Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2018;43(2):371-380
DOI: 10.17826/cumj.341883

ARAŞTIRMA / RESEARCH

Investigation of monoamine oxidase inhibitory activities of new chalcone derivatives

Yeni şalkon türevlerinin monoamin oksidaz enzim inhibitör aktivitelerinin araştırılması

Nafiz Öncü Can

Anadolu University Faculty of Pharmacy, Eskişehir, Turkey

Cukurova Medical Journal 2018;43(2):371-380

Abstract

Purpose: In the present study, it is aimed to investigate monoamine oxidase (MAO) enzyme inhibition potencies of some new chalcone derivatives.

Materials and Methods: Nine novel chalcone derivatives were synthesized and structures of the synthesized compounds (2a-2i) were confirmed by spectroscopic methods. Inhibitory activities of the compounds against MAO-A and MAO-B enzymes have been determined via *in-vitro* fluorometric method. Effects of the compounds on substrate level were measured by an LC-MS/MS method. Enzyme kinetics studies were carried out for the most active compound 2i using Lineweaver–Burk plots.

Results: Compounds 2c, 2e and 2i displayed notable inhibition against *h*MAO-B with lower IC50 values than 1 μM. The most active compound 2i displayed significant inhibition by IC_{50} of 0.13 μM. Results of substrate level quantification study were in an agreement with fluorometric enzyme inhibition assay. Competitive type of inhibition was determined for compound 2i as a result of enzyme kinetic studies.

Conclusion: Synthesized compounds displayed more selectivity towards *h*MAO-B. In this study, the results of inhibitor effects of compounds 2c, 2f and 2i are promising for the synthesis of similar derivatives with enhanced MAO inhibitory activity and therefore for the development of new molecules which may be drug candidates against Parkinson's disease.

Key words: Chalcone, MAO-A, MAO-B, Enzyme inhibition, LC-MS/MS

Öz

Amaç: Bu çalışmada, bazı yeni şalkon bileşiklerinin monoamin oksidaz (MAO) etkinliklerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Dokuz adet yeni şalkon türevi sentezlenmiş ve sentezlenen bileşiklerin (2a-2i) yapıları spektroskopik yöntemler ile aydınlatılmıştır. Bileşiklerin MAO-A ve MAO-B enzimleri üzerine inhibitör etkileri, *in-vitro* florimetrik yöntem ile belirlenmiştir. Bileşiklerin substrat seviyesindeki etkileri bir LC-MS/MS yöntemi ile ölçülmüştür. Enzim kinetik çalışmaları en etkili bileşik olan 2b için Lineweaver–Burk grafiği kullanılarak gerçekleştirilmiştir.

Bulgular: 2c, 2e ve 2i kodlu bileşikler *h*MAO-B'yi 1 μM'den daha düşük IC50 değerlerinde etkin biçimde inhibe etmişlerdir. En aktif bileşik olan 2i, 0.13 μM IC50 değeri ile anlamlı bir inhibisyona neden olmuştur. Substrat seviyesi niceleme çalışmasının sonuçları, florimetrik enzim inhibisyonu testi ile uyumlu bulunmuştur. Enzim kinetik çalışmaları sonucunda 2i kodlu bileşiğin kompetitif tip inhibisyon gösterdiği tespit edilmiştir.

Sonuç: Sentezlenmiş bileşiklerin *h*MAO-B'ye seçiciliği daha yüksektir. Bu çalışmada, 2c, 2f ve 2i kodlu bileşiklerin inhibitör etkilerine ilişkin elde edilen sonuçlar, MAO inhibitörü etkinliği yüksek benzer türevlerin sentezi ve dolayısıyla Parkinson hastalığına karşı ilaç adayı olabilecek yeni moleküllerin geliştirilmesi için umut verici niteliktedir.

Anahtar kelimeler: Şalkon, MAO-A, MAO-B, Enzim inhibisyonu, LC-MS/MS.

Yazışma Adresi/Address for Correspondence: Dr. Nafiz Öncü Can, Anadolu University Faculty of Pharmacy, Eskişehir, Turkey. E-mail: nafizoc@anadolu.edu.tr
Geliş tarihi/Received: 21.05.2017 Kabul tarihi/Accepted: 14.07.2017

INTRODUCTION

Neuropsychiatric and neurodegenerative syndromes are frequently associated to decreased monoamine neurotransmitters levels in the central nervous system (CNS). For instance, reduced levels of serotonin and norepinephrine[1,2] are related to depression, while depletion of dopamine is responsible for Parkinson's disease (PD)[3]. Replacement therapy using metabolic precursors or agonist drugs, and inhibition of neurotransmitter reuptake are leading approaches for reestablishment of synaptic neurotransmission; to increase neurotransmitter levels via blocking their biotransformation in the CNS is another beneficial approach[4].

Monoamine oxidase (MAO), including the flavin adenine dinucleotide (FAD), is a key enzyme that metabolizes monoamine neurotransmitters in peripheral tissues and in the CNS[5]. MAO involves two isoforms, *i.e.* MAO-A and MAO-B. MAO-A catalyzes the degradation of serotonin, epinephrine and norepinephrine[6], and therefore MAO-A inhibitors constitute a well-known class of antidepressant drugs. Iproniazide, nialamide, isocarboxazid, phenelzine, mebenazine, moclobemide, pirlindole, bifemelane and toloxatone are the major agents in this group. Iproniazid is the prototype of the group, but it has been replaced by later-synthesized derivatives due to its toxicity. Many of these drugs have lost their clinical use due to their subsequently detected adverse effects. Moclobemide, pirlindole, bifemelane and toloxatone are currently in clinical use and well tolerated MAO-A inhibitors with low toxicity potential[7]. MAO-A inhibitors have also been found to be effective in the treatment of atypical depression and severe depression having hypochondriac or hysterical symptoms as well as social phobia, rather than routine treatment of major depression[8]. More importantly, these drugs are in use to break refractoriness in cases of depression, especially those that do not respond to tricyclic and non-tricyclic drugs[8,9]. Although there are a large number of antidepressant drugs currently in clinical use, refractoriness that emerges in an important part of the patients is a question that has not yet been surpassed in the treatment of depression. In order to overcome this problem, it is of great importance to develop new drugs with high therapeutic efficacy but low risk of side effect. From this point of view, it would be useful to synthesis and evaluate the therapeutic potential of new compounds with a structure similar to that of MAO inhibitory drugs, which is a drug group that can treat refractory depression.

Similar to MAO-A, inhibition of MAO-B enzyme is known to be able to induce a significant therapeutic effect. Some MAO-B inhibitors such as selegiline and rasagiline are used in the treatment of PD, since they block the metabolism of dopamine in the nigrostriatal pathway[10,11]. Combination of L-Dopa, the metabolic precursor of dopamine, with a MAO-B inhibitor increases central dopamine levels[12,13]. MAO inhibitors may also decrease the levels of harmful by-products of MAO-catalyzed reactions. One of these by-products is hydrogen peroxide, which is produced from the re-oxidation of the FAD cofactor by molecular oxygen. Other by-products are aldehydes, which are generated when the imine product of some amine substrates are hydrolyzed. Since these by-products may contribute to the neurodegenerative processes in Alzheimer's disease (AD) and PD, MAO inhibitors may be shown as neuroprotective agents[14,15]. In this respect, MAO-B inhibitors have importance towards age-related disorders such as AD and PD[16]. Therefore, synthesis of new agents with MAO-B enzyme inhibitory potential may be useful for the development of new drug candidates for the treatment of these type of diseases.

Chalcones (1,3-diphenyl-2-propen-1-ones) are open chain flavonoids that are ubiquitous in edible plants and have a broad range of biological activities[17,18] which include anti-inflammatory and neuroprotective effects[19,20]. Furthermore, chalcones constitute a class of compounds that have been shown to inhibit the MAO enzymes[21-27]. In these studies, it has been pointed out that chalcones possess more selectivity towards MAO-B. Based on these considerations, six novel chalcone derivatives were prepared to investigate their inhibitory potential on MAO-A and MAO-B enzymes, in the current work.

MATERIALS AND METHODS

All chemicals were purchased from either Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by using an MP90 digital melting

point apparatus (Mettler Toledo, Ohio, USA) and were presented as uncorrected. IR spectra were recorded using an Affinity 1S model spectrophotometer from Shimadzu (Shimadzu, Kyoto, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded by using a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-*d6*, respectively. HRMS studies were performed on a Shimadzu LCMS-IT-TOF system (Shimadzu, Kyoto, Japan).

Preparation of 4-(substituedpiperazine-1-yl)benzaldehyde derivatives (1a-c)

4-Fluorobenzaldehyde (10 mmol, 1.075 mL), K_2CO_3 (10 mmol, 1.38 g), piperazine derivatives (20 mmol), and DMF (5 mL) were added into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 200°C and 10 bars for 15 min. After checking the end of a reaction by TLC, the mixture was poured into iced-water, the precipitated product was washed with water, dried, and recrystallized from EtOH.

General synthesis procedure for 1-(3,4-disubstitutedphenyl)-3-[4-(substituted piperazine-1-yl)-phenyl]-prop-2-en-1-one derivatives (2a-2i)

The compound 1a-1c (10 mmol), appropriate acetophenone derivative (10 mmol) and potassium hydroxide (10 mmol, 0.56 g) in 10 mL MeOH) were stirred at room temperature for 12 h. After TLC screening, the resulting solid was filtered, washed with water, dried, and recrystallized from EtOH. Isolated compounds were subjected to spectral analyses for structure conformation. The following spectral data for 1-(3,4-dichlorophenyl)-3-(4-(4-phenylpiperazin-1-yl)phenyl) prop-2-en-1-one (2b) was observed and presented as an example: ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.28 (4H, t, *J*= 5.00 Hz, piperazine –CH_2), 3.55 (4H, t, *J*= 5.00 Hz, piperazine –CH_2), 6.82 (1H, t, *J*= 7.20 Hz, monosubstituted benzene -CH-), 6.99 (2H, d, *J*= 8.20 Hz, monosubstituted benzene -CH-), 7.11 (2H, d, *J*= 8.80 Hz, 1,4-disubstituted benzene), 7.25 (2H, t, *J*= 7.85 Hz, monosubstituted benzene -CH-), 7.48 (1H, dd, *J*=8,50 Hz - 2.00 Hz), 7.56 (1H, d, *J*=8.50 Hz), 7.66 (1H, d, *J*= 15.60 Hz, ethylene -CH-), 7.75 (2H, d, *J*= 8.80 Hz, 1,4-disubstituted benzene), 7.91 (1H, d, *J*= 15.60 Hz, ethylene -CH-), 7.98 (1H, d, *J*=2.00 Hz). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 46.78, 48.37, 113.85, 116.04, 119.65, 121.54, 126.90, 127.91, 129.48, 130.64, 131.29, 131.98, 135.48, 138,76, 140.11, 142.58, 151.15, 155.02, 186.88. HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{22}N_2OCl_2$: 437.1182; found 437.1193.

Fluorometric MAO-A and MAO-B inhibition assay

MAO enzyme inhibitory effects of chalcone derivatives were determined via a fluorometric method using BioVision Monoamine Oxidase A/B (MAO-A/B) Inhibitor Screening Kit (USA) according to the manufacturer's guidelines[28,29]. All pipetting processes were carried by a Biotek Precision XS robotic system (USA). Measurements were carried out by a BioTek-Synergy H1 multimode microplate reader (USA) based on the fluorescence generated (excitation at 535 nm, emission at 587 nm) over a 20 min period. Clorgiline and selegiline were used as reference, since they are specific inhibitors of *h*MAO-A and *h*MAO-B, respectively.

Reference drugs and synthesized compounds were prepared at 10^{-3}-10^{-9} M concentrations using 2% DMSO. Recombinant enzymes and developer were diluted in reaction buffer. Substrate was diluted in double-distilled water. To prepare a MAO working solution, 37 µL assay buffer, 1 µL developer solution, 1 µL substrate solution and 1 µL OxiRed probe were mixed for each well.

The solutions of inhibitors, reference drugs (10 µL/well) and recombinant enzyme solution (50 µL/well) were added to a black flat-bottom 96-well microplate, and incubated for 10 min at 25°C and 37°C for MAO-A and MAO-B assay, respectively. After the incubation period, the reaction was started by adding the working solution (40 µL/well). The mixture was incubated for 30 min at proper temperature. Fluorescence was measured using excitation at 535 nm and emission at 587 nm at 5 min. intervals. Control experiments were carried out simultaneously by replacing the inhibitor solution with 2% DMSO (10 µL).

The specific fluorescence values (used to obtain the final results) were calculated after subtraction of the background activity, which was determined from the wells containing all components except for the *h*MAO isoforms, which were replaced by phosphate buffer (50 µL/well).

Determination of substrate level

Substrate (tyramine) level was determined by using the final mixture of fluorometric assay. After fluorometric measurements, enzyme-substrate reaction was terminated by 2 N NaOH (50 µL/well) and samples (1 µL) from the wells of the most active compounds were injected into LC-MS/MS system; the mass spectrometric analyses were achieved by utilizing a Nexera XR series of UFLC system coupled to an LCMS-8040 model triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Nexera XR series UFLC system used in the analysis consisted of two pumps (LC-20ADxr), an autosampler (SIL-20ADxr), a column heater (CTO-10ASvp), and a degasser (DGU-20A5R). The instrument was equipped with an ESI ion source.

Chromatographic separation was performed using a Shimadzu Shimpack FC-ODS C18 column (150 mm × 2.0 mm, packed with 3 µm particles) at a flow rate of 0.2 mL/min in ESI source. The isocratic mobile phase consisted of acetonitrile-water 0.1% formic acid (50:50, v/v). The mass spectrometer operating parameters were optimized as follows: Nebulizer gas flow, 3 L/min; drying gas flow, 15 L/min; desolvation line (DL) temperature, 250 °C; heat block temperature, 400 °C in ESI source; other parameters were tuned automatically. MRM method was optimized using 200 mM tyramine standard solution. Labsolutions LCMS software (Version 5.86, Shimadzu) was used to control the instrument and process the data.

Enzyme kinetics studies

The same materials were used in the fluorometric MAO inhibition assay. The most active product in the series against the *h*MAO-B enzyme, the compound **2e**, was tested at five different concentrations (IC_{50}/2, IC_{50}, and 2xIC_{50}). The inhibitor (10 µL/well) and *h*MAO-B enzyme solution (50 µL/well) were added to the black flat-bottom 96-well microplate, and incubated at 37°C for 10 min. After the incubation period, the working solution, including reaction buffer, developer solution, OxiRed and various concentrations (20, 10, 5, 2.5, 1.25, and 0.625 µM) of tyramine (40 µL/well) were added. The increase of the fluorescence (Ex/Em = 535/587 nm) was kinetically recorded for 30 min. A parallel experiment was carried out without inhibitor. All processes were assayed in quadruplicate[27].

Statistical analysis

In the enzymatic assay; blank, control and all concentrations of inhibitors were analyzed in quadruplicate and inhibition percentage was calculated by using the following equation:

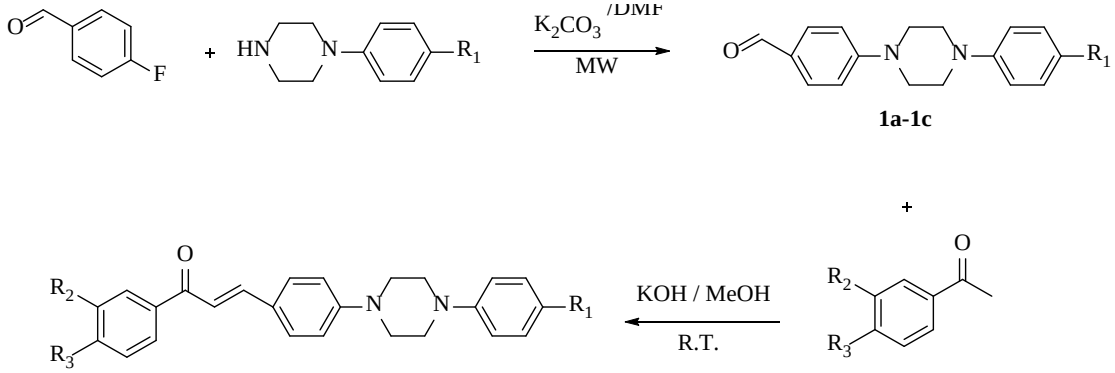
$$\%\ \text{Inhibition} = \frac{(FC_{t2}\text{-}FC_{t1}) - (FI_{t2}\text{-}FI_{t1})}{FC_{t2}\text{-}FC_{t1}} \times 100$$

FC_{t2}: Fluorescence of a control well measured at t_2 time; FC_{t1}: Fluorescence of a control well measured at t_1 time; FI_{t2}: Fluorescence of an inhibitor well measured at t_2 time; FI_{t1}: 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 Microsoft Office Excel 2013; the results were displayed as mean ± standard deviation (SD). Selectivity index (SI) was calculated as IC_{50}(hMAO-A)/ IC_{50}(hMAO-B). The Lineweaver-Burk plots were created using Microsoft Office Excel 2013. K_m and V_{max} values belonging to inhibitor and control were calculated using junction points on the x- and y-axis. The slopes of the Lineweaver-Burk plots were replotted versus the inhibitor concentration and inhibitory constant (K_i) was calculated[30,31]. In the substrate level determination studies, tyramine quantity in blank well was regarded as 100% and substrate percent of compound including wells were calculated as mean ± SD.

RESULTS

In the present study, nine novel 1-(3,4-disubstituedphenyl)-3-[4-(substituted piperazine-1-yl)-phenyl]-prop-2-en-1-one compounds (2a-2i) were synthesized. Synthetic pathway was presented in the **Scheme 1**. Structures of prepared compounds (2a-2i) were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopic methods. The inhibition potencies of the compounds 2a-2i against *h*MAO-A and *h*MAO-B enzymes were evaluated by an *in-vitro* fluorometric method being able to sensitively detect MAO activity.



Scheme 1. Synthesis pathway of the chalcone derivatives (2a-2i).

Compounds **2a-2i** were tested in the concentration range of 10^{-3} to 10^{-9} M. Table 1 presents *h*MAO-A and *h*MAO-B inhibitory potencies of the synthesized compounds. Non-significant inhibition was determined against *h*MAO-A. In contrast, all compounds indicated higher inhibition profile towards *h*MAO-B isoform. Compounds **2c, 2e** and **2i** displayed good inhibition against *h*MAO-B with lower IC_{50} values than 1 μM. The most active compound **2i** displayed significant IC_{50} of 0.13 μM, while reference agent selegiline had an IC_{50} of 0.089 μM. Selectivity indexes (SI) were calculated as IC_{50}(MAO-A) / IC_{50}(MAO-B). Selectivity towards MAO-A increases as the corresponding SI decreases, while selectivity towards MAO-B isoform increases as the corresponding SI increases. It was noted that all synthesized compounds have selective inhibition potency against *h*MAO-B. Compound **2i** displayed very significant SI of 275.27.

Table 1. IC_{50} (μM) and selectivity of the compounds 2a-2i against *h*MAO isoforms.

Compound	IC_{50} (μM) *h*MAO-A	IC_{50} (μM) *h*MAO-B	*SI	Selectivity
2a	38.65±2.27	3.36±0.27	11.50	MAO-B
2b	56.49±2.71	6.42±0.88	8.80	MAO-B
2c	42.88±3.56	0.28±0.03	153.14	MAO-B
2d	28.70±2.43	3.56±0.31	8.06	MAO-B
2e	38.26±1.85	3.49±0.59	10.96	MAO-B
2f	41.64±2.87	0.25±0.08	166.56	MAO-B
2g	44.27±2.75	1.36±0.16	32.55	MAO-B
2h	36.73±1.69	1.73±0.24	21.23	MAO-B
2i	41.29±2.90	0.13±0.06	275.27	MAO-B
Clorgiline	0.0092±0.0006	-	-	MAO-A
Selegiline	-	0.089±0.004	-	MAO-B

Table 2. Optimized MRM parameters for tyramine.

Precusor m/z	Product m/z	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
137.95	121.10	-22.0	-13	-21.0
137.95	77.05	-10.0	-29	-13.0
137.95	103.05	-24.0	-25	-18.0

Comp.	R_1	R_2	R_3	Comp.	R_1	R_2	R_3	Comp.	R_1	R_2	R_3
2a	-H	-Cl	-Cl	**2d**	-F	-Cl	-Cl	**2g**	$-OCH_3$	-Cl	-Cl
2b	-H	-F	-F	**2e**	-F	-F	-F	**2h**	$-OCH_3$	-F	-F
2c	-H	$-OCH_3$	$-OCH_3$	**2f**	-F	$-OCH_3$	$-OCH_3$	**2i**	$-OCH_3$	$-OCH_3$	$-OCH_3$

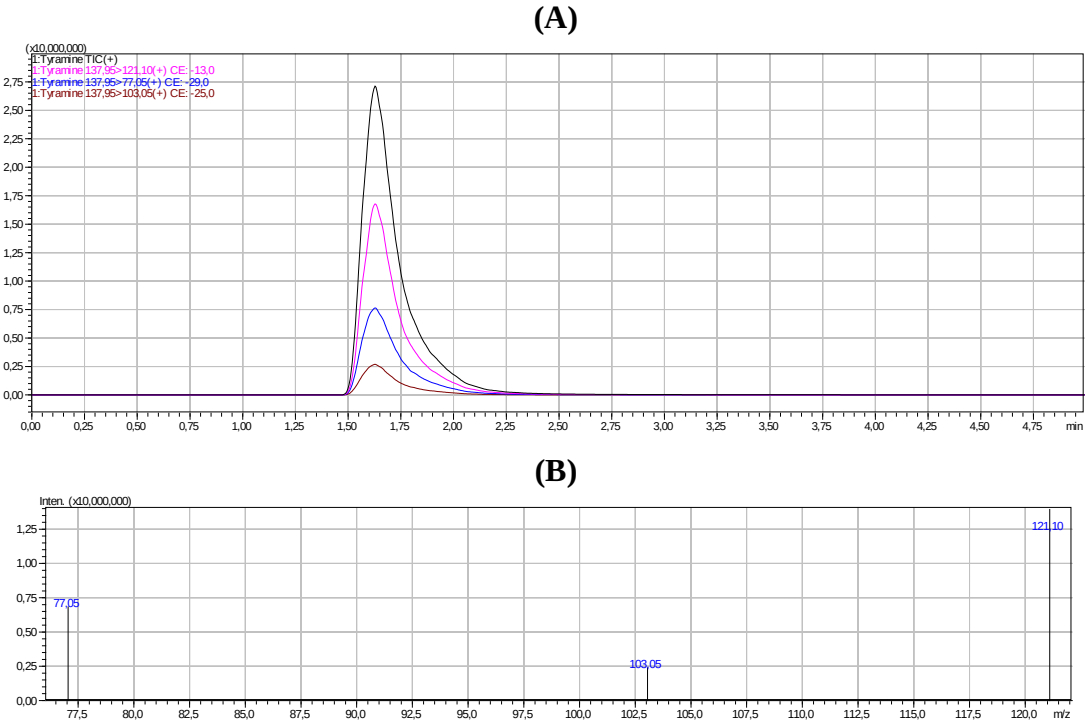


Figure 1. (A) MS Chromatogram of tyramine treated with compound 2i. The outer peak in chromatogram belongs to total ion while the others show the fragment ions of tyramine; (B) MRM spectra of fragment ions of tyramine.

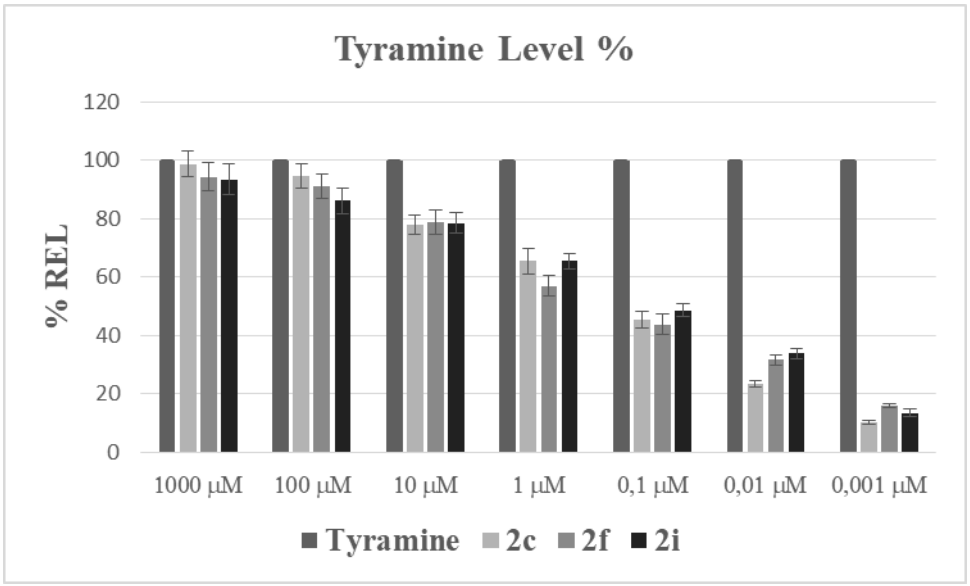
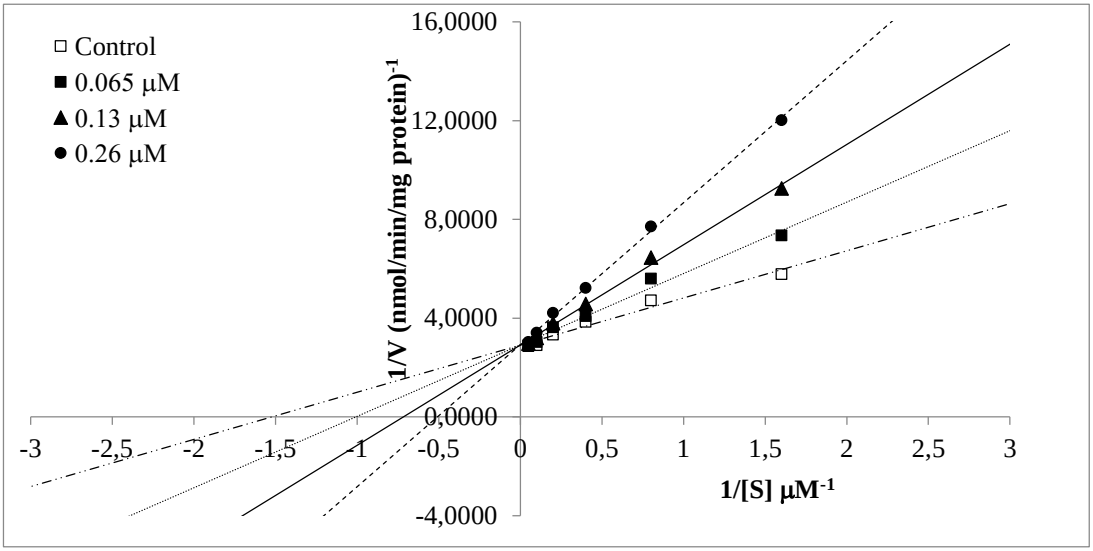


Figure 2. Relative level (REL) of tyramine after treatment with compounds 2c, 2f and 2i.

(A)



(B)

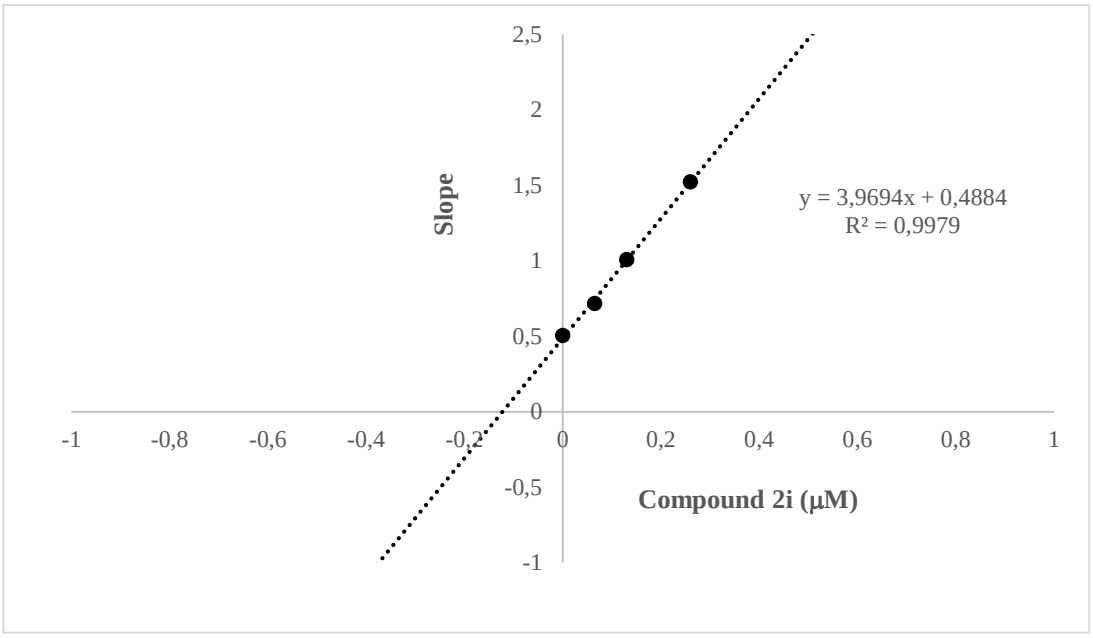


Figure 3. (A) Lineweaver–Burk plots for the inhibition of *h*MAO-B by compound 2i. [S], substrate concentration [µM]; V, reaction velocity [nmol/min/mg]. Inhibitor concentrations are shown at the left. V_{max} value for competitive inhibition was calculated as 0.34. Respective K_m values from 0.26 µM to control: 1.97, 1.39, 0.99 and 0.66 µM (B) Replots of the slopes of the Lineweaver–Burk plots versus inhibitor concentration. K_i was calculated as 0.1230 µM.

In order to support the results of enzyme inhibition assay, quantification of substrate (tyramine) level was performed by an LC-MS/MS instrument using MRM method. Optimized MRM parameters were given in the Table 2. The peaks belonging to fragments of tyramine was presented in Figure 1.

Effects of the compounds **2c**, **2e** and **2i** on tyramine level was given in Figure 2. It was observed that lower inhibitor concentration causes a decrease in the substrate level.The mechanism of *h*MAO-B inhibition was investigated by enzyme kinetics, following the similar procedure applied for the MAO inhibition assay. The linear Lineweaver-Burk graphics were used to estimate the type of inhibition. Enzyme kinetics were analyzed by recording substrate-velocity curves in absence and presence of the most potent compound, **2i**, which was prepared at concentrations of $IC_{50}/2$ (0.065 μM), IC_{50} (0.13 μM), and $2xIC_{50}$ (0.26 μM). In each case, the initial velocity measurements were gained at different substrate (tyramine) concentrations ranging from 20 μM to 0.625 μM. The K_i (intercept on the x-axis) value of compound 2i was determined from the secondary plot of the slope versus varying concentrations. The graphical analysis of steady-state inhibition data for compound **2i** is shown in Figure 3, which demonstrates the compound **2i** as a competitive inhibitor of *h*MAO-B with a K_i value of 0.123 μM.

DISCUSSION

In the present study nine novel chalcone derivatives (**2a-2i**) were synthesized and their chemical structures were clarified by using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS spectral data. Inhibitory potentials of these compounds against MAO-A and MAO-B enzymes have been examined by *in-vitro* fluorometric method. LC-MS/MS method was used to evaluate the effects of test compounds on the substrate level. Finally, enzyme kinetics studies were carried out for the most active compound **2i** by using Lineweaver–Burk plots.

Enzyme inhibition studies suggested that the synthesized compounds have selectivity only towards *h*MAO-B enzyme. Obtained data indicated that compounds **2c**, **2e** and **2i** displayed significant inhibition against *h*MAO-B with lower IC_{50} values than 1 μM. It is determined that the compounds **2c**, **2e** and **2i**, showing these notable *h*MAO-B inhibititory action, bear dimethoxyphenyl substructure. On the other hand, the other compounds, carrying dichloro or difluorophenyl substructures, could not display the same potency. Thus, it can be suggested that dimethoxyphenyl substructure is very important for *h*MAO-B inhibition in terms of structure-activity relationships. Compound **2i**, the most active compound in the serial, displayed significant inhibition by IC_{50} of 0.13 μM. 4-methoxyphenyl substitution of piperazine ring in compound **2i** seems to cause additional enhancement in the *h*MAO-B inhibition when compared to 4-chlorophenyl and 4-fluorophenyl substitution in compounds **2c** and **2f**.

Inhibitors usually react with the enzyme and change it chemically. The binding of an inhibitor can stop entry of a substrate to the enzyme's active site and inhibit the enzyme-substrate reaction. Hence, in the presence of an inhibitor, the enzyme-substrate complex does not form routinely. From this point of view, the quantification of tyramine level was performed by an LC-MS/MS method. It was found out that tyramine level increases in the higher concentrations of compounds **2c**, **2i** and **2f**. Hence, it can be concluded that the enzyme-substrate reaction can occur limitedly in the presence of compounds **2c**, **2i** and **2f** between 1000-10 μM concentrations. Since the enzyme inhibition percentage reduce with the decreasing concentration of the compounds (between 1-0.001 μM), enzyme reaction can occur easier and level of substrate decreases (**Figure 2**). Thus, it can be concluded that results obtained from the substrate level quantification assay are in accordance to the results of the fluorometric enzyme inhibition study.

In the enzyme kinetics studies, the Lineweaver–Burk plot presents the inhibition style: "competitive", "non-competitive" or "mixed-type". In the mixed-typed inhibition, the lines cross neither x- nor y-axis at the same point. Non-competitive inhibition has plots with the same intercept on x-axis but there are different slopes and intercepts on y-axis. Competitive inhibitors possess the same intercept on y-axis but there are diverse slopes and intercepts on x-axis between the two data sets. Our enzyme kinetic study results indicated that pattern of compound **2i** is similar to the pattern of competitive inhibitors (**Figure 3**) and compound **2i** seem to bind the enzyme in a competitive manner with its substrate tyramine.

In summary, among the synthesized compounds **2c**, **2f** and **2i** have inhibitory potentials towards *h*MAO-B enzyme isotype. With its competitive inhibitory nature, it seems that especially compound **2i** has a promising potential for further drug development studies. Advanced detailed preclinical and clinical studies may confirm the therapeutic potencies of these chalcone derivative compounds. For instance, in vivo efficacy of these chalcone derivatives may be

evaluated by using various animal models of Parkinson's disease.

Considering that a large amount of the currently prescribed drugs used in clinic are synthetic, it is crucial to investigate the pharmacological effects of the novel chemical compounds that synthesized for a specific purpose. In this study, some chalcone derivatives were synthesized with the aim of obtaining novel MAO inhibitory compounds, since MAO inhibitors are known to have therapeutic potential for the treatment of various diseases associated with the central nervous system. MAO-B inhibitors, in particular, have a special significance for the pharmacotherapy of Parkinson's disease, for which a radical treatment is not available yet.

In the present study, MAO-B inhibitory activities of chalcone derivative compounds **2c**, **2f** and **2i** were displayed by using some *in vitro* techniques. Observed inhibitory activities of these three compounds seem to be promising for further *in vivo* studies for developing new anti-Parkinson drug candidates. Further toxicity studies are also needed to exhibit safety of these compounds for human being.

Acknowledgment

The author would like to thank Dr. Yusuf ÖZKAY for synthesis of the compounds 2a-2i, Dr. Özgür Devrim CAN and Ümide DEMİR ÖZKAY for their valuable comments on the evaluation of the pharmacological activities, and Serkan LEVENT for the application steps of the instrumental analysis.

REFERENCES

1. Moret C, Briley M. The importance of norepinephrine in depression. Neuropsychiatr Dis Treat. 2011;7:9-13.
2. Stahl SM, Lee-Zimmerman C, Cartwright S, Morrissette DA. Serotonergic drugs for depression and beyond. Curr Drug Targets. 2013;14:578-85.
3. Cavanagh JF, Mueller AA, Brown DR, Janowich JR, Story-Remer JH, Wegele A et al. Cognitive states influence dopamine-driven aberrant learning in Parkinson's disease. Cortex. 2017;90:115-24.
4. Ng J, Papandreou A, Heales SJ, Kurian MA. Monoamine neurotransmitter disorders-clinical advances and future perspectives. Nat Rev Neurol. 2015;11:567-84.
5. Lee J, Lee Y, Park SJ, Lee J, Kim YS, Suh YG et al. Discovery of highly selective and potent monoamine oxidase B inhibitors: Contribution of additional phenyl rings introduced into 2-aryl-1,3,4-oxadiazin-5(6H)-one. Eur J Med Chem. 2017;130:365-78.
6. Zhang HJ, Wang SB, Quan ZS. Synthesis and antidepressant activities of 4-(substituted-phenyl)tetrazolo[1,5-a]quinazolin-5(4H)-ones and their derivatives. Mol Divers. 2015;19:817-28.
7. López-Muñoz F, Alamo C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr Pharm. Des.;2009;15:1563-86..
8. Kayaalp O.S., Rasyonel Tedavi Yönünden Tıbbi Farmakoloji, Pelikan Tıp ve Teknik Kitapçılık Tic. Ltd. Şti., Ankara. 2009;770-95.
9. Yüksel N., Psikofarmakoloji. MN Medikal&Nobel Ltd. Şti., Ankara. 2007;215-6.
10. Tzvetkov NT, Stammler HG, Neumann B, Hristova S, Antonov L, Gastreich M. Crystal structures, binding interactions, and ADME evaluation of brain penetrant N-substituted indazole-5-carboxamides as subnanomolar, selective monoamine oxidase B and dual MAO-A/B inhibitors. Eur J Med Chem. 2017;127:470-92.
11. Van der Walt MM, Terre'Blanche G, Petzer JP, Petzer A. Benzyloxynitrostyrene analogues-A novel class of selective and highly potent inhibitors of monoamine oxidase B. Eur J Med Chem. 2017;125:1193-9.
12. Pathak A, Srivastava AK, Singour PK, Gouda P. Synthetic and natural monoamine oxidase inhibitors as potential lead compounds for effective therapeutics. Cent Nerv Syst Agents Med Chem. 2016;16:81-97.
13. Robakis D, Fahn S. Defining the role of the monoamine oxidase-B inhibitors for Parkinson's disease. CNS Drugs. 2015;29:433-41.
14. Liu Z, Cai W, Lang M, Yan R, Li Z, Zhang G et al. Neuroprotective effects and mechanisms of action of multifunctional agents targeting free radicals, monoamine oxidase B and cholinesterase in Parkinson's disease model. J Mol Neurosci. 2017;61:498-510.
15. Marco-Contelles J, Unzeta M, Bolea I, Esteban G, Ramsay RR, Romero A et al. ASS234, as a new multi-target directed propargylamine for Alzheimer's disease therapy. Front Neurosci. 2016;10:294.
16. Youdim MB. Multi target neuroprotective and neurorestorative anti-Parkinson and anti-Alzheimer drugs ladostigil and m30 derived from rasagiline. Exp Neurobiol. 2013;22:1-10.
17. Go ML, Wu X, Liu XL. Chalcones: an update on cytotoxic and chemoprotective properties. Curr Med Chem. 2005;12:481-99.
18. Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol. 2010;5:1-29.
19. Kim SS, Lim J, Bang Y, Gal J, Lee SU, Cho YC et al. Licochalcone E activates Nrf2/antioxidant response element signaling pathway in both neuronal and

microglial cells: therapeutic relevance to neurodegenerative disease. J Nutr Biochem. 2012;23:1314-23.
20. Nobre-Júnior HV, Oliveira RA, Maia FD, Nogueira MA, de Moraes MO, Bandeira MA et al. Neuroprotective effects of chalcones from Myracrodruon urundeuva on 6-hydroxydopamine-induced cytotoxicity in rat mesencephalic cells. Neurochem Res. 2009;34:1066-75.
21. Chimenti F, Fioravanti R, Bolasco A, Chimenti P, Secci D, Rossi F et al. Chalcones: a valid scaffold for monoamine oxidases inhibitors. J Med Chem. 2009;52:2818-24.
22. Tanaka S, Kuwai Y, Tabata M. Isolation of monoamine oxidase inhibitors from Glycyrrhiza uralensis roots and the structure-activity relationship. Planta Med. 1987;53:5-8.
23. Haraguchi H, Tanaka Y, Kabbash A, Fujioka T, Ishizu T, Yagi A. Monoamine oxidase inhibitors from Gentiana lutea. Phytochemistry. 2004;65:2255-60.
24. Pan X, Kong LD, Zhang Y, Cheng CH, Tan RX. In vitro inhibition of rat monoamine oxidase by liquiritigenin and isoliquiritigenin isolated from Sinofranchetia chinensis. Acta Pharmacol Sin. 2000;21:949-53.
25. Gao GY, Li DJ, Keung WM. Synthesis of potential antidipsotropic isoflavones: inhibitors of the mitochondrial monoamine oxidase-aldehyde dehydrogenase pathway. J Med Chem. 2001;44:3320-8.
26. Morales-Camilo N, Salas CO, Sanhueza C, Espinosa-Bustos C, Sepúlveda-Boza S, Reyes-Parada M et al. Synthesis, biological evaluation, and molecular simulation of chalcones and aurones as selective MAO-B inhibitors. Chem Biol Drug Des. 2015;85:685-95.
27. Mathew B, Mathew GE, Uçar G, Baysal I, Suresh J, Vilapurathu JK et al. Development of fluorinated methoxylated chalcones as selective monoamine oxidase-B inhibitors: Synthesis, biochemistry and molecular docking studies. Bioorg Chem. 2015;62:22-9.
28. Fluorometric Monoamine Oxidase A (MAO-A) Inhibitor Screening Kit, Catalog No: K796-100, Data sheet BioVision, USA.
29. Fluorometric Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit, Catalog No: K797-100, Data sheet BioVision, USA.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
31. Can ÖD, Osmaniye D, Demir Özkay Ü, Sağlık BN, Levent S, Ilgın S et al. MAO enzymes inhibitory activity of new benzimidazole derivatives including hydrazone and propargyl side chains. Eur J Med Chem. 2017;131:92-106.