SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF NEW THIAZOLYLHYDRAZINE DERIVATIVES

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Abstract - Monoamine oxidases (MAO) are enzymes that catalyze the oxidative deamination of monoamines such as dopamine, noradrenaline, adrenaline and serotonin. MAOs are located in the mitochondrial outer membrane of neuronal, glial and other cells. This enzyme consists of two isoforms, MAO-A and MAO-B. While MAO-A selective inhibitors became important in the treatment of depression, MAO-B inhibitors were introduced as promising drugs in the treatment of parkinson's. Recent studies have shown that numerous thiazolylhydrazine derivatives exhibit hMAO inhibitory activity in the micromolar concentration range. Thus, in this study six new thiazolylhydrazine-piperazine derivatives were designed and synthesized to able to have good inhibition profile as cholinesterase inhibitors. The structures of synthesized compounds were confirmed via ^{1}H and ^{13}C NMR spectroscopic methods. The monoamineoxidase inhibitor activity of final compounds was performed. According to enzyme inhibition assay, none of the compounds displayed significant inhibitory activity against MAO-B enzyme. On the other hand, compound 2b showed promising inhibition potency against MAO-A enzyme with IC_{50} values of 0.198±0.009 μM.

Keywords - Monoamine Oxidase Inhibitors, Enzyme Activity, Thiazolylhydrazine, Piperazine

I. INTRODUCTION

The mammalian monoamine oxidases (MAOs) involved in the oxidation of xenobiotic and endogenous monoamines containing monoamine neurotransmitters such as adrenaline, noradrenaline serotonin and dopamine consist of two isoforms, MAO-A and MAO-B. Differences between these two isoforms are their selectivity versus substrates and inhibitors. MAO-A primarily uses serotonin (5HT) as substrate whereas MAO-B uses phenylethylamine and benzylamine as substrate. Other substrates like epinephrine, dopamine, tryptamine and tyramine are oxidized by both isoforms. [1-3]. MAOA, which plays an important role in the catabolism of food-based monoamines, is found in the central nervous system (CNS), lungs, liver and gastrointestinal tract. In contrast MAOB is exclusively expressed in the CNS were it inactivates monoamine-based neurotransmitters. Therefore, MAO dysfunction has been related to several neurological disease such as Parkinson disease (PD) and Alzheimer disease (AD). The bioactivity of MAO-B is considerably improved in the hippocampus regions and the cerebral cortex of AD patients, representing more than 80% of the total MAOs activity in the brain, and the gene expression and activity of MAO-A are also up-regulated in different brain areas. When PD is examined, it is seen that the emergence of oxidative stress contributes to the degeneration of dopaminergic neurons that play an important role in the progression of the disease. Monoamine oxidase B (MAO-B) catalyses the oxidative deamination of dopamine in order to yield hydroperoxide, which participates in Fenton-type reactions with Fe(II) to generate reactive oxygen species. Additionally, MAO-B inhibitors are used in the treatment of PD while MAO-A inhibitors are used in order to treat depression and anxiety, which are also common symptoms related to be risk factors in AD [4-6].

The recent description of the crystal structure of the two isoforms of hMAO helps us to clarify the essential mechanisms. The most important structural difference between these two isozymes is that human MAO-A crystallizes as a monomer while human MAO-B crystallizes as a dimer. The substrate binding regions of both isoforms are substantially hydrophobic, which are substantially covered by aromatic and aliphatic residues. A notable exception is a conserved lysine that interacts with a water molecule that also binds the N5 atom of the flavin cofactor. (Lys296 in MAO B and Lys305 in MAO A) [7,8].

Recent studies have shown that numerous thiazolylhydrazine derivatives exhibit hMAO inhibitory activity in the micromolar concentration range. In addition, the benzylidene-hydrazinothiazole structure was reported to be most active, the activity being further enhanced by substitution at position 4 of the thiazole core. Thiazole and piperazin rings are important pharmacophore groups in medicinal chemistry. For inhibition of MAOs, these groups are generally could be selected and used in the studies [9-12].

For these reasons, there has been a desire to develop a new MAOs inhibitor. Compounds containing thiazolylhydrazine and piperazine rings with known MAO inhibitor activities were synthesized. Therefore, this study was carried out to elucidate the effect of compounds containing these two rings on MAO enzymes.

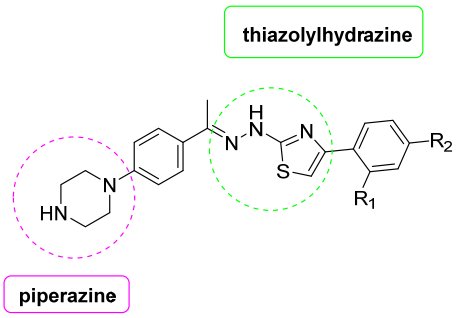


Fig 1: Molecular structure of target compounds

II. DETAILS EXPERIMENTAL

A. Chemistry

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA).

The reactions and the purities of the compounds were observed by thin layer chromatography (TLC) on silica gel 60 F254aluminum sheets obtained from Merck (Darmstadt, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (J) were reported as Hertz.

A1. Synthesis of the 2-(1-(4-(piperazin-1-yl)phenyl)ethylidene)hydrazine-1-carbothioamide (1)

1-(4-(piperazin-1-yl)phenyl)ethan-1-one (0.019 mol, 4gr) and thiosemikarbazide (0.019 mol, 1.73 gr) were dissolved in ethanol (100 mL). This mixture was allowed to stir at 100 °C. After completion of the reaction the mixture was cooled in an ice-bath, precipitated product was filtered, dried, and recrystallized from EtOH.

A2. General synthesis procedure for 4-(2,4-substitutedlphenyl)-2-(2-(1-(4-(piperazin-1-yl) phenyl)ethylidene)hydrazineyl)thiazole derivatives (2a-f)

2-(1-(4-(piperazin-1-yl)phenyl)ethylidene)hydrazine-1-carbothioamide (1) (10 mmol) were refluxed with appropriate 2-bromoacetophenone (10 mmol) in ethanol for 7 h. After TLC screening, the mixture was filtered and the obtained crude products were washed with cold-ethanol, then crystallized from ethanol.

4-(4-nitrophenyl)-2-(2-(1-(4-(piperazin-1-yl)phenyl)ethylidene)hydrazineyl)thiazole (2b)

Yield: 75 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.29 (3H, s, -CH_3), 3.29 (4H, br.s., piperazine), 3.44-3.47 (4H, m, piperazine), 7.04 (2H, d, J=9.0 Hz, 1,4-disubstitutedbenzene), 7.69-7.72 (3H, m, 1,4-disubstitutedbenzene, thiazole), 8.13 (2H, d, J=8.9 Hz, 1,4-disubstitutedbenzene), 8.29 (2H, d, J=9.0 Hz, 1,4-disubstitutedbenzene), 11.24 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.39, 43.03, 45.37, 109.20, 115.70, 124.60, 126.76, 127.24, 128.91, 129.51, 141.34, 146.58, 147.55, 149.08, 150.68, 170.81. (Figure 2 and 3).

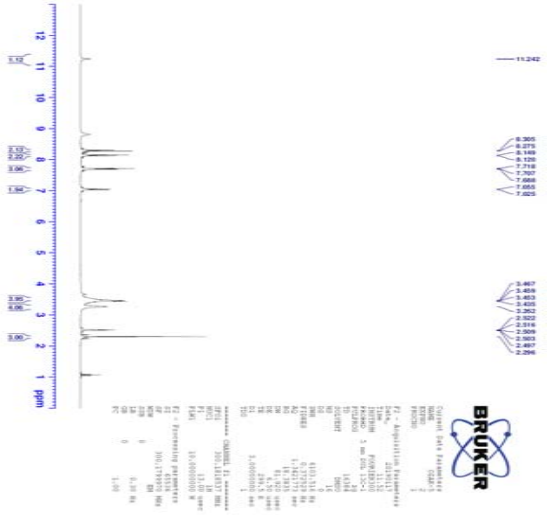


Fig. 2. ^{1}H-NMR spectrum of compound 2b.

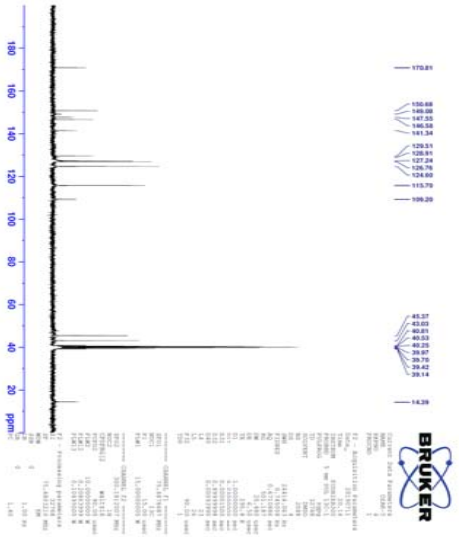


Figure. 3. ^{13}C-NMR spectrum of compound 2b.

B.MAO-A and MAO-B Inhibition Assay

Enzyme inhibition assay was performed according to our recent studies [9, 13-15]. The inhibitor (20 µL / well) and hMAO-A (100 µL / well) or hMAO-B solution (100 µL / well) was added to the fine black bottom 96 well microtiter plate ad the mixture was incubated at 37 ° C for 30 minutes. After the incubation period, the reaction was started by adding a working solution (100 µL/well) (200 mM Amplex Red reagent, 1 U/mL horseradish peroxidase and 1 mM p-tyramine) and all pipetting processes were

performed using a Biotek Precision XS robotic system (USA).

The production of H_2O_2 and, subsequently, of resorufin was quantified at 37 °C in a BioTek-Synergy H1 microplate reader based on the fluorescence generated (excitation, 535 nm, emission, 587 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the inhibitor solution with 2% DMSO (20 µL). To check the probable inhibitory effect of inhibitors on horseradish peroxidase, a parallel reading was achieved by replacing enzyme solutions with %3 H_2O_2 solution (20 mM 100 µL/well). Also, the probable capacity of the inhibitors to modify the fluorescence generated in the reaction mixture due to non-enzymatic inhibition was determined by mixing inhibitor and working solutions. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by phosphate buffer (100 µL/well). Blank, control and all concentrations of inhibitors were analyzed in quadruplicate and inhibition percent was calculated by using following equation:

$$\%\ \text{Inhibition} = \frac{(FC_{t_2} - FC_{t_1}) - (FI_{t_2} - FI_{t_1})}{FC_{t_2} - FC_{t_1}} \times 100$$

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.

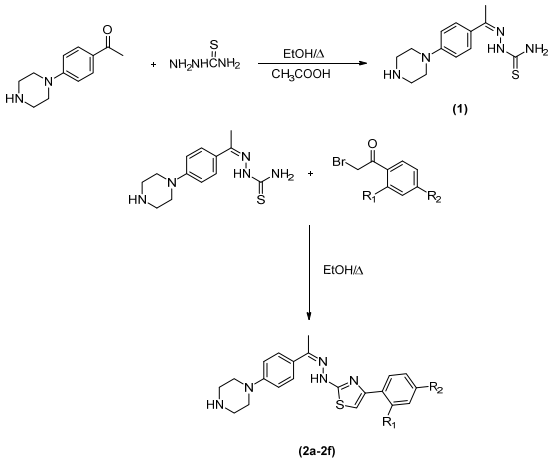
The IC_{50} values were calculated from a dose-response curve obtained by plotting the percentage inhibition versus the log concentration with the use of GraphPad 'PRISM' software (version 5.0). The results were displayed as mean ±standard deviation (SD).

III. RESULTS AND DISCUSSION

A. Chemistry

The compounds 2a-f were synthesized as outlined in Scheme 1. Firstly, 2-(1-(4-(piperazin-1-yl)phenyl)ethylidene)hydrazine-1-carbothioamide (1) was synthesized by a reaction of 1-(4-(piperazin-1-yl)phenyl)ethan-1-one and hydrazinecarbothioamide. Then, obtained thiosemikarbazon (1) and appropriate 2-bromoasetphenone were reacted in order to obtained final compounds (2a-2f). The synthesized compounds were purified by means of crystallization.

The final compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR). In the ^{1}H NMR spectrum, methyl proton had singlet peak at 2.29 ppm. Piperazine protons had multiplet and bronsted singlet peaks between 3.29 ppm and 3.47 ppm. 1,4-disubstituted benzene protons had doublet peaks at 7.04 ppm, 8.13 ppm and 8.29 ppm. Thiazole proton and 1,4-disubstituted benzene protons had multiplet peak between 7.69 ppm and 7.72 ppm. Thiazolylhydrazine NH gave singlet peak at 11.24 ppm. In the ^{13}C NMR spectrum, aliphatic peaks belonging to methyl and piperazine were observed between 14.39 ppm and 45.37 ppm. Aromatic carbons were gained between 109.20 ppm and 170.81 ppm.



Scheme 1. Synthesis way of the target compounds 2a-2f.

Compounds	R_1	R_2
2a	-H	$-CH_3$
2b	-H	$-NO_2$
2c	-H	-Br
2d	$-CH_3$	$-CH_3$
2e	$-OCH_3$	$-OCH_3$
2f	-F	-F

Table 1. R groups of the target compounds 2a-2f.

B. Monoaminoxidase Activity Assay

To evaluate the potential monoamineoxidase (MAO) enzyme affinity of the synthesized novel thiazolylhydrazine derivatives, the inhibitory potential of MAO-A and MAO-B was evaluated according to the modified Ellman method using commercially available selegiline and chlorgiline as reference standards. All tested compounds were prepared at 10^{-3} M and 10^{-4} M concentrations for enzyme activity assay. The percent MAO inhibitory results are outlined in Table 2. According to the activity results, it was seen that none of the compounds showed remarkable enzyme activity against MAO-B enzyme. So, it could be suggested that tested compounds showed better inhibitory activity against MAO-A than MAO-B activity. It can be understood from Table 2, compounds 2a-2f indicated more than 50% activity at the 10^{-3} M concentration, while only compound 2b was able to show more than 50% activity at the 10^{-4} M concentration. Therefore, only compound 2b was selected to evaluate the inhibitory potency on MAO-A enzyme in further concentrations to calculate its IC_{50}

value. The IC_{50} value of the compound 2b was calculated as 0.198±0.009 µM for MAO-A enzyme. The IC_{50} value of standard drug selegiline was 0.040±0.002 µM. According to these findings, compound 2b was found as the most active derivative in the series. But these compounds showed lower inhibition profile than selegiline. Also, it could be suggested by analyzing the enzyme activity results that the 4th position of phenyl ring (R_2 substituent) is important for enzyme inhibition profile. Th presence of an electron withdrawing group such as NO_2 at this position enhance the enzyme activity positively.

Compounds	MAO-A % Inhibition		MAO-B % Inhibition	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
2a	66.28 ±0.98	45.27 ±0.84	44.28 ±0.88	22.16 ±0.69
2b	89.26 ±1.04	82.26 ±1.11	67.29 ±0.91	41.75 ±0.47
2c	69.12 ±0.84	43.20 ±0.70	58.26 ±0.75	39.16 ±0.70
2d	68.29 ±0.81	38.28 ±0.44	40.58 ±0.90	30.16 ±0.81
2e	70.29 ±0.99	42.89 ±0.87	60.28 ±0.90	34.75 ±0.60
2f	67.68 ±0.99	49.20 ±0.72	57.12 ±0.98	41.79 ±0.74
Selegiline	99.85 ±1.10	97.21 ±1.09	-	-
Clorgiline	-	-	98.33 ±1.03	97.19 ±1.09

Table 2. % Inhibition of all compounds 2a–f against MAO-A and MAO-B enzymes.

IV. CONCLUSIONS

Neurodegenerative disease is becoming more common day by day. The low number of clinical drugs used in these diseases and the side effect profiles reveal the need for new drug discovery in this field. As a result, six new thiazolylhydrazine-piperazine derivatives (2a-f) were designed and synthesized through efficient synthetic procedure and screened for their inhibition power toward MAOs. Generally, synthesized compounds demonstrated selective MAO-A enzyme inhibition profile. None of the compounds showed significant inhibitory activity against MAO-B enzyme. In the series compound 2b displayed remarkable MAO-A enzyme inhibition profile compare with the other derivatives.

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