ORIGINAL RESEARCH

Biological evaluation of some triazole and triazolothiadiazine derivatives

Usama Abu Mohsen

ABSTRACT: Triazole and triazoles fused with six-membered ring systems are found to possess diverse applications in the field of medicine, agriculture and industry. The 1,2,4-triazole and 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines derivatives were synthesized as cholinesterase inhibitors. The reaction of 1H-indol-3-acetic acid with thiocarbohydrazide gave the 4-amino-3-mercapto-5-[(1H-indol-3-yl)methyl]-4H-1,2,4-triazole. The reaction of triazole with arylaldehydes in ethanol gave the 4-arylideneamino-3-mercapto-5-[(1H-indol-3-yl)methyl]-4H-1,2,4-triazoles. The 3-[(1H-indol-3-yl)methyl]-6-aryl-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines were obtained by condensing triazole with phenacylbromides in absolute ethanol. The chemical structures of the compounds were elucidated by IR, 1H-NMR and FAB+-MS spectral data and elemental analysis. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method. Compounds 1b and 1c can be identified as promising anticholinesterase agents due to their inhibitory effect on AChE with IC50 value of 96.45±8.14 and 76.24±6.42 μM respectively when compared with Donepezil (IC50 =0.056±0.001μM).

KEY WORDS: Indole, triazole, triazolothiadiazine, cholinesterase inhibitors

AFFILIATIONS
Al -Azhar University, Faculty of Pharmacy, Gaza, Filistin

CORRESPONDENCE
Usama Abu Mohsen
E-mail:
usamapharmacy@gmail.com

Received:
04.07.2012
Revision:
30.07.2012
Accepted:
30.07.2012

INTRODUCTION

In drug design; for a more efficient and beneficial therapy for the patients, it has become more important to discover novel and improved drugs, in means of selectivity and potency, through enzyme inhibition (1,2). Cholinesterase inhibitors (ChEIs) have appealed a great deal of interest among researchers owing to their importance in the treatment of myasthenia gravis, glaucoma and Alzheimer's disease (1–3). It becomes more important since the frequency of these diseases, especially Alzheimer's disease, have been increasing in the world. As known, in humans two cholinesterases are present: acetylcholinesterase (AChE), which selectively hydrolyses acetylcholine, and butyrylcholinesterase (BuChE), which is a non-specific cholinesterase. The main difference between two types of cholinesterase is the respective preferences for substrates: the former hydrolyses acetylcholine more quickly; the latter hydrolyses butyrylcholine more quickly. The main function of AChE is the termination of cholinergic neurotransmission, but the function of BuChE is not so clear (1,4). Acetylcholinesterase inhibitors (AChEIs) exert their therapeutic action by inhibiting AChE, which results in the enhancement of cholinergic action. Especially, in medication of the most common age-related neurodegenerative disorder, Alzheimer's disease, AChEIs play a leading role in the first-line treatment against its symptoms (5–7).

1,2,4-Triazole derivatives are well-known with their different biological activities, therefore various 1,2,4-triazole derivatives and their *N*-bridged heterocyclic analogs have been extensively studied. Also, triazole fused six-membered ring systems are found to possess diverse applications in the fields of medicine (8-12). The commonly known systems are triazole fused with pyridines (8), pyridazines (9), pyrimidines (10), pyrazines (11) and triazines (12). The literature survey reveals that there are not many examples of triazoles fused with thiadiazines. Tri-

azolothiadiazines bear a nucleus incorporating the pharmacophoric N-C-S linkage as in the skeleton of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine, that plays a main role in cholinesterase inhibition (13-14).

On the other hand, cholinesterase inhibitor activity has also been reported to be associated with the indolic nucleus (15, 16).

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles.

In the present study, prompted by these observations, the synthesis and cholinesterase inhibitor activity of 1,2,4-triazoles and 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines as hybrid molecules including different pharmacophores were aimed.

EXPERIMENTAL

Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using a Gallenkamp apparatus and are uncorrected. IR: Shimadzu IR-435 spectrophotometer; 1H NMR: Bruker 250 MHz spectrometer; MS: fast atom bombardment mass spectra (FAB-MS) were obtained by VG Quattro mass spectrometer. Microanalytical data were obtained by the Microanalytical Section of Service Center (CNRS, Ecole Normale de Chimie de Montpellier, France).

4-Amino-3-mercapto-5-[(1H-indol-3-yl)methyl]-1,2,4-triazole (A). Equimolar mixture of thiocarbohydrazide (0.1 mol) and 1H-indol-3-acetic acid was heated in an oil-bath at 160–170°C for 2 h. The fused mass thus obtained was dispersed with hot water to obtain the triazole. The product was recrystallized from methanol (17).

IR (KBr) υ_{max} (cm^{-1}): *3305-3181 (N-H), 1574-1423 (C=C and C=N), 1338 (C=S).*

% Anal. Calc. for $C_{11}H_{11}N_5S$: C, 53.86; H, 4.52; N, 28.55, Found: *C, 53.82; H, 4.50; N, 28.52.*

***General procedure for the preparation of* 5-[(1*H*-indol-3-yl) methyl]-4-arylideneamino-3-mercapto-1,2,4-triazoles (1a-e).** To a suspension of aryl aldehyde (0.005 mol) in ethanol (10 ml), was added an equimolar amount of triazole. The suspension was heated until a clear solution was obtained. A few drops of conc. sulfuric acid were added as a catalyst and the solution was refluxed for 3 h on a water bath. The precipitated solid was filtered off and recrystallized from ethanol (18).

***General procedure for the preparation of* 3-[(1*H*-indol-3-yl) methyl]-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (2a-e).** A solution of triazole (0.005 mol) and phenacyl bromide (0.005 mol) in absolute ethanol (30 ml) was heated under reflux for 1 h, cooled to room temperature and then neutralized with ammonium hydroxide. The product thus obtained was recrystallized from ethanol (18).

1a: R:-H, Yield: 60%, M.p.:185-7 °C, IR (KBr) υ_{max} (cm^{-1}): *3401-3131 (N-H),* 1595–1442 (C=C and C=N), 1372 (C=S). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.45 (2H, s, CH_2), 6.95-8.05 (10H, m, aromatic protons), 10.30 (1H, s, N=CH), 11.10 (1H, s, indole NH), 13.85 (1H, s, triazole NH). MS (FAB); *m/z:* 334 [M+1].

% Anal. Calc. $C_{18}H_{15}N_5S$: C, 64.84; H, 4.53; N, 21.00. Found: C, 64.85; H, 4.56; N, 20.97.

1b: R:-Cl, Yield: 72%, M.p.:195-6 °C, IR (KBr) υ_{max} (cm^{-1}): *3389-3105 (N-H),* 1611–1435 (C=C and C=N), 1370 (C=S). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.40 (2H, s, CH_2), 7.10-7.50 (5H, m, indole protons), 8.05-8.45 (4H, m, phenyl protons), 10.30 (1H, s, N=CH), 11.20 (1H, s, indole NH), 13.75 (1H, s, triazole NH). MS (FAB); *m/z:* 368 [M+1].

% Anal. Calc. $C_{18}H_{14}ClN_5S$: C, 58.77; H, 3.84; N, 19.04. Found: C, 58.73; H, 3.87; N, 19.02.

1c: R:-CH_3, Yield: 70%, M.p.:205-8 °C, IR (KBr) υ_{max} (cm^{-1}): *3415-3150 (N-H),* 1601–1435 (C=C and C=N), 1378 (C=S). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3), 4.35 (2H, s, CH_2), 7.05-8.20 (9H, m, aromatic protons), 10.25 (1H, s, N=CH), 11.05 (1H, s, indole NH), 13.85 (1H, s, triazole NH).

MS (FAB); *m/z:* 348 [M+1].

% Anal. Calc. $C_{19}H_{17}N_5S$: C, 65.68; H, 4.93; N, 20.16. Found: C, 65.71; H, 4.96; N, 20.20.

1d: R:-NO_2, Yield: 81%, M.p.:237-9 °C, IR (KBr) υ_{max} (cm^{-1}): *3396-3120 (N-H),* 1630–1430 (C=C and C=N), 1380 (C=S). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.35 (2H, s, CH_2), 6.95-7.50 (5H, m, indole protons), 8.05-8.45 (4H, dd J=8.75 Hz and 8.71 Hz, phenyl protons), 10.20 (1H, s, N=CH), 10.90 (1H, s, indole NH), 13.85 (1H, s, triazole NH). MS (FAB); *m/z:* 379 [M+1].

% Anal. Calc. $C_{18}H_{14}N_6O_2S$: C, 57.13; H, 3.73; N, 22.21. Found: C, 57.17; H, 3.76; N, 22.24.

1e: R:-$N(CH_3)_2$, Yield: 61%, M.p.:200-1 °C, IR (KBr) υ_{max} (cm^{-1}): *3412-3141 (N-H),* 1571–1439 (C=C and C=N), 1365 (C=S). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.10 (6H, s, $N(CH_3)_2$), 4.25 (2H, s, CH_2), 6.85-7.85 (9H, m, aromatic protons), 10.25 (1H, s, N=CH), 11.00 (1H, s, indole NH), 13.75 (1H, s, triazole NH). MS (FAB); *m/z:* 377 [M+1].

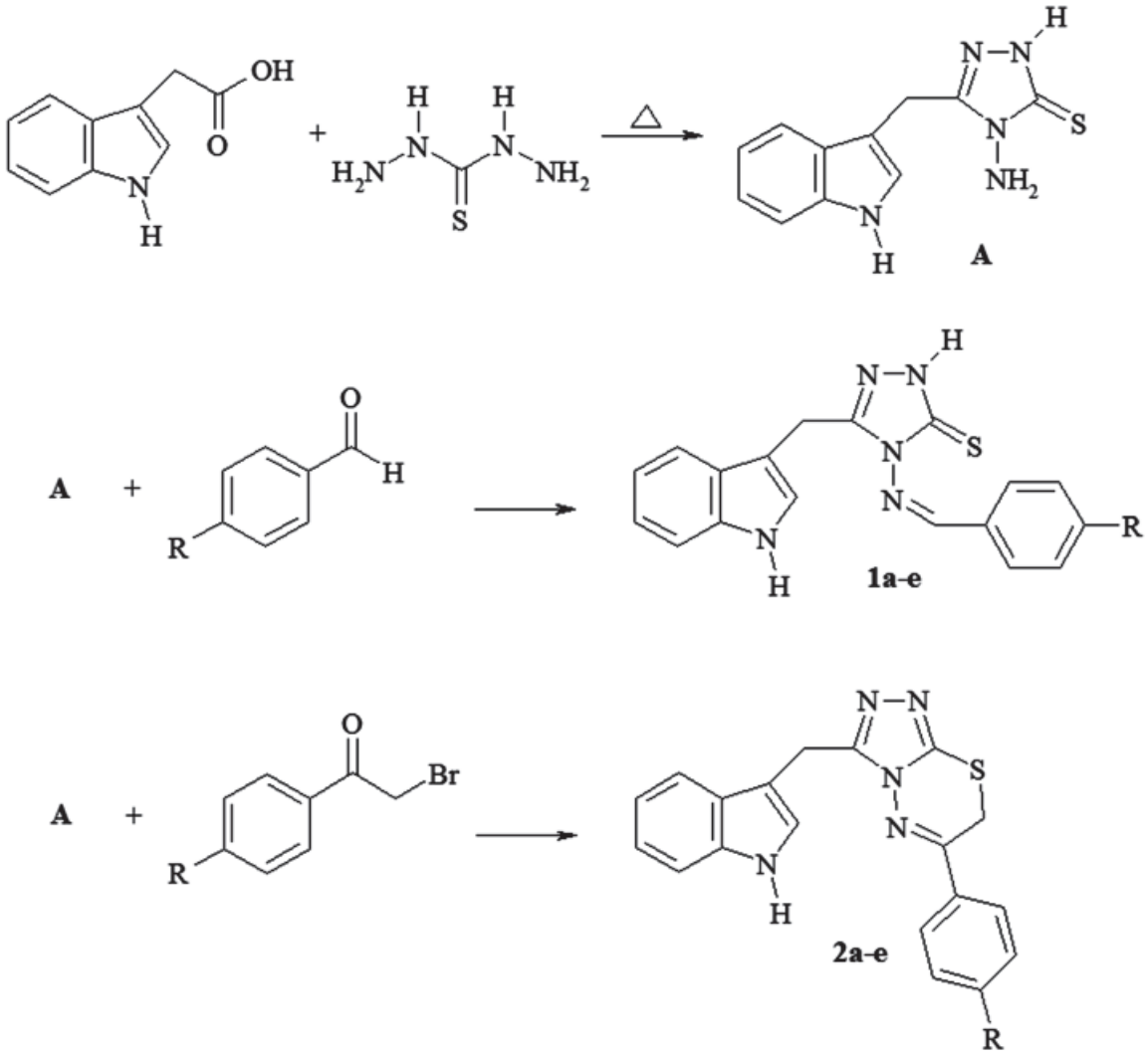
% Anal. Calc. $C_{20}H_{20}N_6S$: C, 63.81; H, 5.35; N, 22.32. Found: C, 63.80; H, 5.35; N, 22.29.

2a: R:-H, Yield: 50%, M.p.:162-3 °C, IR (KBr) υ_{max} (cm^{-1}): *3425-3301 (N-H),* 1615–1444 (C=C and C=N). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.35-4.60 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.85-8.05 (10H, m, aromatic protons), 10.90 (1H, s, indole NH). MS (FAB); *m/z:* 346 [M+1].

% Anal. Calc. $C_{19}H_{15}N_5S$: C, 66.07; H, 4.38; N, 20.27. Found: C, 66.09; H, 4.34; N, 20.31.

2b: R:-Cl, Yield: 45%, M.p.:221-2 °C, IR (KBr) υ_{max} (cm^{-1}): *3431-3291 (N-H),* 1622–1431 (C=C and C=N). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.30-4.45 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.90-8.25 (9H, m, aromatic protons), 11.15 (1H, s, indole NH). MS (FAB); *m/z:* 380 [M+1].

% Anal. Calc. $C_{19}H_{14}ClN_5S$: C, 60.08; H, 3.71; N, 18.44. Found: C, 60.10; H, 3.72; N, 18.43.



SCHEME 1. The synthetic protocol of the compounds.

2c: R:-CH_3, Yield: 45%, M.p.:108-11 °C, IR (KBr) υ_{max} (cm^{-1}): *3405-3311 (N-H),* 1601–1461 (C=C and C=N). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 4.25-4.55 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.75-8.00 (9H, m, aromatic protons), 11.25 (1H, s, indole NH). MS (FAB); *m/z:* 360 [M+1].

% Anal. Calc. $C_{20}H_{17}N_5S$: C, 66.83; H, 4.77; N, 19.48. Found: C, 66.82; H, 4.77; N, 19.51.

2d: R:-NO_2, Yield: 48%, M.p.:225-6 °C, IR (KBr) υ_{max} (cm^{-1}): *3440-3299 (N-H),* 1622–1450 (C=C and C=N). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.30-4.55 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 7.00-8.15 (9H, m, aromatic protons), 11.30 (1H, s, indole NH). MS (FAB); *m/z:* 391 [M+1].

% Anal. Calc. $C_{19}H_{14}N_6O_2S$: C, 58.45; H, 3.61; N, 21.53. Found: C, 58.46; H, 3.64; N, 21.54.

2e: R:-$N(CH_3)_2$, Yield: 39%, M.p.:216-7 °C, IR (KBr) υ_{max} (cm^{-1}): *3417-3320 (N-H),*1631–1431 (C=C and C=N). ^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.10 (6H, s, $N(CH_3)_2$), 4.25-4.40 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 7.05-8.15 (9H, m, aromatic protons), 11.25 (1H, s, indole NH). MS (FAB); *m/z:* 389 [M+1].

% Anal. Calc. $C_{21}H_{20}N_6S$: C, 64.93; H, 5.19; N, 21.63. Found: C, 64.95; H, 5.22; N, 21.62.

Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (19) in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and Donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phos-

TABLE 1. % AChE inbition of the compounds and IC_{50} values.

Comp.	AChE Inhibition (%)			
	100 μM	1 μM	0.01 μM	IC_{50} (μM)
1a	49.17 ±4.35	27.26 ±1.61	13.27 ±1.91	>100
1b	50.19 ±5.24	31.18 ±2.24	14.99 ±1.66	96.45 ±8.14
1c	55.23 ±6.45	33.26 ±2.59	15.23 ±3.22	76.24 ±6.42
1d	45.10 ±3.21	30.26 ±3.14	11.28 ±1.18	>100
1e	47.29 ±1.91	25.02 ±1.98	13.82 ±2.15	>100
2a	29.05 ±4.05	12.65 ±1.99	6.99 ±0.82	>100
2b	28.91 ±3.88	11.49 ±1.27	7.21 ±1.97	>100
2c	19.13 ±2.81	8.16 ±2.16	3.99 ±1.75	>100
2d	37.21 ±3.87	17.19 ±1.97	7.01 ±1.47	>100
2e	40.93 ±1.24	17.26 ±0.96	4.73 ±0.99	>100
Donepezil	96.79 ±5.51	76.45 ±4.75	37.06 ±3.99	0.056 ±0.001

phate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (**1a-e** and **2a-e**) was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (Table 1) (20).

Enzymatic assay

Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2% DMSO at a concentration range of 10^{-1}-10^{-6} mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB) (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

$$\text{Inhibition \%} = (A_C - A_I) / A_C \times 100$$

Where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as Mean ± SD.

RESULTS AND DISCUSSION

In this present work, a series of ten compounds were synthesized (Scheme 1). The structure of the compounds was elucidated by IR, ^{1}H-NMR, FAB$^+$-MS spectral data and elemental analysis. IR spectra of all compounds C=N and C=C bands were observed at about 1630-1430 cm^{-1} region. According to the IR spectroscopic data of the compounds **1a-e** which have triazoline-3-thione structure, the C=S stretching bands observed at about 1380–1365 cm^{-1}. While the NH bands of compounds **1a-e** observed at about 3415-3105 cm^{-1} regions, the NH bands of compounds **2a-e** observed at about 3440-3291 cm^{-1} regions.

In the ^{1}H-NMR spectra of compounds, NH proton of the indole ring was seen as singlet at about 10.90-11.30 ppm. The signal due to indol-CH_2 methylene protons, presented in all compounds, appeared at 4.20–4.60 ppm, as singlets. Due to the electron withdrawing effects of phenyl and triazole ring, the N=CH proton of the compounds (**1a-e)** appeared at 10.20-10.30 ppm as singlet as a result of chemical shift. All the other aromatic and aliphatic protons were observed at the expected regions. Mass spectra (MS (FAB)) of compounds showed M+1 peaks, in agreement with their molecular formula.

The anticholinesterase effects of the compounds (**1a-e** and **2a-e**) were determined by modified Ellman's spectrophotometric method (Table 1). Among these compounds (**1a-e** and **2a-e**), compounds **1b** and **1c** can be identified as promising anticholinesterase agents due to their inhibitory effect on AChE with IC_{50} value of 96.45±8.14 and 76.24±6.42 µM respectively when compared with Donepezil (IC_{50} =0.056±0.001µM). Although compounds **1b** and **1c** include triazole nuclei they showed different levels of anticholinesterase activity. The former bearing Cl atom and methyl group on phenyl ring exhibited the inhibitory effect on AChE with IC_{50} value of 96.45±8.14 and 76.24±6.42 µM, whereas the other substitutions exhibited the inhibitory effect on AChE with >100 µM. On the other hand the trizolothiadiazine derivatives (**2a-e**) did not show notable inhibitory effect on AChE.

As a result, we can say that the triazole derivatives are more active than triazolothiadiazines when their anticholinesterase activity is compared.

ACKNOWLEDGEMENTS

The author would like to thank the staff of Anadolu University Faculty of Pharmacy, Department of Pharmaceutical Chemistry for their valuable suggestions regarding the manuscript.

Bazı triazol ve triazolo tiyadiazin türevlerinin biyolojik değerlendirilmesi

ÖZET: Triazol ve altı üyeli halka sistemleri ile kondense olmuş triazoller; tıp, tarım ve sanayi alanında farklı kullanım alanlarına sahiptir. Bu çalışmada, 1,2,4-triazol ve 1,2,4-triazolo[3,4-b][1,3,4]tiyadiazin türevleri kolinesteraz inhibitörleri olarak sentezlendi. Tiyokarbohidrazid ile 1H-indol-3-asetik asit reaksiyona sokularak, 4-amino-3-merkapto-5-[(1H-indol-3-il)metil]-4H-1,2,4-triazol elde edildi. Arilaldehidler ile triazol etanol içinde reaksiyona sokularak 4-arilidenamino-3-merkapto-5-[(1H-indol-3-il)metil]-4H-1,2,4-triazoller elde edildi. 3-[(1H-indol-3-il)metil]-6-aril-7H-1,2,4-triazolo[3,4-b][1,3,4]tiyadiazinler, triazoller ve fenasilbromürlerin absolu etanol içinde kondensasyonu ile elde edildi. Bileşiklerin kimyasal yapıları, IR, 1H-NMR ve FAB+-MS spektral verileri ve elementel analiz sonuçları yardımı ile aydınlatıldı. Modifiye edilmiş Ellman spektrofotometrik metodu kullanılarak tüm bileşiklerin asetilkolinesteraz (AChE) inhibisyonları incelendi. Bileşikler 1b ve 1c, sırasıyla gösterdikleri 96.45±8.14 ve 76.24±6.42 µM IC50 AChE inhibisyonu değerleri ile Donepezil (IC50 =0.056±0.001µM) ile kıyaslandıklarında ümit verici sonuçlar vermişlerdir.

ANAHTAR SÖZCÜKLER: İndol, triazol, triazolotiyadiazin, kolinesteraz inhibitörleri

REFERENCES

1. Lemke TL, Williams DA. Foye's Principles of Medicinal Chemistry. Lippincott Williams & Wilkins, Baltimore. 2008.
2. Silverman RB. The organic chemistry of drug design and drug action. Elsevier Academic Press, Burlington. 2004.
3. Shen ZX. Brain Cholinesterases: III. Future perspectives of AD research and clinical practice. Med Hypotheses 2004; 63: 298–307.
4. Wilkinson DG, Francis PT, Schwam E, Payne-Parrish J. Cholinesterase inhibitors used in the treatment of Alzheimer's disease: the relationship between pharmacological effects and clinical efficacy. Drugs Aging 2004; 21: 453–78.
5. Grutzendler J, Morris JC. Cholinesterase inhibitors for Alzheimer's disease. Drugs 2001; 61: 41–52.
6. Giacobini E. Cholinesterases: New roles in brain function and in Alzheimer's disease. Neurochem Res 2003; 28: 515–22.
7. Johannsen P. Long-term cholinesterase inhibitor treatment of Alzheimer's disease. CNS Drugs 2004; 18: 757–68.
8. Sadana KA, Mirza Y, Aneja KR, Prakash O. Hypervalent iodine mediated synthesis of 1-aryl/hetryl-1,2,4-triazolo[4,3-a] pyridines and 1-aryl/hetryl 5-methyl-1,2,4-triazolo[4,3-a]quinolines as antibacterial agents. Eur J Med Chem 2003; 38, 533-6.
9. Bussolari JC, Panzica RP. Synthesis and anti-HIV evaluation of 2´,3´-dideoxyimidazo- and nu-triazolo[4,5-d]pyridazine nucleosides. Bioorg Med Chem 1999; 7: 2373-9.
10. Vu CB, Shields P, Peng B, Kumaravel G, Jin X, Phadke D, Wang J, Engber T, Ayyub E, Petter RC. Triamino derivatives of triazolotriazine and triazolopyrimidine as adenosine A2a receptor antagonists. Bioorg Med Chem Lett 2004; 14: 4835-8.
11. Yao G, Haque S, Sha L, Kumaravel G, Wang J, Engber T M, Whalley ET, Conlon PR, Chang H, Kiesman WF, Petter RC. Synthesis of alkyne derivatives of a novel triazolopyrazine as A2a adenosine receptor antagonists. Bioorg Med Chem Lett 2005; 15: 511-5.
12. Vu CB, Peng B, Kumaravel G, Smits G, Jin X, Phadke D, Engber T, Huang C, Reilly J, Tam S, Grant D, Hetu G, Chen L, Zhang J, Petter RC. Piperazine Derivatives of [1,2,4]Triazolo[1,5-a][1,3,5]triazine as Potent and Selective Adenosine A2a Receptor Antagonists. J Med Chem 2004; 47: 4291–9.
13. Shi A, Huang L, Lu C, He F, Li X. Synthesis, biological evaluation and molecular modeling of novel triazole-containing berberine derivatives as acetylcholinesterase and β-amyloid aggregation inhibitors. Bioorg Med Chem 2011; 19: 2298-305.

14. Senapati S, Cheng Y, McCammon JA. In-situ synthesis of a tacrine-triazole-based inhibitor of acetylcholinesterase: configurational selection imposed by steric interactions. J Med Chem 2006; 49: 6222-30.
15. Ismail MM, Kamel MM, Mohamed LW, Faggal SI. Synthesis of New Indole Derivatives Structurally Related to Donepezil and Their Biological Evaluation as Acetylcholinesterase Inhibitors. Molecules 2012; 17: 4811-23.
16. Andradea MT, Limaa JA, Pintoa AC, Rezendea CM, Carvalhob MP, Epifaniob RA. Indole alkaloids from Tabernaemontana australis (Müell. Arg) Miers that inhibit acetylcholinesterase enzyme. Bioorg Med Chem 2005; 13: 4092–5.
17. Misra U, Hitkari A, Saxena AK, Gurtur S, Shanker K. Biologically active indolylmethyl-1,3,4-oxadiazoles, 1,3,4,-thiadiazoles, 4H-1,3-4-triazoles and 1,2,4-triazines. Eur J Med Chem 1996; 31: 629-34.
18. Kaplancıklı ZA, Turan-Zitouni G, Özdemir A, Revial G. New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. Eur J Med Chem 2008; 43: 155-9.
19. Perry NSL, Houghton PJ, Theobald AE, Jenner P, Perry EK. In-vitro inhibition of human erythrocyte acetylcholine esterase by Salvia lavandulae folia essential oil and constituent terpenes. J Pharm Pharmacol 2000; 52: 895-902.
20. Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.