SYNTHESIS AND CHARACTERIZATION OF A NEW SERIES OF THIAZOLYLHYDRAZINE DERIVATIVES CONTAINING DIMETHYLAMINOETHYL MOIETY AS POTENTIAL CHE INHIBITOR

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Abstract - Alzheimer's disease (AD) is the most common of the degenerative brain diseases. Recent studies have shown that numerous thiazolylhydrazine derivatives exhibit cholinesterase (ChE) inhibitory activity in the micromolar concentration range. In addition, the dimethylaminoethyl residue gives the structure similarity to acetylcholine. Thus, in this study five new thiazolylhydrazine containing dimethylaminoethyl derivatives were designed and synthesized to able to have good inhibition profile as cholinesterase inhibitors. The structures of synthesized compounds were confirmed spectroscopic methods (^{1}H NMR and ^{13}C NMR). The ChE inhibitor activity of final compounds was performed. According to enzyme inhibition assay, compound 3e showed promising inhibition potency against AChE enzyme with IC_{50} values of 0.2058±0.007 µM.

Keywords - Alzhmeir, Cholinesterase, Dimethylaminoethyl, Thiazolylhydrazine.

I. INTRODUCTION

Alzheimer's disease (AD) is the most common dementia in the elderly, which is a neurological disease that damages brain cells, causing progressive cognitive impairment such as memory loss, decreased language skills, guidance and judgment [1]. Reports from the Alzheimer's Association estimate that the number of AD patients is currently about 47 million, and the number is estimated to rise to 100 million in 2050 worldwide [2]. For centuries, AD, which has been associated with neurotransmitter acetylcholine deficiency in the brain, can be identified by the deposition of senile plaques consisting mainly of the β-amyloid (Aβ) fragment and neurofibrillary lumps [3]. The current treatment of AD is predominantly called the ''cholinergic hypothesis'' ve and is symptomatic. It is a well-established clinical fact, that in the forebrain of diseased individuals a massive death of cholinergic neurons occurs, leading to reduction of cholinergic innervation in hippocampus and cortical mantle. Partial inhibition of cholinesterases (CEs) followed by increased ACh life compensates for cholinergic deficits. Although AChE was the primary ChE responsible for the hydrolysis of ACh, BChE was found to compensate for AChE, especially when AChE levels were depleted. Inhibition of AChE has a positive effect on the majority of patients [4,5].

Acetylcholine (ACh) is a neurotransmitter associated with cognitive functions and physical awareness. ACh is hydrolyzed with acetylcholine esterase (AChE) or butyrylcholinesterase (BuChE) enzymes, and the imbalance in ACh level is due to AD pathology. It is known that ACh concentration is very low in AD patients compared to healthy people. In CNS BuChE is the primary associated with glial cells and constitutes ~ 10% of ChE activity whereas AChE is found mainly in neurons, constituting ~ 90% of ChE activity in the temporal cortex of normal human brain. Thus, inhibition of AChE enzyme activity is a defined therapeutic approach for the treatment of AD [6,7]. Moreover, recent studies have shown that numerous thiazole derivatives presented as AChE inhibitor [8,9]. Based on this information, compounds having a dimethylaminoethyl group like acetylcholine are designed. The hydrazine group is introduced to bind the dimethylaminoethyl group to the aromatic region. The aromatic part of the structure is the thiazole ring which have been proven to have acetylcholinesterase activity and the phenyl ring attached to it. (Figure 1).

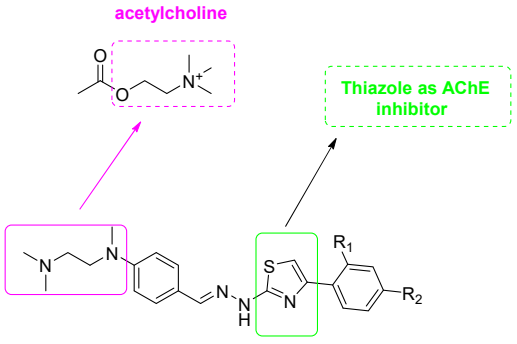


Figure 1. Molecular structure of target compounds

II. MATERIALS AND METHODS

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 4-((2-(Dimethylamino)ethyl)(methyl)amino)benzaldehyde (1)

4-Fluoro benzaldehyde (2.036 mL, 0.019 mol), N^1,N^1,N^2-trimethylethane-1,2-diamine (0.019 mol), and catalytic quanta of potassium carbonate (K_2CO_3) (2.622 g, 0.019 mol) were refluxed in DMF (20 mL) for 24 h. After completion of the reaction, the mixture was poured into ice-water (50 mL), precipitated product was filtered, washed with deionised water, dried, and recrystallized from EtOH.

A2. Synthesis of the 2-(4-((2-(Dimethylamino)ethyl)(methyl)amino)benzylidene)hydrazine-1-carbothioamide (2)

4-((2-(Dimethylamino)ethyl)(methyl)amino)benzaldehyde (0.019 mol, 4gr) and thiosemikarbazide (0.019 mol, 1.73 gr) were dissolved in buthanol (100 mL). This mixture was allowed to stir at 100 ^{o}C. After completion of the reaction the mixture was cooled in an ice-bath and extracted with ethyl acetate as portion. The ethyl acetate phases were combined. And this mixture was evaporated. The residue was scraped off.

A3. General synthesis procedure for target compounds (3a-e)

2-(4-((2-(Dimethylamino)ethyl)(methyl)amino)benzylidene)hydrazine-1-carbothioamide (2) (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.

N^1,N^1,N^2-trimethyl-N^2-(4-((2-(4-(p-tolyl)thiazol-2-yl)hydrazineylidene)methyl)phenyl)ethane-1,2-diamine (3a)

Yield: 79 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.31 (3H, s, -CH_3), 2.84 (3H, s, -CH_3), 2.85 (3H, s, -CH_3), 2.97 (3H, s, -CH_3), 3.21-3.25 (2H, m, -CH_2-), 3.75 (2H, t, J=7.1 Hz, -CH_2-), 6.85 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 7.20-7.23 (3H, m), 7.51 (2H, d, J=8.8 Hz, 1,4-disubstituebenzene), 7.72 (2H, d, J=8.1 Hz, 1,4-disubstituebenzene), 7.96 (1H, s), 11.92 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 21.28, 38.49, 43.07, 46.78, 52.94, 102.71, 112.71, 123.14, 125.98, 128.25, 129.64, 132.14, 137.37, 142.97, 143.09, 149.77, 168.76.

N^1,N^1,N^2-trimethyl-N^2-(4-((2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazineylidene)methyl)phenyl)ethane-1,2-diamine (3b)

Yield: 81 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.84 (3H, s, -CH_3), 2.86 (3H, s, -CH_3), 2.98 (3H, s, -CH_3), 3.40-3.47 (2H, m, -CH_2-), 3.75 (2H, t, J=7.1 Hz, -CH_2-), 6.85 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 7.52 (2H, d, J=8.8 Hz, 1,4-disubstituebenzene), 7.67 (1H, s), 7.96 (1H, s), 8.10 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 8.27 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 12.02 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 38.49, 43.08, 46.79, 52.96, 108.49, 112.70, 123.09, 124.58, 126.77, 128.25, 141.24, 142.95, 146.60, 148.97, 149.79, 169.26.

N^1,N^1,N^2-trimethyl-N^2-(4-((2-(4-(4-(methylsulfonyl)phenyl)thiazol-2-yl)hydrazineylidene)methyl)phenyl)ethane-1,2-diamine (3c)

Yield: 79 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.84 (3H, s, -CH_3), 2.86 (3H, s, -CH_3), 2.98 (3H, s, -CH_3), 3.23-3.29 (5H, m, -CH_2-, -CH_3), 3.75 (2H, t, J=7.0 Hz, -CH_2-), 6.85 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 7.52 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 7.58 (1H, s), 7.94 (2H, d, J=8.5 Hz, 1,4-disubstituebenzene), 7.96 (1H, s), 8.10 (2H, d, J=8.5 Hz, 1,4-disubstituebenzene), 11.99 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 38.49, 43.09, 44.07, 46.79, 52.96, 107.14, 112.71, 123.14, 126.50, 126.50, 127.96, 128.22, 139.58, 139.74, 142.79, 149.33, 149.78, 169.17.

N^1-(4-((2-(4-(2,4-dimethylphenyl)thiazol-2-yl)hydrazineylidene)methyl)phenyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (3d)

Yield: 75 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.28 (3H, s, -CH3), 2.40 (3H, s, -CH3), 2.84 (3H, s, -CH_3), 2.86 (3H, s, -CH_3), 2.98 (3H, s, -CH_3), 3.39-3.48 (2H, m, -CH_2-), 3.75 (2H, t, J=7.1 Hz, -CH_2-), 6.81-6.86 (3H, m), 6.98-7.07 (2H, m), 7.46-7.52 (3H, m), 7.93 (1H, s), 11.81 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 21.14, 21.56, 38.50, 43.09, 46.81, 52.97, 105.64, 112.73, 123.37, 126.82, 128.13, 129.59, 131.86, 132.48, 135.49, 137.07, 142.30, 142.36, 149.67, 167.85.

N^1-(4-((2-(4-(2,4-difluorophenyl)thiazol-2-yl)hydrazineylidene)methyl)phenyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (3e)

Yield: 71 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.79 (3H, s, -CH_3), 2.80 (3H, s, -CH_3), 2.98 (3H, s, -CH_3), 3.18-3.20 (2H, m, -CH_2-), 3.79 (2H, t, J=7.2 Hz, -CH_2-), 6.87 (2H, d, J=8.9 Hz, 1,4-disubstituebenzene), 7.16-7.18 (2H, m), 7.30-7.38 (1H, m, 1,2,4-trisubstituebenzene), 7.50 (2H, d, J=8.8 Hz, 1,4-disubstituebenzene), 7.94 (1H, s), 8.00-8.04 (1H, m, 1,2,4-trisubstituebenzene), 11.93 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 38.44, 42.78, 46.74, 52.63, 104.99, 105.34, 107.73, 112.12, 112.43, 112.64, 123.09, 128.19, 130.71, 142.68, 149.70, 158.39, 161.57, 168.35 (Figure 2 and 3).

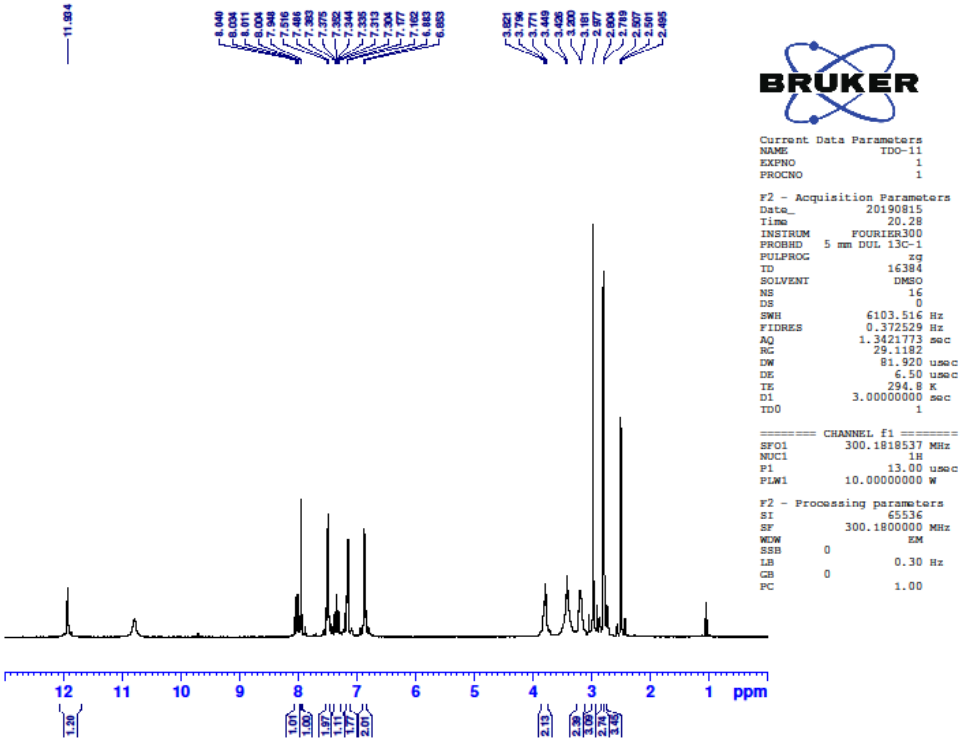


Figure 2. [1]HNMR spectra of compound 3e

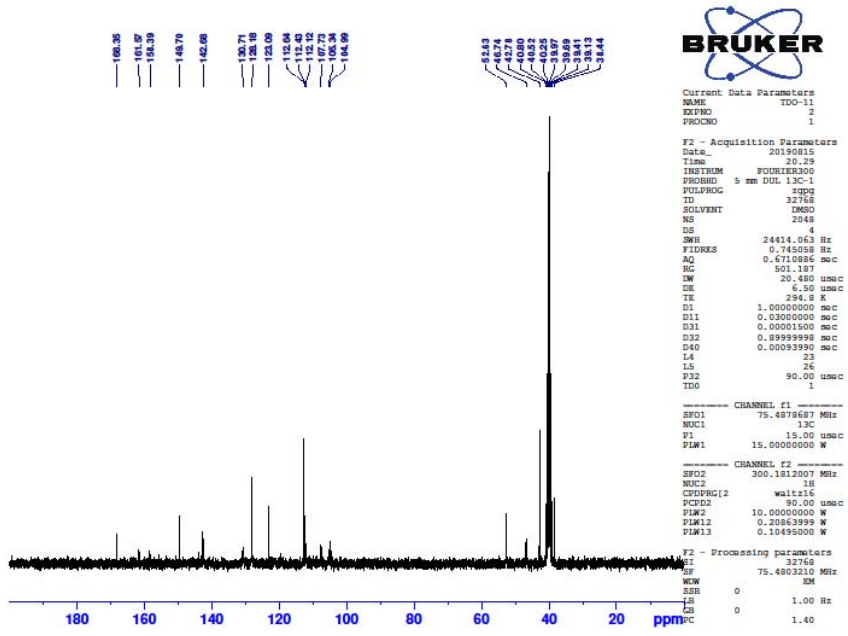


Figure 2. [13]CNMR spectra of compound 3e

B. Anticholinesterase Activity Assay

Modified Ellman's spectrometric method [10] was used for searching the inhibitory affinity of the obtained compounds (3a-3e) on AChE and BChE enzymes as described in our previous studies [11-15]. Donepezil and tacrine were included in the enzyme activity assay as reference drugs. The robotics system, Biotek Precision XS (Winooski, VT, USA), was used for all pipetting procedures in the enzyme inhibition assay. The measurements of percent inhibition at 412 nm were carried out by using a microplate reader, BioTek-Synergy H1 microplate reader (Winooski, VT, USA).

The inhibitory activities of the compounds against AChE and BChE were determined in 96-well plates. Initially, two concentrations (10^{-3} and 10^{-4} M) of the

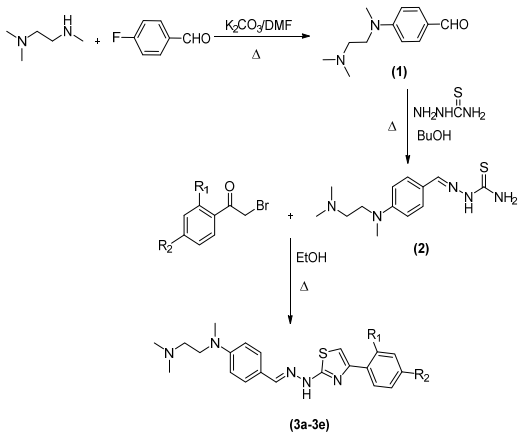
synthesized compounds by using 2% DMSO were used in the first step of enzyme inhibition assay and inhibition potencies were measured. At the second step, the further concentrations (10^{-5}-10^{-9} M) of selected compounds, which showed more than 50% inhibition, were prepared and inhibition assays were performed. The IC_{50} values of the selected compounds were performed as previously demonstrated. Each concentration was analyzed in quadruplicate.

III. RESULT AND DISCUSSION

A. Chemistry

The synthetic route to the target compounds 3a-3e are presented in Scheme 1. 4-((2-(Dimethylamino)ethyl)(methyl)amino)benzaldehyde (1) was obtained with reaction between 4-Fluoro benzaldehyde and N^1,N^1,N^2-trimethylethane-1,2-diamine. Compound 1 were converted into its thiosemicarbazone by reacting 1 with thiosemicarbazide. Finally, target compounds (3a-3i) were prepared via ring closure reaction using compound 2 and an appropriate 2-bromoacetophenone.

The final compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, and LCMSMS).



Compounds	R_1	R_2
3a	-H	$-CH_3$
3b	-H	$-NO_2$
3c	-H	$-SO_2CH_3$
3d	$-CH_3$	$-CH_3$
3e	-F	-F

Scheme 1. Synthetic route of the target compounds

B. Anticholinesterase Activity Assay

The synthesized compounds, which are bearing thiazolylhydrazine moieties, and donepezil and tacrine, which are used as reference drugs, was subjected to modified Ellman method in order to determine their anticholinesterase activity. For first step of enzyme inhibition assay, related compounds were prepared at 10^{-3} M and 10^{-4} M concentrations.

Table 1 presents the percent ChE inhibition of all tested compounds.

Table 1 presented that there is no significant enzyme activity of all synthesized compounds about BChE enzyme. On the other hand, it can be seen that all tested compounds show higher AChE inhibition potency than BChE. At the 10^{-3} M concentration, compounds 3a, 3c and 3e could display more than 50% activity, whereas only compound 3e could show more than 50% enzyme inhibition at the 10^{-4} concentration. So, the second step of enzyme inhibition assay was performed for only compound 3e by preparing its further concentrations (ranging from 10^{-5} M to 10^{-9} M concentrations). This compound showed AChE enzyme inhibition profile with IC_{50} value of 0.2058±0.007 μM. Thus, compound 3e was found as the most active derivative in the series. But, when compared this IC_{50} value with the IC_{50} value of donepezil (0.0287±0.005 μM), compound 3e was not as effective as donepezil on AChE enzyme. This finding was promising in terms of modifying the chemical structures of synthesized compounds in further studies.

Compounds	AChE % Inhibition		BChE % Inhibition	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
3a	58.15 ±0.68	30.18 ±0.56	14.85 ±0.69	10.27 ±0.42
3b	41.21 ±0.97	23.39 ±0.88	18.21 ±0.73	14.76 ±0.60
3c	54.75 ±0.62	39.51 ±0.56	21.65 ±0.66	17.45 ±0.46
3d	47.28 ±0.95	23.41 ±0.41	18.25 ±0.51	11.26 ±0.48
3e	85.30 ±0.88	68.78 ±0.71	20.71 ±0.52	13.62 ±0.50
Donepezil	95.86 ±0.25	88.36 ±1.47	-	-
Tacrine	-	-	98.25 ±1.25	97.16 ±1.06

Table 1. % Inhibition of all compounds 3a–e against AChE and BChE enzymes.

IV.CONCLUSIONS

AD is a progressive neurodegenerative disease related to the presence of extracellular neuritic plaques or senile in the intracellular neurofibrillary confusion and brain. The cholinergic hypothesis is the only theory that explains the nature of Alzheimer's disease and is still accepted. In this study, five new thiazolylhydrazine derivatives including dimethylaminoethyl moiety (3a-e) were designed and synthesized through efficient synthetic procedure and screened for their inhibition power toward ChEs. Generally, synthesized compounds demonstrated selective AChE enzyme inhibition profile. None of the compounds showed significant inhibitory activity

against BChE enzyme. In the series compound 3e displayed remarkable AChE enzyme inhibition profile compare with the other derivatives.

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REFERENCES

[1] M. de Abreu Silva, C.D.B. Sette, A.S. Kiametis, L.A.S. Romeiro, R. Gargano, "Molecular modeling of cardanol-derived AChE inhibitors," Chemical Physics Letters., vol. 731, pp. 136591, July 2019.

[2] N. Jiang, J. Ding, J. Liu, X. Sun, Z. Zhang, Z. Mo, X. Li, H. Yin, W. Tang, S.S. Xie, "Novel chromanone-dithiocarbamate hybrids as multifunctional AChE inhibitors with β -amyloid anti-aggregation properties for the treatment of Alzheimer' s disease," Bioorganic Chemistry., vol. 83, pp. 103027, May 2019.

[3] O. Gungor, M. Kose, T. Taskin Tok, "A biguanide derivative and its cyclic anologue: Structural chracterization, AChE inhibitory effect and docking studies," Journal of Molecular Structure., vol. 1196, pp. 491-498, 2019.

[4] I. Zueva, J. Dias, S. Lushchekina, V. Semenov, M. Mukhamedyarov, T. Pashirova, V. Babaev, F. Nachon, N. Petrova, L. Nurullin, L. Zakharova, V. Ilyin, P. Masson, K. Petrov, "New evidence for dual binding site inhibitors of acetylcholinesterase as improved drugs for treatment of Alzheimer's disease," Neuropharmacology., vol. 155, pp. 131-141, May 2019.

[5] V.K. Nuthakki, R. Mudududdla, A. Sharma, A. Kumar, S.B. Bharate, "Synthesis and biological evaluation of indoloquinoline alkaloid cryptolepine and its bromo-derivative as dual cholinesterase inhibitors," Neuropharmacology., vol. 90, pp. 103062, June 2019.

[6] B. Kumar, V. Kumar, V. Prashar, S. Saini, A.R. Dwivedi, B.Bajaj, D. Mehta, J. Parkash, V. Kumar, "Dipropargyl substituted diphenylpyrimidines as dual inhibitors of monoamine oxidase and acetylcholinesterase," European Journal of Medicinal Chemistry., vol. 177, pp. 221-234, 2019.

[7] N.T. Tzvetkov, H.G. Stammler, M.G. Georgieva, D. Russo, I. Faraone, A.A. Balacheva, S. Hristova, A.G. Atanasov, L. Milella, L. Antonov, M. Gastreich, "Carboxamides vs. methanimines: Crystal structures, binding interactions, photophysical studies, and biological evaluation of (indazole-5-yl)methanimines as monoamine oxidase B and acetylcholinesterase inhibitors," European Journal of Medicinal Chemistry., vol. 179, pp. 404-422, 2019.

[8] Z. Sahin, M. Ertas, C. Bender, E.F. Bülbül, B. Berk, S.N. Biltekin, L. Yurttaş, Ş. Demirayak, ''Thiazole-substituted benzoylpiperazine derivatives as acetylcholinesterase inhibitors.'' Drug Dev. Res. vol. 79, pp.406-425, 2018.

[9] P.A. Channar, M.S. Shah, A. Saeed, S.U. Khan, F.A. Larik, G. Shabir, J. Iqbal, ''Synthesis, characterization and cholinesterase inhibition studies of new arylidene aminothiazolylethanone derivatives.'' Medicinal Chemistry., vol. 13, no.7, pp. 648-653, 2017.

[10] G.L. Ellman, K.D. Courtney, Jr.V. Andres R.M. Featherstone, "A new and rapid colorimetric determination of acetylcholinesterase activity", Biochemical pharmacology, vol. 7, no. 2, pp. 88-95, 1961.

[11] U. Acar Cevik, B.N. Saglik, S. Levent, D. Osmaniye, B. Kaya Cavuşoglu, Y. Ozkay, Z.A. Kaplancikli, "Synthesis and AChE-Inhibitory Activity of New Benzimidazole Derivatives", Molecules, vol. 24, no. 5, pp. 861, 2019.

[12] Ü.D. Özkay, Ö.D. Can, B.N. Sağlık, U. Acar Çevik, S. Levent, Y. Özkay, Ö. Atlı, "Design, synthesis, and AChE inhibitory activity of new benzothiazole–piperazines", Bioorganic & medicinal chemistry letters, vol. 26, no. 22, pp. 5387-5394, 2016.

[13] B.N. Sağlık, S. Ilgın, Y. Özkay, "Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes", European journal of medicinal chemistry, vol. 124, pp. 1026-1040, 2016.

[14] W. Hussein, B.N. Sağlık, S. Levent, B. Korkut, S. Ilgın, Y. Özkay, Z.A. Kaplancıklı, "Synthesis and biological evaluation of new cholinesterase inhibitors for Alzheimer's disease", Molecules, vol. 23, no. 8, pp. 2033, 2018.

[15] F. Tok, B. Koçyiğit-Kaymakçıoğlu, B.N. Sağlık, S. Levent, Y. Özkay, Z.A. Kaplancıklı, "Synthesis and biological evaluation of new pyrazolone Schiff bases as monoamine oxidase and cholinesterase inhibitors", Bioorganic chemistry, vol. 84, pp. 41-50, 2019.

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