 1H, arom. H), 7.0 (d, 1H, arom. H, *J* = 8.1 Hz), 6.98 (d, 1H, olefinic H, *J* = 16.1 Hz), 6.94 (d, 1H, olefinic H, *J* = 16.1 Hz), 6.74 (d, 1H, arom. H, *J* = 7.7 Hz), 6.87 (dd, 1H, arom. H, J_1 = 7.8 Hz, J_2 = 1.1 Hz), 6.63 (dd, 1H, arom. H, J_1 = 7.8 Hz, J_2 = 1.1 Hz), 6.05 (s, 2H, -CH_2-). ^{13}C NMR (DMSO–d_6) δ (ppm) 188.6, 150.3, 148.8, 144.6, 142.8, 141.9, 137.7, 129.8, 128.7, 127.0, 126.8, 126.5, 124.1, 123.2, 122.8, 120.4, 115.9, 115.3, 113.5, 109.3,

107.5, 102.3. HRMS (ESI–MS) m/z $C_{22}H_{15}NO_3S$ calculated $[M + H]^+$ 374.0845; measured 374.0847.

4.2. Biological assays

4.2.1. Anticholinesterase and Butyrylcholinesterase Inhibition Assay

Acetylcholinesterase (AChE, E.C.3.1.1.7, from electric eel), butyrylcholinesterase (BChE from equine serum), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), donepezil hydrochloride and tacrine were purchased from Sigma-Aldrich (Steinheim, Germany). Acetylthiocholine iodide (ATC) and butyrylthiocholine iodide (BTC) were obtained from Fluka (Germany). All pipetting processes were performed using Biotek Precision XS robotic system (USA). Measurements of the percentage inhibition were carried out at 412 nm by using BioTek Synergy H1 microplate reader (USA). The inhibitory activity of compounds against AChE and BChE was determined in 96-well plates by modified Ellman's method[32,34,37] using donepezil and tacrine as reference drugs.

4.2.2. MAO-A and MAO-B Inhibition Assay

Ampliflu™ Red (10-Acetyl-3,7-dihydroxyphenoxazine), peroxidase from horseradish, *h*MAO-A, *h*MAO-B, H_2O_2, tyramine hydrochloride, selegiline and clorgiline were purchased from Sigma-Aldrich (Steinheim, Germany) and retained under the suggested conditions by supplier. All pipetting processes were performed using a Biotek Precision XS robotic system (USA). Measurements were carried out by a BioTek-Synergy H1 microplate reader (USA) based on the fluorescence generated (excitation, 535 nm, emission, 587 nm) over a 30-min period, in which the fluorescence increased linearly[38-40]. Blank, control and all concentrations of inhibitors were analyzed in quadruplicate and inhibition percent was calculated by using the following equation:

$$\%\text{Inhibition} = \frac{(FCt_2 - FCt_1) - (FIt_2 - FIt_1)}{FCt_2 - FCt_1} \times 100$$

FCt_2: Fluorescence of a control well measured at t_2 time, FCt_1: Fluorescence of a control well measured at t_1 time, FIt_2: Fluorescence of an inhibitor well measured at t_2 time, FIt_1: Fluorescence of an inhibitor well measured at t_1 time.

The IC_{50} values were calculated from a dose-response curve obtained by plotting the percentage inhibition versus the log concentration with the use of GraphPad 'PRISM' software (version 5.0). The results were displayed as mean ± standard deviation (SD).

Declaration of Interest

"The authors declared no conflict of interest" in the manuscript.

Captions

Figure 1. Chemical structures of phenothiazine and its derivatives

Figur 2. Lead compounds of the study: **3** (AChE) and **9** (AChE and MAO-B)

Scheme 1. Synthesis of the phenothiazine-based chalcones

Table 1. Cholinesterases (AChE, BuChE) inhibitory effects (% inhibition) of the compounds **1**–**16**

Table 2. % Inhibition and IC_{50} (μM) values of the most potent compounds **3** and **9** against AChE

Table 3. Monoamine oxidases (MAO-A, MAO-B) inhibitory effects (% inhibition) of the compounds **1**–**16**

Table 4. % Inhibition and IC_{50} (μM) value of the most potent compound **9** against MAO-B

Key words: Cholinesterase, monoamine oxidase, chalcone, phenothiazine, Alzheimer's disease, enzyme inhibitors, Claisen-Schmidt

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