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RESEARCH ARTICLE

Design, Synthesis and Evaluation of Some Novel 3 (2*H*) -Pyridazinone-2-yl-Acetohydrazides/ propionohydrazides as Acetylcholinesterase and Butyrylcholinesterase Inhibitors

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Design, Synthesis and Evaluation of Some Novel 3 (2H) -Pyridazinone-2-yl-Acetohydrazides/ propionohydrazides as Acetylcholinesterase and Butyrylcholinesterase Inhibitors

Summary

In order to identify new candidates that may be of value in designing acetylcholinesterase and butyrylcholinesterase inhibitors, we report herein the synthesis of some N'- [(4-Substituedphenyl) sulphonyl] -2- [4- (Substituephenyl) -piperazine] -3 (2H) -pyridazinone-2-yl acetohydrazide/ propionohydrazide V derivatives. The in vitro inhibition of AChE and BChE for the synthesized title compounds was determined by the method of Ellman et al. using galantamine as a reference. Some of V derivatives showed inhibitory activities close to galantamine at 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml concentrations. According to screening data, especially the analogues of N'- [(4-Substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperidine/piperazine] -3 (2H) -pyridazinone-2-yl acetohydrazide/ propionohydrazide V, which possessed CF3 on para position of phenylsulfonyl ring improved anti-AChE activity.

Key Words: *Pyridazinones, Acetohydrazides, Acetylcholinesterase, Butyrylcholinesterase.*

Yeni 3 (2H) -Piridazinon-2-il-asetohidrazit/ propiyonohidrazitlerin Dizaynı, Sentezi ve Asetilkolinesteraz ve Butirilkolinesteraz İnhibitörü Olarak Değerlendirilmesi

Özet

Bu çalışmada asetlkolinesteraz ve butirilkolinesteraz inhibitörü tasarım değeri olabilecek yeni adayları aydınlatmak için bazı türevlerinin sentezini rapor ettik. Sentezlenen bileşiklerin in vitro AChE ve BChE inhibisyonları Ellman ve arkadaşlarının metodu ile galantamin referans olarak kullanılarak yapılmıştır. Bazı V türevleri 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml konsantrasyonlarda galantamine yakın aktiviteler göstermişlerdir. Tarama testleri sonuçlarına göre özellikle fenilsülfonil halkasının para pozisyonunda CF3 taşıyan N'- [(4-Substituefenil) sülfonil] -2- [4- (sübstitüefenil) -piperazin] -3 (2H) -piridazinon-2-il asetohidrazit/propiyonohidrazit V analogları anti- AChE aktiviteyi artırmaktadır.

Anahtar Kelimeler: *Piridazinonlar, Asetohidrazit, Asetilkolinesteraz, Butirilkolinesteraz.*

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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

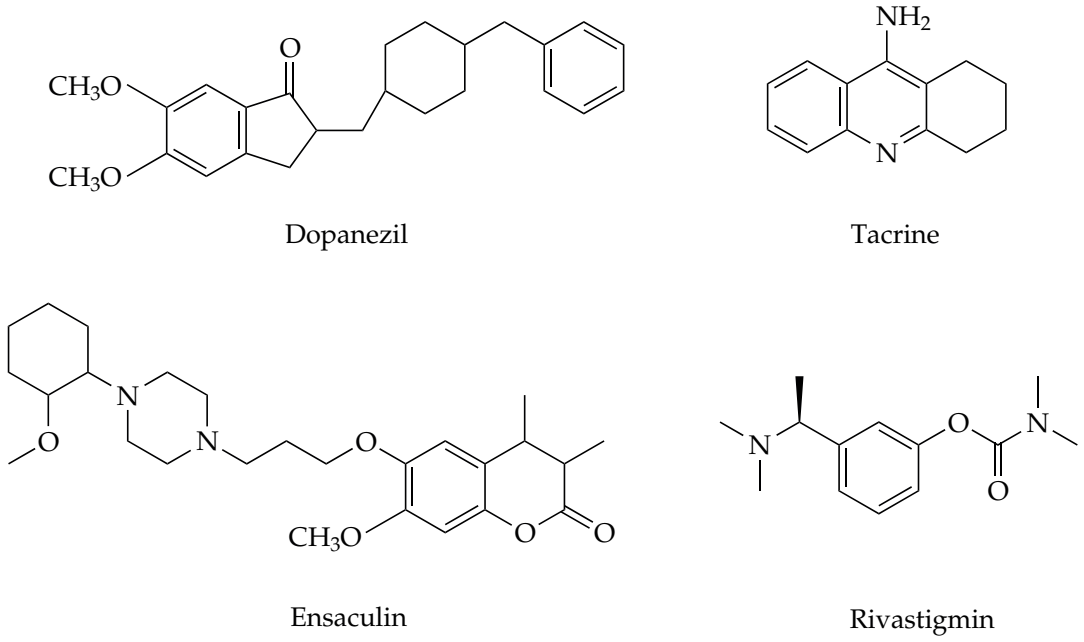


Figure 1. Acetylcholinesterase inhibitor drugs as FDA approved Alzheimer's disease therapeutics

treating AD as AChEI for 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).

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 N'- [(4-substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/propionohydrazide **V** derivatives might have provided structural requirements for AChEI and BuChEI 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-substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/ propionohydrazide **V** derivatives.

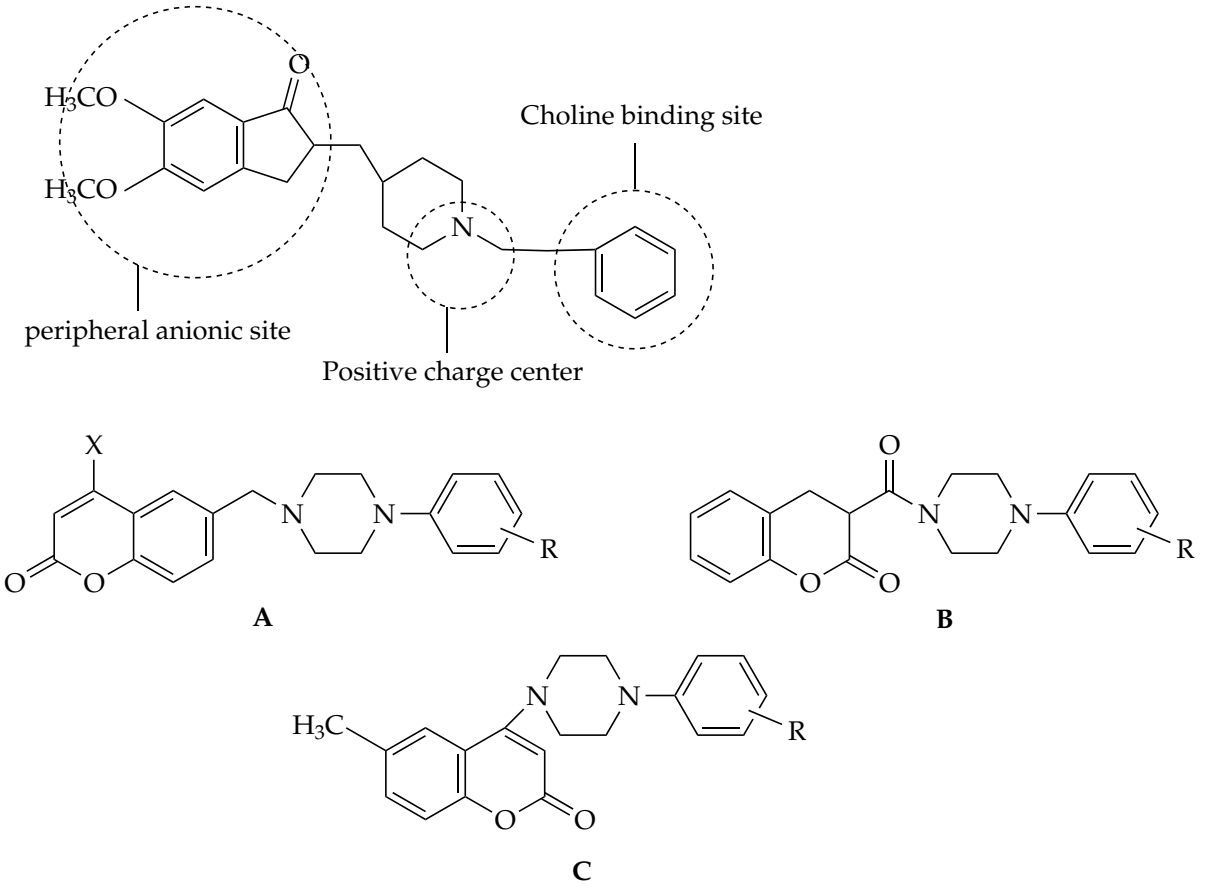
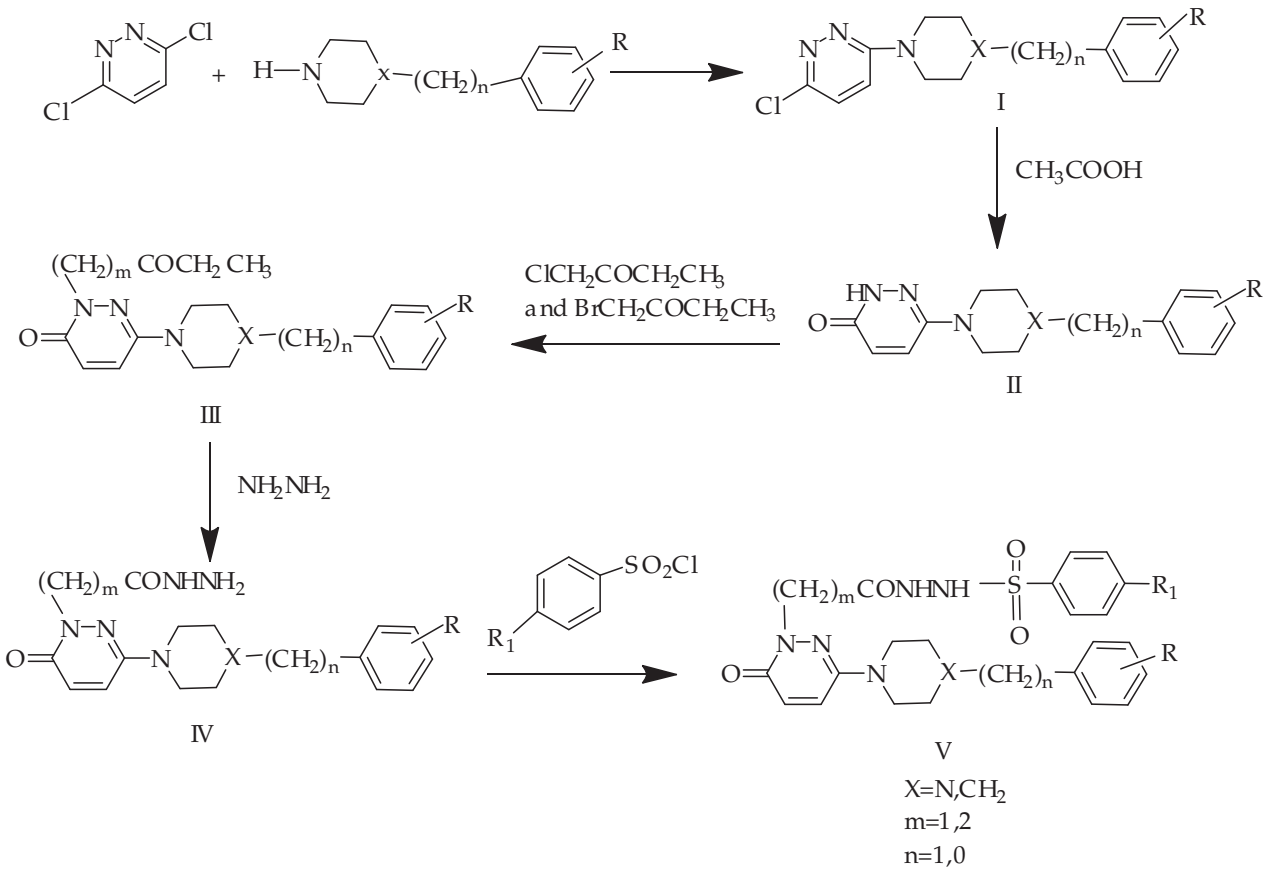


Figure 2. Structural hypothesis for AChEIs and some designed compounds from the literature (12).



Scheme 1 Synthesis of N'- [(4-substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperidine/piperazine] -3 (2H) -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 and **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-acetate/propionate derivatives III

A mixture of required 6-substituted-3 (2*H*) -pyridazinones **II** (0.01 mole), ethyl 3-bromo-acetate / 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-aceto/propionohydrazide derivatives IV

To methanolic solution of ethyl 6-substituted-3 (2*H*) -pyridazinone-2-yl-acetate/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 N'- [(4-substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperidine/piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/ propionohydrazide V derivatives

Substituted benzenesulfonyl chlorides (0.001 mol) were added to the solution of 6-substituted-3 (2*H*) -pyridazinone-2-yl aceto/propionohydrazide derivatives **IV** (0.001 mol) in pyridine (10 mL) at 0°C. The resulting mixture was stirred at room temperature for 5 h. At the end of this period, the reaction mixture was poured into ice water. The precipitate was filtered, dried, and crystallized from an appropriate solvent.

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 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).

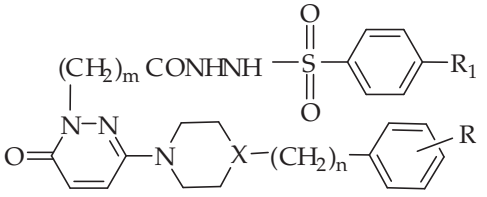
RESULTS and DISCUSSION

Chemistry

N'- [(4-Substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/ propionohydrazide **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-substituted-pyridazine **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*) -pyridazinone-2-ylacetate **IIIa** and ethyl 6-substituted-3 (2*H*) -pyridazinone-2-ylpropionate **IIIb** derivatives were obtained by the reaction of **II** with ethylchloroacetate and ethyl 3-bromopropionate respectively in the presence of K_2CO_3 in acetone. 6-Substituted-3 (2*H*)

-pyridazinone-2-yl acetohydrazide derivatives **IVa** were synthesized by the condensation reaction of ethyl6-substituted-3(2*H*)-pyridazinone-2-ylacetate **IIIa** derivatives with hydrazine hydrate (99%). Similarly **IVb** derivatives were synthesized by the condensation reaction of ethyl 6-substituted-3 (2*H*) -pyridazinone-2-propionate **IIIb** with hydrazine hydrate (99%). N′-[(4-Substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperidine/piperazine] -3 (2*H*) -pyridazinone-2-yl acetohydrazide/propionohydrazide **V** which are target compounds of this study, were synthesized by the condensation of **IV** derivatives with nonsubstituted/p-substi tutedbenzenesulphonylchlorides. The synthesis method of **II**, **III, IV** derivatives has been reported in our previous study (15-20). All of the **V** derivatives were reported for the first time in this study.

Table 1. Physical constant of N′- [(4-substituedphenyl) sulphonyl] -2- [4- (substituedphenyl) -piperidine/ piperazine] -3 (2H) -pyridazinone-2-yl acetohydrazide/propionohydrazide **V** derivatives.



V

Com.	R	R_1	X	m	n	Yield (%)	Mp (°C)	Molecular Formula (MW)
Va	H	H	CH_2	1	1	56	139	$C_{24}H_{27}N_5O_4S$ (Calc.: 482.1862; Found: 482.1849)
Vb	H	4-Cl	CH_2	1	1	52	198	$C_{24}H_{26}ClN_5O_4S$ (Calc.: 516.0123; Found:516.0134)
Vc	H	4-F	CH_2	1	1	48	119	$C_{24}H_{26}FN_5O_4S$ (Calc.: 500.1732; Found:500.1719)
Vd	H	4-CH_3	CH_2	1	1	60	153	$C_{25}H_{30}N_5O_4S$ (Calc.: 496.2019; Found:496.2009)
Ve	H	4-CF_3	CH_2	1	1	43	189	$C_{25}H_{27}F_3N_5O_4S$ (Calc.: 550.1736; Found:550.1732)
Vf	3-CF_3	H	N	2	0	67	188	$C_{24}H_{26}F_3N_6O_4S$ (Calc.: 551.1706; Found:551.1702)
Vg	3-CF_3	4-Cl	N	2	0	63	199	$C_{24}H_{24}ClF_3N_6O_4S$ (Calc.: 584.12.24; Found:584.1320)
Vh	3-CF_3	4-F	N	2	0	54	180	$C_{24}H_{26}F_4N_6O_4S$ (Calc.: 568.1506; Found:568.1501)
Vi	3-CF_3	4-CH_3	N	2	0	70	201	$C_{25}H_{27}F_3N_6O_4S$ (Calc.: 564.5799; Found:564.5791)
Vj	3-CF_3	4-CF_3	N	2	0	49	225	$C_{25}H_{24}F_6N_6O_4S$ (Calc.: 619.1473; Found:619.1476)

Table 2. Spectral data of N'-[(4-substituedphenyl)sulphonyl]-2-[4-(substituedphenyl)-piperidine/piperazine]-3(2H)-pyridazinone-2-yl acetohydrazide/propionohydrazide **V** derivatives (see Table 1 for structural formula).

Comp	IR (KBr)cm^{-1}			^{1}H NMR(DMSO-d_6) ppm (δ)
	C=O (Chain)	C=O (pyridazinone ring)	C=N	
Va	1781	1650	1590	1.09-1.61 (1H, m, piperidin proton), 2.42-2.55 (4H piperidin protons), 3.55 (s, 2H, benzyl CH_2) 3.72-3.82 (4H piperidin protons), 4.39 and 4.67 (2H, s, s, CH_2), 6.78–6.86 (1H, d, pyridazinone H_4), 7.10-7.65 (m, 11H, phenyl protons+pyridazinone H_5), 9.70-9.95 (1H, s, s, N=CH), 10.40 (1H, s, $NHSO_2$).
Vb	1782	1655	1595	1.08-1.61 (1H, m, piperidin proton), 2.49-2.89 (4H piperidin protons), 3.50 (s, 2H, benzyl CH_2) 3.73-3.85 (4H piperidin protons), 4.39 and 4.67 (2H, s, s, CH_2), 6.75–6.86 (1H, d, pyridazinone H_4), 7.17-7.68 (m, 10H, phenyl protons+pyridazinone H_5), 9.78-9.98 (1H, s, s, N=CH), 10.43 (1H, s, $NHSO_2$).
Vc	1781	1665	1597	1.09-1.61 (1H, m, piperidin proton), 2.43-2.71 (4H piperidin protons), 3.52 (s, 2H, benzyl CH_2) 3.72-3.83 (4H piperidin protons), 4.39 and 4.67 (2H, s, s, CH_2), 6.69–6.79 (1H, d, pyridazinone H_4), 7.26-7.85 (m, 10H, phenyl protons + pyridazinone H_5), 9.65-9.96 (1H, s, s, N=CH), 10.43 (1H, s, $NHSO_2$).
Vd	1780	1660	1598	1.09-1-62 (1H, m, piperidin proton), 2.36-2.38 (s, 3H, CH_3 protons), 2.44-2.73 (4H piperidin protons), 3.32-3.55 (4H piperidin protons), 3.56 (s, 2H, benzyl CH_2), 4.38 and 4.69 (2H, s, s, CH_2), 6.71–6.87 (1H, d, pyridazinone H_4) , 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.47 (1H, s, $NHSO_2$).
Ve	1781	1665	1599	1.12-1.59 (1H, m, piperidin proton), 2.47-2.73 4H piperidin protons), 3.42-3.75 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2) , 4.39 and 4.66 (2H, s, s, CH_2), 6.71–6.87 (1H, d, pyridazinone H_4) , 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.45 (1H, s, $NHSO_2$).
Vf	1782	1662	1570	3.24-3.30 (m, 4H, piperazine protons), 3.32-3.36 (m, 4H, piperazine protons), 4.01-4.21 (m, 2H, -CH_2-CH_2CO-), 4.18-4.32(t, 2H, -CH_2-CH_2CO-), 6.81-6.91 (d, 1H, pyridazinone H_4), 7.21-7.78 (m, 10 H, phenyl protons + pyridazinone H_5),), 9.84-9.97 (1H, s, s, N=CH), 10.23 (1H, s, $NHSO_2$).
Vg	1781	1664	1580	3.22-3.28(m, 4H, piperazine protons), 3.36-3.42 (m, 4H, piperazine protons), 4.03-4.22 (m, 2H, -CH_2-CH_2CO-), 4.17-4.29 (t, 2H, -CH_2-CH_2CO-), 6.80-6.88 (d, 1H, pyridazinone H_4), 7.19-7.64 (m, 9 H, phenyl protons + pyridazinone H_5),), 9.76-9.86 (1H, s, s, N=CH), 10.10 (1H, s, $NHSO_2$).
Vh	1781	1663	1585	3.28-3.35 (m, 4H, piperazine protons), 3.40-3.46 (m, 4H, piperazine protons), 4.02-4.18 (m, 2H, -CH_2-CH_2CO-), 4.20-4.30 (t, 2H, -CH_2-CH_2CO-), 6.80-6.87 (d, 1H, pyridazinone H_4), 7.20-7.64 (m, 9 H, phenyl protons + pyridazinone H_5),), 9.90-9.97 (1H, s, s, N=CH), 10.13 (1H, s, $NHSO_2$).
Vi	1780	1665	1590	2.35-2.37 (s, 3H, CH_3 protons), 3.27-3.34 (m, 4H, piperazine protons), 3.35-3.42 (m, 4H, piperazine protons), 4.06-4.24 (m, 2H, -CH_2-CH_2CO-), 4.20-4.23 (t, 2H, -CH_2-CH_2CO-), 6.79-6.86 (d, 1H, pyridazinone H_4), 7.24-7.76 (m, 9 H, phenyl protons + pyridazinone H_5), 9.96-10.05 (1H, s, s, N=CH), 10.20 (1H, s, $NHSO_2$).
Vj	1785	1667	1592	3.27-3.35 (m, 4H, piperazine protons), 3.40-3.45 (m, 4H, piperazine protons), 4.06-4.24 (m, 2H, -CH_2-CH_2CO-), 4.22-4.25 (t, 2H, -CH_2-CH_2CO-), 6.79-6.86 (d, 1H, pyridazinone H_4), 7.24-7.76 (m, 9 H, phenyl protons + pyridazinone H_5),), 10.01-10.05 (1H, s, s, N=CH), 10.28 (1H, s, $NHSO_2$).

2.2. Activity

The in vitro inhibition of AChE and BChE for the synthesized title compounds was determined by the method of Ellman et al. using galantamine as reference. Some of **V** showed inhibitory activities close to galantamine at 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml concentrations.

Table 3. Percentage inhibition ±S.E.M. values of N′-[(4-substituedphenyl)sulphonyl]-2-[4-(substituephenyl)-piperidine/ piperazine]-3(2*H*)-pyridazinone-2-yl acetohydrazide/propionohydrazide **V** derivatives against acetylcholinesterase (AChE)

Com.	Percentage inhibition±S.E.M.			
	25 μg/ml	**50 μg/ml**	**100 μg/ml**	**200 μg/ml**
Va	6.71±1.57	18.49±0.19	47.80±4.27	56.98 ±1.34
Vb	12.99±4.83	14.08±0.35	32.42±3.93	63.56 ±1.51
Vc	21.29±0.60	32.99±4.83	34.08±0.35	52.42±3.93
Vd	16.71±1.57	28.49±0.19	47.80±4.27	58.08±9.13
Ve	34.99±0.63	43.56±4.04	67.81±4.53	77.16 ± 1.33
Vf	17.41±2.38	21.18±2.72	22.35±1.35	26.94±.23
Vg	17.16±1.21	33.67±0.70	41.67±3.38	43.21 ± 1.66
Vh	51.33±5.89	70.53±7.71	77.82±1.49	82.46 ± 1.66
Vi	19.57 ± 0.86	23.23 ±1. 39	30.28 ± 1.30	54. 21 ± 1.30
Vj	72.46 ± 1.96	83.38 ± 1.38	88.96 ± 1.03	97. 23 ± 1.54
Galant.	63.36 ± 0.51	78.33 ± 0.75	84.42 ± 0.67	96.45 ± 1.62

According to screening data, especially the analogue of N′-[(4-substituedphenyl)sulphonyl]-2-[4-(substituedphenyl)-piperidine/piperazine]-3(2*H*)-pyridazinone-2-yl acetohydrazide/ propionohydrazide **V,** which possessed CF_3 on para position of phenylsulfonyl ring improved anti-AChE activity. **Ve** and **Vj** derivatives have shown potent AChE inhibitory activity. AChE inhibitory activity of the compound **Vj** is superior to the reference compound galantamine at all of the studied concentrations. Also **Vj** showed better inhibitory effect than galantamine against BChE

Table 4. Percentage inhibition ±S.E.M. values of N′-[(4-substituedphenyl)sulphonyl]-2-[4-(substituedphenyl)-piperidine/ piperazine]-3(2*H*)-pyridazinone-2-yl acetohydrazide/ propionohydrazide **V** derivatives against butylcholinesterase (BChE).

Com.	Percentage inhibition±S.E.M.			
	25 μg/ml	**50 μg/ml**	**100 μg/ml**	**200 μg/ml**
Va	10.39 ± 3.79	25.71 ± 5.11	47.55 ± 3.78	49.65 ± 3.78
Vb	9 .15± 1.39	18.34 ± 3.39	32.78 ± 3.30	47.98 ± 5.90
Vc	8.57 ± 2.34	15.53 ± 0.19	20.84 ± 2.11	29.73 ± 4.77
Vd	10.52 ± 2.02	18.86±2.68	35.70 ± 4.55	53.81 ± 2.75
Ve	18.43 ± 4.21	54.71 ± 2.45	67.71 ± 3.17	72.13 ± 3.76
Vf	9.94±3.10	22.57±4.48	27.17±4.17	36.94± 2.23
Vg	8.34 ±3.10	16.44 ± 4.10	23.11 ± 2.79	44.98 ± 1.07
Vh	36.05 ± 2.71	51.24 ± 1.29	58.44 ± 1.41	59.43 ± 2.01
Vi	1.79 ± 0.48	15.77 ± 3.99	11.87 ± 1.09	17.08 ± 0.92
Vj	21.43 ± 2.14	46.12 ±1.85	50.90±1.03	75.93±5.36
Galant.	51. 36 ± 0.45	61.33 ± 0.75	64.42 ± 0.17	66.45 ± 0.82

at 200 µg/ml concentration. This result, because of the recent findings concerning the role of BChE in AD, makes our compound **Vj** endowed with a well-balanced profile of AChE/BChE inhibition, valuable a candidate for further development. The rest of the N'-[(4-substituedphenyl)sulphonyl]-2-[4-(substituedphenyl)-piperidine/piperazine]-3(2*H*)-pyridazinone-2-yl acetohydrazide/ propionohydrazide **V** derivatives have shown moderate inhibitory activity either AChE or BChe inhibitory activities.

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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.