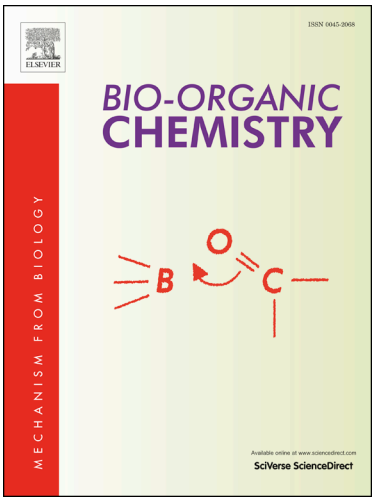


Design and Synthesis of some new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring and the investigation of their inhibitory potential on in-vitro acetylcholinesterase and butyrylcholinesterase

Burcu Kilic, Hayrettin O. Gulcan, Fatma Aksakal, Tugba Ercetin, Nihan Oruklu, E. Umit Bagriacik, Deniz S. Dogruer

PII: S0045-2068(18)30255-4
DOI: https://doi.org/10.1016/j.bioorg.2018.05.006
Reference: YBIOO 2359

To appear in: *Bioorganic Chemistry*

Received Date: 14 March 2018
Revised Date: 4 May 2018
Accepted Date: 7 May 2018

Please cite this article as: B. Kilic, H.O. Gulcan, F. Aksakal, T. Ercetin, N. Oruklu, E. Umit Bagriacik, D.S. Dogruer, Design and Synthesis of some new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring and the investigation of their inhibitory potential on in-vitro acetylcholinesterase and butyrylcholinesterase, *Bioorganic Chemistry* (2018), doi: https://doi.org/10.1016/j.bioorg.2018.05.006

Design and Synthesis of some new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring and the investigation of their inhibitory potential on in-vitro acetylcholinesterase and butyrylcholinesterase

Burcu Kilic[a], Hayrettin O. Gulcan[b], Fatma Aksakal[c], Tugba Ercetin[d], Nihan Oruklu[e], E. Umit Bagriacik[e] and Deniz S. Dogruer[a,*]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Eastern Mediterranean University, Gazimagosa, North Cyprus*
[c]*Department of Chemistry, Hacettepe University, Ankara, Turkey*
[d]*Department of Pharmacognosy, Faculty of Pharmacy, Eastern Mediterranean University, Gazimagosa, North Cyprus*
[e]*Department of Immunology, Faculty of Medicine, Gazi University, Ankara, Turkey*

* Corresponding author E-mail address: denizdogruer2002@yahoo.com (Deniz S. Dogruer)

Keywords: Alzheimer's disease; pyridazine; acetylcholinesterase inhibitory activity; butyrylcholinesterase inhibitory activity; molecular docking.

Abstract

A series of new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring were designed, synthesized and evaluated for their ability to inhibit both cholinesterase enzymes. In addition, a series of carboxamide and propanamide derivatives bearing biphenyl instead of phenylpyridazine were also synthesized to examine the inhibitory effect of pyridazine moiety on both cholinesterase enzymes. The inhibitory activity results revealed that compounds **5b, 5f, 5h, 5j, 5l** pyridazine-3-carboxamide derivative, exhibited selective acetylcholinesterase (AChE) inhibition with IC_{50} values ranging from 0.11 to 2.69 µM. Among them, compound **5h** was the most active one (IC_{50} = 0.11 µM) without cytotoxic effect at its effective concentration against AChE. Additionally, pyridazine-3-carboxamide derivative **5d** (IC_{50} for AChE=0.16 µM and IC_{50} for BChE=9.80 µM) and biphenyl-4-carboxamide derivative **6d** (IC_{50} for AChE=0.59 µM and IC_{50} for BChE=1.48 µM) displayed dual cholinesterase inhibitory activity. Besides, active compounds were also tested for their ability to inhibit Aβ aggregation. Theoretical physicochemical properties of the compounds were calculated by using Molinspiration Program as well. The Lineweaver-Burk plot and docking study showed that compound **5h** targeted both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE.

1. Introduction

With respect to the increase in the average life expectancy, Alzheimer Disease (AD), the most common form of age-related dementia, has become a major threat to the population over the age of 65 during the past several decades. According to the 2015 report of Alzheimer Disease International, the number of patients with dementia is about 46.8 million, and it is expected to increase up to 131.5 million by 2050. Besides, it has been reported that AD is responsible for about more than 50 % of dementia cases and is the third leading cause of death for the high-income countries following ischemic heart disease and stroke [1, 2]. Hence, this situation indicates that AD is a serious health problem.

Although the first AD case was described by Alois Alzheimer in 1906, the precise cause of AD is still unclear due to its complex and multifactorial nature. However, several pathophysiological elements such as amyloid-β (Aβ) aggregates, hyperphosphorylated tau-protein tangles, acetylcholine (ACh) deficiency and oxidative stress are identified to be related to the pathophysiological processes of the disease. Based on these, several hypotheses have been put forward to explain the cause of AD [3-5]. Among them, amyloid β and tau hypotheses have been investigated extensively, however; the drug candidates targeting them could not come into use due to their severe adverse effects or inadequate clinic results [6, 7]. On the other hand, cholinergic hypothesis, the most studied one, continues to be attractive for drug development studies [3-5]. Accordingly, clinical symptoms such as

difficulty in recalling recent events and other cognitive impairments are the consequence of reduced cholinergic transmission, leading to the suggestion that all attempts to increase cholinergic transmission might improve the cognitive functions. In this connection, the strategy for enhancing cholinergic neurotransmission via the inhibition of cholinesterase enzymes, responsible for synaptic cleavage of acetylcholine, has become an important means in the treatment of AD [8-11]. Indeed, there are just three approved drugs called donepezil, galantamine, and rivastigmine, which are cholinesterase inhibitors, and there is one drug called memantine, which is N-methyl-D-aspartic acid (NMDA) receptor antagonist [3-5]. Although this validates the importance of cholinesterase inhibition for the treatment of AD at one hand, it is obvious that the number of these drugs to be used in the treatment of this illness is quite limited.

Acetylcholine is a neurotransmitter of the peripheral and central nervous system (CNS). It provides neural conduction at the neuromuscular junctions in the peripheral nervous system and mainly controls the memory and learning process in the CNS. Two enzymes that break down the acetylcholine to terminate cholinergic transmission are the acetylcholinesterase (AChE) (EC 3.1.1.7) and butyrylcholinesterase (BChE) (EC 3.1.1.8) enzymes. AChE is mainly synthesized in the cholinergic nervous system and muscles, while BChE is primarily synthesized in the liver, and is the dominant cholinesterase in the plasma. Although both can hydrolyze acetylcholine, their kinetics and selectivity for substrate acetylcholine are different [12, 13].

Regarding the current drugs for the treatment of AD, although they differ each other with respect to the organization of their active sites, both AChE and BChE are the potential targets to be focused on. The active site of AChE consists of two primary binding sites, called the catalytic active site (CAS) and the peripheral anionic site (PAS). CAS is located at the bottom of enzyme active-site gorge and reveals the catalytic activity of the enzyme. On the other hand, PAS is located at the entrance of the active site-gorge and enhances the catalytic activity of the enzyme by directing acetylcholine towards the active site [14]. However, recent studies have reported that PAS also has a non-catalytic function in such a way that it can interact with Aβ peptide and cause Aβ plaque formation by triggering Aβ aggregation and these plaques mediate neurodegeneration observed in AD. Thus, the design of the compounds having the ability to bind the both sites of the enzyme may provide both cholinergic enhancement and Aβ aggregation inhibition [15]. Among the three AChEIs drugs, only donepezil has dual binding sites inhibition for AChE. The general structure of BChE enzyme is highly similar to the AChE. However, most of the aromatic residues in the AChE active site replaces with the aliphatic amino acids in the BChE enzyme, which results in BChE active pocket being about 200 $Å^3$ larger in volume. The increase in volume allows binding of the inhibitors to the BChE active pocket in alternative conformations [16]. Moreover, in healthy individuals, the primary enzyme responsible for the acetylcholine hydrolysis in CNS is the AChE. However, as the disease progresses, levels of AChE decrease while the levels of BChE increase [17, 18]. Therefore, simultaneous inhibition of both enzymes should provide additional benefits in the treatment of AD.

Taking into account the aspects stated above, within this research, it was aimed to design novel pyridazine derivatives and corresponding biphenyl derivatives that have the potential to inhibit cholinesterase enzymes.

For many years, our research group has focused on pyridazine derivatives associated with different biological activities [19-22]. For the starting point of our studies, we have employed minaprin, a known pyridazine derivative antidepressant drug. As classical to some of the CNS acting agents, minaprine can interact with various neuroreceptors and it is a weak inhibitor of AChE (IC_{50} = 85 μM) [23]. Considering the structure of minaprine, it is apparent to observe how it also mimics the structure of acetylcholine (Fig. 1). Therefore, a pyridazine derivative, minaprine, is a good tool to design novel molecules that have higher potential to inhibit cholinesterase enzymes. Furthermore, we preferred the bioisosteric replacement of the ester group of acetylcholine with an amide with the aim of improving the hydrolytic stabilities of designed compounds. As a result, on the basis of these findings and as a continuation of our research for new cholinesterase inhibitors [19, 24], we have designed 6-(substitutedphenyl)pyridazine-3-carboxamide and 6-(substitutedphenyl)pyridazine-3-yl propanamide derivatives along with the [1,1'-biphenyl]-4-carboxamide and ([1,1'-biphenyl]-4-yl)propanamide derivatives employing the bioisosteric replacement of acetylcholine in minaprine structure and remembering the Aryl-Spacer-Tertiary amine pharmacophore, which also exist in minaprine, and within the current cholinesterase inhibitor drugs (Fig. 1). Subsequently, the synthesized

compounds were screened for their ability to inhibit both cholinesterase enzymes. Additionally, Aβ aggregation inhibition, cytotoxicity and molecular docking studies were also performed for selected compounds.

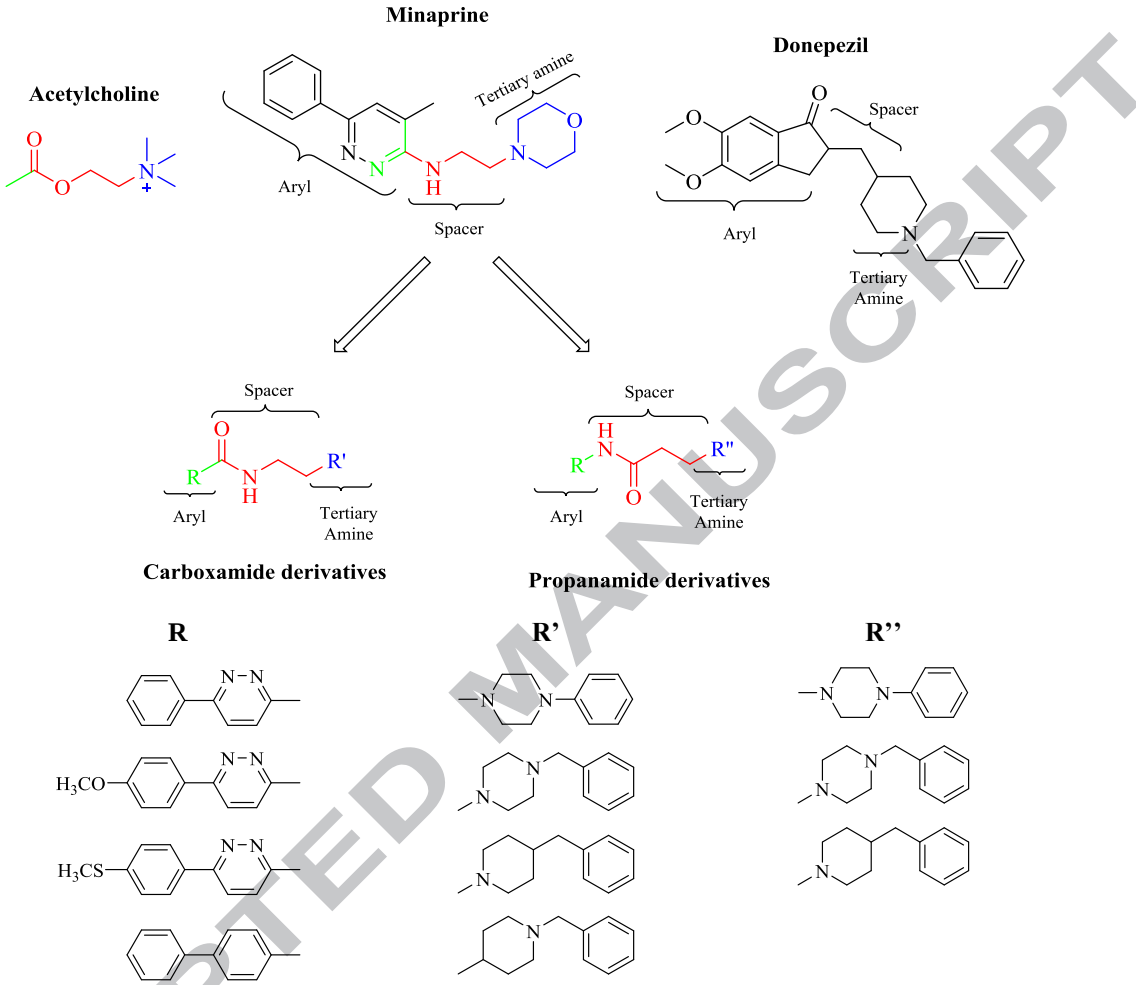


Fig. 1. Design strategy and general structure of synthesized compounds.

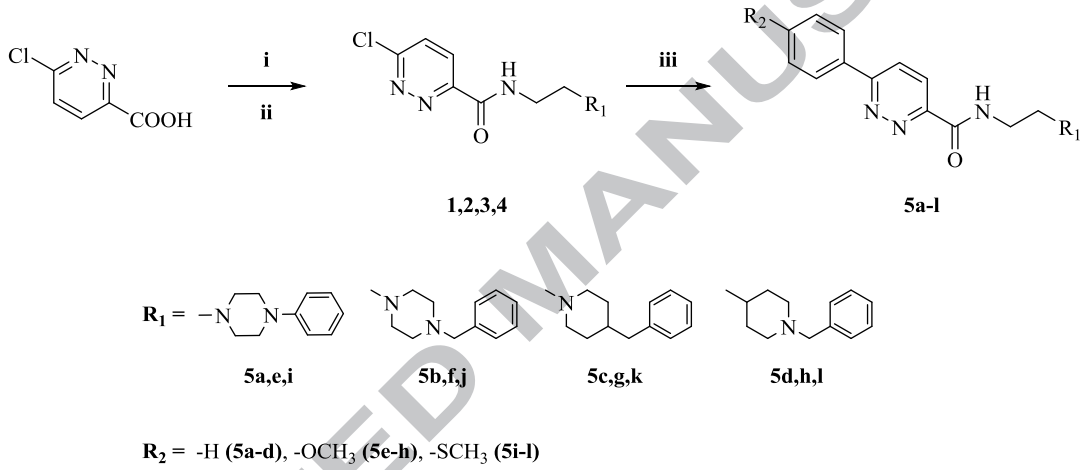
2. Result and discussion

2.1. Chemistry

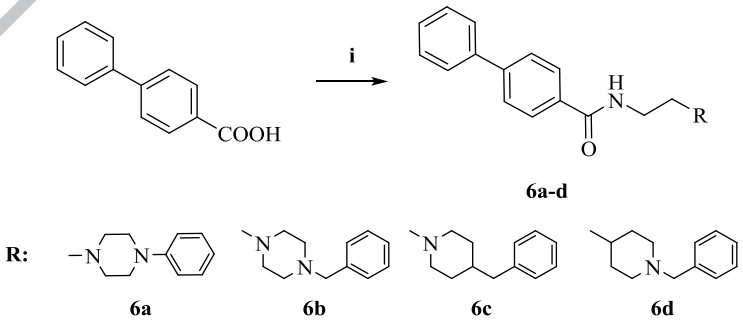
The synthetic scheme for the synthesis of the compounds are shown in Schemes 1, 2, 3, and 4. Initially, 6-chloro-*N*-(2-substitutedethyl)pyridazine-3-carboxamide intermediates **(1-4)** were prepared by commercially available 6-chloropyridazine-3-carboxylic acid and appropriate ethylamine derivatives. It is important to note that the intermediates **1** to **4** have also been originally synthesized compounds within this research. Next, the Suzuki cross-coupling reaction of the prepared intermediate with suitable phenylboronic acid derivative afforded original corresponding *N*-(2-substitutedethyl)-6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl) pyridazine-3-carboxamide derivative **(5a-l)**. Compounds **(6a-d),** bearing biphenyl core, were synthesized by the reaction of commercially available [1,1'-biphenyl]-4-carboxylic acid and appropriate ethylamine derivative in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP). To obtain 3-substituted-*N*-[6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-yl] propanamide derivatives **(13a-i)**, we synthesized both 6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-amine intermediates **(7-9)** and 3-substitutedpropanoic acid intermediates **(10-12)** as starting materials. Intermediates **7-9** were prepared by a previously reported method via the Suzuki cross-coupling reaction using commercially available 6-phenylpyridazin-3-

amine and suitable phenylboronic acid derivative [25]. On the other hand, Michael addition reaction of appropriate secondary amine derivative and methyl acrylate afforded esters which were further hydrolyzed with NaOH to yield intermediates **10-12**. Then, compound **13a-i** were synthesized by the reaction of obtained intermediates in the presence of EDC and DMAP. Finally, the treatment of commercially available [1,1'-biphenyl]-4-amine and 3-chloropropionyl chloride gave the intermediate **14**, which was treated with secondary amine derivatives to give corresponding *N*-([1,1'-biphenyl]-4-yl)-3-substitutedpropanamide derivatives **(15a-c)**.

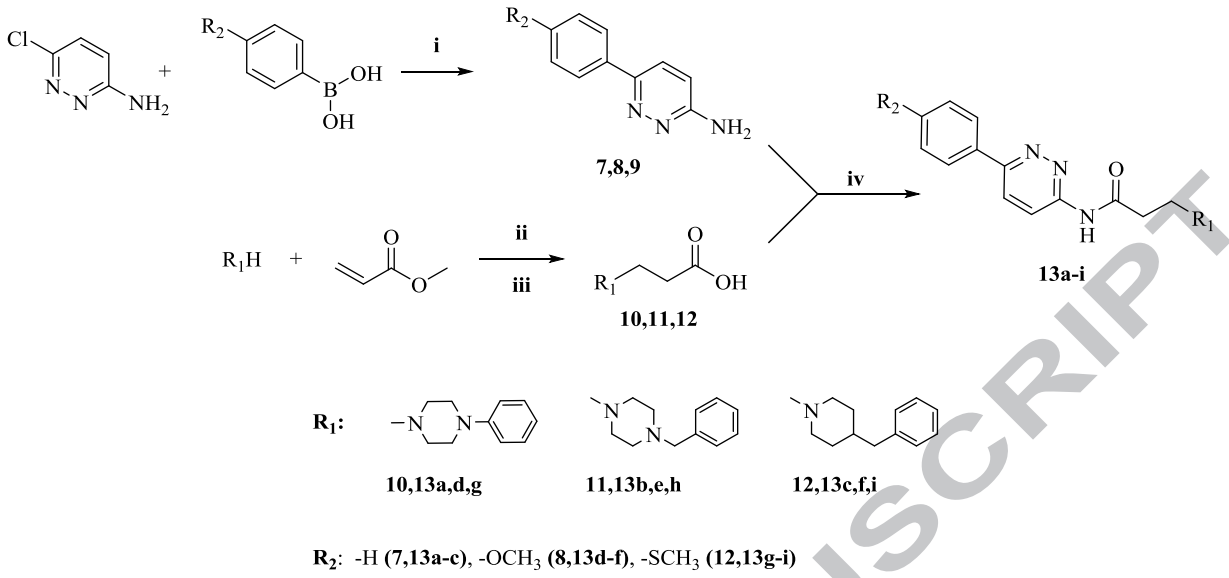
Each of the title compounds synthesized are original except for compounds **6d** and **15b**. **6d** has CAS number 756780-04-8 but there are no registered reference and experimental data for this compound on SciFinder. **15b** is registered with CAS number 191168-70-4 and there is only one reference about this compound [26]. The chemical structures of newly synthesized compounds were verified by ^{1}H NMR, ^{13}C NMR, HRMS and elemental analysis. The ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses data of the compounds are consistent with the proposed structures. Chemical shifts of protons as explained in details under the experimental section.



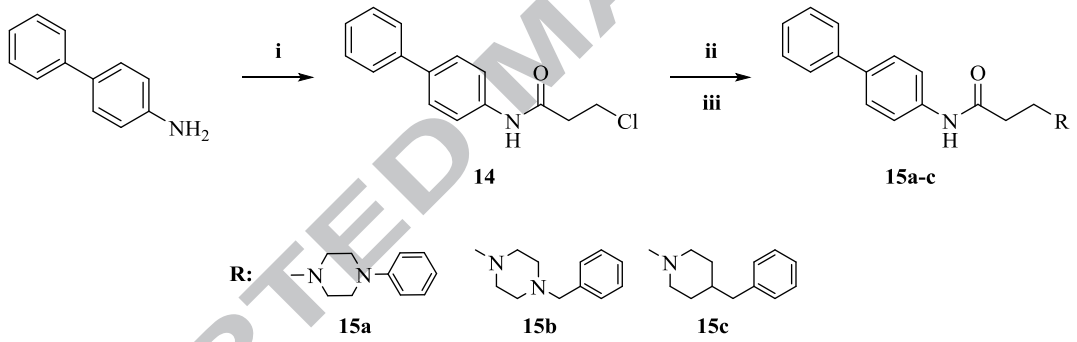
Scheme 1. Synthesis pathway of the compounds **5a-l.** Conditions and reagents: **(i)** Oxalyl chloride, DCM, 2h, rt. **(ii)** Ethylamine derivative, TEA, 1h, rt. **(iii)** Phenylboronic acid derivative, $Pd(PPh_3)_4$, 2M Na_2CO_3, EtOH/toluene, reflux, under argon, 8h.



Scheme 2. Synthesis pathway of the compounds **6a-d.** Conditions and reagents: **(i)** Ethylamine derivative, EDC, DMAP, DCM, overnight, rt.



Scheme 3. Synthesis pathway of the compounds **13a-i.** Conditions and reagents: **(i)** $Pd(PPh_3)_4$, 2M Na_2CO_3, Toluene, reflux, under argon, 24h. **(ii)** DCM, overnight, rt. **(iii)** %5 NaOH, 30 min, 40 °C. **(iv)** EDC, DMAP, DCM, overnight, rt.



Scheme 4. Synthesis pathway of the compounds **15a-c.** Conditions and reagents: **(i)** 3-Chloropropionyl chloride, DMAP, TEA, DMF, 25 min, rt. **(ii)** NaI, ACN, reflux, 2h. **(iii)** Amine derivative, K_2CO_3, 3h, rt.

2.2. Cholinesterase inhibitory activities

The inhibitory activities of the synthesized compounds on AChE (from electric eel) and BChE (from equine serum) were determined by the modified Ellman's method using donepezil and galantamine (10 µM) as the reference compounds. First, the percent inhibitions of the test compounds were investigated. Then, the IC_{50} for those which have shown more than 60% inhibition at 10 µM was measured and the results were reported in Table 1**.**

Regarding inhibitory activity results, the compounds displayed varying results at the percentage inhibition tests. Especially, most of the carboxamide derivatives (**5a-l** and **6a-d**) were found to possess AChE inhibitory activity, but none of the propanamide derivatives (**13a-i** and **15a-c**) displayed remarkable activity on AChE. In addition, the carboxamide derivatives bearing biphenyl core **(6a-d)** rather than phenylpyridazine **(5a-l)** exhibited lower potential to inhibit AChE.

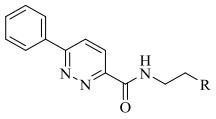
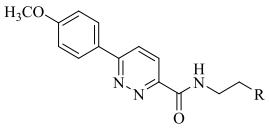
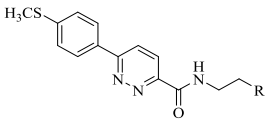
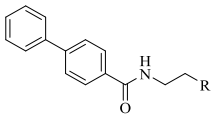
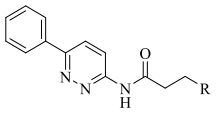
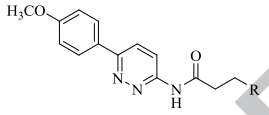
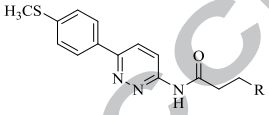
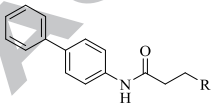
The type of the tertiary amine groups also influenced the activity in varying ways. Compounds, **5h**, **5l**, **5d** and **6d** bearing 1-benzylpiperidine at the side chain were the most active inhibitors for AChE (respectively, IC_{50} = 0.11, 0.12, 0.16 and 0.59 µM). On the other hand, compounds **5f**, **5j**, **5b** and **6b**, bearing 1-benzylpiperazine showed lower AChE inhibition (respectively, IC_{50} = 1.63, 2.66, 2.69 µM and %39 inhibition). However, the introduction of phenylpiperazine (**5a**, **5e**, **5i** and **6a**) or 4-benzylpiperine (**5c**, **5g**, **5k** and **6c**) at the side chain diminished or abolished the activity. These results

indicated and supported that 1-benzylpiperidine and 1-benzylpiperazine moieties are important for optimal AChE inhibitory activity [27]. Furthermore, to improve the interaction with the PAS of AChE [28], electron donating methoxy and methylsulfanyl substituents were introduced to the para position of the phenylpyridazine ring, which resulted in similar AChE inhibitory activity with nonsubstituted phenylpyridazine derivatives.

Among the tested compounds, compound **5h** (IC_{50} = 0.11 µM) was found to be the most active compound. The obtained activity was quite comparable to donepezil (IC_{50}= 0.058 µM), however; it was found to be much more potent in comparison to galantamine. Moreover, the compounds **5d** (IC_{50}= 0.16 µM) and **5l** (IC_{50}= 0.12 µM) exhibited similar potential. On the other hand, the title compounds **5b**, **5f**, **5j**, and **6d** (IC_{50}= 2.69; 1.63; 2.66 and 0.59 µM) displayed a comparable activity when considering the activity of galantamine, although they were not found as active as donepezil (IC_{50}= 0.058 µM). Additionally, all of the active compounds (**5h**, **5l**, **5d**, **6d**, **5f**, **5j**, **5b**, **6b**) showed much more potent activity than minaprine (IC_{50} = 85 µM) [23].

With respect to BChE inhibitory activities, the tested compounds had no effect on BChE at 10 µM concentration, except for compounds **5d** (IC_{50}= 9.80 µM) and **6d** (IC_{50}= 1.48 µM). These compounds containing 1-benzylpiperidine at the side chain are carboxamide derivatives. This indicated that carboxamide structure and 1-benzylpiperidine moiety were also important for BChE inhibition as well as AChE inhibition when compared with the propanamide structure and the other tertiary amine derivatives used in this study. However, unlike AChE inhibition, the replacement of phenylpyridazine (**5d)** with the biphenyl core led to an increase in BChE inhibitory activity (**6d**). Compound **6d** (IC_{50} = 1.48 µM) exhibited a higher BChE activity than donepezil (IC_{50} = 3.70 µM). Moreover, another active compound **5d** (IC_{50} = 9.80 µM) had a similar BChE inhibitory activity compared to galantamine.

Table 1
Cholinesterase inhibitory activities of the synthesized compounds.

	R	Comp	AChE		BChE	
			% inhibition ± SD (10 µM)	IC_{50} (µM) ± SEM	% inhibition ± SD(10 µM)	IC_{50} (µM) ± SEM
	Phenylpiperazine	**5a**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**5b**	85 ± 0.7	2.69 ± 0.001	<%10	n.d.
	4-Benzylpiperidine	**5c**	30 ± 3.4	n.d.	34 ± 4.6	n.d.
	1-Benzylpiperidine	**5d**	97 ± 0.2	0.16 ± 0.001	65 ± 0.9	9.80 ± 0.001
	Phenylpiperazine	**5e**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**5f**	88 ± 0.7	1.63 ± 0.001	<%10	n.d.
	4-Benzylpiperidine	**5g**	<%10	n.d.	23 ± 1.3	n.d.
	1-Benzylpiperidine	**5h**	96 ± 0.4	0.11 ± 0.001	47 ± 1.9	n.d.
	Phenylpiperazine	**5i**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**5j**	79 ± 1.0	2.66 ± 0.001	<%10	n.d.
	4-Benzylpiperidine	**5k**	12 ± 2.5	n.d.	14 ± 1.5	n.d.
	1-Benzylpiperidine	**5l**	91 ± 0.2	0.12 ± 0.001	29 ± 2.9	n.d.
	Phenylpiperazine	**6a**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**6b**	39 ± 4.7	n.d.	27 ± 6.2	n.d.
	4-Benzylpiperidine	**6c**	6 ± 2.5	n.d.	13 ± 3.0	n.d.
	1-Benzylpiperidine	**6d**	95 ± 0.2	0.59 ± 0.001	86 ± 5.2	1.48 ± 0.001
	Phenylpiperazine	**13a**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**13b**	55 ± 3.9	n.d.	<%10	n.d.
	4-Benzylpiperidine	**13c**	<%10	n.d.	<%10	n.d.
	Phenylpiperazine	**13d**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**13e**	25 ± 2.9	n.d.	<%10	n.d.
	4-Benzylpiperidine	**13f**	14 ± 4.9	n.d.	<%10	n.d.
	Phenylpiperazine	**13g**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**13h**	26 ± 2.6	n.d.	<%10	n.d.
	4-Benzylpiperidine	**13i**	<%10	n.d.	<%10	n.d.
	Phenylpiperazine	**15a**	<%10	n.d.	<%10	n.d.
	Benzylpiperazine	**15b**	19 ± 3.7	n.d.	21 ± 3.2	n.d.
	4-Benzylpiperidine	**15c**	7.5 ± 3.9	n.d.	19 ± 3.6	n.d.
		Donepezil	98 ± 1,0	0.058 ± 0.001	90 ± 0.5	3.7 ± 0.001
		Galantamine	89 ± 1,3	n.d.	20 ± 0.5	n.d.

n.d. Not determined.

2.3. Kinetic studies of enzyme inhibition

Kinetic studies were carried out to determine inhibition types of the most active compounds **5h** (for AChE) and **6d** (for BChE). Lineweaver-Burk plots and replots of the slope versus concentration were utilized to obtain the inhibition constants (K_i), as shown in Fig. 2. Accordingly, all compounds

were shown to have mixed-type inhibition. The obtained K_i values were consistent with the measured IC_{50} values.

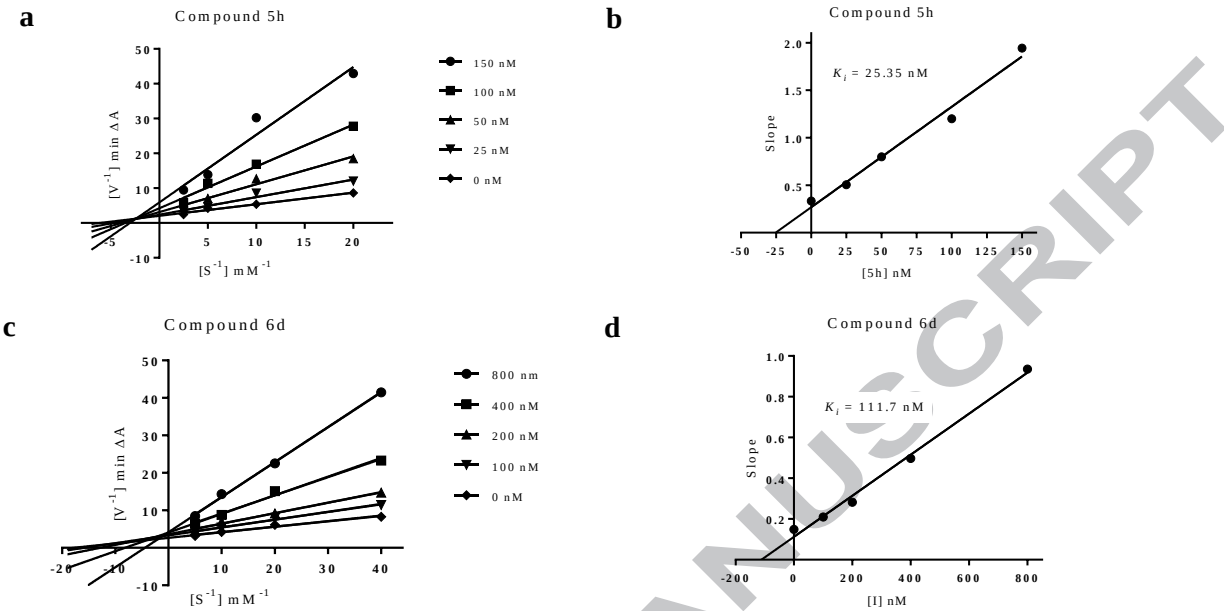


Fig. 2. Lineweaver-Burk plot of **5h** for AChE hydrolysis **(a)** and slope replot vs **5h** concentration **(b)**. Lineweaver-Burk plot of **6d** for BChE hydrolysis **(c)** and slope replot vs **6d** concentration **(d)**.

2.4. Inhibition of self-induced and AChE-induced Aβ aggregation

According to the cholinesterase inhibitory activity results, the most potent six compounds (**5b, 5d, 5f, 5h, 5j,** and **6d**) were selected for Aβ aggregation inhibition assay. To investigate the effect of the selected compounds and references (Donepezil and phenol red) on the aggregation of $A\beta_{1-42}$, the reported thioflavin T-based fluorometric assay was performed at 100 µM concentration [29]. The effects of each compound on the $A\beta_{1-42}$ peptide self-aggregation and the AChE-induced aggregation were summarized as the percent (%) inhibition data in Table 2.

According to the assay results, the test compounds were found to be more active for AChE-induced aggregation than self-induced aggregation. Notably, **5b** (%22.30) and **5j** (%27.58) showed a comparable potency on AChE-induced aggregation when considering the activity of donepezil (%20.30). The results obtained for the reference molecules (donepezil, and phenol red) were consistent with the literature data [30].

Table 2
Inhibition of Aβ aggregation of selected compounds.

Compound (100 µM)	Aβ aggregation % inhibition ± SD Self-induced	Aβ aggregation % inhibition ± SD AChE-induced
5b	8.70 ± 0.04	22.30 ± 0.39
5d	n.i.	10.49 ± 0.13
5f	n.i.	3.38 ± 0.058
5h	n.i.	n.i.
5j	12.72 ± 0.17	27.58 ± 0.10
6d	n.i.	n.i.
Phenol Red	98.40 ± 0.12	92.15 ± 0.05
Donepezil HBr	12.9 ± 0.03	20.3 ± 0.08

(n.i.) no inhibition

2.5. Cytotoxicity test

The cytotoxic effect of compounds **5h** and **6d** was monitored in 3T3 cell lines to gain insight into therapeutic potential of the compounds by using a commercially available 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **5h** was found to be nontoxic at its effective concentration against AChE. On the other hand, **6d** had low cytotoxicity for cells. According to this results, pyridazine ring could be beneficial to improve the safety of compounds (Table 3).

Table 3
Cytotoxicity assay results of **5h** and **6d**.

Compound	Viability (%) of 3T3 cells		
	0.1 µM	**1 µM**	**10 µM**
5h	96.7 ± 6.1	97.9 ± 4.2	80.6 ± 6.1
6d	81.4 ± 3.9	89.0 ± 3.3	102.0 ± 3.9

2.6. Assessment of physicochemical parameters

One of the biggest challenges in drug development is poor pharmacokinetic. Hence, the calculation of molecular properties and BBB penetration scores of compounds were computed employing the Molinspiration and admetSAR online services [31, 32]. Calculated descriptors such as molecular weight, logP, topological polar surface area (tPSA), volume, number of hydrogen donors, number of hydrogen acceptors and number of violations of Lipinski's rule and blood brain barrier (BBB) permeability were presented in Table 4. Molecules violating more than one of these rules may have problems with bioavailability. According to the calculated data, all compounds follow Lipinski's rule. However, **6c**, **6d**, and **15c** have one violation of Lipinski's rule. These three compounds which have logP values over 5 are biphenyl derivatives. Thus it can be stated that phenylpyridazine system enhances drug-likeness properties in addition to the inhibitory activity of the compounds. Based on the predicted BBB values, all compounds are able to penetrate brain with a probability of more than %90, therefore they are considered as CNS-active compounds.

Table 4
Calculated physicochemical parameters of the compounds.

Compound	MW[a]	logP[a]	tPSA[a]	Vol[a]	nON[a]	nOHNH[a]	Vio[a]	BBB[b]
5a	387.49	2.96	61.36	365.47	6	1	0	0.9736
5b	401.51	2.66	61.36	382.27	6	1	0	0.9500
5c	400.53	3.95	58.12	386.32	5	1	0	0.9500
5d	400.53	4.00	58.12	386.32	5	1	0	0.9500
5e	417.51	3.02	70.59	391.02	7	1	0	0.9813
5f	431.54	2.72	70.59	407.82	7	1	0	0.9644
5g	430.55	4.00	67.35	411.86	6	1	0	0.9644
5h	430.55	4.05	67.35	411.86	6	1	0	0.9644
5i	433.58	3.39	61.36	400.16	6	1	0	0.9714
5j	447.61	3.10	61.36	416.96	6	1	0	0.9457
5k	446.62	4.38	58.12	421.01	5	1	0	0.9457
5l	446.62	4.43	58.12	421.01	5	1	0	0.9457
6a	385.51	4.54	35.57	373.78	4	1	0	0.9906
6b	399.54	4.25	35.57	390.58	4	1	0	0.9831
6c	398.55	5.53	32.34	394.63	3	1	1	0.9835
6d	398.55	5.58	32.34	394.63	3	1	1	0.9900
13a	387.49	3.49	61.36	365.47	6	1	0	0.9861
13b	401.51	3.19	61.36	382.27	6	1	0	0.9861
13c	400.53	4.47	58.12	386.32	5	1	0	0.9861
13d	417.51	3.54	70.59	391.02	7	1	0	0.9904
13e	431.54	3.25	70.59	407.82	7	1	0	0.9904
13f	430.55	4.53	67.35	411.86	6	1	0	0.9904
13g	433.58	3.92	61.36	400.16	6	1	0	0.9831
13h	447.61	3.62	61.36	416.96	6	1	0	0.9831
13i	446.62	4.91	58.12	421.01	5	1	0	0.9831
15a	385.51	4.80	35.57	373.78	4	1	0	0.9819
15b	399.54	4.50	35.57	390.58	4	1	0	0.9819
15c	398.55	5.79	32.34	394.63	3	1	1	0.9895

MW: Molecular weight; logP: log octanol/water partition coefficient; tPSA: Total Polar Surface Area; nON: number of Hydrogen acceptors; nOHNH: number of Hydrogen donors; Vol: Molecular volume; Vio: Violation of Lipinski's rule. [a]Calculated with Molinspiration. [b]Calculated with admetSAR.

2.7. Molecular docking

In order to predict the binding mode and affinities of newly synthesized compounds to target enzymes AChE and BChE, docking was carried out. Herein, interaction with AChE and BChE was discussed over the compounds **5h** and **6d**. Three-dimensional (3D) and two-dimensional (2D) representation of the most energetically profitable poses of compounds **5h** and **6d** docked in the active site of AChE was given in Fig. 3.

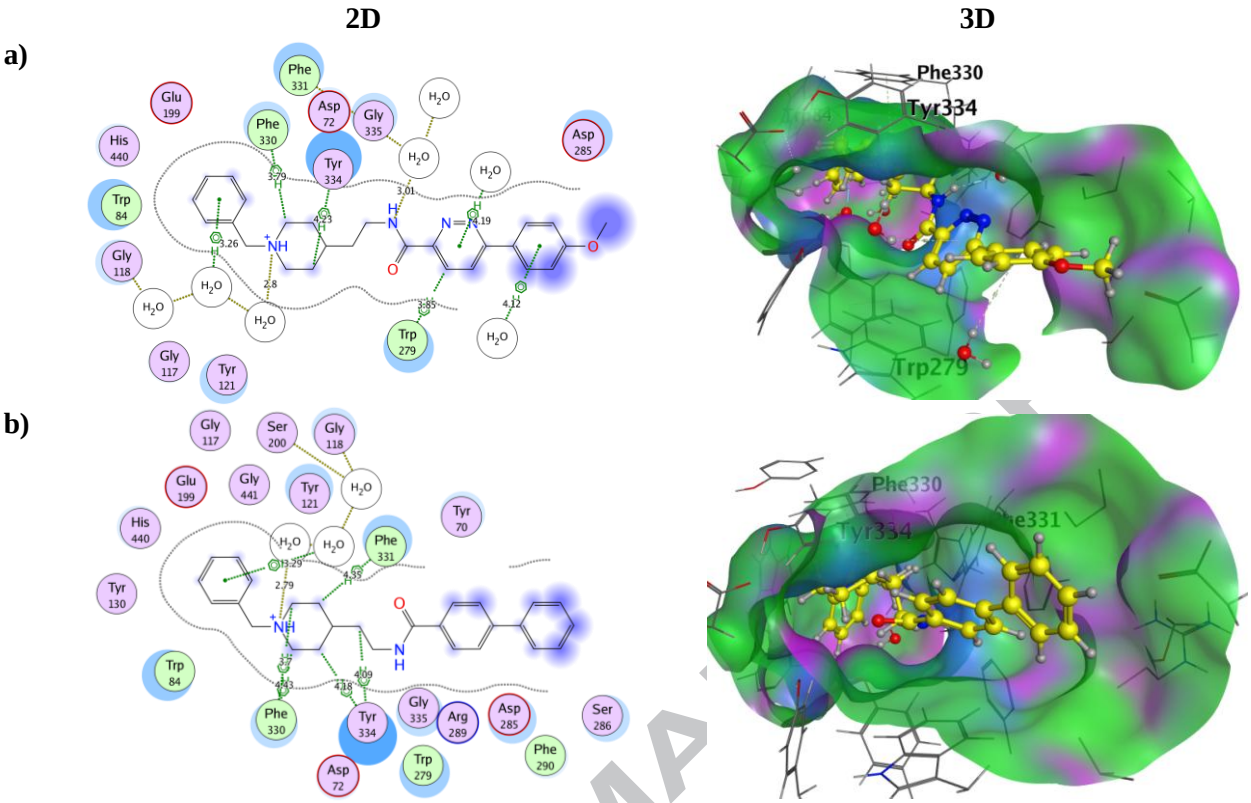


Fig. 3. 2D and 3D representation of docking poses for the compounds **5h** (a) and **6d** (b) in the active site of AChE (PDB code: 1EVE)

AChE contains aromatic residues Trp84 and Phe330 at the catalytic active site and Trp279 at the peripheral anionic site which are very important residues for ligand binding affinity and specificity [27]. As seen from 3D views in Fig. 3, **5h** and **6d** were orientated along the active-site gorge of AChE. **5h** and **6d** docked to AChE with close affinity values of -10.0 and -9.9 kcal mol^{-1}, correspondingly. The interaction of **5h** and **6d** with the active-site gorge of AChE was dominated by hydrogen···π and water-mediated contacts. Pyridazine ring of **5h** interacted with Trp279 residue in the peripheral anionic site of AChE and a water molecule through C–H···π and π···O–H interactions, respectively. Also, π···O–H interaction between anisole ring of **5h** and water molecule contributed to the localization of the compound to the peripheral anionic site. Another C–H···π interaction was predicted between piperazine ring of **5h** and Tyr334 residue inside the PAS. Both of compounds **5h** and **6d** were interacted with catalytic active site over the Phe330, Phe331 residues and water molecules. It was predicted with the docking that compound **6d** interacted only with Tyr334 in the peripheral anionic site. Interaction with Trp279 observed in compound **5h** was disappeared for compound **6d**. Lower AChE inhibitory activity of **6d** contrary to **5h** may be attributed to the absence of interaction with Trp279 residue.

The most energetically profitable pose of compound **6d** in the active site of BChE enzyme was presented in Fig. 4 with 3D and 2D spaces.

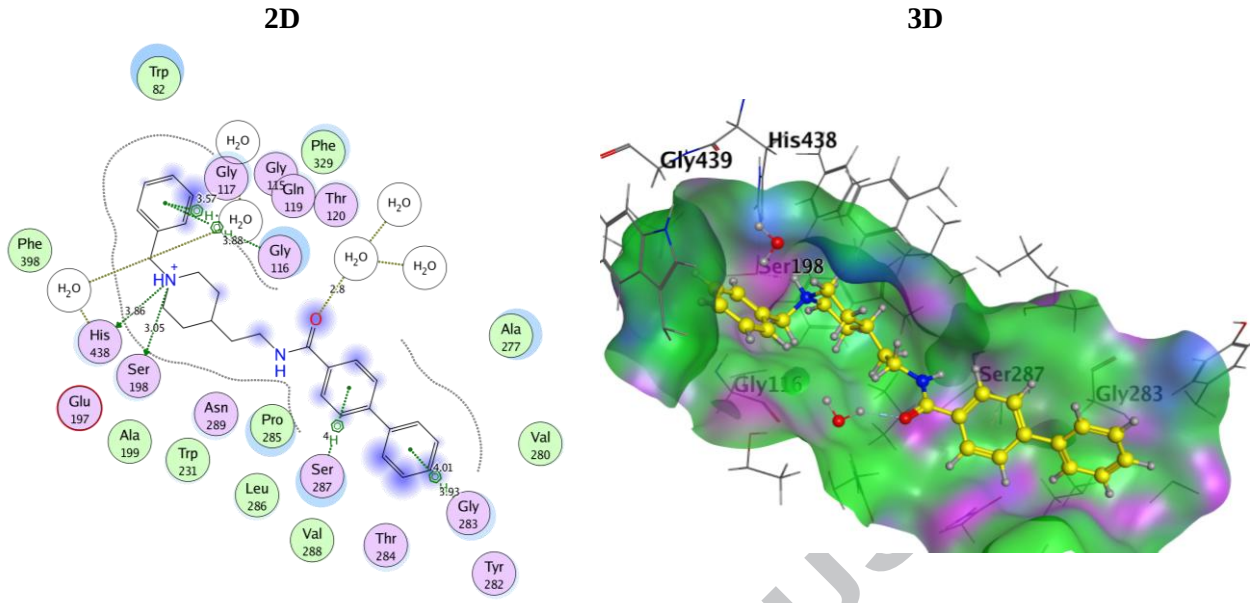


Fig. 4. 2D and 3D representation of docking pose for the compound **6d** in the active site of BChE (PDB code: 1P0I)

The general structure of BChE enzyme is highly similar to the AChE. However, most of the aromatic residues in the AChE active site replaces with the aliphatic amino acids in the BChE enzyme, which results in BChE active pocket being about 200 $Å^3$ larger in volume. This increase in volume allows binding of the inhibitors to the BChE active pocket in alternative conformations [16]. Phe330 and Trp279 residues are conserved in AChE, but absent in BChE which can reflect affinity and noncovalent interactions between BChE and compounds. For the best-scored pose of **6d** to BChE enzyme, binding affinity value of -8.6 kcal mol^{-1} was predicted. For compound **6d**, piperidine group interacted with Ser198 and His438 residues via hydrogen bonding, which is important for BChE inhibition in the catalytic active site. In case of docking of **6d**, π···O–H interaction between benzyl group of compound and water molecule in the same region contributes to the stabilization of BChE. In addition, the phenyl ring of **6d** and Ser287 residue located near the acyl pocket interacted via π···C–H interaction. Another π···C–H interaction was observed between the phenyl group of **6d** and Gly283.

3. Conclusion

The cholinesterase inhibitory activity results revealed that carboxamide series were suitable for both cholinesterase inhibitions. Moreover, 6-phenylpyridazine and biphenyl moieties were found to be optimal for AChE and BChE inhibition, respectively. Additionally, the physicochemical parameter calculations of the synthesized compounds follow Lipinski's rules. Since biphenyl derivatives **6c**, **6d**, and **15c** have only one violation of Lipinski's rule, it can be considered that the pyridazine ring was beneficial for AChE inhibitory activity as well as drug-likeness.

Enzyme kinetics and molecular docking studies indicated that the most potent AChE inhibitor **5h** (IC_{50} = 0.11 µM) could bind simultaneously to the CAS and PAS of AChE and this compound did not show any cytotoxic effect. However, **5h** presented no inhibitory activity for Aβ aggregation. On the other hand, compounds **5b** (%22.30) and **5j** (%27.58) showed a comparable potency on AChE-induced aggregation when considering the activity of donepezil (%20.30). Additionally, compound **6d** exhibited dual cholinesterase inhibitory activity (AChE IC_{50} = 0.59 µM; BChE IC_{50} = 1.48 µM).

As a result, 6-(substitutedphenyl)pyridazine-3-carboxamide and [1,1'-biphenyl]-4-carboxamide can be considered as attractive core structures for the discovery of new multi target directed ligands for AD therapy. Overall, our design studies employing minaprine, a very poor cholinesterase inhibitor, have revealed very potent and original cholinesterase inhibitors derived from pyridazine.

4. Experimental

4.1. Chemistry

All the chemicals used for the synthesis of the compounds were purchased from commercial suppliers. Melting points (mp) were recorded on an Schmelzpunkt SMP-II digital melting point apparatus and values are uncorrected. Thin-layer chromatography (TLC) was performed on Merck 60F254 plates. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded with a Varian Mercury 400 MHz FT-NMR spectrometer in DMSO-d_6 and $CDCl_3$ using tetramethylsilane as the internal standard (Faculty of Pharmacy, Ankara University). All chemical shifts were recorded as δ (ppm) and coupling constants (J) are reported as Hertz. The high resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE Mass Spectrometer also coupled to an AQUITY Ultra Performance Liquid Chromatography system at Faculty of Pharmacy, Gazi University, Ankara, Turkey. Elemental analysis was performed on a Leco 932 CHNS instrument at Faculty of Pharmacy, Ankara University, Ankara, Turkey, and the results were within ± 0.4% of the theoretical values.

*4.1.1. General procedure for the synthesis of 6-chloro-N-(2-substitutedethyl)pyridazine-3-carboxamide intermediates **(1-4)***

6-Chloropyridazine-3-carboxylic acid (1 equivalent) was dissolved in DCM (10 ml). To this solution catalytic amount of DMF and oxalyl choloride (1.1 equivalent) were added and the reaction mixture was stirred at room temperature for 2h. After 2h, reaction mixture cooled at ice bath then, TEA (3 equivalent) and appropriate 2-substituted ethan-1-amine derivative (1.1 equivalent) were added and stirred for additional 1h at room temperature. At the end of this period, reaction mixture was evaporated to dryness then the precipitate boiled with water and petroleum ether respectively. The resulting precipitate was filtered to yield intermediates (1-4), which were used for method A without further purification.

*4.1.1.1. 6-Chloro-N-(2-(4-phenylpiperazin-1-yl)ethyl)pyridazine-3-carboxamide **(1)***

Yield: 74%; mp: 143 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{17}H_{21}ClN_5O$ calculated: 346.1435, found: 346.1438.

*4.1.1.2. N-(2-(4-benzylpiperazin-1-yl)ethyl)-6-chloropyridazine-3-carboxamide **(2)***

Yield: 60%; mp: 145 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{18}H_{23}ClN_5O$ calculated: 360.1591, found: 360.1612.

*4.1.1.3. 3-(4-Benzylpiperidin-1-yl)-N-(6-chloropyridazin-3-yl)propanamide **(3)***

Yield: 40%; mp: 138 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{19}H_{24}ClN_4O$ calculated: 359.1639, found: 359.1614.

*4.1.1.4. N-(2-(1-benzylpiperidin-4-yl)ethyl)-6-chloropyridazine-3-carboxamide **(4)***

Yield: 56%; mp: 147 °C. HRMS (ESI) [M+H]+ m/z for C19H24ClN4O calculated: 359.1639, found: 359.1614.

*4.1.2. General procedure for the synthesis of N-(2-substitutedethyl)-6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazine-3-carboxamide derivatives **(5a-l)** (Method A)*

Corresponding 6-chloro-*N*-(2-substitutedethyl)pyridazine-3-carboxamide intermediate (1 equivalent), appropriate phenyl/4-methoxyphenyl/4-methylsulfanylphenylboronic acid (1.5 equivalent), 2M Na_2CO_3 solution (0.55 ml for 1 mmol intermediate), EtOH (0.37 ml for 1 mmol intermediate) and toluene (4.5 ml for 1 mmol intermediate) were mixed and stirred for a few minutes under inert

atmosphere. Then, tetrakis triphenylphosphine palladium ($Pd(PPh_3)_4$) (0.05 equivalent) were rapidly added to this solution. Reaction mixture was refluxed under inert atmosphere for 8h. After completion of the time, reaction mixture was evaporated to dryness and the obtained residue was purified by column chromatography.

4.1.2.1. 6-Phenyl-N-[2-(4-phenylpiperazin-1-yl)ethyl]pyridazine-3-carboxamide ***(5a)***

Following the method A, reaction of compound **1** (800 mg, 2.31 mmol) with phenylboronic acid (420 mg, 3.47 mmol) in the presence of $Pd(PPh_3)_4$ (130 mg, 0.11 mmol), 2M Na_2CO_3 solution (1.28 ml), toluene (10.0 ml) and EtOH (0.85 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 95:5) and recrystallized from ACN. Yield: 110 mg, 12%; mp: 227 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.14 (t, 1H, *J* = 5.6 Hz, amide H), 8.42 (d, 1H, *J* = 8.6 Hz, pyridazine H), 8.25 (d, 1H, *J* = 8.6 Hz, pyridazine H), 8.23-8.21 (m, 2H, $H^{2'}$, $H^{6'}$), 7.63-7.59 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.20 (t, 2H, *J* = 8.0 Hz, $H^{3''}$, $H^{5''}$), 6.93 (d, 2H, *J* = 8.0 Hz, $H^{2''}$, $H^{6''}$), 6.76 (t, 1H, *J* = 8.0 Hz, $H^{4''}$), 3.55 (q, 2H, *J* = 6.4 Hz, -CONHC<u>H</u>$_2$-), 3.15 (t, 4H, *J* = 4.8 Hz, piperazine H^3, H^5), 2.64-2.61 (m, 6H, piperazine H^2, H^6 and -CONHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR ($CDCl_3$) δ ppm: 163.04, 160.70, 151.01, 135.47, 130.77, 129.21, 129.18, 127.39, 126.22, 124.82, 120.21, 116.39, 56.67, 52.93, 48.56, 36.02. HRMS (ESI) $[M+H]^+$ m/z for $C_{23}H_{26}N_5O$ calculated: 388.2137, found: 388.2126. Anal. Calcd. for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 70.99; H, 6.24; N, 18.29.

4.1.2.2. N-[2-(4-Benzylpiperazin-1-yl)ethyl]-6-phenylpyridazine-3-carboxamide ***(5b)***

Following the method A, reaction of compound **2** (650 mg, 1.81 mmol) with phenylboronic acid (330 mg, 2.71 mmol) in the presence of $Pd(PPh_3)_4$ (105 mg, 0.09 mmol), 2M Na_2CO_3 solution (1.0 ml), toluene (10.0 ml) and EtOH (0.80 ml) gave crude product then, it was purified by column chromatography (EtOAc) and recrystallized from isopropanol. Yield: 135 mg, 19%; mp: 151 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.04 (t, 1H, *J* = 5.6 Hz, amide H), 8.39 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.23-8.18 (m, 3H, pyridazine H, $H^{2'}$, $H^{6'}$), 7.61-7.55 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.31-7.19 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.48-3.43 (m, 4H, -CONHC<u>H</u>$_2$-, piperazine-C<u>H</u>$_2$-phenyl), 2.54-2.37 (m, 10H, piperazine 8H, -NHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 162.21, 159.63, 151.43, 138.17, 135.26, 130.53, 129.04, 128.67, 127.99, 127.11, 126.72, 126.00, 125.31, 61.99, 56.49, 52.59, 52.56, 36.34. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5O$ calculated: 402.2294, found: 402.2299. Anal. Calcd. for $C_{24}H_{27}N_5O$: C, 71.79; H, 6.78; N, 17.44. Found: C, 71.61; H, 6.78; N, 17.37.

4.1.2.3. N-[2-(4-Benzylpiperidin-1-yl)ethyl]-6-phenylpyridazine-3-carboxamide ***(5c)***

Following the method A, reaction of compound **3** (1000 mg, 2.79 mmol) with phenylboronic acid (510 mg, 4.18 mmol) in the presence of $Pd(PPh_3)_4$ (162 mg, 0.14 mmol), 2M Na_2CO_3 solution (1.5 ml), toluene (12.5 ml) and EtOH (1.0 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 95:5) and recrystallized from MeOH. Yield: 590 mg, 53%; mp: 166 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.03 (t, 1H, *J* =5.4 Hz, amide H), 8.41 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.24 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.23-8.21 (m, 2H, $H^{2'}$, $H^{6'}$), 7.62-7.58 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.27 (t, 2H, *J* = 7.6 Hz, $H^{3''}$, $H^{5''}$), 7.18-7.14 (m, 3H, $H^{2''}$, $H^{4''}$, $H^{6''}$), 3.48 (q, 2H, *J* = 6.4 Hz, -CONHC<u>H</u>$_2$-), 2.89 (d, 2H, *J* = 11.0 Hz, piperidine H^{2e}, H^{6e}), 2.53-2.49 (m, 4H, piperidine-C<u>H</u>$_2$-phenyl and -NHCH$_2$C<u>H</u>$_2$-), 1.92 (t, 2H, *J* = 11.0 Hz, piperidine H^{2a}, H^{6a}), 1.56-1.47 (m, 3H, piperidine H^{3e}, H^4, H^{5e}), 1.25-1.19 (m, 2H, piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.19, 159.66, 151.47, 140.32, 135.29, 130.56, 129.08, 128.86, 128.00, 127.13, 126.02, 125.59, 125.34, 56.81, 53.18, 42.29, 37.30, 36.55, 31.73. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{29}N_4O$ calculated: 401.2341, found: 401.2348. Anal. Calcd. for $C_{25}H_{28}N_4O$: C, 74.97; H, 7.05; N, 13.99. Found: C, 74.57; H, 6.92; N, 14.08.

4.1.2.4. N-[2-(1-Benzylpiperidin-4-yl)ethyl]-6-phenylpyridazine-3-carboxamide ***(5d)***

Following the method A, reaction of compound **4** (850 mg, 2.37 mmol) with phenylboronic acid (433 mg, 3.55 mmol) in the presence of $Pd(PPh_3)_4$ (137 mg, 0.12 mmol), 2M Na_2CO_3 solution (1.3 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column

chromatography (EtOAc) and recrystallized from ACN. Yield: 400 mg, 42%; mp: 170 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.20 (t, 1H, *J* = 5.8 Hz, amide H), 8.40 (d, 1H, *J* = 9.2 Hz, pyridazine H), 8.24-8.20 (m, 3H, pyridazine H, $H^{2'}$, $H^{6'}$), 7.63-7.58 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.32-7.20 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.42-3.38 (m, 4H, piperidine-C<u>H</u>$_2$-phenyl and -CONHC<u>H</u>$_2$-), 2.77 (d, 2H, *J* = 11.5 Hz, piperidine H^{2e}, H^{6e}), 1.89 (t, 2H, *J* = 11.5 Hz, piperidine H^{2a}, H^{6a}), 1.69 (d, 2H, *J* = 11.5 Hz, piperidine H^{3e}, H^{5e}), 1.54 (q, 2H, *J* = 7.0 Hz, -NHCH$_2$C<u>H</u>$_2$-), 1.33-1.30 (m, 1H, piperidine H^4), 1.22-1.12 (qd, 2H, *J* = 11.5 Hz and 3.1 Hz piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.32, 159.57, 151.70, 138.66, 135.32, 130.57, 129.10, 128.63, 128.00, 127.12, 126.66, 126.09, 125.32, 62.46, 53.20, 36.66, 35.77, 32.89, 31.81. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{29}N_4O$ calculated: 401.2341, found: 401.2339. Anal. Calcd. for $C_{25}H_{28}N_4O$: C, 74.97; H, 7.05; N, 13.99. Found: C, 75.01; H, 6.85; N, 14.11.

4.1.2.5. 6-(4-Methoxyphenyl)-N-[2-(4-phenylpiperazin-1-yl)ethyl]pyridazine-3-carboxamide ***(5e)***

Following the method A, reaction of compound **1** (770 mg, 2.23 mmol) with 4-methoxyphenylboronic acid (507 mg, 3.34 mmol) in the presence of $Pd(PPh_3)_4$ (128 mg, 0.11 mmol), 2M Na_2CO_3 solution (1.25 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 96:4) and recrystallized from ACN. Yield: 285 mg, 31%; mp: 221 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.07 (bt, 1H, amide H), 8.34 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.21-8.18 (m, 3H, pyridazine H, $H^{2'}$, $H^{6'}$), 7.20 (t, 2H, *J* = 7.6 Hz, $H^{3''}$, $H^{5''}$), 7.14 (d, 2H, *J* = 9.2 Hz, $H^{3'}$, $H^{5'}$), 6.92 (d, 2H, *J* = 7.6 Hz, $H^{2''}$, $H^{6''}$), 6.76 (t, 1H, *J* = 7.6 Hz, $H^{4''}$), 3.86 (s, 3H, -OCH$_3$), 3.54 (q, 2H, *J* = 6.4 Hz -CONH-C<u>H</u>$_2$-), 3.14 (t, 4H, piperazine H^3, H^5), 2.63-2.60 (m, 6H, piperazine H^2, H^6 and -CONHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR ($CDCl_3$) δ ppm: 163.12, 161.95, 160.16, 150.89, 150.51, 129.13, 128.81, 127.85, 126.05, 123.87, 120.08, 116.34, 114.65, 56.74, 55.42, 52.95, 48.63, 36.12. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5O_2$ calculated: 418.2243, found: 418.2241. Anal. Calcd. for $C_{24}H_{27}N_5O_2$: C, 69.04; H, 6.52; N, 16.77. Found: C, 69.02; H, 6.56; N, 16.84.

4.1.2.6. N-[2-(4-Benzylpiperazin-1-yl)ethyl]-6-(4-methoxyphenyl)pyridazine-3-carboxamide ***(5f)***

Following the method A, reaction of compound **2** (550 mg, 1.52 mmol) with 4-methoxyphenylboronic acid (348.5 mg, 2.29 mmol) in the presence of $Pd(PPh_3)_4$ (88 mg, 0.076 mmol), 2M Na_2CO_3 solution (0.84 ml), toluene (9.0 ml) and EtOH (0.7 ml) gave crude product then, it was purified by column chromatography (EtOAc) and recrystallized from EtOH-water. Yield: 330 mg, 50%; mp: 148 °C. ^{1}H NMR (DMSO-d_6) δ: 9.06 (t, 1H, *J* = 5.6 Hz, amide H), 8.36 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.23-8.18 (m, 3H, pyridazine H, $H^{2'}$, $H^{6'}$), 7.34-7.22 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 7.15 (d, 2H, *J* = 8.4 Hz, $H^{3'}$, $H^{5'}$), 3.86 (s, 3H, OCH$_3$), 3.50-3.45 (m, 4H, -CONHC<u>H</u>$_2$-, piperazine-C<u>H</u>$_2$-phenyl), 2.55-2.39 (m, 10H, piperazine 8H, -NHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR (DMSO-d_6) δ: 162.29, 161.34, 159.17, 150.83, 138.16, 135.26, 128.70, 128.63, 128.03, 127.46, 126.76, 125.85, 124.38, 114.54, 62.00, 56.53, 55.31, 52.59, 52.56, 36.27. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{30}N_5O_2$ calculated: 432.2400, found: 432.2404. Anal. Calcd. for $C_{25}H_{29}N_5O_2$: C, 69.58; H, 6.77; N, 16.23. Found: C, 69.34; H, 6.82; N, 16.15.

4.1.2.7. N-[2-(4-Benzylpiperidin-1-yl)ethyl]-6-(4-methoxyphenyl)pyridazine-3-carboxamide ***(5g)***

Following the method A, reaction of compound **3** (750 mg, 2.09 mmol) with 4-methoxyphenylboronic acid (476 mg, 3.13 mmol) in the presence of $Pd(PPh_3)_4$ (121 mg, 0.105 mmol), 2M Na_2CO_3 solution (1.1 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 96:4) and recrystallized from ACN. Yield: 438 mg, 49%; mp: 154 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 8.97 (t, 1H, *J* = 5.4 Hz, amide H), 8.34 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8,21-8,17 (m, 3H, piridazin H, $H^{2'}$, $H^{6'}$), 7,26 (t, 2H, *J* = 7.6 Hz, $H^{3''}$, $H^{5''}$), 7.18-7.13 (m, 5H, $H^{3'}$, $H^{5'}$, $H^{2''}$, $H^{4''}$, $H^{6''}$), 3.86 (s, 3H, -OCH$_3$), 3.47 (q, 2H, *J* = 6.4 Hz, -CONHC<u>H</u>$_2$-), 2.89 (d, 2H, *J* = 11.5 Hz, piperidine H^{2e}, H^{6e}), 2.52-2.49 (m, 4H, piperidine-C<u>H</u>$_2$-phenyl and -CONHCH$_2$C<u>H</u>$_2$-), 1.91 (t, 2H, *J* = 11,5 Hz, piperidine H^{2a}, H^{6a}), 1,54 (d, 2H, *J* = 11,5 Hz, piperidin H^{3e}, H^{5e}), 1,49-1,47 (m, 1H, piperidine H^4), 1.25-1.19 (m, 2H, piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.24, 161.35, 159.17, 150.85, 140.28, 128.82, 128.59, 127.95, 127.49, 125.79, 125.55, 124.34, 114.53, 56.77, 55.30, 53.14, 42.25, 37.26, 36.47, 31.68. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4O_2$ calculated: 431.2447,

found: 431.2441. Anal. Calcd. for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.44; H, 6.76; N, 13.15.

4.1.2.8. N-[2-(1-Benzylpiperidin-4-yl)ethyl]-6-(4-methoxyphenyl)pyridazine-3-carboxamide ***(5h)***

Following the method A, reaction of compound **4** (850 mg, 2.37 mmol) with 4-methoxyphenylboronic acid (539 mg, 3.55 mmol) in the presence of $Pd(PPh_3)_4$ (137 mg, 0.12 mmol), 2M Na_2CO_3 solution (1.3 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 96:4) and recrystallized from ACN. Yield: 370 mg, 36%; mp: 171 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.15 (t, 1H, *J* = 5.8 Hz, amide H), 8.33 (d, 1H, *J* = 8.4 Hz, pyridazine H), 8.21-8.16 (m, 3H, pyridazine H, $H^{2'}$, $H^{6'}$), 7.32-7.21 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 7.14 (d, 2H, *J* = 8.8 Hz, $H^{3'}$, $H^{5'}$), 3.86 (s, 3H, -OCH_3), 3.42-3.37 (m, 4H, piperidine-C<u>H</u>$_2$-phenyl and -CONHC<u>H</u>$_2$-), 2.77 (d, 2H, *J* = 11.5 Hz, piperidine H^{2e}, H^{6e}), 1.89 (t, 2H, *J* = 11.5 Hz, piperidine H^{2a}, H^{6a}), 1.69 (d, 2H, *J* = 11.5 Hz, piperidine H^{3e}, H^{5e}), 1.54 (q, 2H, *J* = 6.8 Hz, -CONHCH$_2$C<u>H</u>$_2$-), 1.32-1.29 (m, 1H, piperidine H^4), 1.22-1.12 (qd, 2H, *J* = 11.5 Hz and 3.4 Hz piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.44, 161.38, 159.14, 151.12, 138.68, 128.67, 128.04, 127.57, 126.70, 125.93, 124.39, 114.60, 62.49, 55.36, 53.24, 36.63, 35.81, 32.92, 31.83. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4O_2$ calculated: 431.2447, found: 431.2463. Anal. Calcd. for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.78; H, 6.92; N, 13.19.

4.1.2.9. 6-[4-(Methylsulfanyl)phenyl]-N-[2-(4-phenylpiperazin-1-yl)ethyl]pyridazine-3-carboxamide ***(5i)***

Following the method A, reaction of compound **1** (800 mg, 2.30 mmol) with 4-(methylsulfanyl)phenylboronic acid (583 mg, 3.47 mmol) in the presence of $Pd(PPh_3)_4$ (133 mg, 0.12 mmol), 2M Na_2CO_3 solution (1.3 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (DCM) and recrystallized from butanol. Yield: 210 mg, 21%; mp: 233 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 8.53 (bt, 1H, amide H), 8.32 (d, 1H, *J* = 9.0 Hz, pyridazine H), 8.06 (d, 2H, *J* = 8.4 Hz, $H^{2'}$, $H^{6'}$), 7.97 (d, 1H, *J* = 9.0 Hz, pyridazine H), 7.38 (d, 2H, *J* = 8.4 Hz, $H^{3'}$, $H^{5'}$), 7.26 (t, 2H, *J* = 7.8 Hz, $H^{3''}$, $H^{5''}$), 6.93 (d, 2H, *J* = 7.8 Hz, $H^{2''}$, $H^{6''}$), 6.85 (t, 1H, *J* = 7.8 Hz, $H^{4''}$), 3.72 (q, 2H, *J* = 6.0 Hz, -CONHC<u>H</u>$_2$-), 3.26 (t, 4H, *J* = 4.8 Hz, piperazine H^3, H^5), 2.75-2.72 (m, 6H, piperazine H^2, H^6 and -CONHCH$_2$C<u>H</u>$_2$-), 2.54 (s, 3H, -SCH_3). ^{13}C NMR ($CDCl_3$) δ ppm: 162.91, 160.06, 150.89, 142.80, 131.72, 129.12, 127.52, 126.28, 126.15, 124.17, 119.92, 116.26, 56.76, 53.00, 48.81, 36.30, 15.13. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5OS$ calculated: 434.2015, found: 434.2010. Anal. Calcd. for $C_{24}H_{27}N_5OS$: C, 66.48; H, 6.28; N, 16.15; S, 7.40. Found: C, 66.13; H, 6.24; N, 16.02; S, 7.28.

4.1.2.10. N-[2-(4-Benzylpiperazin-1-yl)ethyl]-6-[4-(methylsulfanyl)phenyl]pyridazine-3-carboxamide ***(5j)***

Following the method A, reaction of compound **2** (550 mg, 1.52 mmol) with 4-(methylsulfanyl)phenylboronic acid (385 mg, 2.29 mmol) in the presence of $Pd(PPh_3)_4$ (88 mg, 0.076 mmol), 2M Na_2CO_3 solution (0.84 ml), toluene (9.0 ml) and EtOH (0.7 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 97:3) and recrystallized from ACN. Yield: 300 mg, 46%; mp: 171 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.09 (t, 1H, *J* = 5.6 Hz, amide H), 8.40 (d, 1H, *J* = 9.0 Hz, pyridazine H), 8.21 (d, 1H, *J* = 9.0 Hz, pyridazine H), 8.18 (d, 2H, *J* = 8.6 Hz, $H^{2'}$, $H^{6'}$), 7.46 (d, 2H, *J* = 8.6 Hz, $H^{3'}$, $H^{5'}$), 7.34-7.22 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.51-3.45 (m, 4H, -CONHC<u>H</u>$_2$-, piperazine-C<u>H</u>$_2$-phenyl), 2.56-2.39 (m, 13H, -SCH_3, piperazine 8H, -NHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 162.27, 159.15, 151.23, 142.08, 138.22, 131.37, 128.76, 128.09, 127.45, 126.82, 126.02, 125.84, 124.83, 62.06, 56.57, 52.66, 52.63, 36.35, 14.16.^{13}C NMR ($CDCl_3$) δ ppm: 162.91. 160.06. 150.89. 142.80. 131.72. 129.12. 127.52. 126.28. 126.15. 124.17. 119.92. 116.26. 56.76. 53.00. 48.81. 36.30. 15.13. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{30}N_5OS$ calculated: 448.2171, found: 448.2186. Anal. Calcd. for $C_{25}H_{29}N_5OS$: C, 67.08; H, 6.53; N, 15.65; S, 7.16. Found: C, 66.74; H, 6.42; N, 15.49; S, 7.14.

*4.1.2.11. N-[2-(4-Benzylpiperidin-1-yl)ethyl]-6-[4-(methylsulfanyl)phenyl]pyridazine-3-carboxamide **(5k)***

Following the method A, reaction of compound **3** (750 mg, 2.10 mmol) with 4-(methylsulfanyl)phenylboronic acid (528 mg, 3.14 mmol) in the presence of $Pd(PPh_3)_4$ (121 mg, 0.105 mmol), 2M Na_2CO_3 solution (1.2 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (EtOAc) and recrystallized from ACN. Yield: 520 mg, 56%; mp: 183 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 8.96 (t, 1H, *J* =5.6 Hz, amide H), 8.35 (d, 1H, *J* = 9.0 Hz, pyridazine H), 8.19 (d, 1H, *J* = 9.0 Hz, pyridazin H), 8.15 (d, 2H, *J* = 8.6 Hz, $H^{2'}$, $H^{6'}$), 7.44 (d, 2H, *J* = 8.6 Hz, $H^{3'}$, $H^{5'}$), 7.24 (t, 2H, *J* = 7.4 Hz, $H^{3''}$, $H^{5''}$), 7.16-7.12 (m, 3H, $H^{2''}$, $H^{4''}$, $H^{6''}$), 3.45 (q, 2H, *J* = 6.2 Hz, -CONHC$\underline{H}_2$-), 2.86 (d, 2H, *J* = 11.3 Hz, piperidine H^{2e}, H^{6e}), 2.54 (s, 3H, SCH_3), 2.50-2.47 (m, 4H, piperidine-C$\underline{H}_2$-phenyl and -NHCH$_2$C$\underline{H}_2$-), 1.89 (t, 2H, *J* = 11.3 Hz, piperidine H^{2a}, H^{6a}), 1.54-1.45 (m, 3H, piperidine H^{3e}, H^4, H^{5e}), 1.23-1.12 (m, 2H, piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.23, 159.15, 151.26, 142.01, 140.32, 131.46, 128.86, 127.99, 127.42, 125.93, 125.59, 124.76, 56.80, 53.18, 42.29, 36.66, 37.29, 36.56, 31.73, 14.23. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4OS$ calculated: 447.2219, found: 447.2212. Anal. Calcd. for $C_{26}H_{30}N_4OS$: C, 69.92; H, 6.77; N, 12.54; S, 7.18. Found: C, 70.31; H, 6.67; N, 12.58; S, 7.22.

*4.1.2.12. N-[2-(1-Benzylpiperidin-4-yl)ethyl]-6-[4-(methylsulfanyl)phenyl]pyridazine-3-carboxamide **(5l)***

Following the method A, reaction of compound **4** (850 mg, 2.37 mmol) with 4-(methylsulfanyl)phenylboronic acid (598 mg, 3.56 mmol) in the presence of $Pd(PPh_3)_4$ (137 mg, 0.12 mmol), 2M Na_2CO_3 solution (1.3 ml), toluene (11.0 ml) and EtOH (0.9 ml) gave crude product then, it was purified by column chromatography (EtOAc:MeOH, 97:3) and recrystallized from EtOAc. Yield: 610 mg, 58%; mp: 191 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 9.15 (t, 1H, *J* = 6.2 Hz, amide H), 8.35 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.18 (d, 1H, *J* = 8.8 Hz, pyridazine H), 8.15 (d, 2H, *J* = 8.4 Hz $H^{2'}$, $H^{6'}$), 7.44 (d, 2H, *J* = 8.4 Hz, $H^{3'}$, $H^{5'}$), 7.30-7.18 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.40-3.35 (m, 4H, piperidine-C$\underline{H}_2$-phenyl, -CONHC$\underline{H}_2$-), 2.75 (d, 2H, *J* = 11.2 Hz, piperidine H^{2e}, H^{6e}), 2.54 (s, 3H, SCH_3) 1.87 (t, 2H, *J* = 11.5 , piperidine H^{2a}, H^{6a}), 1.66 (d, 2H, *J* = 11.2 Hz, piperidine H^{3e}, H^{5e}), 1.51 (q, 2H, *J* = 7.0 Hz, -NHCH$_2$C$\underline{H}_2$-), 1.37-1.24 (m, 1H, piperidine H^4), 1.20-1.13 (qd, 2H, *J* = 11.2 Hz and 3. 3 Hz piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 162.33, 159.05, 151.47, 141.96, 138.66, 131.48, 128.60, 127.96, 127.38, 126.62, 125.95, 125.92, 124.70, 62.44, 53.17, 36.66, 35.73, 32.91, 31.80, 14.21. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4OS$ calculated: 447.2219, found: 447.2223. Anal. Calcd. for $C_{26}H_{30}N_4OS$: C, 69.92; H, 6.77; N, 12.54; S, 7.18. Found: C, 69.68; H, 6.72; N, 12.44; S, 7.17.

*4.1.3. General procedure for the synthesis of N-(2-substitutedethyl)-[1,1'-biphenyl]-4-carboxamide derivatives **(6a-d)** (Method B)*

A mixture of [1,1'-biphenyl]-4-carboxylic acid (1.1 equivalent) and EDC (1.2 equivalent) in DCM (10 ml) stirred at room temperature for 10 min then, DMAP (0.2 equivalent) was added to stirred mixture and stirred for additional 5 min. To this mixture, appropriate 2-substituted ethan-1-amine derivative (1 equivalent) was added. After stirring overnight at room temperature, the reaction mixture was evaporated to dryness then the precipitate boiled with water and petroleum ether respectively. The resulting precipitate was filtered and crystallized from appropriate solvent.

*4.1.3.1. N-[2-(4-Phenylpiperazin-1-yl)ethyl]-[1,1'-biphenyl]-4-carboxamide **(6a)***

Following the method B, reaction of 2-(4-phenylpiperazin-1-yl)ethan-1-amine (400 mg, 1.95 mmol) with [1,1'-biphenyl]-4-carboxylic acid (425 mg, 2.14 mmol) in the presence of EDC (488 mg, 2.33 mmol) and DMAP (49 mg, 0.39 mmol) gave crude product then, it was recrystallized from butanol. Yield: 300 mg, 40%; mp: 238 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 8.45 (bt, 1H, amide H), 7.94 (d, 2H, *J* = 8.0 Hz, H^2, H^6), 7.74 (d, 2H, *J* = 8.0 Hz, H^3, H^5), 7.72 (d, 2H, *J* = 7.6 Hz, $H^{2'}$, $H^{6'}$), 7.49 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.40 (t, 1H, *J* = 7.6 Hz, $H^{4'}$), 7.20 (t, 2H, *J* = 7.9 Hz, $H^{3''}$, $H^{5''}$), 6.92 (d, 2H, *J* = 7.9

Hz, $H^{2''}$, $H^{6''}$), 6.76 (t, 1H, *J* = 7.9 Hz, $H^{4''}$), 3.45 (q, 2H, *J* = 6.4 Hz, -CONHC<u>H</u>$_2$-), 3.13 (t, 4H, *J* = 4,8 Hz, piperazine H^3, H^5), 2.61-2.54 (m, 6H, piperazine H^2, H^6 and -CONHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR ($CDCl_3$) δ ppm: 167.07, 151.16, 144.20, 140.04, 133.27, 129.14, 128.91, 127.95, 127.42, 127.25, 127.18, 119.87, 116.06, 56.37, 52.87, 49.23, 36.30. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{28}N_3O$ calculated: 386.2232, found: 386.2227. Anal. Calcd. for $C_{25}H_{27}N_3O$: C, 77.89; H, 7.06; N, 10.90. Found: C, 77.99; H, 7.23; N, 10.81.

4.1.3.2. N-[2-(4-Benzylpiperazin-1-yl)ethyl]-[1,1'-biphenyl]-4-carboxamide ***(6b)***

Following the method B, reaction of 2-(4-benzylpiperazin-1-yl)ethan-1-amine (300 mg, 1.37 mmol) with [1,1'-biphenyl]-4-carboxylic acid (298 mg, 1.51 mmol) in the presence of EDC (315 mg, 1.64 mmol) and DMAP (34 mg, 0.28 mmol) gave crude product then, it was recrystallized from isopropanol. Yield: 100 mg, 18%; mp: 141 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 8.39 (t, 1H, *J* = 5.6 Hz, amide H), 7.92 (d, 2H, *J* = 8.2 Hz, H^2, H^6), 7.76 (d, 2H, *J* = 8.2 Hz, H^3, H^5), 7.72 (d, 2H, *J* = 7.6 Hz, $H^{2'}$, $H^{6'}$), 7.49 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.41 (t, 1H, *J* = 7.6 Hz, $H^{4'}$), 7.34-7.22 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.45 (s, 2H, piperazine-CH$_2$-phenyl), 3.39 (q, 2H, *J* = 6.8 Hz, -CONHC<u>H</u>$_2$-), 2.49-2.36 (m, 10H, piperazine and -CONHCH$_2$C<u>H</u>$_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 165.66, 142.50, 139.13, 138.18, 133.34, 128.89, 128.69, 128.01, 127.88, 127.68, 126.74, 126.37, 62.02, 56.86, 52.71, 52.58, 36.84. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{30}N_3O$ calculated: 400.2389, found: 400.2387. Anal. Calcd. for $C_{26}H_{29}N_3O$: C, 78.16; H, 7.32; N, 10.52. Found: C, 77.95; H, 7.42; N, 10.62.

4.1.3.3. N-[2-(4-Benzylpiperidin-1-yl)ethyl]-[1,1'-biphenyl]-4-carboxamide ***(6c)***

Following the method B, reaction of 2-(4-benzylpiperidin-1-yl)ethan-1-amine (350 mg, 1.60 mmol) with [1,1'-biphenyl]-4-carboxylic acid (350 mg, 1.76 mmol) in the presence of EDC (369 mg, 1.92 mmol) and DMAP (39 mg, 0.32 mmol) gave crude product then, it was recrystallized from EtOH-water. Yield: 300 mg, 47%; mp: 155 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 8.42 (t, 1H, *J* = 5.4 Hz, amide H), 7.92 (d, 2H, *J* = 8.6 Hz, H^2, H^6), 7.76 (d, 2H, *J* = 7.6 Hz, H^3, H^5), 7.72 (d, 2H, *J* = 7.6 Hz, $H^{2'}$, $H^{6'}$), 7.49 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.40 (t, 1H, *J* = 7.6 Hz, $H^{4'}$), 7.27 (t, 2H, *J* = 7.4 Hz, $H^{3''}$, $H^{5''}$), 7.19-7.14 (m, 3H, $H^{2''}$, $H^{4''}$, $H^{6''}$), 3.40-3.34 (m, 2H, -CONHC<u>H</u>$_2$-), 2.87 (d, 2H, *J* = 11.8 Hz, piperidine H^{2e}, H^{6e}), 2.48-2.43 (m, 4H, piperidine-CH$_2$-phenyl and -CONHCH$_2$C<u>H</u>$_2$-), 1.89 (t, 2H, *J* = 11,8 Hz, piperidin H^{2a}, H^{6a}), 1,53 (d, 2H, *J* = 11,8 Hz, piperidin H^{3e}, H^{5e}), 1,47-1,42 (m, 1H, piperidine H^4), 1.22-1.17 (m, 2H, piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 165.60, 142.48, 140.29, 139.11, 133.31, 128.91, 128.86, 128.00, 127.90, 127.69, 126.75, 126.38, 125.59, 57.20, 53.31, 42.31, 37.29, 37.02, 31.69. HRMS (ESI) $[M+H]^+$ m/z for $C_{27}H_{31}N_2O$ calculated: 399.2436, found: 399.2432 Anal. Calcd. for $C_{27}H_{30}N_2O$: C, 81.37; H, 7.59; N, 7.03. Found: C, 81.56; H, 7.66; N, 7.23.

4.1.3.4. N-[2-(1-Benzylpiperidin-4-yl)ethyl]-[1,1'-biphenyl]-4-carboxamide ***(6d)***

Following the method B, reaction of 2-(1-benzylpiperidin-4-yl)ethan-1-amine (350 mg, 1.60 mmol) with [1,1'-biphenyl]-4-carboxylic acid (350 mg, 1.76 mmol) in the presence of EDC (369 mg, 1.92 mmol) and DMAP (39 mg, 0.32 mmol) gave crude product then, it was recrystallized from EtOH-water. Yield: 300 mg, 47%; mp: 137 °C. CAS Number: 756780-04-8. ^{1}H NMR (DMSO-d_6) δ ppm: 8.47 (t, 1H, *J* = 5.6 Hz, amide H), 7.93 (d, 2H, *J* = 8.4 Hz, H^2, H^6), 7.75 (d, 2H, *J* = 8.4 Hz, H^3, H^5), 7.72(d, 2H, *J* = 7.6 Hz, $H^{2'}$, $H^{6'}$), 7.49 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.40 (t, 1H, *J* = 7.6 Hz, $H^{4'}$), 7.33-7.21 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.42 (s, 2H, piperidine-C<u>H</u>$_2$-phenyl), 3.32-3.29 (m, 2H, -CONHC<u>H</u>$_2$-), 2.77 (d, 2H, *J* = 11.8 Hz, piperidine H^{2e}, H^{6e}), 1.89 (t, 2H, *J* = 11.8 Hz, piperidine H^{2a}, H^{6a}), 1.67 (d, 2H, *J* = 11.8 Hz, piperidine H^{3e}, H^{5e}), 1.48 (q, 2H, *J* = 7.2 Hz, -CONHCH$_2$C<u>H</u>$_2$-), 1.32-1.29 (m, 1H, piperidin H^4), 1.21-1.14 (qd, 2H, *J* = 11.8 Hz and 3.2 Hz, piperidine H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 165.55, 142.43, 139.14, 138.62, 133.41, 128.91, 128.62, 127.97, 127.88, 127.70, 126.74, 126.64, 126.35, 62.44, 53.19, 36.80, 35.84, 32.93, 31.81. HRMS (ESI) $[M+H]^+$ m/z for $C_{27}H_{31}N_2O$ calculated: 399.2436, found: 399.2417 Anal. Calcd. for $C_{27}H_{30}N_2O$: C, 81.37; H, 7.59; N, 7.03. Found: C, 81.50; H, 7.61; N, 7.25.

*4.1.4. General procedure for the synthesis of 6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-amine intermediates **(7-9)***

6-Phenylpyridazin-3-amine **(7)**, 6-(4-methoxyphenyl)pyridazin-3-amine **(8)** and 6-(4-(methylsulfanyl)phenyl)pyridazin-3-amine **(9)** were synthesized according previously reported method [25]. Intermediates were recrystallized from toluene before using for method C. Yield for **(7)**: 61%.; mp: 153 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{10}H_9N_3$ calculated: 172.0875, found: 172.0869. Yield for **(8)**: 66%; mp: 165 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{11}H_{11}N_3O$ calculated: 202.0980, found: 202.0975. Yield for **(9)**: 68%; mp: 176 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{11}H_{11}N_3S$ calculated: 218.0752, found: 218.0745.

*4.1.5. General procedure for the synthesis of 3-substitutedpropanoic acid intermediates **(10-12)***

To a mixture of 1-phenylpiperazine, 1-benzylpiperazine or 4-benzylpiperidine (2.0 g, 1 equivalent) in DCM (15 ml), methyl acrylate (1.2 equivalent) was added. The mixture stirred at room temperature overnight. After evaporation under reduced pressure, corresponding methyl ester was obtained and hydrolyzed by heating at 40 °C for 30 min with 5% NaOH solution. End of this period, reaction mixture was cooled to room temperature and neutralized with HCl. The resulting precipitate was filtered, dried and used for the method C without further purification. Yield for 3-(4-phenylpiperazin-1-yl)propanoic acid **(10)**: 36%; mp: 196 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{13}H_{18}N_2O_2$ calculated: 235.1447, found: 235.1452. Yield for 3-(4-benzylpiperazin-1-yl)propanoic acid **(11)**: 31%; mp: 99 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{14}H_{20}N_2O_2$ calculated: 249.1603, found: 249.1595. Yield for 3-(4-benzylpiperidin-1-yl)propanoic acid **(12)**: 65%; mp: 150 °C. HRMS (ESI) $[M+H]^+$ m/z for $C_{15}H_{21}N_2O$ calculated: 248.1651, found: 248.1639.

*4.1.6. General procedure for the synthesis of 3-substituted-N-(6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-yl)propanamide derivatives **(13a-i)** (Method C)*

A mixture of appropriate 3-substitutedpropanoic acid **(10-12)** (1.1 equivalent) and EDC (1.2 equivalent) in DCM (10 ml) stirred at room temperature for 10 min then, DMAP (0.2 equivalent) was added to stirred mixture and stirred for additional 5 min. To this mixture, 3-substituted-*N*-(6-(phenyl/4-methoxyphenyl/4-methylsulfanylphenyl)pyridazin-3-yl)propanamide intermediate **(7-9)** (1 equivalent) was added. After stirring overnight at room temperature, the reaction mixture was evaporated to dryness then the precipitate boiled with water and petroleum ether respectively. The resulting precipitate was filtered and crystallized from appropriate solvent.

*4.1.6.1. 3-(4-Phenylpiperazine-1-yl)-N-(6-phenylpyridazin-3-yl)propanamide **(13a)***

Following the method C, reaction of **7** (300 mg, 1.75 mmol) with **10** (415 mg, 1.93 mmol) in the presence of EDC (403 mg, 2.10 mmol) and DMAP (43 mg, 0.35 mmol) gave crude product then, it was recrystallized from butanol. Yield: 210 mg, 31%; mp: 221 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 11.46 (s, 1H, amide H), 8.41 (d, 1H, *J* = 9.4 Hz, pyridazine H), 8.22 (d, 1H, *J* = 9.4 Hz, pyridazine H), 8.09 (d, 2H, *J* = 6.8 Hz, $H^{2'}$, $H^{6'}$), 7.56-7.49 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.20 (t, 2H, *J* = 7.6 Hz, $H^{3''}$, $H^{5''}$), 6.94 (d, 2H, *J* = 7.6 Hz, $H^{2''}$, $H^{6''}$), 6.77 (t, 1H, *J* = 7.6 Hz, $H^{4''}$), 3.16 (t, 4H, *J* = 4.8 Hz, piperazine H^3, H^5), 2.74-2.69 (m, 4H, -COC<u>H</u>$_2$C<u>H</u>$_2$-), 2.61 (t, 4H, *J* = 4.8 Hz, piperazine H^2, H^6). ^{13}C NMR (DMSO-d_6) δ ppm: 171.89, 155.08, 154.51, 150.89, 135.82, 129.42, 128.86, 128.80, 126.21, 125.83, 118.71, 118.44, 115.27, 53.26, 52.26, 48.11, 33.57. HRMS (ESI) $[M+H]^+$ m/z for $C_{23}H_{26}N_5O$ calculated: 388.2137, found: 388.2137. Anal. Calcd. for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 70.91; H, 6.47; N, 18.15.

*4.1.6.2. 3-(4-Benzylpiperazine-1-yl)-N-(6-phenylpyridazin-3-yl)propanamide **(13b)***

Following the method C, reaction of **7** (250 mg, 1.46 mmol) with **11** (399 mg, 1.61 mmol) in the presence of EDC (336 mg, 1.75 mmol) and DMAP (18 mg, 0.15 mmol) gave crude product then, it was recrystallized from butanol. Yield: 150 mg, 31%; mp: 207 °C. ^{1}H NMR (DMSO-d_6) δ: 11.38 (s,

1H, amide H), 8.39 (d, 1H, *J* = 9.6 Hz, pyridazine H), 8.20 (d, 1H, *J* = 9.6 Hz, pyridazine H), 8.09 (d, 2H, *J* = 6.8 Hz, $H^{2'}$, $H^{6'}$), 7.56-7.49 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.31-7.23 (m, 4H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.46 (s, 1H, piperazine-C$\underline{H_2}$-phenyl), 2.69-2.61 (m, 4H, -COC$\underline{H_2}$C$\underline{H_2}$-), 2.46-2.38 (m, 8H, piperazine). ^{13}C NMR (DMSO-d_6) δ ppm: 171.82, 155.06, 154.43, 138.15, 135.85, 129.31, 128.78, 128.61, 127.95, 126.68, 126.16, 125.68, 118.40, 61.90, 53.20, 52.48, 52.24, 33.53. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5O$ calculated: 402.2294, found: 402.2287. Anal. Calcd. for $C_{24}H_{27}N_5O$: C, 71.29; H, 6.78; N, 17.44. Found: C, 71.48; H, 6.39; N, 17.48.

4.1.6.3. 3-(4-Benzylpiperidin-1-yl)-N-(6-phenylpyridazin-3-yl)propanamide ***(13c)***

Following the method C, reaction of **7** (400 mg, 2.33 mmol) with **12** (636 mg, 2.57 mmol) in the presence of EDC (538 mg, 2.81 mmol) and DMAP (58 mg, 0.47 mmol) gave crude product then, it was recrystallized from isobutanol. Yield: 185 mg, 20%; mp: 209 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 12.33 (s, 1H, amide H), 8.53 (d, 1H, *J* = 9.4 Hz, pyridazine H), 8.04 (d, 2H, *J* = 6.8 Hz, $H^{2'}$, $H^{6'}$), 7.84 (d, 1H, *J* = 9.4 Hz, pyridazine H), 7.54-7.44 (m, 3H, $H^{3'}$, $H^{4'}$, $H^{5'}$), 7.31-7.16 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.10 (d, 2H, *J* = 10.8 Hz, piperidin H^{2e}, H^{6e}), 2.73 (t, 2H, *J* = 6.0 Hz, -COCH_2C$\underline{H_2}$-), 2.62-2.58 (m, 4H, -COC$\underline{H_2}$CH_2- and piperidin-C$\underline{H_2}$-phenyl), 2.06 (t, 2H, *J* = 10.8 Hz, piperidin H^{2a}, H^{6a}), 1.74 (d, 2H, *J* = 10.8 Hz, piperidin H^{3e}, H^{5e}), 1.60-1.55 (m, 3H, piperidin H^{3a}, H^4, H^{5a}). ^{13}C NMR ($CDCl_3$) δ ppm: 172.29, 156.23, 154.71, 140.72, 136.36, 129.50, 129.10, 128.93, 128.21, 126.68, 125.82, 125.62, 119.20, 53.63, 53.26, 42.77, 38.27, 33.02, 32.08. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{29}N_4O$ calculated: 401.2341, found: 401.2344. Anal. Calcd. for $C_{25}H_{28}N_4O$: C, 74.97; H, 7.05; N, 13.99. Found: C, 74.66; H, 7.12; N, 13.60.

4.1.6.4. N-[6-(4-Methoxyphenyl)pyridazin-3-yl]-3-(4-phenylpiperazin-1-yl)propanamide ***(13d)***

Following the method C, reaction of **8** (350 mg, 1.74 mmol) with **10** (451 mg, 1.93 mmol) in the presence of EDCI(403 mg, 2.10 mmol) and DMAP (43 mg, 0.35 mmol) gave crude product then, it was recrystallized from butanol. Yield: 225 mg, 31%; mp: 251 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 11.35 (s, 1H, amide H), 8.35 (d, 1H, *J* = 9.6 Hz, pyridazine H), 814 (d, 1H, *J* = 9.6 Hz, pyridazine H), 8.04 (d, 2H, *J* = 8.6 Hz, $H^{2'}$, $H^{6'}$), 7.20 (t, 2H, *J* = 7.6 Hz, $H^{3''}$, $H^{5''}$), 7.08 (d, 2H, *J* = 8.6 Hz, $H^{3'}$, $H^{5'}$), 6.94 (d, 2H, *J* = 7.6 Hz, $H^{2''}$, $H^{6''}$), 6.77 (t, 1H, *J* = 7.6 Hz, $H^{4''}$), 3.83 (s, 3H, -OCH_3), 3.16 (bt, 4H, piperazine H^3, H^5), 2.74-2.68 (m, 4H, -COC$\underline{H_2}$C$\underline{H_2}$-), 2.62 (bt, 4H, piperazine H^2, H^6). ^{13}C NMR ($CDCl_3$) δ: 160.97, 156.00, 154.12, 152.48, 150.88, 129.10, 128.77, 127.97, 125.05, 120.07, 119.21, 116.37, 114.37, 55.34, 53.38, 52.57, 48.94, 32.98. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5O_2$ calculated: 418.2243, found: 418.2235. Anal. Calcd. for $C_{24}H_{27}N_5O_2$: C, 69.04; H, 6.52; N, 16.77. Found: C, 69.20; H, 6.44; N, 16.83.

4.1.6.5. 3-(4-Benzylpiperazin-1-yl)-N-[6-(4-methoxyphenyl)pyridazin-3-yl]propanamide ***(13e)***

Following the method C, reaction of **8** (390 mg, 1.94 mmol) with **11** (530 mg, 2.13 mmol) in the presence of EDC (447 mg, 2.33 mmol) and DMAP (48 mg, 0.39 mmol) gave crude product then, it was recrystallized from butanol. Yield: 295 mg, 35%; mp: 211 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 11.31 (s, 1H, amide H), 8.34 (d, 1H, *J* = 9.2 Hz, pyridazine H), 8.13 (d, 1H, *J* = 9.2 Hz, pyridazine H), 8.05 (d, 2H, *J* = 8.8 Hz, $H^{2'}$, $H^{6'}$), 7.34-7.21 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 7.09 (d, 2H, *J* = 8.8 Hz, $H^{3'}$, $H^{5'}$), 3.84 (s, 3H, -OCH_3), 3.46 (s, 2H, piperazine-C$\underline{H_2}$-phenyl), 2.67-2.61 (m, 4H, -COC$\underline{H_2}$C$\underline{H_2}$-), 2.48-2.38 (t, 8H, piperazine). ^{13}C NMR (DMSO-d_6) δ ppm: 171.77, 160.42, 154.83, 154.02, 138.20, 128.67, 128.29, 128.02, 127.60, 126.75, 125.04, 118.49, 114.29, 61.95, 55.20, 53.28, 52.55, 52.29, 33.53. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{30}N_5O_2$ calculated: 432.2400, found: 432.2386. Anal. Calcd. for $C_{25}H_{29}N_5O_2$: C, 69.58; H, 6.77; N, 16.23. Found: C, 69.56; H, 6.60; N, 16.35.

4.1.6.6. 3-(4-Benzylpiperidin-1-yl)-N-[6-(4-methoxyphenyl)pyridazin-3-yl]propanamide ***(13f)***

Following the method C, reaction of **8** (400 mg, 1.99 mmol) with **12** (541 mg, 2.19 mmol) in the presence of EDC (458 mg, 2.39 mmol) and DMAP (49 mg, 0.40 mmol) gave crude product then, it was recrystallized from isopropanol. Yield: 450 mg, 53%; mp: 200 °C. ^{1}H NMR ($CDCl_3$) δ ppm:

12.23 (s, 1H, amide H), 8.49 (d, 1H, *J* = 9.2 Hz, pyridazine H), 8.01 (d, 2H, *J* = 8.8 Hz, $H^{2'}$, $H^{6'}$), 7.79 (d, 1H, *J* = 9.2 Hz, pyridazine H), 7.30-7.16 (m, 5H, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 7.03 (d, 2H, *J* = 8.8 Hz, $H^{3'}$, $H^{5'}$), 3.88 (s, 3H, -OCH_3), 3.10 (d, 2H, *J* = 11.0 Hz, piperidin H^{2e}, H^{6e}), 272 (t, 2H, *J* = 5.8 Hz, -$COCH_2C\underline{H}_2$-), 2.62-2.58 (m, 4H, -$COC\underline{H}_2CH_2$- and piperidin-$C\underline{H}_2$-phenyl), 2.06 (t, 2H, *J* = 11.0 Hz, piperidin H^{2a}, H^{6a}), 1.74 (d, 2H, *J* = 11.0 Hz, piperidin H^{3e}, H^{5e}), 1.60-1.58 (m, 3H, piperidin H^{3a}, H^4, H^{5a}). ^{13}C NMR ($CDCl_3$) δ ppm: 172.18, 160.88, 155.89, 154.26, 140.72, 129.09, 128.89, 128.20, 127.98, 125.81, 124.96, 119.26, 114.34, 55.37, 53.65, 53.26, 42.76, 38.25, 33.01, 32.06. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4O_2$ calculated: 431.2447, found: 431.2448. Anal. Calcd. for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.52; H, 7.16; N, 12.86.

4.1.6.7. N-{6-[4-(Methylsulfanyl)phenyl]pyridazin-3-yl}-3-(4-phenylpiperazin-1-yl)propanamide ***(13g)***

Following the method C, reaction of **9** (350 mg, 1.61 mmol) with **10** (415 mg, 1.77 mmol) in the presence of EDC (370 mg, 1.93 mmol) and DMAP (39 mg, 0.32 mmol) gave crude product then, it was recrystallized from butanol. Yield: 100 mg, 14%; mp: 243 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 11.75 (s, 1H, amide H), 8.49 (d, 1H, *J* = 9.4 Hz, pyridazine H), 7.92 (d, 2H, *J* = 8.4 Hz, $H^{2'}$, $H^{6'}$), 7.80 (d, 1H, *J* = 9.4 Hz, pyridazine H), 7.35 (d, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.26 (t, 2H, *J* = 8.0 Hz, $H^{3''}$, $H^{5''}$), 6.92-6.87 (m, 3H, $H^{2''}$, $H^{4''}$, $H^{6''}$), 3.41 (bt, 3H, 4H, piperazine H^3, H^5), 3.24-2.88 (m, 8H, piperazine H^2, H^6, -$COC\underline{H}_2C\underline{H}_2$-), 2.50 (s, 3H, -$SCH_3$). ^{13}C NMR ($CDCl_3$) δ ppm: 155.86, 154.39, 141.05, 137.74, 132.63, 129.19, 126.96, 126.41, 125.52, 120.51, 119.38, 116.61, 55.12, 52.47, 48.27, 32.62, 15.30. HRMS (ESI) $[M+H]^+$ m/z for $C_{24}H_{28}N_5OS$ calculated: 434.2015, found: 434.2025. Anal. Calcd. for $C_{24}H_{27}N_5OS$: C, 66.48; H, 6.28; N, 16.15; S, 7.40. Found: C, 66.35; H, 6.23; N, 15.98; S, 7.43.

4.1.6.8. 3-(4-Benzylpiperazin-1-yl)-N-{6-[4-(methylsulfanyl)phenyl]pyridazin-3-yl}propanamide ***(13h)***

Following the method C, reaction of **9** (450 mg, 2.07 mmol) with **11** (566 mg, 2.28 mmol) in the presence of EDC (476 mg, 2.48 mmol) and DMAP (51 mg, 0.41 mmol) gave crude product then, it was recrystallized from EtOH. Yield: 150 mg, 16%; mp: 203 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 11.94 (s, 1H, amide H), 8.51 (d, 1H, *J* = 9.2 Hz, pyridazine H), 7.96 (d, 2H, *J* = 88 Hz, $H^{2'}$, $H^{6'}$), 7.81 (d, 1H, *J* = 9.2 Hz, pyridazine H), 7.40-726 (m, 7H, $H^{3'}$, $H^{5'}$, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.66 (s, 2H, piperazine-$C\underline{H}_2$-phenyl), 2.88-2.62 (m, 12H, piperazine, -$COC\underline{H}_2C\underline{H}_2$-), 2.54 (s, 3H, -$SCH_3$). ^{13}C-NMR ($CDCl_3$) δ ppm: 155.77, 154.48, 140.92, 132.68, 129.53, 128.45, 127.53, 126.89, 126.36, 125.35, 119.30, 62.34, 53.16, 52.50, 51.93, 32.91, 15.36. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{30}N_5OS$ calculated: 448.2171, found: 448.2173. Anal. Calcd. for $C_{25}H_{29}N_5OS$: C, 67.08; H, 6.53; N, 15.65; S, 7.16. Found: C, 67.04; H, 6.47; N, 15.50; S, 7.20.

4.1.6.9. 3-(4-Benzylpiperidin-1-yl)-N-{6-[4-(methylsulfanyl)phenyl]pyridazin-3-yl}propanamide ***(13i)***

Following the method C, reaction of **9** (450 mg, 2.07 mmol) with **12** (566 mg, 2.28 mmol) in the presence of EDC (476 mg, 2.48 mmol) and DMAP (51 mg, 0.41 mmol) gave crude product then, it was recrystallized from MeOH. Yield: 60 mg, 6%; mp: 198 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 11.44 (s, 1H, amide H), 8.36 (d, 1H, *J* = 9.2 Hz, pyridazine), 8.18 (d, 1H, *J* = 9.2 Hz, pyridazine), 8.05 (d, 2H, *J* = 8.6 Hz, $H^{2'}$, $H^{6'}$), 7.41 (d, 2H, *J* = 86 Hz, $H^{3'}$, $H^{5'}$), 7.27 (t, 2H, $H^{3''}$, $H^{5''}$), 718-7.15 (m, 3H, $H^{2''}$, $H^{4''}$, $H^{6''}$), 2.91(d, 2H, *J* = 10,9 Hz, piperidin H^{2a}, H^{6a}), 2.65-2.58 (m, 4H, -$COC\underline{H}_2C\underline{H}_2$-), 254 (s, 3H, -$SCH_3$), 2,51 (d, .2H, piperidin-CH_2-phenyl with DMSO), 1.92 (t, 2H, *J* = 10.9 Hz, piperidin H^{3e}, H^{5e}), 1.59-1.51 (m, 3H, piperidin H^{3e}, H^4, H^{5e}), 1.24-1.21 (m, 2H, piperidin H^{3a}, H^{5a}). ^{13}C NMR (DMSO-d_6) δ ppm: 171.96, 154.58, 154.35, 140.30, 140.22, 132.20, 128.83, 128.01, 126.55, 125.89, 125.60, 125.35, 118.44, 53.56, 52.78, 42.21, 37.29, 33.64, 31.67, 14.30. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{31}N_4OS$ calculated: 447.2219, found: 447.2203. Anal. Calcd. for $C_{26}H_{30}N_4OS$: C, 69.92; H, 6.77; N, 12.54; S, 7.18. Found: C, 69.53; H, 6.86; N, 12.55; S, 7.28.

4.1.7. General procedure for the synthesis of N-([1,1'-biphenyl]-4-yl)-3-substitutedpropanamide derivatives ***(15a-c)*** *(Method D)*

DMAP (72 mg, 0.59 mmol) and TEA (0.62 ml, 4.42) were added to [1,1'-biphenyl]-4-amine (1 g, 5.9 mmol) in dimethylformamide (8 ml) and the mixture cooled in the ice bath. To this cooled solution 3-chloropropionyl chloride (0.85 ml, 8.87 mmol) was added dropwise and then reaction mixture was stirred at room temperature for 25 min. At the end of this period, reaction mixture was poured into ice water and the precipitate was filtered to yield N-([1,1'-biphenyl]-4-yl)-3-chloropropanamide **(14)**, which was used for the next step without further purification.
To solution of N-([1,1'-biphenyl]-4-yl)-3-chloropropanamide (1 equivalent) in 15 mL of ACN, NaI (3 equivalent) was added and the mixture was refluxed for 2 h and cooled to room temperature. Subsequently, anhydrous potassium carbonate (2.5 equivalent) and appropriate amine derivative (2.5 equivalent) were added. Then the mixture was refluxed for 3 h. After evaporation under reduced pressure, the crude product was purified by crystallization from an appropriate solvent.

*4.1.7.1. N-([1,1'-Biphenyl]-4-yl)-3-(4-phenylpiperazin-1-yl)propanamide **(15a)***

Following the method D, reaction of **14** (400 mg, 1.54 mmol) with phenylpiperazine (0.58 ml, 3.84 mmol) in the presence of NaI (692 mg, 4.61 mmol) and anhydrous potassium carbonate (531 mg, 3.84 mmol) gave crude product then, it was recrystallized from butanol. Yield: 420 mg, 70%; mp: 215 °C. ^{1}H NMR ($CDCl_3$) δ ppm: 11.00 (s, 1H, amide H), 7.61-7.52 (m, 6H, H^2, H^3, H^5, H^6, $H^{2'}$, $H^{6'}$), 7.42 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.33-7.29 (m, 3H, $H^{4'}$, $H^{3''}$, $H^{5''}$), 6.98 (d, 2H, *J* = 7.4 Hz, $H^{2''}$, $H^{6''}$), 6.92 (t, 1H, *J* = 7.4 Hz, $H^{4''}$), 3.41 (t, 4H, *J* = 4.8 Hz, piperazine H^3, H^5), 2.84-2.80 (m, 6H, piperazine H^2, H^6 and -$COCH_2C\underline{H}_2$-), 2.61 (t, 2H, *J* = 6.0 Hz, -$COC\underline{H}_2CH_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 170.19, 150.97, 139.70, 138.69, 134.67, 128.86, 128.85, 126.93, 126.88, 126.17, 119.34, 118.75, 115.32, 53.72, 52.42, 48.19, 34.17. HRMS (ESI) $[M+H]^+$ m/z for $C_{25}H_{28}N_3O$ calculated: 386.2232, found: 386.2233. Anal. Calcd. for $C_{25}H_{27}N_3O$: C, 77.89; H, 7.06; N, 10.90. Found: C, 77.71; H, 7.07; N, 10.80.

*4.1.7.2. N-([1,1'-Biphenyl]-4-yl)-3-(4-benzylpiperazin-1-yl)propanamide **(15b)***

Following the method D, reaction of **14** (250 mg, 0.96 mmol) with benzylpiperazine (0.42 ml, 2.40 mmol) in the presence of NaI (432 mg, 2.88 mmol) and anhydrous potassium carbonate (332 mg, 2.40 mmol) gave crude product then, it was recrystallized from butanol. Yield: 300 mg, 78%; mp: 146 °C. CAS Number: 191168-70-4. ^{1}H NMR (DMSO-d_6) δ ppm: 10.18 (s, 1H, amide H), 7.68-7.60 (m, 6H, H^2, H^3, H^5, H^6, $H^{2'}$, $H^{6'}$), 7.44 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.34-7.24 (m, 6H, $H^{4'}$, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.45 (s, 2H, piperazine-$C\underline{H}_2$-phenyl), 2.64 (t, 2H, *J* = 6.8 Hz, -$COCH_2C\underline{H}_2$-), 2.48-2.39 (m, 10H, piperazine and -$COC\underline{H}_2CH_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 170.11, 139.63, 138.61, 138.12, 134.58, 128.77, 128.70, 128.03, 126.85, 126.80, 126.77, 126.10, 119.24, 61.95, 53.64, 52.56, 52.36, 34.04. HRMS (ESI) $[M+H]^+$ m/z for $C_{26}H_{30}N_3O$ calculated: 400.2389, found: 400.2383. Anal. Calcd. for $C_{26}H_{29}N_3O$: C, 78.16; H, 7.32; N, 10.52. Found: C, 77.76; H, 7.20; N, 10.39.

*4.1.7.3. N-([1,1'-Biphenyl]-4-yl)-3-(4-benzylpiperidin-1-yl)propanamide **(15c)***

Following the method D, reaction of **14** (250 mg, 0.96 mmol) with 4-benzylpiperidin (0.42 ml, 2.40 mmol) in the presence of NaI (432 mg, 2.88 mmol) and anhydrous potassium carbonate (332 mg, 2.40 mmol) gave crude product then, it was recrystallized from butanol. Yield: 300 mg, 78%; mp: 168 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 10.18 (s, 1H, amide H), 7.68-7.60 (m, 6H, H^2, H^3, H^5, H^6, $H^{2'}$, $H^{6'}$), 7.44 (t, 2H, *J* = 7.6 Hz, $H^{3'}$, $H^{5'}$), 7.34-7.24 (m, 6H, $H^{4'}$, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''}$), 3.45 (s, 2H, piperazine-$C\underline{H}_2$-phenyl), 2.64 (t, 2H, *J* = 6.8 Hz, -$COCH_2C\underline{H}_2$-), 2.48-2.39 (m, 10H, piperazine and -$COC\underline{H}_2CH_2$-). ^{13}C NMR (DMSO-d_6) δ ppm: 170.11, 139.63, 138.61, 138.12, 134.58, 128.77, 128.70, 128.03, 126.85, 126.80, 126.77, 126.10, 119.24, 61.95, 53.64, 52.56, 52.36, 34.04. HRMS (ESI) $[M+H]^+$ m/z for $C_{27}H_{31}N_2O$ calculated: 399.2436, found: 399.2433. Anal. Calcd. for $C_{27}H_{30}N_2O$: C, 81.37; H, 7.59; N, 7.03. Found: C, 81.75; H, 7.74; N, 7.07.

4.2. Acetylcholinesterase and butyrylcholinesterase inhibition assays

AChE and BChE % inhibitory activities of the 28 test compounds at 10 μM concentration were determined by the modified Ellman's method [19, 33]. The formation of the yellow color was measured at 412 nm using molecular devices, Versamax microplate reader. Electric eel AChE (type-VI-S, EC 3.1.1.7) and equine serum BChE (EC 3.1.1.8), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholine iodide (ATI), butyrylthiocholine iodide (BTI) and Tris HCl were purchased from Sigma Aldrich. Donepezil and Galantamine were used as the reference drug at 10 μM concentration. Sample compounds were dissolved in DMSO, the concentration of DMSO in final reaction mixture was 1%. At this concentration, DMSO has no inhibitory effect on both enzymes [34].

Briefly, AChE inhibitory activities of the compounds, each reaction mixture (final volume of 200 μL) contained 168 μL of 50 mM of Tris HCl buffer (pH 8.0), 10 μL of 6.8 mM DTNB solution, 10 μL of AChE solution (0.4 U/mL), and 2 μL of each sample solution. After incubation for 10 min at 25 °C, the reactions were initiated by the addition of 10 μL ATI solution (10 mM). The change of absorbance was recorded for 10 min at 412 nm. As a control, an identical solution of the reaction mixture without the sample solution was processed following the same protocol. The blank reading contained 178 μL buffer, 10 μL DTNB, 2 μL DMSO and 10 μL ATI solution. BChE inhibitory activities of the test compounds were measured through the same methodology described, except employing 0.8 U/mL BChE solution as the enzyme source and the BTI solution (30 mM) as the substrate of the reaction. Each experiment was performed in triplicate; absorbance values were corrected by subtracting the absorbance of blank and the inhibition rate (%) was calculated.

Compounds displaying more than 60% inhibition were tested for IC_{50} determination. Assays were performed with at least nine concentrations of selected compounds and IC_{50} values were determined graphically from linear curves (log [inhibitor] vs. % inhibition) with the use of GraphPad Prism software (Version 7.0). The results were displayed as mean ± SEM.

4.3. Kinetic studies of enzyme inhibition

Compounds **5h** (for AChE) and **6d** (for BChE) were selected and kinetic studies performed similar to enzyme inhibition assay. To obtain estimates of the inhibition type, reciprocal plots of 1/V versus 1/[S] were constructed by using reported method with minor modifications [29]. The increase of the absorbances was measured with different inhibitor concentrations (**5h**; 25, 50, 100, 150 nM and **6d**; 100, 200, 400, 800 nM) and without inhibitor for proposed substrate concentrations (ATI; 0.05, 0.1, 0.2, 0.4 mM and BTI; 0.025, 0.05, 0.1, 0.2 mM). Slopes of the reciprocal plots were plotted against the concentration of inhibitor, for estimation of *Ki*. All processes were assayed in triplicate and data analysis was performed with GraphPad Prism software (Version 7.0). The results were displayed as mean ± SEM.

4.4. Aβ aggregation inhibition assay/Thioflavin T (ThT) assay

All the chemicals used for the assay purchased from Sigma-Aldrich and measurements carried out at Thermo Biolite 24 well Multidish plate by using Thermo Varioskan Flash Microplate Reader. To investigate the effect of the selected compounds and references (Donepezil and phenol red) on the aggregation of $A\beta_{1-42}$ at 100 mM concentration, a reported thioflavin T-based fluorometric assay was performed [29]. Samples were dissolved in DMSO and analyzed in triplicate.

4.5. Cytotoxicity test

4.5.1. Cell line and culture conditions

Cell line were provided by Immunology Department at Gazi University Medical School. Mouse fibroblast NIH/3T3 (ATCC CRL1658) cell line were included in this study. Cell line was grown in Dulbecco's modified Eagle medium containing fetal bovine serum, 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, and maintained in a humidified atmosphere of 5% CO_2 at 37°C (Sanyo, San Diego, California). Cells were subcultured when they were confluent.

4.5.2. MTT Assay

Cytotoxicity of compounds was assessed by using a commercially available 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche Applied Science, Indianapolis, Indiana). Briefly, target cells ($5x10^3$ in a volume of 0.1ml) were plated in 96-well flat bottom culture plates (Greiner Bio-One, Germany) allowing cells to adhere to the bottom of the plate at 37°C overnight. Cells were treated with various concentrations of **5h** and **6d** for 24 hours. MTT reagent was added in the wells. Plates were incubated for 4 hours at 37°C. Cells were disrupted by adding 0.1 ml of lysis buffer in the wells. After incubating them at 37°C for additional 24 hours, color reaction was measured at 570 nm (with 670-nm reference) using a Synergy HT Multi-mode microplate reader (Biotek, Winnoski, Vermont). All groups were performed in triplicates.

4.6. Assessment of physicochemical parameters

Calculated descriptors (molecular weight, log P, topological polar surface area (tPSA), volume, number of hydrogen donors, number of hydrogen acceptors and number of violations of Lipinski's rule) of the compounds were computed employing the Molinspiration online service [31]. Blood brain barrier (BBB) permeability values were calculated by admetSAR online service [32].

4.7. Molecular docking

The crystal structures of the AChE and BuChE enzymes were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/), under the accession codes 1EVE [27] and 1P0I [35], respectively. Molecular Operating Environment (MOE) software [36] was used for molecular docking of the ligands. The co-crystallized bound compounds and water molecules farther than 4.5 Å from ligand or receptor were deleted from the crystal structure. Enzyme-ligand complexes were energy minimized to a gradient of 0.01 kcal/(mol Å), and protonated by means of the force field AMBER99. Charges on the enzyme and ligands were assigned using the force fields AMBER99 and MMF94X, correspondingly. The active sites of enzymes were identified by the site finder application in MOE. Triangle Matcher Algorithm and two rescoring functions, London dG and GBVI/WSA dG were used to produce 30 poses of each ligand. All poses generated with docking were analyzed and the best-scored pose with the lowest binding energy for each compound was selected for further investigation of interactions with the corresponding enzyme.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

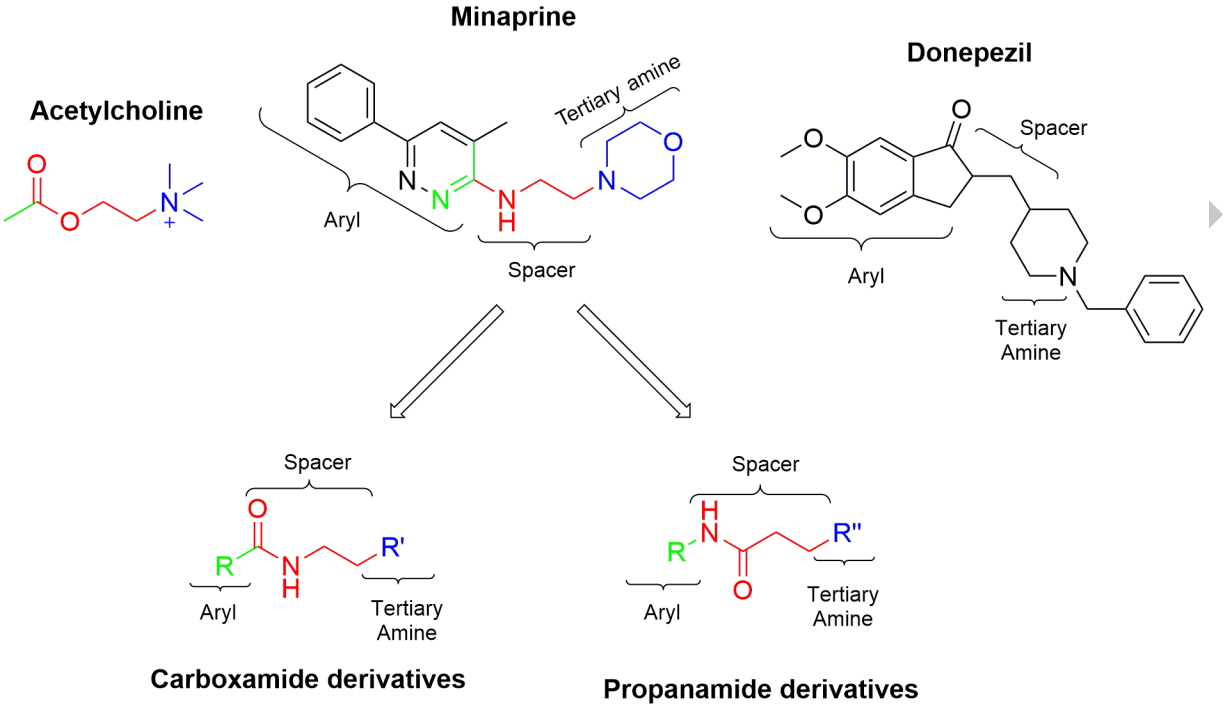
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) [grant number 114S129].

References

[1] M. Prince, A. Wimo, M. Guerchet, G.C. Ali, Y.T. Wu, M. Prina, World Alzheimer Report 2015 The Global Impact of Dementia An Analysis of Prevalence, Incidence, Cost and Trends, Alzheimer's Disease International, Alzheimer's Disease International, London, 2015, pp. 1-82.
[2] Leading causes of death by economy income group. http://www.who.int/mediacentre/factsheets/fs310/en/index1.html. (Accessed March 2017).
[3] S. Salloway, J. Mintzer, M.F. Weiner, J.L. Cummings, Disease-modifying therapies in Alzheimer's disease, Alzheimers Dement. 4(2) (2008) 65-79.
[4] Y. Biran, C.L. Masters, K.J. Barnham, A.I. Bush, P.A. Adlard, Pharmacotherapeutic targets in Alzheimer's disease, J. Cell. Mol. Med. 13(1) (2009) 61-86.
[5] L. Piazzi, A. Cavalli, F. Colizzi, F. Belluti, M. Bartolini, F. Mancini, M. Recanatini, V. Andrisano, A. Rampa, Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds, Bioorg. Med. Chem. Lett. 18(1) (2008) 423-426.
[6] B.D. Greenberg, M.C. Carrillo, J.M. Ryan, M. Gold, K. Gallagher, M. Grundman, R.M. Berman, T. Ashwood, E.R. Siemers, Improving Alzheimer's disease phase II clinical trials, Alzheimers Dement. 9(1) (2013) 39-49.
[7] K. Mullane, M. Williams, Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond?, Biochem. Pharmacol. 85(3) (2013) 289-305.
[8] H.B. Nygaard, Current and emerging therapies for Alzheimer's disease, Clin. Ther. 35(10) (2013) 1480-1489.
[9] M.S. Mega, The cholinergic deficit in Alzheimer's disease: impact on cognition, behaviour and function, Int. J. Neuropsychopharmacol. 3(Supplement 2) (2000) 3-12.
[10] A. Contestabile, The history of the cholinergic hypothesis, Behav. Brain Res. 221(2) (2011) 334-340.
[11] P.T. Francis, A.M. Palmer, M. Snape, G.K. Wilcock, The cholinergic hypothesis of Alzheimer's disease: a review of progress, J. Neurol. Neurosurg. Psychiatr. 66(2) (1999) 137-147.
[12] M. Gentry, B. Doctor, Alignment of amino acid sequences of acetylcholinesterases and butyrylcholinesterases, Cholinesterases: Structure, Function, Mechanism, Genetics and Cell Biology, American Chemical Society, ACS Conference Proceedings, 1991, pp. 394-398.
[13] A. Saxena, A. Redman, X. Jiang, O. Lockridge, B. Doctor, Differences in Active Site Gorge Dimensions of Cholinesterases Revealed by Binding of Inhibitors to Human Butyrylcholinesterase, Biochemistry 36 (1997) 14642-14651.
[14] H. Dvir, I. Silman, M. Harel, T.L. Rosenberry, J.L. Sussman, Acetylcholinesterase: from 3D structure to function, Chem. Biol. Interact. 187(1-3) (2010) 10-22.
[15] P. Munoz-Ruiz, L. Rubio, E. Garcia-Palomero, I. Dorronsoro, M. del Monte-Millan, R. Valenzuela, P. Usan, C. de Austria, M. Bartolini, V. Andrisano, A. Bidon-Chanal, M. Orozco, F.J. Luque, M. Medina, A. Martinez, Design, synthesis, and biological evaluation of dual binding site acetylcholinesterase inhibitors: new disease-modifying agents for Alzheimer's disease, J. Med. Chem. 48(23) (2005) 7223-7233.
[16] A. Saxena, J.M. Fedorko, C.R. Vinayaka, R. Medhekar, Z. Radic, P. Taylor, O. Lockridge, B.P. Doctor, Aromatic amino-acid residues at the active and peripheral anionic sites control the binding of E2020 (AriceptR) to cholinesterases, Eur. J. Biochem. 270(22) (2003) 4447-4458.
[17] E. Giacobini, Selective Inhibitors of Butyrylcholinesterase A Valid Alternative for Therapy of Alzheimer's Disease?, Drugs Aging 18(21) (2001) 891-898.
[18] S. Darvesh, D.A. Hopkins, C. Geula, Neurobiology of butyrylcholinesterase, Nat. Rev. Neurosci. 4(2) (2003) 131-138.
[19] C. Yamali, H.O. Gulcan, B. Kahya, S. Cobanoglu, M.K. Sukuroglu, D.S. Dogruer, Synthesis of some 3(2H)-pyridazinone and 1(2H)-phthalazinone derivatives incorporating aminothiazole moiety and investigation of their antioxidant, acetylcholinesterase, and butyrylcholinesterase inhibitory activities, Med. Chem. Res. 24(3) (2015) 1210-1217.
[20] D. Tiryaki, M. Sukuroglu, D.S. Dogruer, E. Akkol, S. Ozgen, M.F. Sahin, Synthesis of some new 2,6-disubstituted-3(2H)-pyridazinone derivatives and investigation of their analgesic, anti-inflammatory and antimicrobial activities, Med. Chem. Res. 22(6) (2013) 2553-2560.

[21] M. Sukuroglu, C. Yamali, D. Tiryaki, F.K. Onurdag, E. Akkol, D.S. Dogruer, Synthesis of Some New 3(2H)-Pyridazinone Derivatives and Evaluation of their Analgesic-anti-inflammatory and Antimicrobial Activities, Lett. Drug. Des. Discov. 10(6) (2013) 507-514.
[22] D.S. Dogruer, S. Urlu, T. Onkol, B. Ozcelik, M.F. Sahin, Synthesis of some pyridazine derivatives carrying urea, thiourea, and sulfonamide moieties and their antimicrobial activity, Turk. J. Chem 34(1) (2010) 57-65.
[23] J.M. Contreras, Y.M. Rival, S. Chayer, J.J. Bourguignon, C.G. Wermuth, Aminopyridazines as acetylcholinesterase inhibitors, J. Med. Chem. 42(4) (1999) 730-741.
[24] B. Kılıc, H. Ozan Gulcan, M. Yalcın, F. Aksakal, A. Dimoglo, M. Fethi Sahin, D. Songul Dogruer, Synthesis of Some New 1 (2H)-Phthalazinone Derivatives and Evaluation of Their Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities, Lett. Drug. Des. Discov. 14(2) (2017) 159-166.
[25] B.U.W. Maes, G.L.F. Lemie`re, R. Dommisse, K. Augustyns, A. Haemers, A New Approach Towards the Synthesis of 3-Amino-6-(hetero)arylpyridazines Based on Palladium Catalyzed Cross-coupling Reactions, Tetrahedron 56 (2000) 1777-1781.
[26] Y. El-Ahmad, P. Maillet, E. Laurent, A. Talab, G. Tran, R. Ollivier, Synthesis of 1-[ω-[(arylamino) carbonyl] alkyl]-4-(venzocycloalkyl) piperazines, Heterocycles 4(45) (1997) 723-734.
[27] G. Kryger, I. Silman, J. Sussman, Structure of acetylcholinesterase complexed with E2020 (Aricep): implications for the design of new anti-Alzheimer drugs, Structure 7(3) (1999) 297-307.
[28] G. Johnson, S.W. Moore, The peripheral anionic site of acetylcholinesterase: structure, functions and potential role in rational drug design, Curr. Pharm. Des. 12(2) (2006) 217-225.
[29] M.L. Bolognesi, V. Andrisano, M. Bartolini, R. Banzi, C. Melchiorre, Propidium-based polyamine ligands as potent inhibitors of acetylcholinesterase and acetylcholinesterase-induced amyloid-beta aggregation, J. Med. Chem. 48(1) (2005) 24-27.
[30] M. Bartolini, C. Bertucci, V. Cavrini, V. Andrisano, β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies, Biochem. Pharmacol. 65(3) (2003) 407-416.
[31] Molinspiration, Molinspiration Cheminformatics Nova ulica SK-900 26 Slovensky Grob Slovak Republic.
[32] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties, (2012).
[33] G.L. Ellman, K.D. Courtney, V.J. Andres, R.M. Feather-Stone, A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity, Biochem. Pharmacol. 7 (1961) 88-95.
[34] A.D.C. Obregon, M.R.C. Schetinger, M.M. Correa, V.M. Morsch, J.E.P.d. Silva, M.A.P. Martins, H.G. Bonacorso, N. Zanatta, Effects per se of Organic Solvents in the Cerebral Acetylcholinesterase of Rats, Neurochem. Res. 30(3) (2005) 379-384.
[35] Y. Nicolet, O. Lockridge, P. Masson, J.C. Fontecilla-Camps, F. Nachon, Crystal structure of human butyrylcholinesterase and of its complexes with substrate and products, J. Biol. Chem. 278(42) (2003) 41141-41147.
[36] Molecular Operating Environment (MOE), C.C.G.I., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.

Graphical abstract



Highlights

- A series of new carboxamide and propanamide derivatives bearing phenylpyridazine or biphenyl as a core ring were synthesized.
- Most of the carboxamide derivatives were selective for AChE.
- The most active compounds inhibit human AChE at submicromolar/micromolar concentration.
- Kinetic and docking studies showed that most active compound was mixed type inhibitor and binding both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE.