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RESEARCH ARTICLE

Synthesis and Acetylcholinesterase/ Butyrylcholinesterase Inhibitory Activities of (Substituted/Nonsubstituted Benzal)Hydrazone Derivatives of 3-(6-Substituted-3(2*H*)-Pyridazinon-2-yl)propionohydrazides

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Synthesis and Acetylcholinesterase/ Butyrylcholinesterase Inhibitory Activities of (Substituted/Nonsubstituted Benzal)Hydrazone Derivatives of 3-(6-Substituted-3(2H)-Pyridazinon-2-yl) propionohydrazides

Summary

*In this study thirteen new substituted/nonsubstituted benzalhydrazone **V** derivatives of 3-(6-substituted-3(2H)-pyridazinon-2-yl)propionohydrazide derivatives **IV** were synthesized as acetylcholinesterase and butyrylcholinesterase inhibitors. The structures of compounds **V** were elucidated by IR, ^{1}H-NMR and MASS spectra. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChe) inhibitory activity of **V** derivatives was measured using Ellman's method. Only (4-chlorophenylbenzal)hydrazone derivatives of 3-(6-(4-fluorophenyl)-3(2H)-pyridazinon-2-yl)propionohydrazide **Vi** showed an excellent inhibitory effect against AChE. In addition, **Vi** showed a significant inhibitory effect against BChE.*

***Key Words:** Pyridazinone, Benzalhydrazone, AChE inhibitory, BChe inhibitory.*

3-(6-Sübstitüe -3(2H)-Piridazinon-2-il) Propiyionohidrazit Türevlerinin (Sübstitüe/ Nonsübsitüe Benzal) Hidrazon bileşiklerinin Sentezi ve Asetilkolinesteraz/ Butirilkolinesteraz İnhibitörü Aktivitesi

Özet

*Bu çalışmada 3-(6-sübstitüe-3(2H)-piridazinon-2-il) propiyonohidrazit **IV** türevlerinin on üç tane yeni sübstitüe/ nonsübstitüe benzalhidrazon **V** türevi asetilkolinesteraz ve butirilkolinesteraz intihibitörü olarak sentezlenmiştir. Bileşik V türevlerinin yapısı IR, ^{1}H-NMR and MASS spektroskopisi ile aydınlatılmıştır. Bileşik **V** türevlerinin AChE ve BChE inhibitor aktivitesi Ellman ve arkadaşlarının metodu kullanılarak ölçülmüştür. Sadece 3-(6-(4-florofenil)-3(2H)-piridazinon-2-l)propiyionohidrazit **Vi**'nin (4-klorofenilbenzal)hidrazon türevi AChE üzerinde çok iyi aktivite göstermiştir. **Vi** aynı zamanda belirgin BChE inhibitor aktivite göstermiştir.*

***Anahtar Kelimeler:** Piridazinonlar, Benzalhidrazon, AChE inhibitör, BChe inhibitör.*

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system, characterized by loss of cognitive ability and severe behavior abnormalities, which ultimately results in degradation of intellectual and mental activities (1). Three main stages can be clinically characterized in AD (2). The first stage is the so-called amnesia stage, which involves initial loss of short-term memory and lack of emotional spontaneity. In the second stage, the confusion stage, the patient exhibits time and space disorientation, severe mental confusion, and personality changes. The last stage, the dementia stage, involves the total mental incapacity and full dependence of the patient. While the disease itself is not fatal, medical complications associated with AD, usually viral or bacterial infections, lead to the death of the patient (3). Thus, AD is the third largest cause of death in the western world after cardiovascular diseases and cancer. Taking into account the increase in life expectancy and the fact that the incidence of AD increases with advancing age, the devastating effects of this illness are found on rise. AD is currently a major public health problem and will presumably be the most important pathology of this century in developed/developing countries (4).

Despite an enormous amount of work, many aspects of both the etiology and physiological pathways of the disease still remain unclear. To date, the majority of current drug therapeutic approaches to AD have followed the cholinergic hypothesis (5-7). The acetylcholinesterase (AChE) has received important attention as a drug design target for the palliative treatment of the Alzheimer's disease (AD). On this basis, acetylcholinesterase inhibitors (AChEIs) have become the leading strategy for the development of anti-AD agents. The current interest in these drugs has received considerable attention too (8,9). Some anti-AChE agents, such as tacrine, donepezil, rivastigmine, and ensaculin (Fig. 1), show to improvement in memory and cognitive functions, (10) and have been used to treat AD clinically for a long time. However ensaculin, a coumarin derivative, has appeared to prevent or slow down the progressive neurodegeneration in these compounds. Following these considerations, we report the synthesis of novel 3 (2H) -pyridazinones as acetylcholinesterase and butyrylcholinesterase inhibitors.

As seen in Figure 1 ensaculin, a coumarin analogue, composed of a benzopyran with a piperazine substituted moiety has been used clinically for treating AD as AChEIfor a long time. (11) Recently, three series of coumarin analogues (A, B, C) with phenylpiperazine functions as substitution have been designed and synthesized by Zhou et. al. (12) in order to study their potential for treating Alzheimer's (AD) disease (Fig. 2).

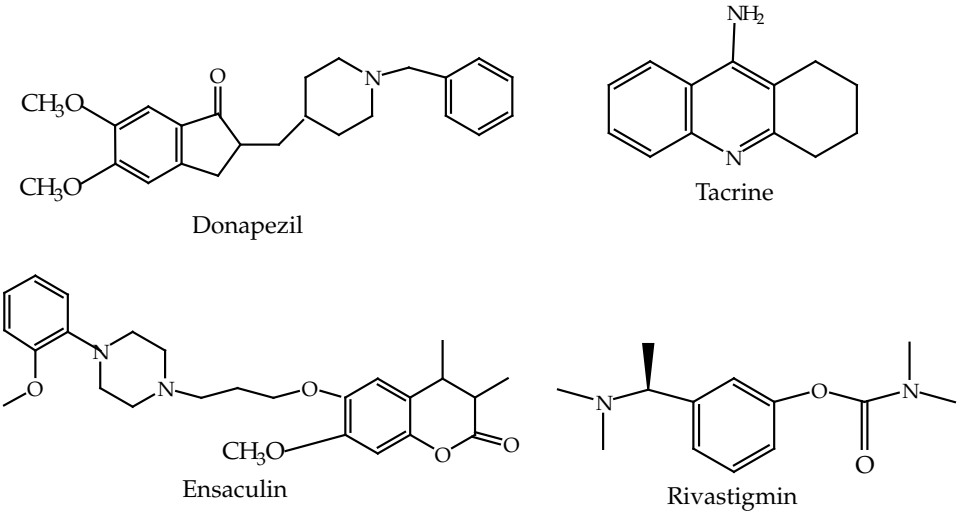


Figure 1. Acetyl cholinesterase inhibitor drugs as FDA approved Alzheimer's disease therapeutics

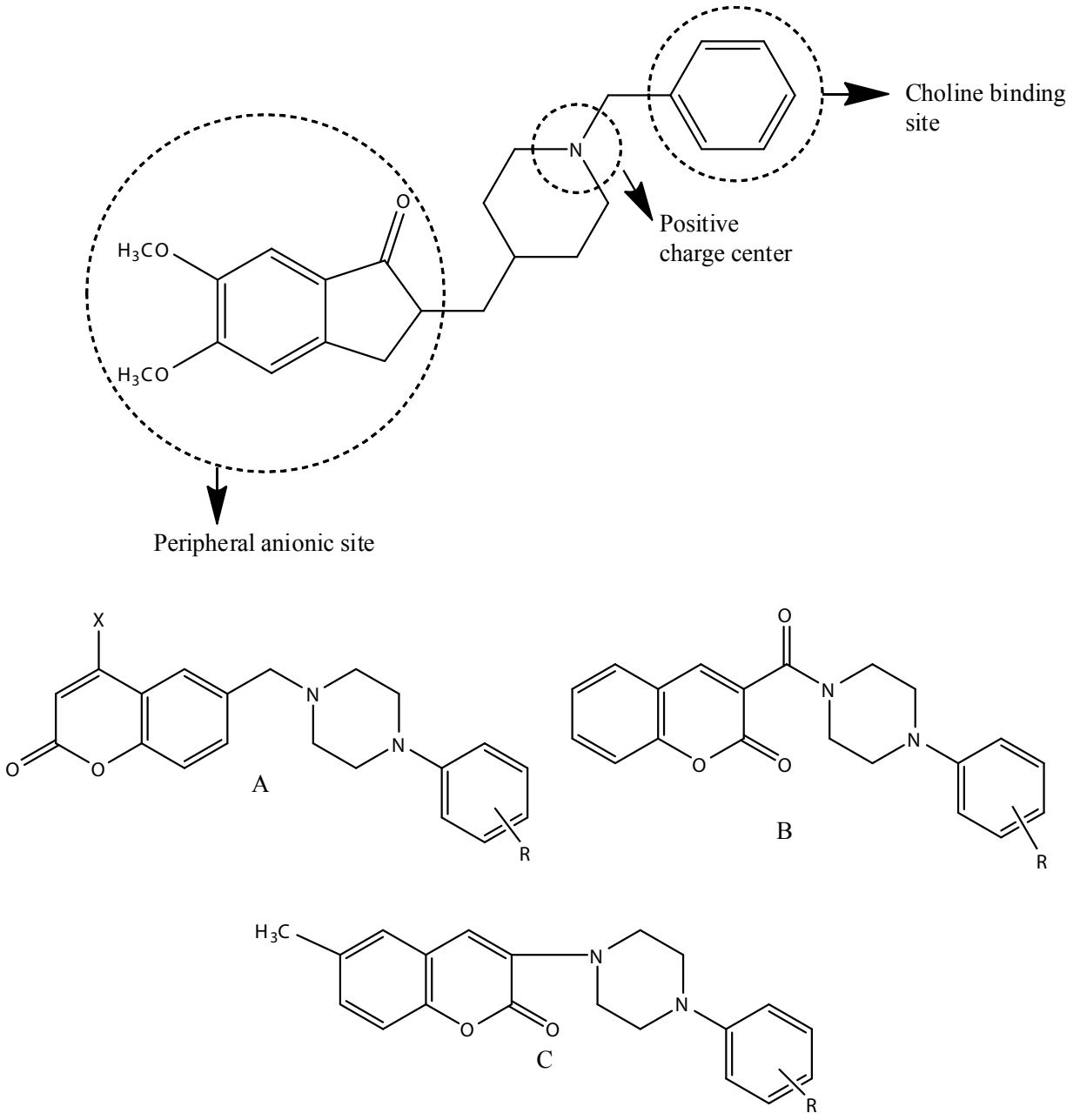
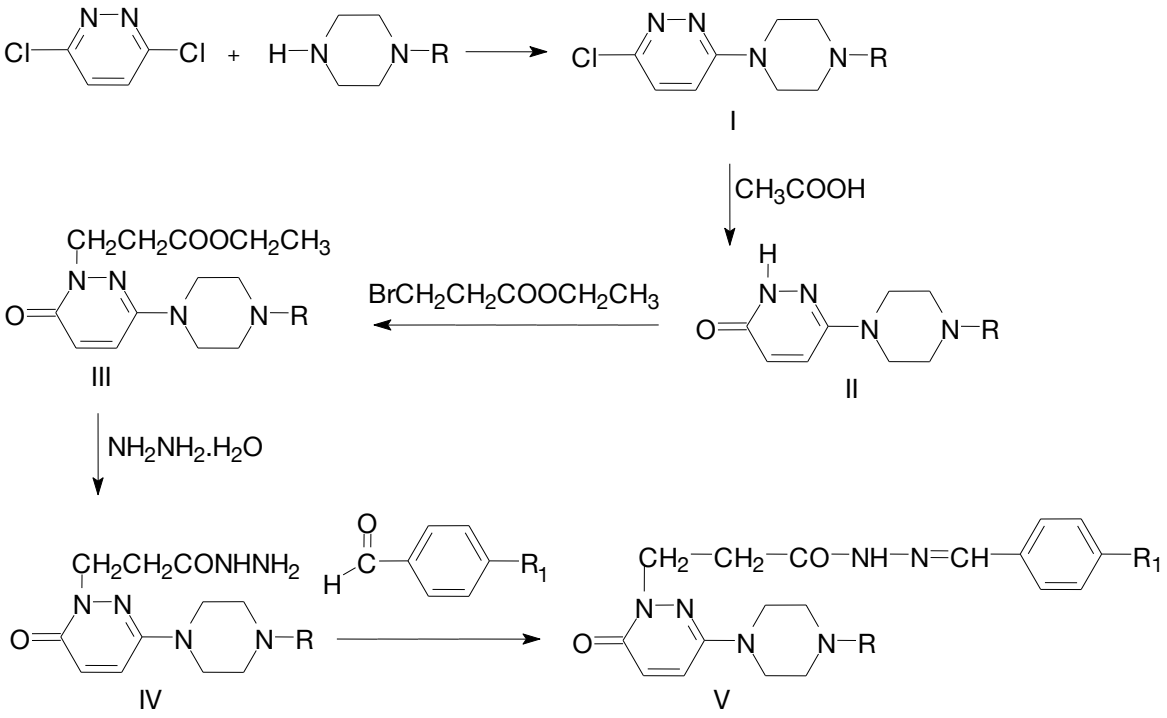


Figure 2. Structural hypothesis for AChEIs and some designed compounds from the literature

Zhou et. al also reported three hypotheses for AChEI activity (12): 1) the coumarin ring, 2H-chromen-2-one heterocycle, a heterocyclic moiety comprising of ensaculin with cognitive functions, demonstrated to be compatible with a high anti-AChE potency, and acted as the peripheral anonic site, which can interact with the peripheral binding site; 2) the nitrogen atom from the phenylpiperazine groups acted as the positive charge center presented in many potent AChE inhibitors, which can interact with the catalytic center of AChE demonstrated by the X-ray crystallographic studies of the AChE/ donepezil and AChE/galantamine complexes and 3) the phenyl ring connecting with the piperazine ring acted as the choline binding site as shown in Figure 2. In addition, a linking chain bearing different amounts of carbon atoms might have the chance to line the wall of the AChE gorge (12). As seen in Scheme 1, title compounds (substituted/nonsubstituted benzal) hydrazone derivatives of 3- (6-substituted-3 (2*H*)



Scheme 1: Synthesis pathway of substituted/nonsubstituted benzalhydrazone **V** derivatives of 3- (6-substituted-3 (2*H*) -pyridazinon-2-yl) propionohydrazide **IV** derivatives.

-pyridazinon-2-yl) propionohydrazide **V** derivatives might have provided structural requirements for AChEI and BChEI activities.

In view of the above mentioned pharmacological active 3 (2*H*) -pyridazinones (13-14) and as a continuation of our effort (15-20) to identify new candidates that may be of value in designing acetylcholinesterase and butyrylcholinesterase inhibitors, we report herein the synthesis of some N'- [(4-Substituephenyl) sulphonyl] -2- [4- (Substituephenyl) -piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/ propionohydrazide **V** derivatives.

MATERIAL and METHODS

Chemistry

The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points of the compounds were determined using an Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected. The IR spectra of the compounds were recorded on a Bruker Vector 22 IR spectrophotometer (Bruker Analytische Messtechnik, Karlrure, Germany). The ^{1}H-NMR of the compounds spectra were recorded on a Bruker 400 MHz-NMR Spectrometer (Rheinstetten, Karlrure, Germany) using tetramethylsilane as an internal standard. All the chemical shifts were recorded as δ (ppm).

High resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in either ESI (+) methods, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA,USA).

6-Substituted-3-chloropyridazines **I** were synthesized in our laboratory according to the reports in the literature (13,14). The synthesis method of **II,** ethyl 6-substituted-3 (2*H*) -pyridazinone-2-ylpropionate **III** and 6-Substituted-3 (2*H*)

-pyridazinone-2-ylpropionohydrazide derivatives **IV** have been reported in our previous study (15-20).

Synthesis of ethyl 6-substituted-3 (2*H*) -pyridazinone-2-yl-propionate derivatives III

A mixture of required 6-substitited-3 (2*H*) -pyridazinones **II** (0.01 mole), ethyl 3-bromo-propionate (0.02 mole) and potassium carbonate (0.02 mole) in acetone (40 ml) was refluxed overnight. After the mixture was cooled, the organic salts were filtered off, the solvent evaporated, and the residue was purified by recrystallization with appropriate alcohol to give the esters.

Synthesis of 6-substituted-3 (2*H*) -pyridazinone-2-yl-propionhydrazide derivatives IV

To methanolic solution of ethyl 6-substituted-3 (2*H*) -pyridazinone-2-yl-propionate derivatives **III** (25 ml, 0.01 mol) hydrazine hydrate (99%) (3 ml) was added and stirred for 3 h at room temperature. The obtained precipitate was filtered off, washed with water, dried and recrystallized from ethanol.

Synthesis of substituted/nonsubstituted benzalhydrazone derivatives of 3-(6-substituted-3 (2*H*) -pyridazinon-2-yl) propionohydrazide V derivatives

Mixture of 6-substituted-3 (2*H*) -pyridazinone-2-yl-propionohydrazide derivatives **IV** (0.01 mol) and appropriate benzaldehyde (0.01 mol) was refluxed in ethanol (15 ml) for 6 h. Then the mixture was poured into ice-water. The formed precipitate was recrystallized from ethanol.

Determination of AChE and BChE inhibitor activities

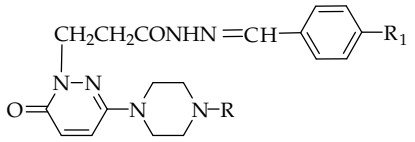
AChE/BChE inhibitor activity was assayed by the spectrophotometric method of Ellman, Courtney et al. (21) Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma) and horse serum BChE (EC 3.1.1.8, Sigma) were employed as the enzyme sources, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) as substrates and 5,5´-dithio-bis (2-nitrobenzoic) acid (DTNB) were also used in the anti-cholinesterase activity determination. All reagents and conditions were the same as described recently (22). Briefly, in this method, 140 µl of 0.1 mM sodium phosphate buffer (pH 8.0), 20 µl of DTNB, 20 µl of test solution and 20 µl of AChE/BChE solution were added with a multichannel automatic pipette (Gilson pipetman, France) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 µl of acetylthiocholine iodide/ butyryl-thiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (VersaMax Molecular Devices, CA, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. The percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH = 8) using the formula *(E-S) /E x 100*, where *E* is the activity of enzyme without the test sample and *S* is the activity of enzyme with the test sample. The experiments were done in triplicate and the results were expressed as average values with S.E.M. (Standard Error Mean).

RESULTS AND DISCUSSION

Chemistry

New 6-substituted-3 (2*H*) -pyridazinone-2-propyl-3-(p-substituted/ nonsubstituted benzal) hydrazone **V** derivatives were synthesized according to Scheme 1. Initially, nucleophilic displacement reaction of commercial 3, 6-dichloropyridazine with arylpiperazines in ethanol afforded 3-chloro-6-substitutedpyridazines **I**. The physical and spectral properties of 3-chloro-6-substitutedpyridazine **I** were accordance with the literature (13,14). Therefore, the next steps of the reaction were carried out without any further analysis. The hydrolysis of 3-chloro-6-substitutedpyridazines **I** was carried out upon heating in glacial acetic acid to afford 6-substituted-3 (2*H*) -pyridazinone **II** derivatives. The formation of these compounds was confirmed by IR spectra of a C = O signal at about 1660 cm^{-1}. Ethyl 6-substituted-3 (2*H*)

Table 1. Physical constant of substituted/nonsubstituted benzalhydrazone **V** derivatives of 3- (6-substituted-3 (2*H*) -pyridazinon-2-yl) propionohydrazide **IV** derivatives



V

Comp.	R	R_1	Yield (%)	Mp (°C)	Molecular formula (M. W)
Va	Phenyl	H	61	198	$C_{24}H_{27}N_6O_2$ MS (ESI+) Calculated: 431.2195 Found: 431.2180
Vb	Phenyl	4-Br	70	219	$C_{24}H_{26}BrN_6O_2$ MS (ESI+) Calculated: 509.1301 Found: 509.1302
Vc	Phenyl	4-Cl	55	217	$C_{24}H_{26}ClN_6O_2$ MS (ESI+) Calculated: 465.1806 Found: 465.1809
Vd	Phenyl	4-F	64	176	$C_{24}H_{26}FN_6O_2$ MS (ESI+) Calculated: 449.2101 Found: 449.2082
Ve	Phenyl	4-CH_3	72	232	$C_{25}H_{29}N_6O_2$ MS (ESI+) Calculated: 445.2352 Found: 445.2337
Vf	Phenyl	3-OCH_3	76	89	$C_{25}H_{29}N_6O_3$ MS (ESI+) Calculated: 461.2301 Found: 461.2300
Vg	Phenyl	4-OCH_3	65	203	$C_{25}H_{29}N_6O_3$ MS (ESI+) Calculated: 461.2301 Found: 461.2317
Vh	4-Fluorophenyl	H	68	164	$C_{24}H_{26}FN_6O_2$ MS (ESI+) Calculated: 449.2101 Found: 449.2089
Vi	4-Fluorophenyl	Cl	71	209	$C_{24}H_{25}ClFN_6O_2$ MS (ESI+) Calculated: 483.1712 Found: 483.1704
Vj	4-Fluorophenyl	F	66	220	$C_{24}H_{25}F_2N_6O_2$ MS (ESI+) Calculated: 467.2007 Found: 467.2003
Vk	4-Fluorophenyl	4-CH_3	73	205	$C_{25}H_{28}FN_6O_2$ MS (ESI+) Calculated: 467.2007 Found: 467.2003
Vm	4-Fluorophenyl	3-OCH_3	69	199	$C_{25}H_{28}FN_6O_3$ MS (ESI+) Calculated: 479.2207 Found: 479.2198
Vn	4-Fluorophenyl	4-OCH_3	67	125	$C_{25}H_{28}FN_6O_3$ MS (ESI+) Calculated: 479.2207 Found: 479.2183

Table 2. Spectral data of substituted/nonsubstituted benzalhydrazone derivatives **V** of 3- (6-substituted-3 (2*H*) -pyridazinon-2-yl) propionohydrazide **IV** derivatives (see Table 1)

Comp	IR (KBr) cm^{-1}				^{1}H NMR (DMSO-d_6) ppm (δ)
	N-H	C = O (hydrazone)	C = O (ring)	C = N	
Va	3187	1695	1649	1595-1515	3.17–3.27 (m, 4H, piperazine b+b'), 3.34–3.44 (m, 4H, piperazine a+a'), 4.04-4.31 (t, -CH_2CH_2-) 6.70-6.83 (d, 1H, pyridazinone H_4), 6.86–7.50 (m, 10H, phenyl protons), 7.64-7.56 (d, 1H, pyridazinone H_5), 7.90-8.10 (1H, s, s N = CH), 11.41-11.30 (1H, s, s NH).
Vb	3188	1690	1654	1593-1510	3.16–3.27 (m, 4H, piperazine b+b'), 3.29–3.37 (m, 4H, piperazine a+a'), 4.05-4.31 (t, -CH_2CH_2-) 6.68-6.78 (d, 1H, pyridazinone H_4), 6.82–7.48 (m, 9H, phenyl protons), 7.52-7.60 (d, 1H, pyridazinone H_5), 7.90 and 7.08 (1H, s, s N = CH), 11.38-11.48 (1H, s, s NH).
Vc	3186	1694	1653	1596-1514	3.16–3.27 (m, 4H, piperazine b+b'), 3.29–3.37 (m, 4H, piperazine a+a'), 4.05-4.31 (t, -CH_2CH_2-) 6.68-6.78 (d, 1H, pyridazinone H_4), 6.82–7.48 (m, 9H, phenyl protons), 7.52-7.60 (d, 1H, pyridazinone H_5), 7.90 and 7.08 (1H, s, s N = CH), 11.38-11.48 (1H, s, s NH).
Vd	3187	1699	1651	1595-1516	3.05–3.23 (m, 4H, piperazine b+b'), 3.27-3.53 (m, 4H, piperazine a+a'), 4.10-4.33 (t, -CH_2CH_2-) 6.70-6.82 (d, 1H, pyridazinone H_4), 6.85–7.50 (m, 9H, phenyl protons), 7.60-7.71 (d, 1H, pyridazinone H_5), 7.89-8.09 (1H, s, s N = CH), 11.31-11.41 (1H, s, s NH).
Ve	3185	1699	1650	1597-1516	2.27-2.25 (s, 3H, CH3), 3.03–3.28 (m, 4H, piperazine b+b'), 3.46-3.57 (m, 4H, piperazine a+a'), 4.06-4.32 (t, -CH_2CH_2-) 6.67-6.77 (d, 1H, pyridazinone H_4), 6.80–7.40 (m, 9H, phenyl protons), 7.44-7.53 (d, 1H, pyridazinone H_5), 7.85-8.04 (1H, s, s N = CH), 11.21-11.33 (1H, s, s NH).
Vf	3183	1698	1654	1590-1510	3.04-3.23 (m, 4H, piperazine b+b'), 3.29–3.39 (m, 4H, piperazine a+a'), 3.52-3.65 (s, 3H, OCH_3), 4.06-4.33 (t, -CH_2CH_2-) 6.71-6.82 (d, 1H, pyridazinone H_4), 6.83–7.33 (m, 9H, phenyl protons), 7.40-7.51 (d, 1H, pyridazinone H_5), 7.86-8.07 (1H, s, s N = CH), 11.23-11.32 (1H, s, s NH).
Vg	3185	1696	1649	1595-1515	3.06-3.24 (m, 4H, piperazine b+b'), 3.27–3.40 (m, 4H, piperazine a+a'), 3.69-3.74 (s, 3H, OCH_3), 4.05-4.31 (t, -CH_2CH_2-) 6.71-6.85 (d, 1H, pyridazinone H_4), 6.95–7.45 (m, 9H, phenyl protons), 7.51-7.60 (d, 1H, pyridazinone H_5), 7.84-8.04 (1H, s, s N = CH), 11.17-11.27 (1H, s, s NH).
Vh	3185	1697	1650	1592-1513	3.29–3.37 (m, 4H, piperazine b+b'), 3.42-3.55 (m, 4H, piperazine a+a'), 4.04-4.31 (t, -CH_2CH_2-), 6.76-6.87 (d, 1H, pyridazinone H_4), 6.91–7.41 (m, 8H, phenyl protons), 7.58-7.65 (d, 1H, pyridazinone H_5), 7.90-8.09 (1H, s, s N = CH), 11.31-11.41 (1H, s, s NH).
IVi	3190	1694	1653	1595-1515	3.22–3.27 (m, 4H, piperazine b+b'), 3.29-3.42 (m, 4H, piperazine a+a'), 4.02-4.30 (t, -CH_2CH_2-), 6.72-6.85 (d, 1H, pyridazinone H_4), 6.89–7.50 (m, 8H, phenyl protons), 7.57-7.67 (d, 1H, pyridazinone H_5), 7.91-8.08 (1H, s, s N = CH), 11.36-11.46 (1H, s, s NH).
IVj	3189	1694	1653	1590-1510	3.21–3.28 (m, 4H, piperazine b+b'), 3.36-3.41 (m, 4H, piperazine a+a'), 4.04-4.28 (t, -CH_2CH_2-), 6.76-6.86 (d, 1H, pyridazinone H_4), 6.87–7.47 (m, 8H, phenyl protons), 7.50-7.57 (d, 1H, pyridazinone H_5), 7.87-8.06 (1H, s, s N = CH), 11.37-11.47 (1H, s, s NH).
Vk	3190	1695	1650	1595-1515	2.26-2.28 (s, 3H, CH_3), 3.22–3.29 (m, 4H, piperazine b+b'), 3.30-3.38 (m, 4H, piperazine a+a'), 4.03-4.30 (t, -CH_2CH_2-), 6.73-6.83 (d, 1H, pyridazinone H_4), 6.86–7.21 (m, 8H, phenyl protons), 7.43-7.50 (d, 1H, pyridazinone H_5), 7.87-8.06 (1H, s, s N = CH), 11.24-11.24 (1H, s, s NH).
Vm	3186	1689	1650	1590-1510	3.22–3.29 (m, 4H, piperazine b+b'), 3.30-3.38 (m, 4H, piperazine a+a'), 3.70-3.78 (s, 3H, OCH_3), 4.01-4.20 (t, -CH_2CH_2-), 6.70-6.78 (d, 1H, pyridazinone H_4), 6.82–7.40 (m, 8H, phenyl protons), 7.43-7.50 (d, 1H, pyridazinone H_5), 7.85-8.06 (1H, s, s N = CH), 11.31-11.41 (1H, s, s NH).
Vn	3188	1685	1649	1595-1515	3.17–3.27 (m, 4H, piperazine b+b'), 3.30-3.41 (m, 4H, piperazine a+a'), 3.71-3.76 (s, 3H, OCH_3), 4.00-4.25 (t, -CH_2CH_2-), 6.72-6.85 (d, 1H, pyridazinone H_4), 6.87–7.44 (m, 8H, phenyl protons), 7.49-7.58 (d, 1H, pyridazinone H_5), 7.85-8.02 (1H, s, s N = CH), 11.16-11.26 (1H, s, s NH).

-pyridazinone-2-ylpropionate **III** derivatives were obtained by the reaction of **II** with ethyl 3-bromopropionate in the presence of K_2CO_3 in aceton. 6-Substituted-3 (2H) -pyridazinone-2-yl propionohydrazide derivatives **IV** were synthesized by the condensation reaction of ethyl 6-substituted-3 (2*H*) -pyridazinone-2-ylpropionate **III** derivatives with hydrazine hydrate (99%). All of the substituted/nonsubstituted benzalhydrazone derivatives of 3- (6-substituted-3 (2*H*) -pyridazinon-2-yl) propionohydrazide **V** derivatives were reported for the first time in this study. Synthesized **V** derivatives are given in Table 1. The physical data, yield and molecular formula of all compounds are reported in Table 1.

Pharmacology

The in vitro inhibition of AChE and BChE for the new synthesized title compounds was determined by the method of Ellman et al. (21) using galantamine as a reference. Only compound **Vi** showed an inhibitory effect against AChE having 98.63 ±1.07% and 99.76 ±1.54% inhibition at 0.1 mM and 0.2 mM concentrations while galantamine exhibited 80.3 **±**1.14 and 92.6 **±**0.1% inhibition at the same concentrations. In addition, **Vi** showed an inhibitory effect against BChE having 32.30 ±1.26% and 75.67 ±1.55% of inhibition at 0.1 mM and 0.2 mM concentrations, respectively. The rest of **V** derivatives have been found non active against either AChE or BChe. This result, because of the recent findings concerning the role of BChE in AD, makes our compound **Vi** endowed with a well-balanced profile of AChE/BChE inhibition, a valuable candidates for further development.

Acknowledgements

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REFERENCES

1. Frank RA, Galasko D, Hampel H, Hardy J, de Leon M J, Mehta PD, Rogers J, Siemers E, Trojanowski QJ. Biological markers for therapeutic trials in Alzheimer's disease: Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. *Neurobiol Aging*. 24: 521-536, 2003.
2. Mosconi L, Brys M, Glodzik-Sobanska L, De Santi S, Rusinek H, de Leon MJ. Early detection of Alzheimer's disease using neuroimaging. *Exp Gerontology*. 42:129-138, 2007.
3. Beard CM, Kokmen E, Sigler C, Smith GE, Petterson T, O'Brien PC. Cause of death in Alzheimer's disease. *Ann Epidemiol*. 6: 195-200, 1996.
4. Sadashiva CT, Chandra JN, Ponnappa KC, Veerabasappa T. Synthesis and efficacy of 1-bis (4-fluorophenyl) -methyl] piperazine derivatives for acetylcholineesterase inhibition, as a stimulant of central cholinergic neurotransmission in Alzheimer's disease. *Bioorg Med Chem Lett*. 16: 3932-3936, 2006.
5. Pakaski M, Kalman J. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochem Internation.* 53: 103-111, 2008.
6. Auld DS, Kornecook TJ, Bastianetto S, Quirion R. Alzheimer's disease and the basal forebrain cholinergic system: relations to β-amyloid peptides, cognition, and treatment strategies. *Prog Neurobiol.* 68:209-245, 2002.
7. Ingram DK, Spangler EL, Iijima S, Ikari H, Kuo H, Greig NH, London ED. Rodent models of memory dysfunction in Alzheimer's disease and normal aging: Moving beyond the cholinergic hypothesis. *Life Sci.* 55: 2037-2049, 1994.
8. Masterman D. Cholinesterase inhibitors in the treatment of Alzheimer's disease and related dementias. *Clin Geriat Med*. 20: 59-68, 2004.
9. Zhang N, Casida JE. Novel Irreversible Butyrylcholinesterase Inhibitors: 2-Chloro-1-(substitutedphenyl) ethylphosphonic Acids. *Bioorg Med Chem*. 10 (5): 1281-1290, 2002.
10. Reale M, Iarlori C, Gambi F, Feliciani C, Isabella L, Gambi D. The acetylcholinesterase inhibitor, Donepezil, regulates a Th2 bias in Alzheimer's disease patients.*Neuropharmacol*. 50: 606-613, 2006.
11. Shen Q, Peng Q, Shao J, Liu X, Huang Z, Pu X, Ma L, Li Y, Chan ASC, Gu L, Synthesis and biological evaluation of functionalized coumarins as

acetylcholinesterase inhibitors. *Eur J Med Chem*. 40: 1307-1315, 2005.

12. X. Zhou, X. Wang, T. Wangb, L. Kong. Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues. *Bioor Med Chem*. 16: 8011–8021, 2008.
13. Boissier JR, Ratouis R, Dumont C. Synthesis and Pharmacological Study of New Piperazine Derivatives. Benzylpiperazines. *J Med Chem*. 6 (5): 541-544, 1963.
14. Elvio B, Parravicini F, Emilio T. Hydrazino Pyridazines Containing Basic Groups in the 6-position and Possessing Hypotensive Activity. *Farmaco de Sci*. 24 (11): 919-929, 1969.
15. Gökçe M, Doğruer D, Şahin MF. Synthesis and Antinociceptive Activity of 6-Substituted-3 (2H) -Pyridazinone Derivatives. *Farmaco*. 56**:** 221-224, 2001.
16. Gökçe M, Şahin MF, Küpeli E, Yeşilada E. Synthesis and Analgesic and Anti-inflammatory Activity of New 3 (2H) -pyridazinone Derivatives. *Arzneim Forsch Drug Res*. 54: 396-401, 2004.
17. Şahin MF, Badıçoğlu B, Gökçe M, Küpeli E, Yeşilada E. Synthesis and analgesic and antiinflammatory activity of methyl [6-substitue-3 (2H) -piridazinone-2-yl] acetate derivatives. *Arch Pharm Med Chem*. 337: 445-452, 2004.
18. Gökçe M, Bakır G, Şahin MF, Küpeli E, Yeşilada E. Synthesis of New Mannich Bases of Arylpyridazinones as Analgesic and Anti-inflammatory Agents. *Arzneim-Forsch Drug Res*. 55: 318-325, 2005.
19. Dündar Y, Gökçe M, Küpeli E, Şahin, MF. Synthesis and Analgesic and Antiinflammatory Activity of Ethyl 6-Substituted-3 (2*H*) -pyridazinone-2-ylacetate Derivatives. *Arzneim-Forsch Drug Res*. 57: 777-781, 2007.
20. Özçelik AB, Gökçe M, Orhan I, Kaynak F, Şahin MF. Synthesis and Antimicrobial, Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities of Novel Ester and Hydrazide Derivatives of 3 (2*H*) -pyridazinone. *Arzneim-Forsch Drug Res*. 60 (7): 452-458, 2010.
21. Elman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharm*. 7: 88-95, 1961.
22. Orhan I, Aslan S, Kartal M, Şener B, Başer KHC. Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes, *Food Chem*. 108: 663-668, 2008.