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Design, synthesis and evaluation of some novel 3(2H)-pyridazinone-2-yl acetohydrazides as acetylcholinesterase and butyrylcholnesterase inhibitors

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Abstract: In this study eighteen new N'-[(4-Substituephenyl)sulfonyl]-2-[4-(Substituephenyl)-piperazine]-3(2H)-pyridazinone-2-yl acetohydrazide **V** derivatives were synthesized as acetylcholinesterase and butyrylcholinesterase inhibitors. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChe) inhibitory activity of **V** derivatives was measured using Ellman's method. Some of N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazides **V** showed inhibitory activities close galantamine at 0.05 mM 0.1 mM and 0.2 mM concentrations. According to screening data, the analog of derivatives of **V** which possessed CF_3 on para position of phenylsulfonyl ring improved anti-AChE activity. Also antimicrobial activity of the synthesized compounds have been evaluated. In general **V** Derivatives showed weak antibacterial activity when compared reference compounds. Also all the compounds are less potent than fluconazole against yeast like fungi.

Keywords: 3(2H)-Pyridazinone; p-substituted sulfonylchloride acetohydrazides; acetylcholinesterase (AChE) inhibitor; butyrylcholinesterase (BChE) inhibitor.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system, characterized by loss of cognitive ability and severe behaviour 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

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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 follow 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 a modest induce of the improvement in memory and cognitive functions[10] and have been used to treat AD clinically for a long time. But only 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.

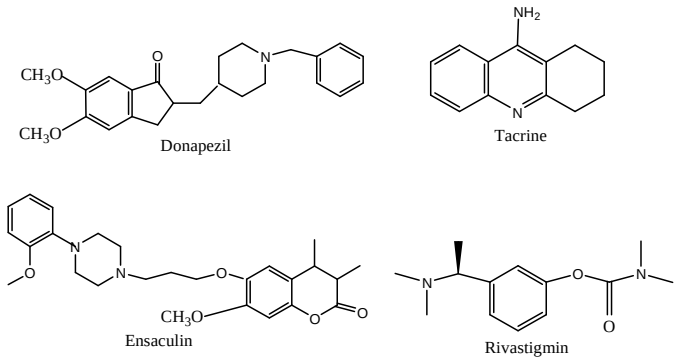


Figure 1. Acetyl cholinesterase inhibitor drugs as FDA approved Alzheimer's disease therapeutics.

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

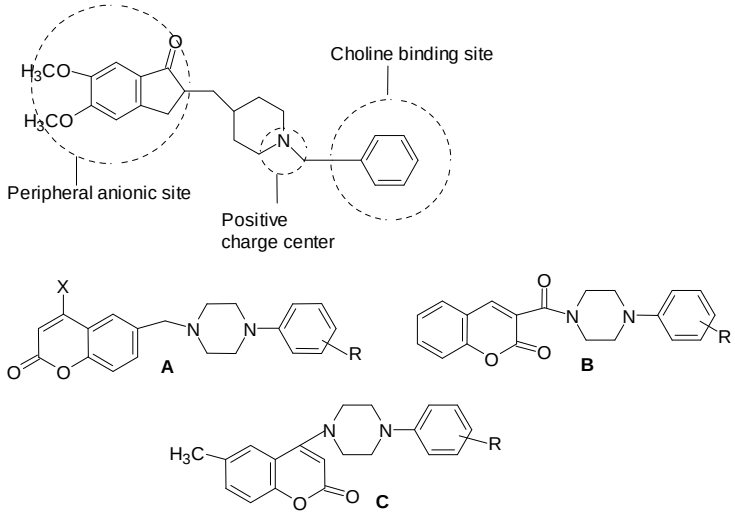


Figure 2. Structural hypothesis for AChEIs and some designed compounds from the literature.[12]

Zhou et al.[12] also reported that three hypothesis for AChEI activity; (1) the coumarin ring, 2H-chromen-2-one heterocycle, a heterocyclic moiety comprising in ensaculin with cognitive functions, demonstrated to be compatible with a high anti-AChE potency, 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. As it seen in Figure 3, title compounds N'-[(4-substituted-phenyl)sulfonyl]-2-[4-(substitututed-phenyl)-piperazine]-3(2H)-pyridazinone-2-yl acetohydrazide **V** derivatives might have been provided structural requirements for AChEI and BuChEI activities.

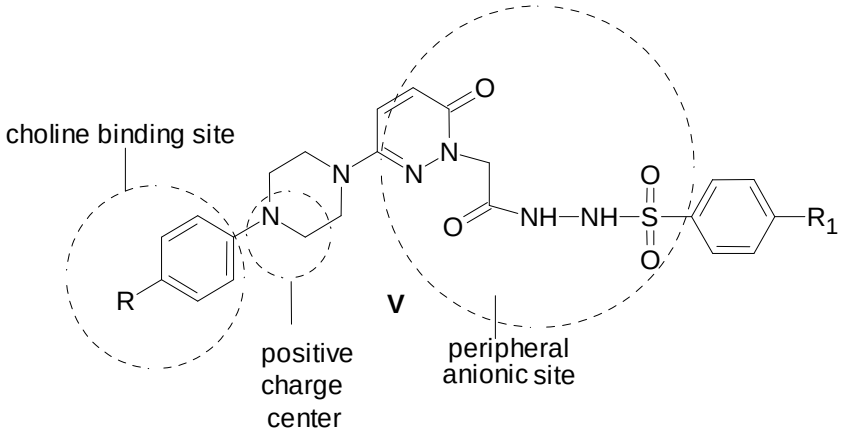


Figure 3. Design strategy of N'-[(4-Substituephenyl)sulfonyl]-2-[4-(Substituephenyl)-piperazine]-3(2H)-pyridazinone-2-yl acetohydrazide**V** derivatives

Furthermore, a number of hydrazide-hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial-antifungal[13], anticonvulsant[14], antiinflammatory[15], antimalarial[16], analgesic[17,18], antiplatelets[19], antituberculosis[20] and anticancer activities[21]. Aroylhydrazide-hydrazones containing hetero-ring such as pyridine[15,22], indole[23], 1,2,4-oxadiazole[5], 1,2,3-triazole[18] and imidazo[2,1-b]thiadiazole ring[20] have attracted special attention. A few of pyrazole carbohydrazide hydrazone derivatives have also been reported.[24,25] However, there has been no report in the literature on the synthesis and biological evaluation of 3(2*H*)-pyridazinone-2-yl acetohydrazide derivatives.

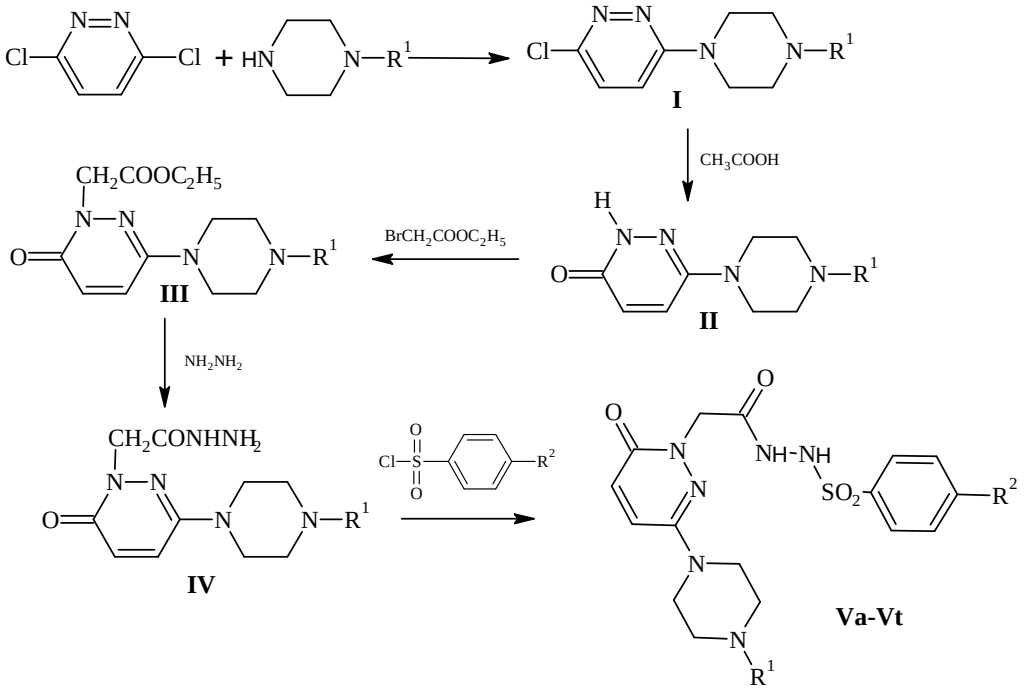
In view of the above mentioned findings and as continuation of our effort[26,27] to identify new candidates that may be of value in designing acetylcholinesterase and butyrylcholinesterase inhibitors and antimicrobial agents, we report herein the synthesis of some eigthteen new N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl) acetohydrazide **V** derivatives.

2. Results and discussion

New N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2*H*)-pyridazinone-2-yl)ace-tohydrazide **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.[28, 29] Therefore we carried out the next steps of the reaction without any further analysis. Hydrolysis of 3-chloro-6-substitutedpyridazines **I** were carried out upon heating in glacial acetic acid to afford 6-substituted-3(2H)-pyridazinone **II** derivatives.[32] The formation of these compounds were confirmed by IR spectra of a C=O signal at about 1660 cm^{-1}. Ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate **III** derivatives[32] were obtained

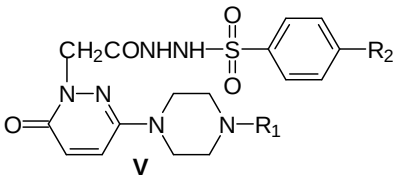
by the reaction of **II** with ethyl bromoacetate in the presence of K_2CO_3 in acetone. 6-Substituted-3(2H)-pyridazinone-2-yl acetohydrazide derivatives **IV** were synthesized by the condensation reaction of ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate **III** derivatives with hydrazine hydrate (99%).

N'-[(substituted-phenyl)sulfonyl]-2-(6-substituted-3(2*H*)-pyridazinone-2-yl)acetohydrazide **V** derivatives which are target compounds of this study, were synthesized by the condensation of **IV** derivatives with nonsubstituted/p-substituted-benzenesulphonyl chlorides. Synthesis method of 6-substituted-3(2H)-pyridazinone **II,** ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate **III** and 6-substituted-3(2H)-pyridazinone-2-ylacetohydrazide derivatives **IV** have been reported in our previous study.[30-33] All of the N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2*H*)-pyridazinone-2-yl)acetohydrazide **V** derivatives were reported first time in this study. The physical data, yield and molecular formula of all compounds are reported Table 1.



Scheme 1. Synthesis of N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide(**Va-Vt)** derivatives

Table 1. Physical constant ofN'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide**V**derivatives



V

3.

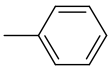
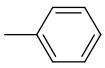
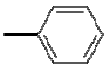
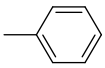
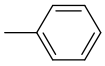
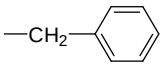
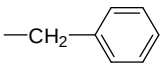
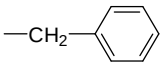
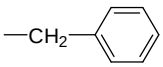
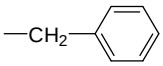
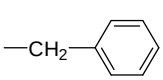
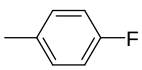
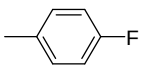
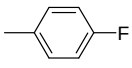
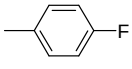
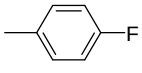
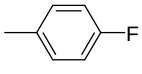
	R_1	R_2	Yield (%)	Molecular Formula	Formula Weight	Mp (°C)
Va		H	65	$C_{22}H_{24}N_6O_4S$	468.528	208
Vb		Cl	60	$C_{22}H_{23}ClN_6O_4S$	502.973	243
Vc		F	60	$C_{22}H_{23}FN_6O_4S$	486.519	245
Vd		CH_3	61	$C_{23}H_{26}N_6O_4S$	482.555	235
Ve		OCH_3	39	$C_{23}H_{26}N_6O_5S$	498.554	213
Vf		CF_3	56	$C_{23}H_{23}F_3N_6O_4S$	536.526	271
Vg		H	37	$C_{23}H_{26}N_6O_4S$	482.555	195
Vh		Cl	52	$C_{23}H_{25}ClN_6O_4S$	517.000	203
Vi		F	48	$C_{23}H_{25}FN_6O_4S$	500.545	202
Vj		CH_3	43	$C_{24}H_{28}N_6O_4S$	496.581	189
Vk		OCH_3	49	$C_{24}H_{28}N_6O_5S$	512.581	173
Vm		CF_3	51	$C_{24}H_{25}F_3N_6O_4S$	550.553	220
Vn		H	89	$C_{22}H_{23}FN_6O_4S$	486.519	242
Vo		Cl	84	$C_{22}H_{22}ClFN_6O_4S$	520.964	240
Vp		F	68	$C_{22}H_{22}F_2N_6O_4S$	504.509	251
Vr		CH_3	92	$C_{23}H_{25}FN_6O_4S$	500.545	246
Vs		OCH_3	86	$C_{23}H_{25}FN_6O_5S$	516.545	228
Vt		CF_3	80	$C_{23}H_{22}F_4N_6O_4S$	554.517	268

Table 2. Percentage inhibition ±S.E.M. values of N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide**V**against acetylcholinesterase (AChE)

Compounds	**Percentage inhibition±S.E.M.**		
	0.05 mM	**0.1 mM**	**0.2 mM**
Va	NA	NA	NA
Vb	NA	NA	NA
Vc	19.46 ± 1.25	34.78 ± 0.67	49.75 ± 0.14
Vd	NA	NA	NA
Ve	NA	29.46 ± 1.25	34.38 ± 1.18
Vf	72.46 ± 1.96	83.38 ± 1.38	88.96 ± 1.03
Vg	NA	NA	66.57±1.54
Vh	NA	16.76 ± 1.12	38.93 ± 1.36
Vi	19.57 ± 0.86	23.23 ±1. 39	70.28 ± 1.30
Vj	NA	NA	NA
Vk	27.99 ± 1.25	39.71 ± 0.91	53.75 ± 1.64
Vm	80.30±1.14	92.63±2.06	95.39 ± 1.28
Vn	NA	NA	NA
Vo	NA	NA	NA
Vp	38.86 ± 1.45	54.27 ± 0.78	68.96 ± 1.03
Vr	NA	NA	NA
Vs	17.87 ± 1.00	60.62 ± 1.62	88.40 ± 1.43
Vt	84.07 ± 1.23	98.63 ± 1.07	99.76 ± 1.54
Galantamine	13.36 ± 0.51	96.33 ± 0.75	97.20 ± 0.67

NA: non active

In order to determine the antibacterial activity of the N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide **V** derivatives derivatives two Gram positive, two Gram negative bacteria species and clinical isolates were screened. Also two *Candida* species were used for antifungal activity. The assessment of the antimicrobial activities of the synthesized compounds was performed using the broth microdilution test in Mueller-Hinton Broth medium. Ampicillin, gentamycin sulphate, ofloxacin, rifampicin, tetracyclin, ceftriaxon, meropenem, eritromycin, vancomycin, ampicillin/sulbactam, amoxicillin/clavulonic acid, fluconazole and amphotericin B were used as reference compounds. Antibacterial and antifungal results of compounds are given in Table 3.

In general N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide **V** derivatives showed weak activity towards Gram negative bacteria and Gram positive bacteria when compared reference compounds. Also all the compounds are less potent than fluconazole against yeast like fungi. It is worth mentioning that compounds **Ve** and **Vg** exhibited equal antibacterial activity with gentamicin and ceftriaxon against *P. aeruginosa* clinicalisolate. Furthermore **Ve** and **Vg** have been found more active than rifampicin and tetracyclin against same isolate. Similarly **Vo** have been found more active than gentamycin against *S. aureus* clinical isolate.

Table 3. Antibacterial and antifungal activity of N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide**V**derivatives.

Compound	A	B	C	D	E	F	G	H	I	J	K
Va	256	256	256	128	128	256	128	256	256	128	128
Vb	256	256	256	256	128	256	128	256	256	128	128
Vc	256	256	256	128	128	256	128	128	256	128	128
Vd	256	256	256	128	128	256	128	128	256	128	128
Ve	256	256	256	128	64	256	128	128	256	128	128
Vf	256	256	256	128	128	256	128	128	256	64	128
Vg	256	256	128	128	64	256	128	256	256	128	128
Vh	256	256	256	128	128	256	128	256	256	128	128
Vi	256	256	256	256	128	256	128	256	256	128	128
Vj	256	256	256	256	128	256	128	256	256	128	128
Vk	256	256	256	256	128	256	128	256	256	128	128
Vm	256	256	256	256	128	256	128	256	256	128	128
Vn	256	256	256	128	128	256	128	256	256	128	128
Vo	256	256	256	128	128	256	64	128	128	64	128
Vp	256	256	256	256	128	256	128	256	256	128	256
Vr	256	256	256	256	128	256	128	256	256	128	256
Vs	256	256	256	256	128	256	128	256	256	128	1024
Vt	256	256	256	128	128	256	128	256	256	64	128
Ampicillin	2	-	>1024	-	-	0.5	-	0.5	0.5	-	-
Gentamycin	0.25	-	512	1	64	0.5	128	8	8	-	-
Ofloxacin	0.015	-	32	1	2	0.25	0.5	1	4	-	-
Rifampicin	16	-	256	32	128	0.004	2	0.5	4	-	-
Tetracyclin	0.5	-	256	8	128	0.25	8	8	16	-	-
Ceftriaxon	0.125	-	512	64	64	2	-	-	-	-	-
Meropenem	0.008	-	<0.25	1	0.015	0.03	-	4	8	-	-
Eritromycin	-	-	-	-	-	0.25	16	1	0.25	-	-
Vancomycin	-	-	-	-	-	0.5	1	1	8	-	-
Ampicillin Sulbactam	-	16	-	-	-	-	-	-	-	-	-
Amoxicillin clavulonic acid	-	16	-	-	-	-	-	-	-	-	-
Fluconazol	-	-	-	-	-	-	-	-	-	0.0625	32
Amphotericin B	-	-	-	-	-	-	-	-	-	<0.03	0.5

A: *E.coli* ATCC 25922, **B**: *E.coli* ATCC 35218, **C**: *E.coli* isolat, **D**: *Pseudomonas aeruginosa* ATCC 27853, **E**: *P. aeruginosa* isolat, **F**: *Staphylococcus aureus* ATCC 29213, **G**: *S.aureus* isolat, **H**: *Enterococcus faecalis* ATCC 29212, **I**: *E.faecalis* isolat, **J**: *Candida albicans* ATCC 10231, **K**: *C.krusei* ATCC 6258

3. Experimental

3.1. Materials and Methods

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 on 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). Elemental analyses were performed with Leco-932 (C,H,N,S,O-Elemental analyzer, St. Joseph, USA) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara-Turkey) and within ± 0.4 % of the theoretical values.

3.2. Chemistry

Synthesis of 6-substituted-3(2H)-pyridazinone derivatives II: The compounds **II** (R_1: phenyl, benzyl, 4-fluorophenyl) were prepared as described in our previous paper.[32]

Synthesis of ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate derivatives III: The compounds **III** (R_1: phenyl, benzyl, 4-fluorophenyl) were prepared as described in our previous paper.[32]

Synthesis of 6-substituted-3(2H)-pyridazinone-2-yl acetohydrazide derivatives IV: To methanolic solution of ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate derivatives **III** (25 mL, 0.01 mol) was added hydrazine hydrate (99%) (3 ml) and stirred for 3 h in the room temperature. The precipitate obtained was filtered off, washed with water, dried and recrystallized from ethanol.[33] 6-[(4-Benzyl)piperidine)]-3(2H)-pyridazinone-2-yl acetohydrazide derivative have been synthesized for the first time in this study (mp. 132 °C).

General methods of N'-[(substituted phenyl)sulfonyl]-2-(6-substituted-3(2H)-pyridazinone-2-yl)acetohydrazide V derivatives: Substituted benzenesulfonyl chlorides (0.001 mol) were added to the solution of 6-substituted-3(2H)-pyridazinone-2-yl acetohydrazide 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.

N'-[(Phenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide(Va): IR (KBr) ν_{max} (cm^{-1}): 1705 (C=O ring), 1648 (C=O chain) 1348, 1168 (SO_2). ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.24-3.38 (m, 8H, piperazine protons), 4.47 and 4.78 (2H, s, s, CH_2), 6.85 (1H, d, pyridazinone H_4;J=8.7 Hz), 6.88–7.85 (m, 10 H, phenyl protons+pyridazinone H_5), 9.78 and 10.01 (1H, s, s, HN=CO), 11.40 (1H, s, $NHSO_2$). Anal. Calc. for $C_{22}H_{24}N_6O_4S$: C: 56.40.26, H: 5.16, N: 17.94. Found: C: 56.19, H: 5.05, N: 17.68.

N'-[(4-Chlorophenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vb): IR (KBr) ν_{max} (cm^{-1}): 1704 (C=O ring), 1651 (C=O chain), 1343, 1165(SO_2). ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.24-3.38 (m, 8H, piperazine protons), 4.47 and 4.80 (2H, s, s, CH_2), 6.82 (1H, d, pyridazinone H_4 ; J=8.9 Hz), 6.90-7.84 (m, 10 H, phenyl protons+pyridazinone H_5), 9.74 and 10.03 (1H, s, s, HN=CO), 10.38 (1H, s, NH SO_2). Anal. Calc. for $C_{22}H_{23}ClN_6O_4S$: C: 52.53.26, H: 4.61, N: 16.71. Found: C: 52.50, H: 4.54, N: 16.59.

N'-[(4-Fluorophenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vc): IR (KBr) ν_{max} (cm^{-1}): 1703 (C=O ring), 1648 (C=O chain) , 1353, 1171 (SO_2). ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.24-3.39 (m, 8H, piperazine protons), 4.46 and 4.77 (2H, s, s, CH_2), 6.86 (1H, d, pyridazinone H_4, J=8.9 Hz), 7.01-7.90 (m, 10 H, phenyl protons+pyridazinone H_5), 9.77 and 10.03 (1H, s, s, HN=CO), 10.39 (1H, s, NH SO_2). Anal. Calc. for $C_{22}H_{23}FN_6O_4S$: C: 54.31, H: 4.76, N: 17.27. Found: C: 54.35, H: 4.76, N: 17.18.

N'-[(4-Methylphenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vd): IR (KBr) ν_{max} (cm^{-1}): 1704 (C=O ring), 1649 (C=O chain), 1348, 1168 (SO2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.36-2.38 (s, 3H, CH_3protons), 3.24-3.39 (m, 8H, piperazine protons), 4.46 and 4.77 (2H, s, s, CH_2), 6.86 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.01-7.90 (m, 10 H, phenyl protons+pyridazinone H_5), 9.77 and 10.03 (1H, s, s, HN=CO), 10.39 (1H, s, NH SO_2). Anal. Calc. for $C_{23}H_{26}N_6O_4S$: C: 54.31, H: 4.76, N: 17.27. Found: C: 54.35, H: 4.76, N: 17.18.

N'-[(4-Methoxyphenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Ve): IR (KBr) ν_{max} (cm^{-1}): 1700 (C=O ring), 1652 (C=O chain), 1343, 1168 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.26-3.36 (m, 8H, piperazine protons), 3.85- 3.88 (s, 3H, OCH_3), 4.46 and 4.79 (2H, s, s, CH_2), 6.85 (1H, d, pyridazinone H_4;J=8.9 Hz), 6.90-7.77 (m, 10 H, phenyl protons+pyridazinone H_5), 9.80 and 9.88 (1H, s, s, HN=CO), 10.37 (1H, s, NH SO_2). Anal. Calc. for $C_{23}H_{26}N_6O_5S$: C: 55.41, H: 5.26, N: 16.86. Found: C: 55.29, H: 5.36, N: 16.59.

N'-[(4-Trifluoromethylphenyl)sulfonyl]-2-(6-(4-phenyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vf): IR (KBr) ν_{max} (cm^{-1}): IR (KBr) ν_{max} (cm^{-1}): 1700 (C=O ring), 1652 (C=O chain), 1344, 1176 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.29-3.38 (m, 8H, piperazine protons), 4.47 and 4.82 (2H, s, s, CH_2), 6.85 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.00-8.06 (m, 10 H, phenyl protons+pyridazinone H_5), 9.77 and 9.86 (1H, s, s, HN=CO), 10.44 (1H, s, NH SO_2). Anal. Calc. for $C_{23}H_{23}F_3N_6O_4S$: C: 51.49, H: 4.32, N: 15.66. Found: C: 51.12, H: 4.22, N: 15.40.

N'-[(4-Phenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vg): IR (KBr) ν_{max} (cm^{-1}): 1704 (C=O ring), 1649 (C=O chain), 1348, 1168 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.30-3.38 (m, 8H, piperazine protons), 4.19 and 4.27 (s, 2H, phenyl-CH_2-piperazine), 4.45 and 4.78 (2H, s, s, CH_2), 6.82 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.38-7.83 (m, 11 H, phenyl protons+pyridazinone H_5), 9.70 and 10.01 (1H, s, s, HN=CO), 10.34 (1H, s, $NHSO_2$). Anal. Calc. For $C_{23}H_{26}N_6O_4S$: C: 57.25, H: 5.43, N: 17.42. Found: C: 56.99, H: 5.47, N: 17.42.

N'-[(4-Chlorophenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vh): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1648 (C=O chain), 1353, 1165 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.29-3.38 (m, 8H, piperazine protons), 4.45 and 4.50 (2H, s, s, CH_2), 4.79 and 4.83 (s, s, 2H, phenyl-CH_2-piperazine), 6.87 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.37-7.84 (m, 10 H, phenyl protons+pyridazinone H_5), 9.75 and 9.95 (1H, s, s, HN=CO), 10.37 (1H, s, $NHSO_2$). Anal. Calc. for $C_{23}H_{25}ClN_6O_4S$: C: 53.43, H: 4.87, N: 16.26. Found: C: 53.30, H: 4.61, N: 16.07.

N'-[(4-Fluorophenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vi): IR (KBr) ν_{max} (cm^{-1}): 1706 (C=O ring), 1650 (C=O chain), 1340, 1172 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.30-3.39 (m, 8H, piperazine protons), 4.45 and 4.49 (2H, s, s, CH_2), 4.78 and 4.83 (s, s, 2H, phenyl-CH_2-piperazine), 6.87 (1H, d, pyridazinone H_4 ; J=8.9 Hz), 7.39-7.89 (m, 10 H, phenyl protons+pyridazinone H_5), 9.72 and 10.01 (1H, s, s, HN=CO), 10.38 (1H, s, $NHSO_2$). Anal. Calc. for $C_{23}H_{25}FN_6O_4S$: C: 55.19, H: 5.03, N: 16.79. Found: C: 55.01, H: 5.01, N: 16.56.

N'-[(4-Methylphenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl)acetohydrazide (Vj): IR (KBr) ν_{max} (cm^{-1}): 1704 (C=O ring), 1653 (C=O chain), 1350, 1166 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.34 (s, 3H, CH_3 protons) 3.30-3.38 (m, 8H, piperazine protons), 4.45 (2H, s, CH_2), 4.76 (s, 2H, phenyl-CH_2-piperazine), 6.85 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.30-7.72 (m, 10 H, phenyl protons+pyridazinone H_5), 9.61 and 9.86 (1H, s, s, HN=CO), 10.38 (1H, s, NH SO_2). Anal. Calc. for $C_{24}H_{28}N_6O_4S$: C: 58.05, H: 5.68, N: 16.92. Found: C: 57.71, H: 5.37, N: 16.67.

N'-[(4-Methoxyphenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl) acetohydrazide(Vk): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1666 (C=O chain), 1340, 1165 (SO_2). ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.32-3.38 (m, 8H, piperazine protons), 3.81 (s, 3H, OCH_3), 4.45 (2H, s, CH_2), 4.73 (s, 2H, phenyl-CH_2-piperazine), 6.85 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.30-7.75 (m, 10 H, phenyl protons+pyridazinone H_5), 9.85 and 9.90 (1H, s, s, HN=CO), 10.24 (1H, s, NH SO_2). Anal. Calc. for $C_{24}H_{28}N_6O_5S$: C: 56.24, H: 5.51, N: 16.40. Found: C: 56.13, H: 5.56, N: 16.20.

N'-[(4-Trifluoromethylphenyl)sulfonyl]-2-(6-(4-benzyl)piperazine-3(2H)-pyridazinone-2-yl) acetohydrazide (Vm): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1649 (C=O chain), 1348, 1166 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.32-3.38 (m, 8H, piperazine protons), 4.46 (2H, s, CH_2), 4.75 (s, 2H, phenyl-CH_2-piperazine), 6.85 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.37-8.06 (m,

10 H, phenyl protons + pyridazinone H_5), 9.87 and 9.92 (1H, s, s, HN=CO), 10.43 (1H, s, NH SO_2). Anal. Calc. for $C_{24}H_{25}F_3N_6O_4S$: C: 52.36, H: 4.58, N: 15.26. Found: C: 52.14, H: 4.55, N: 15.09.

N'-[(4-Phenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl) acetohydrazide (Vn): IR (KBr) ν_{max} (cm^{-1}): 1705 (C=O ring), 1649 (C=O chain), 1348, 1168 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.34-3.38 (m, 8H, piperazine protons), 4.46 and 4.79 (2H, s, s, CH_2), 6.88 (1H, d, pyridazinone H_4), 7.05-7.85 (m, 10 H, phenyl protons+pyridazinone H_5), 9.83 and 9.87 (1H, s, s, HN=CO), 10.32 (1H, s, NH SO_2). Anal. Calc. for $C_{22}H_{23}FN_6O_4S$: C: 54.31, H: 4.76, N: 17.27. Found: C: 54.53, H: 4.45, N: 17.14.

N'-[(4-Chlorophenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl) acetohydrazide (Vo): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1653 (C=O chain), 1349, 1164 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.35-3.39 (m, 8H, piperazine protons), 4.46 and 4.81 (2H, s, s, CH_2), 6.89 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.05-7.84 (m, 9H, phenyl protons+pyridazinone H_5), 9.84 and 9.87 (1H, s, s, HN=CO), 10.39 (1H, s, NH SO_2). Anal. Calc. for $C_{22}H_{22}ClFN_6O_4S$: C: 50.72, H: 4.26, N: 16.13. Found: C: 51.09, H: 4.04, N: 15.86.

N'-[(4-Fluorophenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl) acetohydrazide (Vp): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1653 (C=O chain), 1349, 1164 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.34-3.39 (m, 8H, piperazine protons), 4.47 and 4.80 (2H, s, s, CH_2), 6.88 (1H, d, pyridazinone H_4 J=8.9 Hz), 7.04-7.90 (m, 9H, phenyl protons+pyridazinone H_5), 9.85 and 9.88 (1H, s, s, HN=CO), 10.36 (1H, s, NH SO_2). Anal. Calc. for $C_{22}H_{22}F_2N_6O_4S$: C: 52.37, H: 4.40, N: 16.16. Found: C: 52.39, H: 4.17, N: 16.45.

N'-[(4-Methylphenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl) acetohydrazide (Vr): IR (KBr) ν_{max} (cm^{-1}): 1701 (C=O ring), 1666 (C=O chain), 1345, 1167 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.37 (s, 3H, methyl protons), 3.32-3.37 (m, 8H, piperazine protons), 4.45 and 4.76 (2H, s, s, CH_2), 6.88 (1H, d, pyridazinone H_4; J=8.9 Hz), 7.00-7.73 (m, 9H, phenyl protons+pyridazinone H_5), 9.85 and 9.88 (1H, s, s, HN=CO), 10.20 (1H, s, NH SO_2). Anal. Calc. for $C_{23}H_{25}FN_6O_4S$: C: 55.19, H: 5.03, N: 16.79. Found: C: 55.25, H: 4.86, N: 16.59.

N'-[(4-Methoxylphenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl) acetohydrazide (Vs): IR (KBr) ν_{max} (cm^{-1}): 1700 (C=O ring), 1651 (C=O chain), 1345, 1172 (SO_2). ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.29-3.37 (m, 8H, piperazine protons), 3.85 (s, 3H, OCH_3), 4.45 and 4.76 (2H, s, s, CH_2), 6.87 (1H, d, pyridazinone H_4 ; J=8.7 Hz), 7.00-7.77 (m, 9H, phenyl protons+pyridazinone H_5), 9.80 and 9.93 (1H, s, s, HN=CO), 10.25 (1H, s, NH SO_2). Anal. Calc. for $C_{23}H_{25}FN_6O_5S$: C: 53.48, H: 4.88, N: 16.27. Found: C: 53.64, H: 4.84, N: 16.17.

N'-[(4-Trifluoromethylphenyl)sulfonyl]-2-(6-(4-(4-fluorophenyl)piperazine)-3(2H)-pyridazinone-2-yl)acetohydrazide (Vt): IR (KBr) ν_{max} (cm^{-1}): 1707 (C=O ring), 1648 (C=O chain), 1339, 1166 (SO_2).^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.32-3.39 (m, 8H, piperazine protons), 4.47and 4.82 (2H, s, s, CH_2), 6.88 (1H, d, pyridazinone H_4 ; J=8.6 Hz), 7.02-8.07 (m, 9H, phenyl protons+pyridazinone H_5), 9.81 and 9.86 (1H, s, s, HN=CO), 10.43(1H, s, $NHSO_2$). Anal. Calc. for $C_{23}H_{22}F_4N_6O_4S$: C: 49.82, H: 4.00, N: 15.16. Found: C: 49.57, H: 3.60, N: 15.00.

4. Determination of AChE and BChE inhibitor activities

The in vitro inhibition of AChE and BChE for the new synthesized title compounds was determined by the method of Ellman et al.[34] using galantamine as reference.

Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma St. Louis, MO, USA) were employed as the enzyme sources, while acetylthiocholine iodide and 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 same as described recently.[35] 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 by multichannel automatic pipette (Gilson pipetman, Middleton, USA) 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, North Carolina, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Softmax Moleculer Devices, Downingtown, USA). 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 test sample. The experiments were done in triplicate and the results were expressed as average values with S.E.M. (Standard error mean). AChE and BChE inhibitor activitiesof synthesized title compounds compounds are given in Table 2.

4.1. Evaluation of Antibacterial and Antifungal Activities

Standard strains of *E.coli* ATCC 25922, *E.coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 6258 and clinical isolates of these microorganisms that are known to be resistant to various antimicrobial agents were included in the study. Strains were provided from Gazi University Faculty of Medicine Department of Medical Microbiology. Standard powders of ampicillin, gentamycin sulphate, ofloxacin, rifampicin, tetracyclin, ceftriaxon, meropenem, eritromycin, vancomycin, ampicillin/sulbactam, amoxicillin/clavulonic acid, fluconazole and amphotericin B were obtained from the manufacturers.
Stock solutions of the tested compounds were dissolved in DMSO. Standard antibiotic solutions were dissolved in appropriate solvents recommended by CLSI guidelines.[36, 37]
All bacterial isolates were subcultured in Mueller Hinton Agar (MHA) plates and incubated overnight at 37 °C and all Candida isolates were subcultured in Sabouraud Dextrose Agar (SDA) plates at 35 °C for 24-48 hours. Stock solutions of the tested compounds and standard drugs were diluted two-fold in the wells of the microplates so the solution of the synthesized compounds were prepared at 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 µg/mL concentrations and standard drugs were prepared at 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03, 0.015, 0.008, 0.004, 0.002, 0.001 µg/mL concentrations.
Bacterial susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).[36] Mueller Hinton Broth (MHB) was added each well of the microplates. The bacterial suspensions used for inoculation were prepared at 10^5 colony forming unit (CFU)/mL by diluting fresh cultures at McFarland 0.5 density (10^7CFU/mL). Suspensions of the bacteria at 10^5CFU/mL concentration were inoculated to the two fold diluted solution of the compounds. There were 10^4 CFU/mL bacteria in the wells after inoculations. A 10 µL bacteria inoculum was added to each well of the microplates. Microplates were incubated at 37 °C overnight. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MICs).
Fungal susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).[37] RPMI-1640 medium with L-glutamine buffered to pH 7 with MOPS was added each well of the microplates. The yeast suspensions used for inoculation were prepared at 10^4 CFU/mL by diluting fresh cultures at McFarland 0.5 density (10^6 CFU/mL). Suspensions of the yeast at 10^4 CFU/mL concentration were inoculated to the two fold diluted solution of the compounds. There were 10^3 CFU/mL yeast in the wells after inoculations. A 10 µL yeast inoculum was added to each well of the microplates. Microplates were incubated at 37 °C for 24-48 hours. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MICs). All solvents and diluents, pure microorganisms and pure media were used in control wells. All the experiments were done in three

parallel series. Antibacterial and antifungal tests results of synthesized title compounds are given in Table 3.

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