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Research paper

Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes

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ABSTRACT

Donepezil (DNP), an acetylcholinesterase (AChE) inhibitor, is one of the most preferred choices in Alzheimer diseases (AD) therapy. In the present study, 38 new DNP analogues were synthesized. Structures of the synthesized compounds (**1**–**38**) were elucidated by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic methods and elemental analysis. Inhibitory potential of the compounds on cholinesterase enzymes was investigated. None of the compounds displayed significant activity on butyrylcholinesterase (BChE) enzyme. On the other hand, compounds **26**–**29** indicated important inhibitory activity on AChE enzyme. Kinetic studies were performed in order to observe the effects of the most active compounds on substrate-enzyme relationship. Cytotoxicity studies and theoretical calculation of pharmacokinetic properties were also carried out to get an information about toxicity and pharmacokinetic profiles of the compounds. The compounds **26**–**29** were found to be nontoxic at their effective concentrations against AChE. A good pharmacokinetic profile was predicted for these compounds. Docking studies were performed for the most active compounds **26**–**29** and interaction modes with enzyme active sites were determined. Docking studies revealed that there is a strong interaction between the active sites of AChE enzyme and analyzed compounds.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative process, characterized by age-related loss of memory [1]. Language disorders [2], visual deficiency [3], orientation disorders [4], shortage of decision-making, management function [5] and memory impairment [6] are the primary symptoms of the disease. Its etiology has not been enlightened yet. It is thought that accumulation of β amyloid in the senile plaques [7], neurofibrillary tangle composed by hyperphosphorylation of tau protein [8], loss of cholinergic activity in certain part of brain [9], cerebrovascular disorders [10], oxidative stress [11], functional loss of neuron and synapse [12] and absence of acetylcholine (ACh) [13] are responsible for the pathophysiology of AD [14]. Although numerous therapeutic approaches have been offered, only non-competitive *N*-methyl-D-aspartate receptor antagonist, memantine, and acetylcholinesterase (AChE) inhibitors, tacrine, donepezil (DNP), rivastigmine and galantamine have been approved by the European and United States regulatory authorities [15,16]. Among these drugs, DNP is the most preferred AChE inhibitor because it gives the most positive response in AD treatment. Furthermore, DNP has some advantages as blood-brain barrier permeability, non-hepatotoxicity, the least side efficacy and usage once-daily [17].

Cholinergic hypothesis reveals that there is a loss in cholinergic activity because of the decreased level of Ach, which is hydrolyzed by cholinesterase enzymes in synaptic gap [18,19]. There are two types of cholinesterase enzymes in the central nervous system (CNS): AChE and butyrylcholinesterase (BChE) [20]. These two isoenzymes are responsible for hydrolyzing ACh. Although AChE has more hydrolytic activity than BChE, it is reported that BChE plays a key role in the regulation of AChE activity [21]. Thus, AChE inhibitors are preferred in the treatment of AD to keep up ACh normal levels in the CNS and to eliminate the symptoms of disease.

According to the X-ray crystallographic structure of AChE (PDB ID:4EY7), two main binding sites has been determined: the catalytic anionic site (CAS) including Ser203, Glu334, His447, Trp86,

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Tyr130, Tyr133, Tyr337, Phe338 and the peripheral anionic site (PAS) consisting of Tyr72, Asp74, Tyr124, Tyr341, Trp286 [22–25]. It has been reported that DNP interacts with both CAS and PAS and thus it is situated in the gorge concordantly owing to the feature of dual binding site (DBS) [26–28]. Analyses of binding modes of the DNP indicate that the benzyl moiety is in a π- π interaction with the indole of Trp86 in the CAS. The formation of hydrogen bond between the oxygen atom of the carbonyl group in the 1-indanone and the amino group of Phe338 is a very significant interaction in terms of binding to the active site. The 1-indanone constitutes a π-π interaction with the indole of Trp286 in the PAS region. The piperidine has a position in the gorge to interact with Tyr337 and Tyr341 by doing a hydrogen bond. It also set up a van der Waals interaction with amino acids in both CAS and PAS [29–31].

As stated above, receptor-ligand interactions and mechanism of molecular recognition of the DNP have been clarified well. Observed data clearly indicate that both 1-indanone and piperidine on the chemical structure of DNP are responsible for inhibition of the AChE. Therefore, there are too many studies including anticholinesterase activity evaluation of piperidine and/or 1-indanone compounds [32–40]. There are also many reports about cholinesterase inhibitory potential of novel compounds, including bioisosteric replacement of piperidine with another basic center like piperazine, pyrrolidine etc. [41–47]. In the view of chemical structure of DNP, it has been reported that new AChE inhibitor candidates should bear a core ring system that interacts with PAS, a basic center that binds to CAS and a linker as –O–, CH_2, CONH, CONH(CH_2)n, etc. between the core ring system and basic center [48–50]. For example, a strong AChE inhibitor BYYT-25, containing an indanone core ring, an oxygen linker and a 4-(pyrrolydin-1-ylmethyl)phenyl basic center, has been synthesized as a result of described chemical requirements [49].

Based on the pharmacological profile of DNP and the information about its molecular interaction with AChE, in the present study, a novel series of DNP analogues was synthesized (Fig. 1) to investigate their inhibitory potency against cholinesterase enzymes. Thus, it is aimed to gain new cholinesterase inhibitors with enhanced biological activity.

2. Result and discussion

2.1. Chemistry

The compounds **1**–**14** and **15**–**38** were synthesized as shown in Scheme 1 and Scheme 2. Initially, 2-chloro-*N*-arylacetamide derivatives were prepared via the acetylation reaction using chloroacetyl chloride. In the second step, substitution reaction between 5,6-dimethoxy-2-[(piperidin-4-yl)-methyl]-2,3-dihydro-1*H*-inden-1-one and 2-chloro-*N*-arylacetamide derivatives gave the compounds **1**–**14**. The reaction of 4-fluorobenzaldehyde and appropriate secondary amine afforded 4-substitutedbenzaldehyde derivatives, which were treated with corresponding 2,3-dihydro-1*H*-inden-1-one to gain compounds **15**–**38** by Claisen-Schmidt condensation.

The structure of the newly synthesized compounds was elucidated by IR, ^{1}H NMR, ^{13}C NMR, HRMS spectroscopic methods (Electronic Supporting Information) and elemental analysis.

2.2. Anticholinesterase activity assay

All synthesized compounds were assessed as the AChE and BChE inhibitors by using in vitro modified Ellman's spectrophotometric method [51]. The assay was performed in two steps. First of all, the compounds **1**–**38** were tested at 10^{-3} and 10^{-4} M concentrations. Second step was performed by using 10^{-5}-10^{-9} M concentrations of the selected compounds that indicate more than 50% inhibitory activity at initial concentrations.

Table 1 presents the anticholinesterase activity of compounds **1**–**38** at initial concentrations. None of the compounds displayed remarkable inhibitor activity on BChE enzyme. Even the most effective compound **17** could show the inhibition of 19.23% against BChE at 10^{-3} M concentration. It was noted that all synthesized compounds showed more potent inhibition profile on AChE rather than BChE. The compounds **1**–**14** displayed lower inhibition against

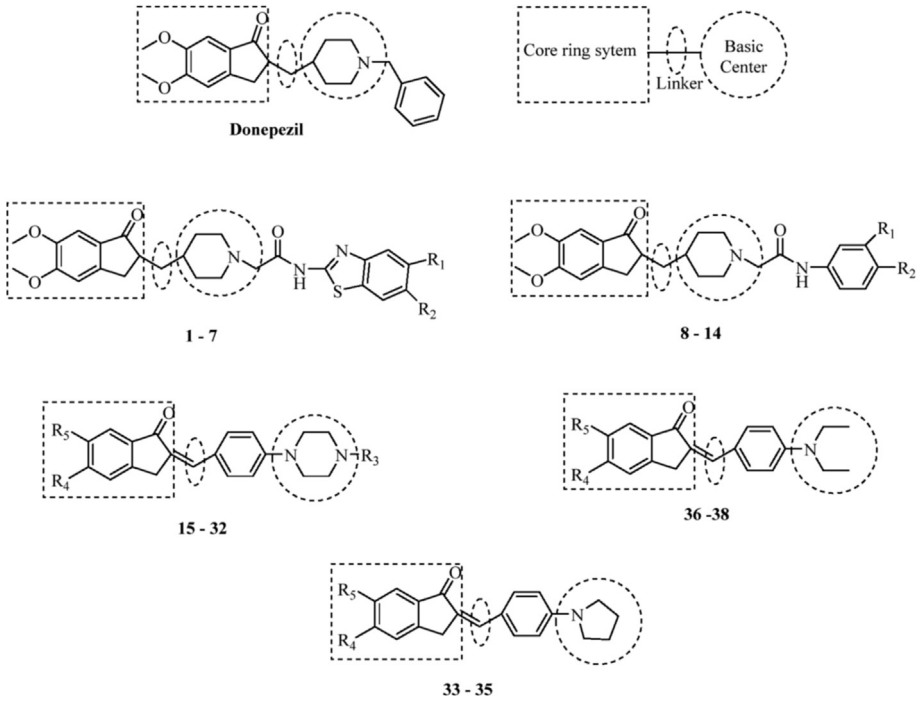
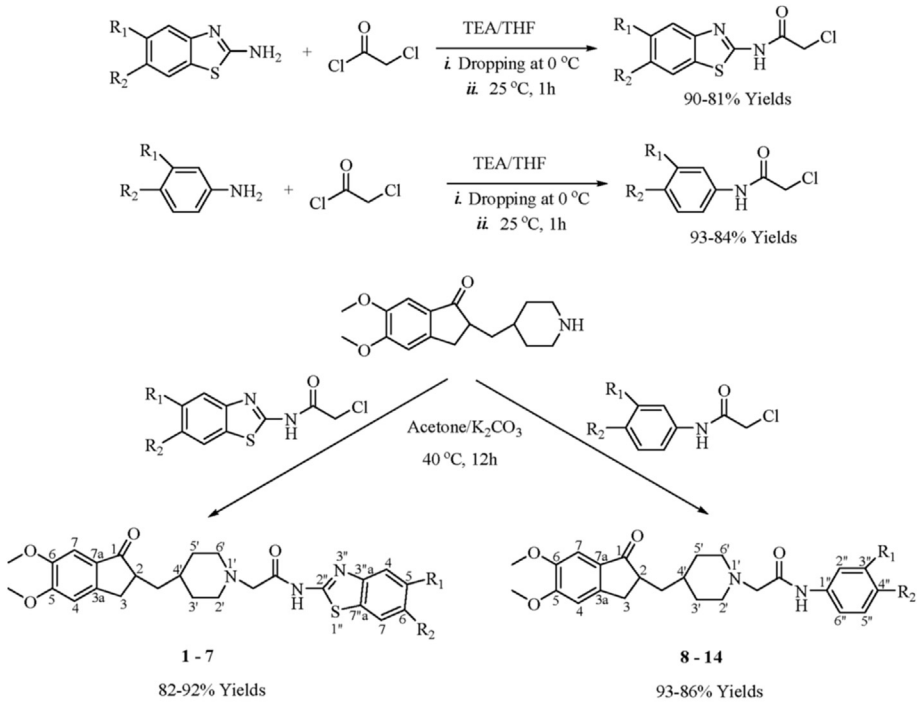


Fig. 1. Structures of donepezil and synthesized compounds **1**–**38**.



	1	2	3	4	5	6	7
	8	9	10	11	12	13	14
R_1	-H	-H	-H	-H	-H	-H	$-OCH_3$
R_2	-H	$-CH_3$	$-OCH_3$	-F	-Cl	$-NO_2$	$-OCH_3$

Scheme 1. Synthesis pathway of the compounds **1**–**14**.

AChE when compare with the compounds **15**–**38**. At 10^{-3} M concentration, the compounds **17**, **20**, **24**–**29** possessed inhibition profile over 50%, whereas at 10^{-4} M concentration the compounds **26**–**29** indicated more than 50% activity. Thus, the compounds **26**–**29** were evaluated in further concentrations (10^{-5}-10^{-9} M) against AChE along with reference drugs DNP and tacrine (Table 2). The IC_{50} values on AChE were recorded as 0.2197 and 0.3715 μM for DNP and tacrine, respectively. This result confirms that DNP is more potent inhibitor then tacrine against AChE. It was determined that the compound **29** is the most active derivative in the series. The compound **29** displayed 90.31% inhibition potency at a concentration of 10^{-3} M. IC_{50} values of the compounds **26**, **27**, **28** and **29** were calculated as 6.382 μM, 8.540 μM, 6.056 μM and 4.895 μM, respectively (Table 2). As a result of anticholinesterase activity, it can be clearly declared that the compounds **26**–**29** are selective AChE inhibitors.

2.3. Kinetic studies of enzyme inhibition

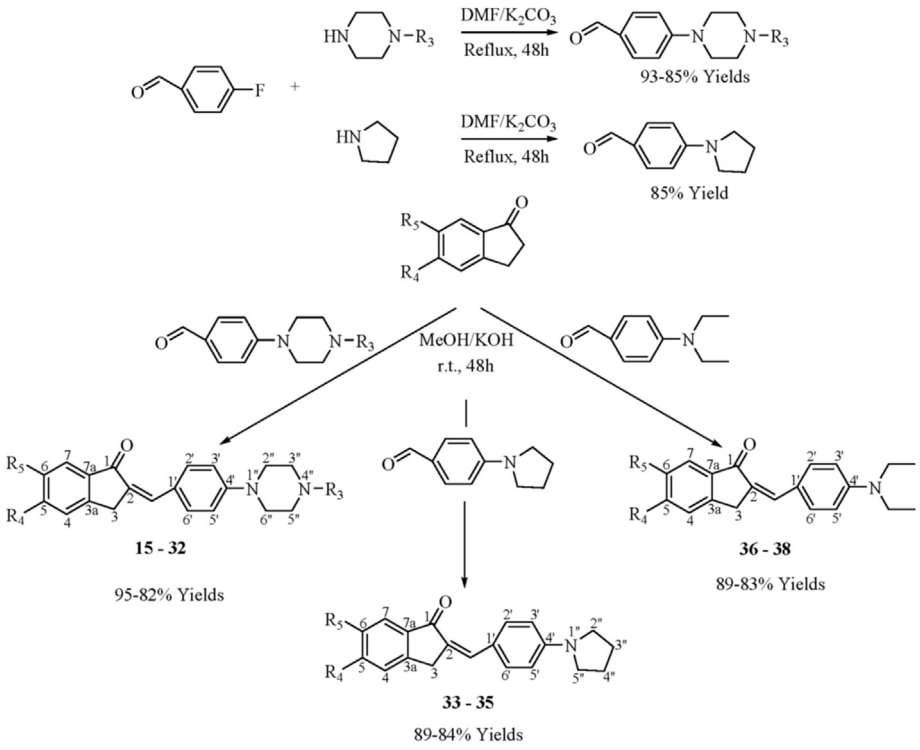
The mechanism of AChE inhibition was investigated by enzyme kinetic [52] using Ellman's spectrophotometric method [51]. The linear Limeweaver-Burk graphics were used to estimate the type of inhibition. Enzyme kinetic was analyzed by recording substrate-velocity curves in absence and presence of the most potent compound **29**, which was prepared at IC_{50} (4.895 μM) and 2 × IC_{50} (9.790 μM) concentrations. In each case, the initial velocity measurements were gained at different substrate (ATC) concentrations ranging from 150 μM to 0.2929 μM.

The graphical analysis of steady-state inhibition data for the compound **29** is shown in Fig. 2. The Lineweaver–Burk plot establishes the inhibition type; competitive, non-competitive or mixed-type. Competitive inhibitors possess the same intercept on y-axis but there are diverse slopes and intercepts on x-axis between the two data sets. Non-competitive inhibition has plots with the same intercept on x-axis but there are different slopes and intercepts on y-axis. Mix-typed inhibition causes different intercepts on both y- and x-axis as presented in Fig. 2. Therefore, this pattern indicates that the mechanism of AChE inhibition of **29** is mix-typed.

2.4. Cytotoxicity test

New drug development is a long and arduous process, which includes studies regarding chemistry, physical properties and biological evaluation. Biological evaluation covers pharmacology, ADME and toxicology. Hence, the development of novel pharmaceuticals requires toxicity studies to be performed on candidate drugs.

In the present study toxicity of the compounds **26**–**29** was investigated by MTT assay, which is based on the reduction of yellow MTT dye by metabolically active eukaryotic and prokaryotic cells to form the purple formazan product. This assay is mainly preferred to form a view about cell viability and to observe the growth of cell culture [53,54]. MTT assay was carried out using healthy NIH/3T3 mouse embryonic fibroblast cell lines (ATCC CRL1658), which is recommended for cytotoxicity screening by ISO (10993-5, 2009) [55]. Cytotoxicity test resulted with following IC_{50}; 320 ± 22.10 μM (for compound **26**), 230 ± 20.86 μM (for compound **27**), 78 ± 19.47 μM (for compound **28**), 78 ± 5.70 μM (for compound **29**) (Table 3). IC_{50} of the compounds **26**, **27**, **28** and **29** against NIH/3T3 is about 50, 27, 13 and 16 folds higher than IC_{50} against AChE (Table 2). This finding enhances the importance of the compounds



Bileşik	R_3	R_4	R5
15	CH_3	OCH_3	H
16	CH_3	H	OCH_3
17	CH_3	OCH_3	OCH_3
18	CH_2CH_3	OCH_3	H
19	CH_2CH_3	H	OCH_3
20	CH_2CH_3	OCH_3	OCH_3
21	CH_2CH_2OH	OCH_3	H
22	CH_2CH_2OH	H	OCH_3
23	CH_2CH_2OH	OCH_3	OCH_3
24	$CH_2CH_2N(CH_3)_2$	OCH_3	H
25	$CH_2CH_2N(CH_3)_2$	H	OCH_3
26	$CH_2CH_2N(CH_3)_2$	OCH_3	OCH_3
27	$CH_2CH_2CH_2N(CH_3)_2$	OCH_3	H
28	$CH_2CH_2CH_2N(CH_3)_2$	H	OCH_3
29	$CH_2CH_2CH_2N(CH_3)_2$	OCH_3	OCH_3
30	C_6H_5	OCH_3	H
31	C_6H_5	H	OCH_3
32	C_6H_5	OCH_3	OCH_3
33	-	OCH_3	H
34	-	H	OCH_3
35	-	OCH_3	OCH_3
36	-	OCH_3	H
37	-	H	OCH_3
38	-	OCH_3	OCH_3

Scheme 2. Synthesis pathway of the compounds **15**–**38**.

26–**29** as AChE inhibitors. It is easy to declare that the compounds are nontoxic at their effective concentrations against AChE.

2.5. Theoretical calculation of ADME parameters

Poor pharmacokinetic is one of the important causes of costly late-stage failures in drug development. Pharmacokinetic profiles of the new drug candidates should be evaluated as early as possible in the drug development process. In recent years, combinatorial chemistry has significantly increased the number of compounds for which early data on absorption, distribution, metabolism and excretion (ADME) are needed [56]. Hence, ADME properties of the synthesized compounds (**1**–**38**) were calculated by online Molinspiration property program [57]. This program was coded to give the data of Lipinski's rule, which evaluates the ADME properties of drug like compounds and is important for the optimization of a biologically active compound. The rule requires that an orally active drug has no more than one violation [58].

The theoretical calculations of ADME parameters (molecular weight, log P, topological polar surface area (tPSA), number of hydrogen donors, number of hydrogen acceptors, number of rotatable bonds and volume) are presented in Table 4 along with the violations of Lipinski's rule. According to these data only the compound **5** does not abide to the rule with two violations. On the other hand, all the other compounds (**1**–**4**, **6**–**38**) follow Lipinski's rule by causing no more than one violation. For the most active

Table 1
Initial screening and inhibition percent of the compounds **1**–**38** against AChE and BChE at 10^{-3}-10^{-4} M concentrations. Inhibitions rates, being higher than 50%, are presented in bold numbers.

Comp.	AChE Inhibition %		BChE Inhibition %		Comp.	AChE Inhibition %		BChE Inhibition %	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M		10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
1	4.62 ± 0.39	2.19 ± 0.13	5.23 ± 0.46	2.31 ± 0.19	**20**	**86.87 ± 2.38**	47.44 ± 1.63	16.88 ± 0.86	8.56 ± 0.42
2	2.13 ± 0.41	1.43 ± 0.16	4.29 ± 0.70	2.08 ± 0.24	**21**	10.54 ± 0.75	8.09 ± 0.47	7.63 ± 0.19	5.91 ± 0.31
3	5.82 ± 0.61	3.93 ± 0.39	5.33 ± 0.47	3.19 ± 0.56	**22**	2.30 ± 0.14	1.45 ± 0.08	3.45 ± 0.13	1.25 ± 0.07
4	5.90 ± 0.30	4.65 ± 0.62	4.89 ± 0.38	3.10 ± 0.71	**23**	45.50 ± 1.95	1.85 ± 0.12	18.23 ± 1.09	7.55 ± 0.43
5	3.72 ± 0.26	2.32 ± 0.42	6.21 ± 0.70	3.09 ± 0.09	**24**	**70.62 ± 2.83**	22.32 ± 1.14	15.23 ± 0.72	9.56 ± 0.34
6	3.20 ± 0.19	2.48 ± 0.33	4.18 ± 0.38	1.13 ± 0.16	**25**	**74.38 ± 2.18**	29.67 ± 1.83	17.23 ± 1.21	10.55 ± 0.83
7	1.24 ± 0.19	0.13 ± 0.23	3.77 ± 0.44	2.05 ± 0.63	**26**	**89.14 ± 1.33**	**68.09 ± 1.12**	16.98 ± 0.83	12.14 ± 0.66
8	5.81 ± 0.21	2.15 ± 0.34	5.00 ± 0.68	2.58 ± 0.50	**27**	**87.05 ± 1.52**	**74.94 ± 1.07**	15.99 ± 0.77	11.27 ± 0.65
9	2.74 ± 0.11	2.17 ± 0.45	5.89 ± 0.72	3.20 ± 0.59	**28**	**88.05 ± 1.33**	**77.44 ± 1.21**	16.15 ± 0.27	10.08 ± 0.63
10	13.91 ± 0.82	2.49 ± 0.14	4.28 ± 0.27	1.33 ± 0.11	**29**	**90.31 ± 1.20**	**70.09 ± 1.18**	18.67 ± 0.77	13.95 ± 0.49
11	8.67 ± 0.78	6.98 ± 0.49	4.75 ± 0.38	1.99 ± 0.23	**30**	6.88 ± 0.19	1.19 ± 0.06	5.20 ± 0.31	2.38 ± 0.23
12	12.81 ± 0.45	0.41 ± 0.03	5.39 ± 0.44	2.77 ± 0.19	**31**	1.90 ± 0.11	0.62 ± 0.04	1.73 ± 0.12	1.06 ± 0.07
13	7.33 ± 0.66	0.76 ± 0.02	4.88 ± 0.36	2.69 ± 0.21	**32**	4.76 ± 0.18	0.88 ± 0.03	3.22 ± 0.12	1.08 ± 0.09
14	6.19 ± 0.43	1.34 ± 0.09	5.85 ± 0.22	1.29 ± 0.07	**33**	3.09 ± 0.10	2.72 ± 0.14	3.08 ± 0.21	1.25 ± 0.06
15	17.87 ± 1.83	9.97 ± 0.93	12.88 ± 0.88	7.56 ± 0.69	**34**	5.88 ± 0.26	1.53 ± 0.09	5.70 ± 0.26	3.18 ± 0.31
16	15.52 ± 1.28	14.00 ± 1.33	14.66 ± 1.56	9.63 ± 0.97	**35**	5.10 ± 0.34	2.09 ± 0.17	4.98 ± 0.22	2.77 ± 0.13
17	**54.66 ± 2.27**	34.99 ± 2.65	19.23 ± 2.08	8.55 ± 0.92	**36**	12.15 ± 0.43	0.54 ± 0.03	10.28 ± 0.54	5.27 ± 0.28
18	18.61 ± 1.73	9.23 ± 0.83	15.09 ± 1.39	8.77 ± 0.77	**37**	2.35 ± 0.17	0.42 ± 0.02	2.80 ± 0.19	1.65 ± 0.08
19	6.59 ± 0.47	4.53 ± 0.23	5.05 ± 0.42	4.20 ± 0.56	**38**	3.31 ± 0.15	0.24 ± 0.07	3.76 ± 0.22	1.96 ± 0.16

Table 2
Inhibition percent and IC_{50} (μM) of the compounds **26**–**29** against AChE at 10^{-3}-10^{-9} M concentrations.

Compound	Concentrations							
	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	IC_{50} (μM)
26	89.14 ± 1.33	68.09 ± 1.12	56.31 ± 0.98	10.81 ± 0.35	7.50 ± 0.24	7.42 ± 0.20	5.76 ± 0.15	6.382 ± 0.32
27	87.05 ± 1.52	74.94 ± 1.07	51.43 ± 1.00	13.65 ± 0.39	9.75 ± 0.28	8.47 ± 0.22	7.22 ± 0.16	8.540 ± 0.56
28	88.05 ± 1.33	77.44 ± 1.21	60.53 ± 1.29	13.44 ± 0.48	12.25 ± 0.37	9.91 ± 0.35	9.11 ± 0.28	6.056 ± 0.29
29	90.31 ± 1.20	70.09 ± 1.18	61.22 ± 0.88	11.53 ± 0.32	7.48 ± 0.29	3.75 ± 0.12	3.34 ± 0.11	4.895 ± 0.14
Donepezil	99.37 ± 1.24	98.63 ± 1.18	93.82 ± 1.06	90.67 ± 0.87	37.90 ± 0.91	30.18 ± 0.73	10.85 ± 0.42	0.2197 ± 0.06
Tacrine	99.16 ± 1.36	98.90 ± 1.19	95.95 ± 1.22	75.05 ± 0.96	36.64 ± 0.67	20.40 ± 0.53	17.05 ± 0.39	0.3715 ± 0.05

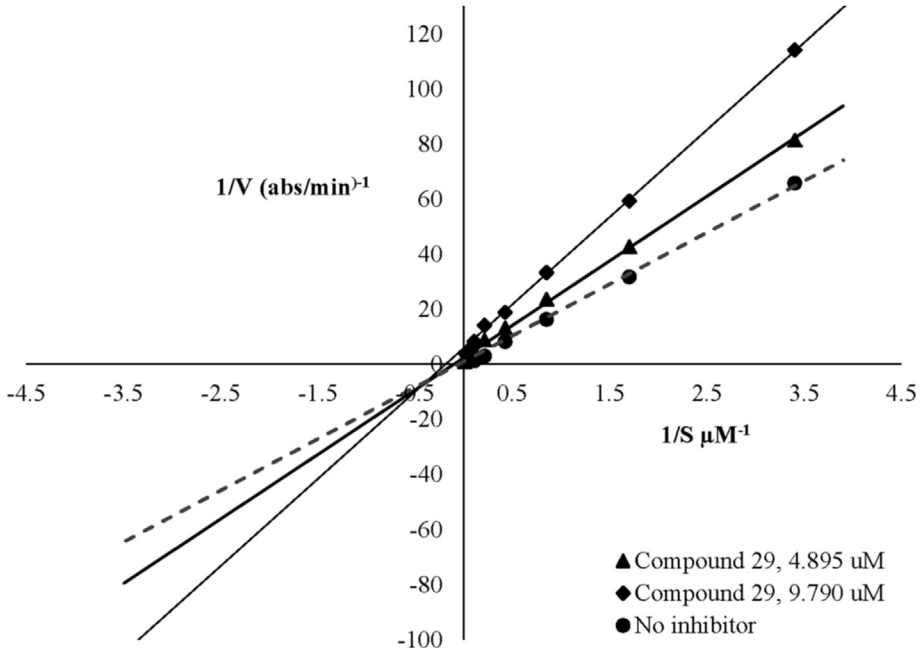


Fig. 2. Lineweaver–Burk graphs of the compounds **29**. Respective V_{max} $(abs/min)^{-1}$ values for control, 4.895 μM and 9.790 μM lines: 0.88, 0.41 and 0.16. Respective K_m (μM) values: 27.96, 9.71 and 3.07.

Table 3
IC_{50} (μM) of compounds **26**–**29** on NH3T3 cells.

	IC_{50} (μM)
26	320 ± 22.10
27	230 ± 20.86
28	78 ± 19.47
29	78 ± 5.70

Table 4
Some physicochemical parameters of the compounds **1**–**38** used in prediction of ADME profiles.

Comp.	MW	logP	tPSA	nON	nOHNH	Vol	nrotb	Vio
1	479.60	4.43	80.77	7	1	429.83	7	0
2	493.63	4.86	80.77	7	1	446.39	7	0
3	509.63	4.46	90.00	8	1	455.37	8	1
4	497.59	4.57	80.77	7	1	434.76	7	0
5	514.05	5.08	80.77	7	1	443.36	7	2
6	524.60	4.37	126.59	10	1	453.16	8	1
7	539.65	4.05	99.23	9	1	480.92	9	1
8	422.52	3.87	67.88	6	1	399.28	7	0
9	436.55	4.32	67.88	6	1	415.84	7	0
10	452.55	3.93	77.11	7	1	424.83	8	0
11	440.51	4.04	67.88	6	1	404.21	7	0
12	456.97	4.55	67.88	6	1	412.82	7	0
13	467.52	3.83	113.70	9	1	422.62	8	0
14	482.58	3.52	86.34	8	1	450.37	9	0
15	348.45	3.77	32.78	4	0	331.85	3	0
16	348.45	3.77	32.78	4	0	331.85	3	0
17	378.47	3.36	42.02	5	0	357.40	4	0
18	362.47	4.14	32.78	4	0	348.65	4	0
19	362.47	4.14	32.78	4	0	348.65	4	0
20	392.50	3.73	42.02	5	0	374.20	5	0
21	378.47	3.14	53.01	5	1	356.91	5	0
22	378.47	3.14	53.01	5	1	356.91	5	0
23	408.50	2.73	62.24	6	1	382.46	6	0
24	405.54	3.80	36.02	5	0	394.80	6	0
25	405.54	3.80	36.02	5	0	394.80	6	0
26	435.57	3.39	45.25	6	0	420.35	7	0
27	419.57	4.07	36.02	5	0	411.60	7	0
28	419.57	4.07	36.02	5	0	411.60	7	0
29	449.60	3.66	45.25	6	0	437.15	8	0
30	410.52	5.47	32.78	4	0	386.70	4	1
31	410.52	5.47	32.78	4	0	386.70	4	1
32	440.54	5.05	42.02	5	0	412.25	5	1
33	319.40	4.28	29.54	3	0	302.51	3	0
34	319.40	4.28	29.54	3	0	302.51	3	0
35	349.43	3.87	38.78	4	0	328.05	4	0
36	321.42	4.63	29.54	3	0	312.87	5	0
37	321.42	4.63	29.54	3	0	312.87	5	0
38	351.45	4.22	38.78	4	0	338.41	6	0

MW: Molecular weight; log P: log octanol/water partition coefficient; tPSA: Total Polar Surface Area; nON: number of Hydrogen acceptors; nOHNH: number of Hydrogen donors; Vol: Molecular volume; nrotb: number of rotatable bonds; Vio: Violation.

compounds **26**–**29**, all calculated physicochemical parameters are compatible with Lipinski's rule. Thus, it can be declared that the compounds **26**–**29** may have a good pharmacokinetic profile, which enhances their biological importance.

2.6. Molecular docking

Docking studies were performed in order to gain more insight into the binding mode of the compounds **26**–**29** and to evaluate the effects of structural modifications on the inhibitory activity against AChE enzyme. Studies were carried out by using the X-ray crystal structure of *Homo sapiens* AChE (*h*AChE PDB ID:4EY7) obtained from Protein Data Bank server (www.pdb.org).

The docking poses of the compounds **26**–**29**, showing remarkable activity in the series, are presented in Electronic Supporting Information. According to poses, it is clearly understood that the compounds bind to AChE enzyme in a similar position with DNP due to the DBS. The compounds **26**–**29** mainly carry the lipophilic benzene and 1-indanone ring systems, piperazine moiety as a polar group and a basic dimethylaminoalkyl side chain. The docking poses indicate that lipophilic groups interact with the PAS region, whereas the polar and basic groups bind to the CAS region.

The compounds **26**–**29** bear methoxy or dimethoxy substituents at fifth and/or sixth position of 1-indanone. The oxygen atom of the methoxy group is very significant in terms of polar interactions. The docking poses reveals that the methoxy substituents interact with His287, Ser293, Gln291, Leu289 and Glu292 by formation of hydrogen bond. Besides, the compounds **26** and **29**, bearing dimethoxy substituents, shows more potent binding to the active site owing to the more polar interactions. A hydrogen bond is also established between 1-indanone carbonyl and the amino group of Phe295 for the compounds **26** and **29**, while the same formation occurs with Arg296 for the compound **28**.

The other important factor of binding with the active site is π-π interaction between the 1,4-disubstituted phenyl of the compound **26**, **28** and **29** and Tyr341. However, this interaction is observed with Trp286 for the compound **27**.

The formation of cation-π interaction between the nitrogen atom of the dimethylamino group and Trp86 provides an efficient binding. Furthermore, for the compounds **26**, **28** and **29**, there is a formation of hydrogen bond between nitrogen of dimethyamino group and the carboxyl of Glu202. The piperazine ring system establishes van der Waals interaction with the amino acids in the cavity, and therefore, it consolidates the binding with the active region of the enzyme.

The compound **26** carries dimethylaminoethyl side chain at fourth position of piperazine, whereas the compounds **27**–**29** include dimethylaminopropyl group at same position. Docking results reveal that extended carbon chain raises the van der Waals interaction with the amino acids in the gorge and intensifies the proper binding.

2.7. Structure-activity relationships

As a result of enzyme inhibition and docking studies, following points about structure activity relationships of the synthesized compounds may be recommended;

- In the compounds **1**–**14**, substitution of piperidine with *N*-arylacetamides causes a dramatic activity loss when compared with DNP that carries benzyl at the same position.
- In the compounds **15**–**38**, dimethoxy substitution of 1-indanone at fifth and sixth positions enhances the activity when compared with mono substitutions at the same positions.
- *Para* substitution of benzylidene fragment with a basic center as piperazine, pyrrolidine or diethylamino is not enough to create important activity.
- Side chains as methyl, ethyl, hydroxyethyl and benzyl at fourth position of piperazine do not increase the activity.
- On the other hand, the side chains as dimethylaminoethyl and dimethylaminopropyl, which bear second basic center, at fourth position of piperazine cause a significant activity.
- Dimethylaminopropyl substitution intensifies the activity more than dimethylaminoetyl group.

3. Conclusion

Modifying the structure of existing drugs is an important approach in the development of novel agents. Prompted from this strategy, in the present study, 38 new DNP analogues were

evaluated as anticholinesterase agents. Pharmacological, toxicological and ADME studies revealed the potency of the compounds **26**–**29**. The docking studies clearly explained the molecular interactions between the compounds and AChE. Consequently, all these data may have an impact on medicinal chemists to synthesize more potent compounds as inhibitors against AChE.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (M.p.) were determined on Mettler Toledo-MP90 Melting Point System and were uncorrected. The TLC was performed on silica gel 60 F_{254} (Merck) layer using petroleum ether: ethyl acetate (3:1 or 1:1 v/v) as eluents. IR spectra were recorded on an IR Affinity-1S Infrared spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on a Bruker, UltraShield 500 MHz NMR and ^{13}C NMR spectra were recorded on a Bruker, UltraShield 100 MHz NMR spectrometer (Bruker, USA) using DMSO-d_6. Chemical shifts (δ) were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. Mass spectra were recorded on a LCMS-IT-TOF (Shimadzu, Kyoto, Japan) by using ESI method. Elemental analyses were performed on a Leco CHNS-932 analyzer (USA).

4.1.1. General procedure for the synthesis of the compounds

4.1.1.1. 2-Chloro-N-arylacetamide derivatives. Chloroacetyl chloride (33 mmol, 2.63 mL) was added dropwise with stirring to a mixture of triethylamine (33 mmol, 4.63 mL) and 2-aminobenzothiazole or aniline derivative (30 mmol) in THF (100 mL) at 0 °C. After the completion of dropping, the mixture was allowed to stir for 1 h. The solvent was evaporated under reduced pressure. The residue was washed with water to remove trimethylamine hydrochloride, dried and recrystallized from EtOH [59,60].

*4.1.1.2. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl) methyl)piperidin-1-yl)-N-acetamide derivatives (**1**–**14**).* A mixture of 5,6-dimethoxy-2-(piperidin-4-yl-methyl)-2,3-dihydro-1*H*-inden-1-one (1 mmol, 0.289 g) and 2-chloro-*N*-arylacetamide derivatives (1 mmol) in acetone (10 mL) was stirred at 40 °C for 12 h in the presence of potassium carbonate (1 mmol, 0.138 g). The solvent was evaporated. The residue was washed with water, dried and crystallized from ethanol [61].

*4.1.1.2.1. N-(Benzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)acetamide (**1**).* Yield: 89%. Mp: 193.1 °C. IR (ATR) V_{max} (cm^{-1}): 3263 (N-H stretching), 1697 (indanone C=O stretching), 1681 (amide C=O stretching), 1593-1446 (C=C and C=N stretchings), 1265 (C-N stretching), 1033 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.22–1.30 (3H, m, $H_{3'}$+$H_{5'}$), 1.47 (1H, s, $H_{3'}$+$H_{5'}$), 1.63–1.71 (1H, m, $H_{4'}$), 1.72–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.18–2.22 (2H, m, $H_{2'}$+$H_{6'}$), 2.66–2.69 (2H, m, H_3), 2.90 (2H, t, J = 11,60 Hz, -CH_2-), 3.19 (2H, s, -CH_2CO-), 3.34 (1H, s, H_2), 3.80 (3H, s, C_5-OCH_3), 3.88 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.32 (1H, t, J = 7.20 Hz, $H_{5''}$), 7.45 (1H, t, J = 7.60 Hz, $H_{6''}$), 7.75 (1H, d, J = 7.95 Hz, $H_{4''}$), 7.99 (1H, d, J = 7.95 Hz, $H_{7''}$), 9.72 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.82 (1C, C_3), 33.05 (2C, $C_{3'}$+$C_{5'}$), 33.82 (1C, $C_{4'}$), 38.66 (1C, -CH_2-), 45.22 (1C, C_2), 53.72 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 62.21 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.68 (1C, C_7), 120.98 (1C, $C_{4''}$), 122.18 (1C, $C_{7''}$), 124.03 (1C, $C_{6''}$), 126.59 (1C, $C_{5''}$), 128.91 (1C, C_{7a}), 131.95 (1C, $C_{7''a}$), 148.96 (1C, C_{3a}), 149.19 (1C, C_6), 149.59 (1C, $C_{3''a}$), 155.72 (1C, C_5), 158.00 (1C, $C_{2''}$), 170.24 (1C, -NH-CO-), 207.07 (1C, C_1). HRMS (ESI) $(M+H)^+$(m/z): For $C_{26}H_{29}N_3O_4S$ Calculated: 480.1952. Found: 480.1959. Elemental analyses: $C_{26}H_{29}N_3O_4S$, Calculated (%): C, 65.11; H, 6.10; N, 8.76; S, 6.68. Found (%): C, 65.09; H, 6.11; N, 8.74; S, 6.66.

*4.1.1.2.2. N-(6-Methylbenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl) acetamide (**2**).* Yield: 87%. Mp: 205.5 °C. IR (ATR) V_{max} (cm^{-1}): 3273 (N-H stretching), 1695 (indanone C=O stretching), 1680 (amide C=O stretching), 1593-1429 (C=C and C=N stretchings), 1257 (C-N stretching), 1029 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.20–1.31 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.70–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.17–2.22 (2H, m, $H_{2'}$+$H_{6'}$), 2.42 (3H, s, -CH_3), 2.64–2.68 (2H, m, H_3), 2.88 (2H, t, J = 11.65 Hz, -CH_2-), 3.26 (2H, s, -CH_2-CO-), 3.35 (1H, s, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.26 (1H, dd, J = 1.30 Hz and J = 8.25 Hz, $H_{5''}$), 7.63 (1H, d, J = 8.25 Hz, $H_{4''}$), 7.77 (1H, d, J = 1.30 Hz, $H_{7''}$), 9.72 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 21.44 (1C, -CH_3), 31.83 (1C, C_3), 33.05 (2C, $C_{3'}$+$C_{5'}$), 33.15 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.22 (1C, C_2), 53.72 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 61.20 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.67 (1C, C_7), 120.62 (1C, $C_{4''}$), 121.78 (1C, $C_{7''}$), 127.91 (1C, $C_{5''}$), 128.91 (1C, C_{7a}), 131.10 (1C, $C_{7''a}$), 133.49 (1C, $C_{6''}$), 149.19 (1C, C_6), 149.93 (1C, C_{3a}), 150.76 (1C, $C_{3''a}$), 155.71 (1C, C_5), 158.07 (1C, $C_{2''}$), 170.08 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$(m/z): For $C_{27}H_{31}N_3O_4S$ Calculated: 494.2108. Found: 494.2109. Elemental analyses: $C_{27}H_{31}N_3O_4S$, Calculated (%): C, 65.70; H, 6.33; N, 8.51; S, 6.49. Found (%): C, 65.68; H, 6.31; N, 8.48; S, 6.50.

*4.1.1.2.3. N-(6-Methoxybenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl) acetamide (**3**).* Yield: 92%. Mp: 175 °C. IR (ATR) V_{max} (cm^{-1}): 3275 (N-H stretching), 1697 (indanone C=O stretching), 1681 (amide C=O stretching), 1602-1500 (C=C and C=N stretchings), 1259 (C-N stretching), 1029 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.21–1.31 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.70–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.17–2.22 (2H, m, $H_{2'}$+$H_{6'}$), 2.64–2.69 (2H, m, H_3), 2.90 (2H, t, J = 11.50 Hz, -CH_2-), 3.39 (1H, s, H_2), 3.27 (2H, s, -CH_2-CO-), 3.80 (3H, s, C_5-OCH_3), 3.84 (3H, s, $C_{6''}$-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.03 (1H, dd, J = 2.60 Hz and J = 8.80 Hz, $H_{5''}$), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.57 (1H, d, J = 2.55 Hz, $H_{7''}$), 7.64 (1H, d, J = 8.80 Hz, $H_{4''}$), 9.76 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.84 (1C, C_3), 33.16 (2C, $C_{3'}$+$C_{5'}$), 33.82 (1C, $C_{4'}$), 39.86 (1C, -CH_2-), 45.22 (1C, C_2), 53.73 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.11 (1C, $C_{6''}$-OCH_3), 56.70 (1C, C_6-OCH_3), 61.17 (1C, -CH_2-CO-), 104.46 (1C, C_4), 105.26 (1C, $C_{5''}$), 108.68 (1C, C_7), 115.38 (1C, $C_{7''}$), 121.58 (1C, $C_{4''}$), 128.91 (1C, C_{7a}), 133.26 (1C, $C_{7''a}$), 143.04 (1C, $C_{6''}$), 149.19 (1C, C_6), 149.59 (1C, C_{3a}), 155.72 (1C, $C_{3''a}$), 155.94 (1C, C_5), 156.64 (1C, $C_{2''}$), 169.93 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{27}H_{31}N_3O_5S$ Calculated: 510.2057. Found: 510.2054. Elemental analyses: $C_{27}H_{31}N_3O_5S$, Calculated (%): C, 63.63; H, 6.13; N, 8.25; S, 6.29. Found (%): C, 63.61; H, 6.12; N, 8.24; S, 6.30.

*4.1.1.2.4. N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)acetamide (**4**).* Yield: 88%. Mp: 212.5 °C. IR (ATR) V_{max} (cm^{-1}): 3265 (N-H stretching), 1701 (indanone C=O stretching), 1676 (amide C=O stretching), 1604-1456 (C=C and C=N stretchings), 1199 (C-N stretching), 1124 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.20–1.28 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.65–1.66 (1H, m, $H_{4'}$), 1.70–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.17–2.21 (2H, m, $H_{2'}$+$H_{6'}$), 2.64–2.68 (2H, m, H_3), 2.89 (2H, t, J = 11.55 Hz, -CH_2-), 3.22 (2H, s, -CH_2CO-), 3.39 (1H, s, H_2), 3.79 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.06 (1H, s, H_4), 7.09 (1H, s, H_7), 7.27–7.32 (1H, m, $H_{7''}$), 7.74–7.77 (1H, m, $H_{4''}$), 7.89–7.91 (1H, m, $H_{5''}$), 9.75 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.80 (1C, C_3), 33.09 (2C, $C_{3'}$+$C_{5'}$), 33.80 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.21 (1C, C_2), 53.71

(2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.39 (1C, C_6-OCH_3), 61.14 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.53 (1C, C_7), 120.04 (1C, $C_{4''}$), 122.12 (1C, $C_{7''}$), 123.59 (1C, $C_{5''}$), 128.91 (1C, C_{7a}), 131.79 (1C, $C_{7''a}$), 133.15 (1C, $C_{6''}$), 148.03 (1C, C_5), 149.19 (1C, C_6), 149.59 (1C, C_{3a}), 155.71 (1C, $C_{3''a}$), 158.74 (1C, $C_{2''}$), 170.31 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{26}H_{28}N_3O_4FS$ Calculated: 498.1857. Found: 498.1862. Elemental analyses: $C_{26}H_{28}N_3O_4FS$, Calculated (%): C, 62.76; H, 5.67; N, 8.44; S, 6.44. Found (%): C, 62.75; H, 5.66; N, 8.42; S, 6.43.

*4.1.1.2.5. N-(6-Chlorobenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl) acetamide (**5**).* Yield: 84%. Mp: 171.7 °C. IR (ATR) V_{max} (cm^{-1}): 3292 (N-H stretching), 1705 (indanone C=O stretching), 1676 (amide C=O stretching), 1593-1444 (C=C and C=N stretchings), 1261 (C-N stretching), 1033 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.21–1.30 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.70–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.18–2.22 (2H, m, $H_{2'}$+$H_{6'}$), 2.65–2.69 (2H, m, H_3), 2.90 (2H, t, J = 11.70 Hz, -CH_2-), 3.22 (2H, s, -CH_2CO-), 3.35 (1H, s, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.46 (1H, dd, J = 2.20 Hz and J = 8.60 Hz, $H_{5''}$), 7.74 (1H, d, J = 8.60 Hz, $H_{4''}$), 8.14 (1H, d, J = 2.15 Hz, $H_{7''}$), 9.74 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.78 (1C, C_3), 33.08 (2C, $C_{3'}$+$C_{5'}$), 33.80 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.21 (1C, C_2), 53.70 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.39 (1C, C_6-OCH_3), 61.15 (1C, -CH_2-CO-), 104.45 (1C, C_4), 108.66 (1C, C_7), 121.91 (1C, $C_{4''}$), 122.18 (1C, $C_{7''}$), 126.94 (1C, $C_{5''}$), 128.91 (1C, C_{7a}), 131.33 (1C, $C_{7''a}$), 133.66 (1C, $C_{6''}$), 147.66 (1C, C_5), 149.18 (1C, C_6), 149.59 (1C, C_{3a}), 155.71 (1C, $C_{3''a}$), 158.90 (1C, $C_{2''}$), 170.43 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{26}H_{28}N_3O_4SCl$ Calculated: 514.1562. Found: 514.1562. Elemental analyses: $C_{26}H_{28}N_3O_4SCl$, Calculated (%): C, 60.75; H, 5.49; N, 8.17; S, 6.24. Found (%): C, 60.73; H, 5.48; N, 8.18; S, 6.25.

*4.1.1.2.6. N-(6-Nitrobenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)acetamide (**6**).* Yield: 89%. Mp: 132.4 °C. IR (ATR) V_{max} (cm^{-1}): 3296 (N-H stretching), 1712 (indanone C=O stretching), 1695 (amide C=O stretching), 1506-1440 (C=C and C=N stretchings), 1126 (C-N stretching), 1037 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.25–1.31 (3H, m, $H_{3'}$+$H_{5'}$), 1.48 (1H, s, $H_{3'}$+$H_{5'}$), 1.64–1.67 (1H, m, $H_{4'}$), 1.70–1.77 (2H, m, $H_{2'}$+$H_{6'}$), 2.26–2.30 (2H, m, $H_{2'}$+$H_{6'}$), 2.66–2.69 (2H, m, H_3), 2.96 (2H, t, J = 11.20 Hz, -CH_2-), 3.25 (2H, s, -CH_2CO-), 3.46 (1H, s, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.87 (1H, d, J = 8.95 Hz, $H_{4''}$), 8.28 (1H, dd, J = 2.40 Hz and J = 8.90 Hz, $H_{5''}$), 9.03 (1H, d, J = 2.40 Hz, $H_{7''}$), 9.77 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.71 (1C, C_3), 33.84 (2C, $C_{3'}$+$C_{5'}$), 33.87 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.19 (1C, C_2), 53.60 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 59.98 (1C, -CH_2-CO-), 103.76 (1C, C_4), 108.67 (1C, C_7), 120.83 (1C, $C_{4''}$), 122.16 (1C, $C_{7''}$), 127.31 (1C, $C_{5''}$), 128.84 (1C, C_{7a}), 131.48 (1C, $C_{7''a}$), 133.42 (1C, $C_{6''}$), 148.61 (1C, C_5), 149.36 (1C, C_6), 149.63 (1C, C_{3a}), 155.67 (1C, $C_{3''a}$), 156.28 (1C, $C_{2''}$), 177.53 (1C, -NH-CO-), 207.58 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{26}H_{28}N_4O_6S$ Calculated: 525.1802. Found: 525.1806. Elemental analyses: $C_{26}H_{28}N_4O_6S$, Calculated (%): C, 59.53; H, 5.38; N, 10.68; S, 6.11. Found (%): C, 59.51; H, 5.39; N, 10.66; S, 6.10.

*4.1.1.2.7. N-(5,6-Dimethoxybenzo[d]thiazol-2-yl)-2-(4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl) acetamide (**7**).* Yield: 82%. Mp: 136.5 °C. IR (ATR) V_{max} (cm^{-1}): 3263 (N-H stretching), 1695 (indanone C=O stretching), 1683 (amide C=O stretching), 1539-1435 (C=C and C=N stretchings), 1201 (C-N stretching), 1159 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.20–1.30 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.70–1.77 (2H, m, $H_{2'}$+$H_{6'}$), 2.17–2.21 (2H, m, $H_{2'}$+$H_{6'}$), 2.65–2.69 (2H, m, H_3), 2.89 (2H, t, J = 11.60 Hz, -CH_2-), 3.18 (2H, s, -CH_2CO-), 3.31 (1H, s, H_2), 3.80 (3H, s, C_5-OCH_3), 3.81 (3H, s, $C_{5''}$-OCH_3), 3.83 (3H, s, $C_{6''}$-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10 (1H, s, H_7), 7.30 (1H, s, $H_{4''}$), 7.55 (1H, s, $H_{7''}$), 9.78 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.85 (1C, C_3), 33.08 (2C, $C_{3'}$+$C_{5'}$), 33.15 (1C, $C_{4'}$), 39.68 (1C, -CH_2-), 45.22 (1C, C_2), 53.73 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.12 (1C, $C_{5''}$-OCH_3), 56.33 (1C, $C_{6''}$-OCH_3), 56.46 (1C, C_6-OCH_3), 61.12 (1C, -CH_2-CO-), 104.03 (1C, C_4), 104.26 (1C, $C_{4''}$), 104.46 (1C, $C_{7''}$), 108.67 (1C, C_7), 123.38 (1C, $C_{7''a}$), 128.91 (1C, C_{7a}), 142.47 (1C, $C_{5''}$), 142.95 (1C, $C_{6''}$), 148.56 (1C, C_6), 149.42 (1C, C_{3a}), 150.28 (1C, $C_{3''a}$), 155.72 (1C, C_5), 156.40 (1C, $C_{2''}$), 169.61 (1C, -NH-CO-), 207.07 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{28}H_{33}N_3O_6S$ Calculated: 540.2163. Found: 540.2163. Elemental analyses: $C_{28}H_{33}N_3O_6S$, Calculated (%): C, 62.32; H, 6.16; N, 7.79; S, 5.94. Found (%): C, 62.30; H, 6.17; N, 7.80; S, 5.93.

*4.1.1.2.8. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-phenylacetamide (**8**).* Yield: 92%. Mp: 174.1 °C. IR (ATR) V_{max} (cm^{-1}): 3290 (N-H stretching), 1697 (indanone C=O stretching), 1681 (amide C=O stretching), 1589-1442 (C=C and C=N stretchings), 1037 (C-N stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.25–1.38 (3H, m, $H_{3'}$+$H_{5'}$), 1.46 (1H, s, $H_{3'}$+$H_{5'}$), 1.63–1.65 (1H, m, $H_{4'}$), 1.71–1.78 (2H, m, $H_{2'}$+$H_{6'}$), 2.08–2.15 (2H, m, $H_{2'}$+$H_{6'}$), 2.50–2.51 (2H, m, H_3), 2.70 (2H, t, J = 11.45 Hz, -CH_2-), 3.05 (2H, s, -CH_2CO-), 3.30–3.33 (1H, m, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.08 (1H, s, H_4), 7.10 (1H, s, H_7), 7.29 (1H, m, $H_{4''}$), 7.31–7.33 (2H, m, $H_{3''}$+$H_{5''}$), 7.64 (2H, dd, J = 2.40 Hz and J = 8.60 Hz, $H_{2''}$+$H_{6''}$), 9.66 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 33.03 (2C, $C_{3'}$+$C_{5'}$), 33.14 (1C, C_3), 33.82 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.22 (1C, C_2), 53.94 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.46 (1C, C_6-OCH_3), 60.93 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.68 (1C, C_7), 119.88 (2C, $C_{2''}$+$C_{6''}$), 123.84 (1C, $C_{4''}$), 129.13 (2C, $C_{3''}$+$C_{5''}$), 128.91 (1C, C_{7a}), 139.03 (1C, $C_{1''}$), 149.20 (1C, C_{3a}), 155.65 (1C, C_6), 155.72 (1C, C_5), 168.70 (1C, -NH-CO-), 207.07 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{25}H_{30}N_2O_4$ Calculated: 423.2278. Found: 423.2298. Elemental analyses: $C_{25}H_{30}N_2O_4$, Calculated (%): C, 71.07; H, 7.16; N, 6.63. Found (%): C, 71.05; H, 7.15; N, 6.62.

*4.1.1.2.9. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(p-tolyl)acetamide (**9**).* Yield: 86%. Mp: 144.5 °C. IR (ATR) V_{max} (cm^{-1}): 3273 (N-H stretching), 1697 (indanone C=O stretching), 1668 (amide C=O stretching), 1589-1498 (C=C and C=N stretchings), 1120 (C-N stretching), 810 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.24–1.36 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.71–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.09–2.16 (2H, m, $H_{2'}$+$H_{6'}$), 2.26 (3H, s, -CH_3), 2.65–2.69 (2H, m, H_3), 2.87 (2H, t, J = 11.425 Hz, -CH_2-), 3.07 (2H, s, -CH_2CO-), 3.22–3.27 (1H, m, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.07 (1H, s, H_4), 7.10–7.12 (3H, m, H_7+$H_{3''}$+$H_{5''}$), 7.52 (2H, d, J = 8.35 Hz, $H_{2''}$+$H_{6''}$), 9.56 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 20.90 (-CH_3), 31.76 (1C, C_3), 33.03 (2C, $C_{3'}$+$C_{5'}$), 33.14 (1C, $C_{4'}$), 39.68 (1C, -CH_2-), 45.22 (1C, C_2), 53.96 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 62.67 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.67 (1C, C_7), 119.88 (2C, $C_{2''}$+$C_{6''}$), 129.49 (2C, $C_{3''}$+$C_{5''}$), 128.90 (1C, C_{7a}), 132.74 (1C, $C_{1''}$), 136.52 (1C, $C_{4''}$), 149.19 (1C, C_{3a}), 159.59 (1C, C_6), 155.71 (1C, C_5), 168.77 (1C, -NH-CO-), 207.07 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{26}H_{32}N_2O_4$ Calculated: 437.2435. Found: 437.2454. Elemental analyses: $C_{26}H_{32}N_2O_4$, Calculated (%): C, 71.53; H, 7.39; N, 6.42. Found (%): C, 71.51; H, 7.38; N, 6.40.

*4.1.1.2.10. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(p-methoxyphenyl)acetamide (**10**).* Yield: 89%. Mp: 138.2 °C. IR (ATR) V_{max} (cm^{-1}): 3278 (N-H stretching), 1697 (indanone C=O stretching), 1674 (amide C=O stretching), 1591-1498 (C=C and C=N stretchings), 1236 (C-N stretching), 1122 (C-N stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.24–1.39 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.63–1.65

(1H, m, $H_{4'}$), 1.71–1.76 (2H, m, $H_{2'}$+$H_{6'}$), 2.17–2.18 (2H, m, $H_{2'}$+$H_{6'}$), 2.65–2.69 (2H, m, H_3), 2.89 (2H, s, -CH_2-), 3.10 (2H, s, -CH_2CO-), 3.22–3.25 (1H, m, H_2), 3.73 (3H, s, $C_{4''}$-OCH_3), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 6.88 (2H, d, J = 2.15 Hz, $H_{3''}$+$H_{5''}$), 7.06 (1H, s, H_4), 7.10 (1H, s, H_7), 7.54 (2H, d, J = 2.67 Hz, $H_{2''}$+$H_{6''}$), 9.56 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 33.05 (2C, $C_{3'}$+$C_{5'}$), 33.13 (1C, C_3), 33.14 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.21 (1C, C_2), 53.93 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 62.15 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.67 (1C, C_7), 114.26 (2C, $C_{3''}$+$C_{5''}$), 121.50 (2C, $C_{2''}$+$C_{6''}$), 128.90 (1C, C_{7a}), 132.18 (1C, $C_{1''}$), 149.19 (1C, C_{3a}), 149.60 (1C, C_6), 155.72 (1C, $C_{4''}$), 155.83 (1C, C_5), 168.90 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{26}H_{32}N_2O_5$ Calculated: 453.2384. Found: 453.2389. Elemental analyses: $C_{26}H_{32}N_2O_5$, Calculated (%): C, 69.01; H, 7.13; N, 6.19. Found (%): C, 69.00; H, 7.11; N, 6.18.

*4.1.1.2.11. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(p-fluorophenyl)acetamide (**11**).* Yield: 91%. Mp: 155.8 °C. IR (ATR) V_{max} (cm^{-1}): 3309 (N-H stretching), 1703 (indanone C=O stretching), 1689 (amide C=O stretching), 1525-1498 (C=C and C=N stretchings), 1222 (C-N stretching), 1124 (C-O stretching), 827 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.24–1.33 (3H, m, $H_{3'}$+$H_{5'}$), 1.45 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.64 (1H, m, $H_{4'}$), 1.71–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.09–2.16 (2H, m, $H_{2'}$+$H_{6'}$), 2.65–2.68 (2H, m, H_3), 2.87 (2H, t, J = 11.45 Hz, -CH_2-), 3.09 (2H, s, -CH_2CO-), 3.21–3.27 (1H, m, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.06 (1H, s, H_4), 7.10 (1H, s, H_7), 7.13–7.16 (2H, m, $H_{3''}$+$H_{5''}$), 7.66–7.68 (2H, m, $H_{2''}$+$H_{6''}$), 9.73 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.98 (2C, $C_{3'}$+$C_{5'}$), 33.14 (1C, C_3), 33.85 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.22 (1C, C_2), 53.96 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 62.67 (1C, -CH_2-CO-), 104.45 (1C, C_4), 108.67 (1C, C_7), 115.63 (2C, $C_{3''}$+$C_{5''}$), 121.76 (2C, $C_{2''}$+$C_{6''}$), 128.91 (1C, C_{7a}), 135.48 (1C, $C_{1''}$), 149.19 (1C, C_{3a}), 149.59 (1C, C_6), 155.72 (1C, C_5), 159.51 (1C, $C_{4''}$), 168.99 (1C, -NH-CO-), 207.07 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{25}H_{29}N_2O_4F$ Calculated: 441.2184. Found: 441.2190. Elemental analyses: $C_{25}H_{29}N_2O_4F$, Calculated (%): C, 68.16; H, 6.64; N, 6.36. Found (%): C, 68.15; H, 6.62; N, 6.35.

*4.1.1.2.12. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(p-chlorophenyl)acetamide (**12**).* Yield: 90%. Mp: 156.6 °C. IR (ATR) V_{max} (cm^{-1}): 3309 (N-H stretching), 1714 (indanone C=O stretching), 1691 (amide C=O stretching), 1587-1490 (C=C and C=N stretchings), 1091 (C-N stretching), 825 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.24–1.35 (3H, m, $H_{3'}$+$H_{5'}$), 1.46 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.71–1.75 (2H, m, $H_{2'}$+$H_{6'}$), 2.09–2.15 (2H, m, $H_{2'}$+$H_{6'}$), 2.65–2.69 (2H, m, H_3), 2.87 (2H, s, -CH_2-), 3.11 (2H, s, -CH_2CO-), 3.22–3.25 (1H, m, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.06 (1H, s, H_4), 7.10 (1H, s, H_7), 7.36 (2H, d, J = 8.85 Hz, $H_{3''}$+$H_{5''}$), 7.69 (2H, d, J = 8.90 Hz, $H_{2''}$+$H_{6''}$), 9.82 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 33.03 (2C, $C_{3'}$+$C_{5'}$), 33.14 (1C, C_3), 33.82 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.21 (1C, C_2), 53.92 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 61.12 (1C, -CH_2-CO-), 104.46 (1C, C_4), 108.68 (1C, C_7), 121.51 (2C, $C_{2''}$+$C_{6''}$), 129.00 (2C, $C_{3''}$+$C_{5''}$), 127.41 (1C, C_{7a}), 128.91 (1C, $C_{4''}$), 138.03 (1C, $C_{1''}$), 149.19 (1C, C_{3a}), 149.60 (1C, C_6), 155.72 (1C, C_5), 169.62 (1C, -NH-CO-), 207.05 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{25}H_{29}N_2O_4Cl$ Calculated: 457.1889. Found: 457.1887. Elemental analyses: $C_{25}H_{29}N_2O_4Cl$, Calculated (%): C, 65.71; H, 6.40; N, 6.13. Found (%): C, 65.70; H, 6.41; N, 6.12.

*4.1.1.2.13. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(p-nitrophenyl)acetamide (**13**).* Yield: 93%. Mp: 182.8 °C. IR (ATR) V_{max} (cm^{-1}): 3255 (N-H stretching), 1712 (indanone C=O stretching), 1695 (amide C=O stretching), 1598-1492 (C=C and C=N stretchings), 1109 (C-N stretching), 852 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.25–1.31 (3H, m, $H_{3'}$+$H_{5'}$), 1.46 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.71–1.76 (2H, m, $H_{2'}$+$H_{6'}$), 2.14–2.18 (2H, m, $H_{2'}$+$H_{6'}$), 2.66–2.69 (2H, m, H_3), 2.88 (2H, t, J = 11.50 Hz, -CH_2-), 3.18 (2H, s, -CH_2CO-), 3.22–3.27 (1H, m, H_2), 3.80 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_6-OCH_3), 7.06 (1H, s, H_4), 7.10 (1H, s, H_7), 7.93 (2H, d, J = 2.45 Hz, $H_{2''}$+$H_{6''}$), 8.23 (2H, d, J = 2.375 Hz, $H_{3''}$+$H_{5''}$), 10.30 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.67 (2C, $C_{3'}$+$C_{5'}$), 33.13 (1C, C_3), 33.82 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.21 (1C, C_2), 53.88 (2C, $C_{2'}$+$C_{6'}$), 56.08 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 62.73 (1C, -CH_2-CO-), 104.45 (1C, C_4), 108.67 (1C, C_7), 119.60 (2C, $C_{2''}$+$C_{6''}$), 125.53 (2C, $C_{3''}$+$C_{5''}$), 128.90 (1C, C_{7a}), 142.78 (1C, $C_{4''}$), 145.30 (1C, $C_{1''}$), 149.19 (1C, C_{3a}), 149.59 (1C, C_6), 155.72 (1C, C_5), 168.70 (1C, -NH-CO-), 207.06 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{25}H_{29}N_3O_6$ Calculated: 468.2129. Found: 468.2131. Elemental analyses: $C_{25}H_{29}N_3O_6$, Calculated (%): C, 64.23; H, 6.25; N, 8.99. Found (%): C, 64.22; H, 6.24; N, 8.98.

*4.1.1.2.14. 2-(4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)-N-(3,4-dimethoxyphenyl)acetamide (**14**).* Yield: 87%. Oil. ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.24–1.33 (3H, m, $H_{3'}$+$H_{5'}$), 1.46 (1H, s, $H_{3'}$+$H_{5'}$), 1.62–1.65 (1H, m, $H_{4'}$), 1.71–1.76 (2H, m, $H_{2'}$+$H_{6'}$), 2.11–2.16 (2H, m, $H_{2'}$+$H_{6'}$), 2.66–2.69 (2H, m, H_3), 2.87 (2H, t, J = 11.40 Hz, -CH_2-), 3.06 (2H, s, -CH_2CO-), 3.21–3.27 (1H, m, H_2), 3.72 (3H, s, C_5-OCH_3), 3.74 (3H, s, $C_{3''}$-OCH_3), 3.78 (3H, s, $C_{4''}$-OCH_3), 3.87 (3H, s, C_6-OCH_3), 6.88 (1H, d, J = 8.70 Hz, $H_{5''}$), 7.06 (1H, s, H_4), 7.09 (1H, s, H_7), 7.17 (1H, dd, J = 2.30 Hz and J = 8.70 Hz, $H_{6''}$), 7.34 (1H, d, J = 2.30 Hz, $H_{2''}$), 9.50 (1H, s, -NH-). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.72 (2C, $C_{3'}$+$C_{5'}$), 33.12 (1C, C_3), 33.86 (1C, $C_{4'}$), 40.02 (1C, -CH_2-), 45.22 (1C, C_2), 54.00 (2C, $C_{2'}$+$C_{6'}$), 55.94 (1C, $C_{3''}$-OCH_3), 55.97 (1C, $C_{4''}$-OCH_3), 56.08 (1C, C_5-OCH_3), 56.46 (1C, C_6-OCH_3), 62.17 (1C, -CH_2-CO-), 104.45 (1C, C_4), 105.22 (1C, $C_{2''}$), 108.67 (1C, C_7), 111.84 (1C, $C_{5''}$), 112.54 (1C, $C_{6''}$), 128.91 (1C, C_{7a}), 132.73 (1C, $C_{1''}$), 145.43 (1C, $C_{4''}$), 149.05 (1C, $C_{3''}$), 149.19 (1C, C_{3a}), 149.59 (1C, C_6), 155.72 (1C, C_5), 168.60 (1C, -NH-CO-), 207.08 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{27}H_{34}N_2O_6$ Calculated: 483.2490. Found: 483.2497. Elemental analyses: $C_{27}H_{34}N_2O_6$, Calculated (%): C, 67.20; H, 7.10; N, 5.81. Found (%): C, 67.21; H, 7.09; N, 5.80.

4.1.1.3. 4-Substitued benzaldehyde derivatives. A mixture of 4-fluorobenzaldehyde (30 mmol, 3.21 mL) and appropriate secondary amine derivative (60 mmol) in dimethylformamide (DMF) (10 mL) was refluxed for 48 h in the presence of potassium carbonate (30 mmol, 4.14 g). The raw product was precipitated by pouring into ice water. The residue was filtered, washed with water, dried and recrystallized from EtOH [62].

*4.1.1.4. 5-Methoxy, 6-methoxy or 5,6-dimethoxy-2-(4-substituedbenzilidene)-2,3-dihydro-1H-inden-1-one derivatives (**15**–**38**).* A mixture of appropriate indan-1-one derivative (2 mmol), benzaldehyde derivative (2 mmol) and potassium hydroxide (2 mmol, 0.112 g) in methanol (10 mL) was stirred at room temperature for 48 h. The resulting colored solid was filtered, dried and crystallized from EtOH [63].

*4.1.1.4.1. 2-(4-(4-Methylpiperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**15**).* Yield: 90%. Mp: 184.7 °C. IR (ATR) V_{max} (cm^{-1}): 1678 (C=O stretching), 1597-1487 (C=C stretching), 1222 (C-N stretching), 1103 (C-O stretching), 810 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.23 (3H, s, -N-CH_3), 2.44–2.46 (4H, m, $H_{3''}$+$H_{5''}$), 3.29–3.33 (4H, m, $H_{2''}$+$H_{6''}$), 3.89 (3H, s, -OCH_3), 4.01 (2H, s, H_3), 7.01–7.04 (3H, m, $H_{3'}$+$H_{5'}$+H_6), 7.19 (1H, s, H_4), 7.38 (1H, s, =CH-), 7.62 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$), 7.70 (1H, d, J = 8.50 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.62 (1C, C_3), 46.21 (1C,

-N-CH_3), 47.33 (2C, $C_{3''}$+$C_{5''}$), 54.83 (2C, $C_{2''}$+$C_{6''}$), 56.22 (1C, C_5-OCH_3), 110.65 (1C, C_6), 114.95 (2C, $C_{3'}$+$C_{5'}$), 115.63 (1C, C_4), 125.12 (1C, $C_{1'}$), 125.56 (1C, C_7), 131.53 (1C, C_{7a}), 131.86 (1C, $C_{4'}$), 132.57 (1C, =CH-), 132.67 (2C, $C_{2'}$+$C_{6'}$), 151.98 (1C, C_2), 152.97 (1C, C_{3a}), 165.01 (1C, C_5), 193.35 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{22}H_{24}N_2O_2$ Calculated: 349.1911. Found: 349.1911. Elemental analyses: $C_{22}H_{24}N_2O_2$, Calculated (%): C, 75.83; H, 6.94; N, 8.04. Found (%): C, 75.82; H, 6.93; N, 8.05.

*4.1.1.4.2. 2-(4-(4-Methylpiperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**16**).* Yield: 87%. Mp: 198.7 °C. IR (ATR) V_{max} (cm^{-1}): 1683 (C=O stretching), 1597-1489 (C=C stretching), 1240 (C-N stretching), 1103 (C-O stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.23 (3H, s, -N-CH_3), 2.44–2.46 (4H, m, $H_{3''}$+$H_{5''}$), 3.30–3.33 (4H, m, $H_{2''}$+$H_{6''}$), 3.84 (3H, s, -OCH_3), 3.97 (2H, s, H_3), 7.04 (2H, d, J = 8.90 Hz, $H_{3'}$+$H_{5'}$), 7.23 (1H, d, J = 2.40 Hz, H_5), 7.27 (1H, dd, J = 2.50 Hz and J = 8.30 Hz, H_7), 7.46 (1H, s, =CH-), 7.58 (1H, d, J = 8.50 Hz, H_4), 7.64 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.86 (1C, C_3), 46.21 (1C, -N-CH_3), 47.25 (2C, $C_{3''}$+$C_{5''}$), 54.82 (2C, $C_{2''}$+$C_{6''}$), 55.96 (1C, C_6-OCH_3), 105.97 (1C, C_7), 114.89 (2C, $C_{3'}$+$C_{5'}$), 123.18 (1C, C_5), 124.91 (1C, $C_{1'}$), 127.86 (1C, C_4), 132.05 (1C, $C_{4'}$), 132.26 (1C, =CH-), 132.56 (2C, $C_{2'}$+$C_{6'}$), 139.56 (1C, C_2), 142.61 (1C, C_{3a}), 152.15 (1C, C_{7a}), 159.59 (1C, C_6), 193.35 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{22}H_{24}N_2O_2$ Calculated: 349.1911. Found: 349.1909. Elemental analyses: $C_{22}H_{24}N_2O_2$, Calculated (%): C, 75.83; H, 6.94; N, 8.04. Found (%): C, 75.81; H, 6.95; N, 8.03.

*4.1.1.4.3. 2-(4-(4-Methylpiperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**17**).* Yield: 88%. Mp: 195.6 °C. IR (ATR) V_{max} (cm^{-1}): 1680 (C=O stretching), 1585-1498 (C=C stretching), 1120 (C-N stretching), 1091 (C-O stretching), 815 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.23 (3H, s, -N-CH_3), 2.44–2.46 (4H, m, $H_{3''}$+$H_{5''}$), 3.28–3.30 (4H, m, $H_{2''}$+$H_{6''}$), 3.84 (3H, s, C_5-OCH_3), 3.91 (3H, s, C_6-OCH_3), 3.94 (2H, s, H_3), 7.03 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.21 (1H, s, =CH-), 7.22 (1H, s, H_4), 7.35 (1H, s, H_7), 7.61 (2H, d, J = 8.80 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.22 (1C, C_3), 46.22 (1C, -N-CH_3), 47.34 (2C, $C_{3''}$+$C_{5''}$), 54.84 (2C, $C_{2''}$+$C_{6''}$), 56.13 (1C, C_5-OCH_3), 56.42 (1C, C_6-OCH_3), 105.00 (1C, C_7), 108.61 (1C, C_4), 114.98 (2C, $C_{3'}$+$C_{5'}$), 125.19 (1C, $C_{1'}$), 130.94 (1C, $C_{4'}$), 132.12 (1C, =CH-), 132.34 (1C, C_2), 132.58 (2C, $C_{2'}$+$C_{6'}$), 144.99 (1C, C_{3a}), 149.71 (1C, C_{7a}), 152.96 (1C, C_6), 155.34 (1C, C_5), 192.26 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{23}H_{26}N_2O_3$ Calculated: 379.2016. Found: 379.2021. Elemental analyses: $C_{23}H_{26}N_2O_3$, Calculated (%): C, 72.99; H, 6.92; N, 7.40. Found (%): C, 72.98; H, 6.93; N, 7.39.

*4.1.1.4.4. 2-(4-(4-Ethylpiperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**18**).* Yield: 82%. Mp: 157.6 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1597-1581 (C=C stretching), 1195 (C-N stretching), 1101 (C-O stretching), 810 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.04 (3H, t, J = 3.55 Hz, -N-CH_2-CH_3), 2.35–2.38 (6H, m, -N-CH_2-CH_3+$H_{3''}$+$H_{5''}$), 3.28–3.34 (4H, m, $H_{2''}$+$H_{6''}$), 3.89 (3H, s, -OCH_3), 4.04 (2H, s, H_3), 7.02–7.04 (3H, m, $H_{3'}$+$H_{5'}$+H_6), 7.19 (1H, s, H_4), 7.38 (1H, s, =CH-), 7.61 (2H, d, J = 8.70 Hz, $H_{2'}$+$H_{6'}$), 7.70 (1H, d, J = 8.45 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.42 (1C, -N-CH_2-CH_3), 32.62 (1C, C_3), 39.52 (1C, -N-CH_2-CH_3), 47.44 (2C, $C_{3''}$+$C_{5''}$), 52.06 (2C, $C_{2''}$+$C_{6''}$), 56.21 (1C, C_5-OCH_3), 110.64 (1C, C_6), 114.91 (2C, $C_{3'}$+$C_{5'}$), 115.63 (1C, C_4), 125.10 (1C, $C_{1'}$), 125.56 (1C, C_7), 131.54 (1C, C_{7a}), 131.83 (1C, $C_{4'}$), 132.58 (1C, =CH-), 132.66 (2C, $C_{2'}$+$C_{6'}$), 152.01 (1C, C_2), 152.96 (1C, C_{3a}), 165.00 (1C, C_5), 191.96 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{23}H_{26}N_2O_2$ Calculated: 363.2067. Found: 363.2067. Elemental analyses: $C_{23}H_{26}N_2O_2$, Calculated (%): C, 76.21; H, 7.23; N, 7.73. Found (%): C, 76.20; H, 7.22; N, 7.72.

*4.1.1.4.5. 2-(4-(4-Ethylpiperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**19**).* Yield: 88%. Mp: 179.4 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1597-1489 (C=C stretching), 1238 (C-N stretching), 1103 (C-O stretching), 812 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.04 (3H, t, J = 7.15 Hz, -N-CH_2-CH_3), 2.37–2.39 (6H, m, -N-CH_2-CH_3+ $H_{3''}$+$H_{5''}$), 3.84 (3H, s, -OCH_3), 3.31–3.34 (4H, m, $H_{2''}$+$H_{6''}$), 3.97 (2H, s, H_3), 7.04 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.23 (1H, d, J = 2.45 Hz, H_5), 7.28 (1H, dd, J = 2.50 Hz and J = 8.25 Hz, H_7), 7.46 (1H, s, =CH-), 7.58 (1H, d, J = 8.35 Hz, H_4), 7.64 (2H, d, J = 8.80 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.42 (1C, -N-CH_2-CH_3), 31.85 (1C, C_3), 47.36 (2C, $C_{3''}$+$C_{5''}$), 51.84 (1C, -N-CH_2-CH_3), 52.06 (2C, $C_{2''}$+$C_{6''}$), 55.96 (1C, C_6-OCH_3), 105.97 (1C, C_7), 114.85 (2C, $C_{3'}$+$C_{5'}$), 123.54 (1C, C_5), 124.95 (1C, $C_{1'}$), 127.85 (1C, C_4), 132.03 (1C, $C_{4'}$), 132.95 (2C, $C_{2'}$+$C_{6'}$), 133.54 (1C, =CH-), 139.70 (1C, C_2), 142.60 (1C, C_{3a}), 152.19 (1C, C_{7a}), 159.62 (1C, C_6), 197.96 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{23}H_{26}N_2O_2$ Calculated: 363.2067. Found: 363.2067. Elemental analyses: $C_{23}H_{26}N_2O_2$, Calculated (%): C, 76.21; H, 7.23; N, 7.73. Found (%): C, 76.22; H, 7.24; N, 7.71.

*4.1.1.4.6. 2-(4-(4-Ethylpiperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**20**).* Yield: 84%. Mp: 138.5 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1600-1498 (C=C stretching), 1211 (C-N stretching), 1118 (C-O stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.04 (3H, t, J = 3.70 Hz, -N-CH_2-CH_3), 2.35–2.39 (6H, m, -N-CH_2-CH_3 +$H_{3''}$+$H_{5''}$), 3.27–3.29 (4H, m, $H_{2''}$+$H_{6''}$), 3.83 (3H, s, C_5-OCH_3), 3.90 (3H, s, C_6-OCH_3), 3.92 (2H, s, H_3), 7.01–7.02 (2H, m, H_4+=CH-), 7.20 (2H, d, J = 4.25 Hz, $H_{3'}$+$H_{5'}$), 7.35 (1H, s, H_7), 7.59 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.42 (1C, -N-CH_2-CH_3), 32.21 (1C, C_3), 47.45 (2C, $C_{3''}$+$C_{5''}$), 51.52 (1C, -N-CH_2-CH_3), 52.06 (2C, $C_{2''}$+$C_{6''}$), 56.11 (1C, C_5-OCH_3), 56.41 (1C, C_6-OCH_3), 104.98 (1C, C_7), 108.58 (1C, C_4), 114.92 (2C, $C_{3'}$+$C_{5'}$), 125.18 (1C, $C_{1'}$), 130.94 (1C, $C_{4'}$), 131.91 (1C, =CH-), 132.28 (1C, C_2), 132.56 (2C, $C_{2'}$+$C_{6'}$), 144.96 (1C, C_{3a}), 149.70 (1C, C_{7a}), 153.69 (1C, C_6), 155.33 (1C, C_5), 192.24 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{24}H_{28}N_2O_3$ Calculated: 393.2173. Found: 393.2170. Elemental analyses: $C_{24}H_{28}N_2O_3$, Calculated (%): C, 73.44; H, 7.19; N, 7.14. Found (%): C, 73.43; H, 7.20; N, 7.13.

*4.1.1.4.7. 2-(4-(4-(2-Hydroxyethyl)piperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**21**).* Yield: 91%. Mp: 162.9 °C. IR (ATR) V_{max} (cm^{-1}): 3390 (O-H stretching), 1670 (C=O stretching), 1593-1514 (C=C stretching), 1192 (C-N stretching), 1064 (C-O stretching), 819 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.45 (2H, t, J = 6.20 Hz, -CH_2-CH_2-OH), 2.55–2.57 (4H, m, $H_{2''}$+$H_{6''}$), 3.28–3.30 (4H, m, $H_{3''}$+$H_{5''}$), 3.55 (2H, t, J = 6.00 Hz, -CH_2-CH_2-OH), 3.89 (3H, s, -OCH_3), 4.00 (2H, s, H_3), 4.45 (1H, s, -OH), 7.01–7.04 (3H, m, $H_{3'}$+$H_{5'}$+ = CH-), 7.20 (1H, s, H_6), 7.38 (1H, s, H_4), 7.61 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$), 7.70 (1H, d, J = 8.45 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.62 (1C, C_3), 47.45 (2C, $C_{3''}$+$C_{5''}$), 53.40 (2C, $C_{2''}$+$C_{6''}$), 56.22 (1C, C_5-OCH_3), 59.07 (1C, -CH_2-CH_2-OH), 60.70 (1C, -CH_2-CH_2-OH), 110.65 (1C, C_6), 114.90 (2C, $C_{3'}$+$C_{5'}$), 115.63 (1C, C_4), 125.09 (1C, $C_{1'}$), 125.56 (1C, C_7), 131.54 (1C, C_{7a}), 131.83 (1C, $C_{4'}$), 132.59 (1C, =CH-), 132.67 (2C, $C_{2'}$+$C_{6'}$), 152.02 (1C, C_2), 152.97 (1C, C_{3a}), 165.01 (1C, C_5), 191.97 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{23}H_{26}N_2O_3$ Calculated: 379.2016. Found: 379.2017. Elemental analyses: $C_{23}H_{26}N_2O_3$, Calculated (%): C, 72.99; H, 6.92; N, 7.40. Found (%): C, 72.97; H, 6.91; N, 7.41.

*4.1.1.4.8. 2-(4-(4-(2-Hydroxyethyl)piperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**22**).* Yield: 94%. Mp: 164 °C. IR (ATR) V_{max} (cm^{-1}): 3381 (O-H stretching), 1681 (C=O stretching), 1593-1517 (C=C stretching), 1180 (C-N stretching), 1016 (C-O stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.45 (2H, t, J = 6.20 Hz,

-CH_2-CH_2-OH), 2.55–2.57 (4H, m, $H_{2''}$+$H_{6''}$), 3.29–3.33 (4H, m, $H_{3''}$+$H_{5''}$), 3.55 (2H, t, J = 6.15 Hz, -CH_2-CH_2-OH), 3.84 (3H, s, -OCH_3), 3.97 (2H, s, H_3), 4.46 (1H, s, -OH), 7.03 (2H, d, J = 8.85 Hz, $H_{3'}$+$H_{5'}$), 7.23 (1H, d, J = 2.35 Hz, H_5), 7.28 (1H, dd, J = 2.40 Hz and J = 8.35 Hz, H_7), 7.49 (1H, s, =CH-), 7.58 (1H, d, J = 8.35 Hz, H_4), 7.64 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.85 (1C, C_3), 47.37 (2C, $C_{3''}$+$C_{5''}$), 53.38 (2C, $C_{2''}$+$C_{6''}$), 55.96 (1C, C_6-OCH_3), 59.07 (1C, -CH_2-CH_2-OH), 60.68 (1C, -CH_2-CH_2-OH), 105.97 (1C, C_7), 114.83 (2C, $C_{3'}$+$C_{5'}$), 123.17 (1C, C_5), 124.87 (1C, $C_{1'}$), 127.85 (1C, C_4), 131.60 (1C, $C_{4'}$), 132.02 (2C, $C_{2'}$+$C_{6'}$), 132.59 (1C, =CH-), 139.56 (1C, C_2), 142.60 (1C, C_{3a}), 152.19 (1C, C_{7a}), 159.59 (1C, C_6), 193.34 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{23}H_{26}N_2O_3$ Calculated: 379.2016. Found: 379.2017. Elemental analyses: $C_{23}H_{26}N_2O_3$, Calculated (%): C, 72.99; H, 6.92; N, 7.40. Found (%): C, 73.00; H, 6.93; N, 7.39.

*4.1.1.4.9. 2-(4-(4-(2-Hydroxyethyl)piperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**23**).* Yield: 95%. Mp: 176 °C. IR (ATR) V_{max} (cm^{-1}): 3392 (O-H stretching), 1674 (C=O stretching), 1598-1581 (C=C stretching), 1222 (C-N stretching), 1093 (C-O stretching), 817 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.45 (2H, t, J = 6.20 Hz, -CH_2-CH_2-OH), 2.55–2.57 (4H, m, $H_{2''}$+$H_{6''}$), 3.28–3.30 (4H, m, $H_{3''}$+$H_{5''}$), 3.55 (2H, t, J = 6.10 Hz, -CH_2-CH_2-OH), 3.84 (3H, s, C_5-OCH_3), 3.91 (3H, s, C_6-OCH_3), 3.94 (2H, s, H_3), 4.45 (1H, s, -OH), 7.03 (2H, d, J = 8.85 Hz, $H_{3'}$+$H_{5'}$), 7.21 (1H, s, =CH-), 7.22 (1H, s, H_4), 7.36 (1H, s, H_7), 7.61 (2H, d, J = 8.60 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.22 (1C, C_3), 47.47 (2C, $C_{3''}$+$C_{5''}$), 53.41 (2C, $C_{2''}$+$C_{6''}$), 56.13 (1C, C_5-OCH_3), 56.43 (1C, C_6-OCH_3), 59.07 (1C, -CH_2-CH_2-OH), 60.70 (1C, -CH_2-CH_2-OH), 105.00 (1C, C_7), 108.61 (1C, C_4), 114.92 (2C, $C_{3'}$+$C_{5'}$), 125.16 (1C, $C_{1'}$), 130.94 (1C, $C_{4'}$), 131.91 (1C, C_2), 132.28 (1C, =CH-), 132.58 (2C, $C_{2'}$+$C_{6'}$), 144.98 (1C, C_{3a}), 148.61 (1C, C_{7a}), 151.96 (1C, C_6), 155.33 (1C, C_5), 199.30 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{24}H_{28}N_2O_4$ Calculated: 409.2122. Found: 409.2126. Elemental analyses: $C_{24}H_{28}N_2O_4$, Calculated (%): C, 70.57; H, 6.91; N, 6.86. Found (%): C, 70.56; H, 6.90; N, 6.85.

*4.1.1.4.10. 2-(4-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**24**).* Yield: 90%. Mp: 109.8 °C. IR (ATR) V_{max}(cm^{-1}): 1681 (C=O stretching), 1589-1516 (C=C stretching), 1193 (C-N stretching), 1087 (C-O stretching), 815 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.15 (6H, s, 2x-CH_3), 2.36–2.38 (2H, m, -N-CH_2-CH_2-), 2.39–2.42 (2H, m, -N-CH_2-CH_2-), 2.51–2.55 (4H, m, $H_{2''}$+$H_{6''}$), 3.27–3.29 (4H, m, $H_{3''}$+$H_{5''}$), 3.89 (3H, s, -OCH_3), 4.00 (2H, s, H_3), 7.01–7.04 (3H, m, $H_{3'}$+$H_{5'}$+ = CH-), 7.19 (1H, s, H_6), 7.38 (1H, s, H_4), 7.61 (2H, d, J = 8.75 Hz, $H_{2'}$+$H_{6'}$), 7.68 (1H, m, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.62 (1C, C_3), 45.92 (2C, 2x-CH_3), 47.76 (2C, $C_{3''}$+$C_{5''}$), 53.21 (2C, $C_{2''}$+$C_{6''}$), 56.21 (1C, C_5-OCH_3), 56.29 (1C, -N-CH_2-CH_2-), 57.17 (1C, -N-CH_2-CH_2-), 110.65 (1C, C_6), 114.90 (2C, $C_{3'}$+$C_{5'}$), 115.63 (1C, C_4), 125.11 (1C, $C_{1'}$), 125.56 (1C, C_7), 131.54 (1C, C_{7a}), 131.84 (1C, $C_{4'}$), 132.59 (1C, =CH-), 132.66 (2C, $C_{2'}$+$C_{6'}$), 152.00 (1C, C_2), 152.96 (1C, C_{3a}), 165.01 (1C, C_5), 191.96 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{25}H_{31}N_3O_2$ Calculated: 406.2489. Found: 406.2493. Elemental analyses: $C_{25}H_{31}N_3O_2$, Calculated (%): C, 74.04; H, 7.71; N, 10.36. Found (%): C, 74.03; H, 7.70; N, 10.35.

*4.1.1.4.11. 2-(4-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**25**).* Yield: 93%. Mp: 130.3 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1593-1485 (C=C stretching), 1232 (C-N stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.15 (6H, s, 2x-CH_3), 2.38–2.39 (2H, m, -N-CH_2-CH_2-), 2.42–2.44 (2H, m, -N-CH_2-CH_2-), 2.50–2.55 (4H, m, $H_{2''}$+$H_{6''}$), 3.28–3.30 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, -OCH_3), 3.97 (2H, s, H_3), 7.03 (2H, d, J = 8.90 Hz, $H_{3'}$+$H_{5'}$), 7.23 (1H, d, J = 2.55 Hz, H_5), 7.27 (1H, dd, J = 2.60 Hz and J = 8.30 Hz, H_7), 7.45 (1H, s, =CH-), 7.58 (1H, d, J = 8.40 Hz, H_4), 7.64 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.85 (1C, C_3), 46.03 (2C, 2x-CH_3), 47.38 (2C, $C_{3''}$+$C_{5''}$), 53.30 (2C, $C_{2''}$+$C_{6''}$), 55.96 (1C, C_6-OCH_3), 56.28 (1C, -N-CH_2-CH_2-), 57.17 (1C, -N-CH_2-CH_2-), 105.98 (1C, C_7), 114.84 (2C, $C_{3'}$+$C_{5'}$), 123.17 (1C, C_5), 124.89 (1C, $C_{1'}$), 127.85 (1C, C_4), 132.03 (1C, $C_{4'}$), 132.95 (2C, $C_{2'}$+$C_{6'}$), 133.28 (1C, =CH-), 139.56 (1C, C_2), 142.60 (1C, C_{3a}), 152.17 (1C, C_{7a}), 159.59 (1C, C_6), 192.50 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{25}H_{31}N_3O_2$ Calculated: 406.2489. Found: 406.2493. Elemental analyses: $C_{25}H_{31}N_3O_2$, Calculated (%): C, 74.04; H, 7.71; N, 10.36. Found (%): C, 74.05; H, 7.72; N, 10.37.

*4.1.1.4.12. 2-(4-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**26**).* Yield: 89%. Mp: 135.7 °C. IR (ATR) V_{max} (cm^{-1}): 1676 (C=O stretching), 1579-1498 (C=C stretching), 1298 (C-O stretching), 1091 (C-O stretching). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.15 (6H, s, 2x-CH_3), 2.36–2.39 (2H, m, -N-CH_2-CH_2-), 2.42–2.43 (2H, m, -N-CH_2-CH_2-), 2.51–2.55 (4H, m, $H_{2''}$+$H_{6''}$), 3.26–3.28 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, C_5-OCH_3), 3.90 (3H, s, C_6-OCH_3), 3.93 (2H, s, H_3), 7.01–7.03 (2H, m, =CH- + H_4), 7.22 (2H, d, J = 6.10 Hz, $H_{3'}$+$H_{5'}$), 7.36 (1H, s, H_7), 7.60 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.21 (1C, C_3), 46.03 (2C, 2x-CH_3), 47.38 (2C, $C_{3''}$+$C_{5''}$), 53.32 (2C, $C_{2''}$+$C_{6''}$), 56.12 (1C, C_5-OCH_3), 56.29 (1C, C_6-OCH_3), 56.42 (1C, -N-CH_2-CH_2-), 57.17 (1C, -N-CH_2-CH_2-)), 105.00 (1C, C_7), 108.59 (1C, C_4), 114.92 (2C, $C_{3'}$+$C_{5'}$), 125.18 (1C, $C_{1'}$), 130.94 (1C, $C_{4'}$), 132.13 (1C, C_2), 132.20 (1C, =CH-), 132.57 (2C, $C_{2'}$+$C_{6'}$), 144.97 (1C, C_{3a}), 149.71 (1C, C_{7a}), 152.21 (1C, C_6), 155.34 (1C, C_5), 192.25 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{26}H_{33}N_3O_3$ Calculated: 436.2595. Found: 436.2595. Elemental analyses: $C_{26}H_{33}N_3O_3$, Calculated (%): C, 71.70; H, 7.64; N, 9.65. Found (%): C, 71.69; H, 7.65; N, 9.64.

*4.1.1.4.13. 2-(4-(4-(3-(Dimethylamino)propyl)piperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**27**).* Yield: 85%. Mp: 70.4 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1666-1516 (C=C stretching), 1192 (C-N stretching), 1085 (C-O stretching), 823 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.56–1.62 (2H, m, -N-CH_2-CH_2-CH_2), 2.12 (6H, s, 2x-CH_3), 2.23 (2H, t, J = 7.25 Hz, -N-CH_2-CH_2-CH_2-), 2.33 (2H, t, J = 7.40 Hz, -N-CH_2-CH_2-CH_2-), 2.48–2.51 (4H, m,$H_{3''}$+$H_{5''}$), 3.28–3.29 (4H, m, $H_{2''}$+$H_{6''}$), 3.89 (3H, s, -OCH_3), 4.00 (2H, s, H_3), 7.02 (3H, m, $H_{3'}$+$H_{5'}$+=CH-), 7.19 (1H, s, H_6), 7.37 (1H, s, H_4), 7.60–7.62 (2H,d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$), 7.69–7.70 (1H, m, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 24.95 (1C, -N-CH_2-CH_2-CH_2-), 32.62 (1C, C_3), 45.67 (2C, 2x-CH_3), 48.66 (2C, $C_{3''}$+$C_{5''}$), 53.04 (2C, $C_{2''}$+$C_{6''}$), 56.21 (1C, C_5-OCH_3), 56.46 (1C, -N-CH_2-CH_2-CH_2-), 57.77 (1C, -N-CH_2-CH_2-CH_2-), 110.65 (1C, C_6), 114.90 (2C, $C_{3'}$+$C_{5'}$), 115.62 (1C, C_4), 125.11 (1C, $C_{1'}$), 125.56 (1C, C_7), 131.54 (1C, C_{7a}), 131.84 (1C, $C_{4'}$), 132.58 (1C, =CH-), 132.66 (2C, $C_{2'}$+$C_{6'}$), 152.00 (1C, C_2), 152.96 (1C, C_{3a}), 165.00 (1C, C_5), 191.96 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{26}H_{33}N_3O_2$ Calculated: 420.2646. Found: 420.2647. Elemental analyses: $C_{26}H_{33}N_3O_2$, Calculated (%): C, 74.43; H, 7.93; N, 10.02. Found (%): C, 74.42; H, 7.92; N, 10.01.

*4.1.1.4.14. 2-(4-(4-(3-(Dimethylamino)propyl)piperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**28**).* Yield: 88%. Mp: 126.5 °C. IR (ATR) V_{max} (cm^{-1}): 1681 (C=O stretching), 1593-1485 (C=C stretching), 1232 (C-N stretching), 1103 (C-O stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.57–1.62 (2H, m, -N-CH_2-CH_2-CH_2-),2.12 (6H, s, 2x-CH_3), 2.22 (2H, t, J = 7.20 Hz, -N-CH_2-CH_2-CH_2-), 2.33 (2H, t, J = 7.40 Hz, -N-CH_2-CH_2-CH_2-), 2.48–2.50 (4H, m, $H_{2''}$+$H_{6''}$), 3.29–3.31 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, -OCH_3), 3.96 (2H, s, H_3), 7.02–7.04 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.23 (1H, d, J = 2.45 Hz, H_5), 7.27 (1H, dd, J = 2.50 Hz and J = 8.30 Hz, H_7), 7.45 (1H, s, =CH-), 7.58 (1H, d, J = 8.35 Hz, H_4), 7.64 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 24.99 (1C, -N-CH_2-CH_2-CH_2-), 31.86 (1C, C_3), 45.71 (2C, 2x-CH_3), 48.47 (2C,

$C_{3''}$+$C_{5''}$), 53.04 (2C, $C_{2''}$+$C_{6''}$), 55.96 (1C, C_6-OCH_3), 56.47 (1C, -N-CH_2-CH_2-CH_2-), 57.79 (1C, -N-CH_2-CH_2-CH_2-), 105.98 (1C, C_7), 114.84 (2C, $C_{3'}$+$C_{5'}$), 123.16 (1C, C_5), 124.89 (1C, $C_{1'}$), 127.85 (1C, C_4), 132.03 (1C, $C_{4'}$), 133.18 (2C, $C_{2'}$+$C_{6'}$), 133.75 (1C, =CH-), 139.57 (1C, C_2), 142.59 (1C, C_{3a}), 152.18 (1C, C_{7a}), 159.59 (1C, C_6), 193.33 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{26}H_{33}N_3O_2$ Calculated: 420.2646. Found: 420.2642. Elemental analyses: $C_{26}H_{33}N_3O_2$, Calculated (%): C, 74.43; H, 7.93; N, 10.02. Found (%): C, 74.44; H, 7.94; N, 10.00.

4.1.1.4.15. 2-(4-(4-(3-(Dimethylamino)propyl)piperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (***29***). Yield: 92%. Mp: 145.6 °C. IR (ATR) V_{max} (cm^{-1}): 1658 (C=O stretching), 1591-1573 (C=C stretching), 1190 (C-N stretching), 1095 (C-O stretching), 819 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.56–1.61(2H, m, -N-CH_2-CH_2-CH_2-), 2.12 (6H, s, 2x-CH_3), 2.22 (2H, t, J = 7.20 Hz, -N-CH_2-CH_2-CH_2-), 2.33 (2H, t, J = 7.375 Hz, -N-CH_2-CH_2-CH_2-), 2.48–2.51 (4H, m, $H_{2''}$+$H_{6''}$), 3.27–3.29 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, C_5-OCH_3), 3.91 (3H, s, C_6-OCH_3), 3.94 (2H, s, H_3), 7.01–7.03 (2H, m, =CH-+H_4), 7.22 (2H, d, J = 6.65 Hz, $H_{3'}$+$H_{5'}$), 7.35 (1H, s, H_7), 7.60 (2H, d, J = 9.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 24.99 (1C, -N-CH_2-CH_2-CH_2-), 32.22 (1C, C_3), 45.55 (2C, 2x-CH_3), 47.54 (2C, $C_{3''}$+$C_{5''}$), 53.06 (2C, $C_{2''}$+$C_{6''}$), 56.13 (1C, C_5-OCH_3), 56.42 (1C, C_6-OCH_3), 56.48 (1C, -N-CH_2-CH_2-CH_2-), 57.79 (1C, -N-CH_2-CH_2-CH_2-), 105.00 (1C, C_7), 108.60 (1C, C_4), 114.93 (2C, $C_{3'}$+$C_{5'}$), 125.18 (1C, $C_{1'}$), 130.94 (1C, $C_{4'}$), 131.91 (1C, C_2), 132.13 (1C, =CH-), 132.58 (2C, $C_{2'}$+$C_{6'}$), 144.98 (1C, C_{3a}), 149.70 (1C, C_{7a}), 151.95 (1C, C_6), 155.34 (1C, C_5), 192.37 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{27}H_{35}N_3O_3$ Calculated: 450.2751. Found: 450.2752. Elemental analyses: $C_{27}H_{35}N_3O_3$, Calculated (%): C, 72.13; H, 7.85; N, 9.35. Found (%): C, 72.12; H, 7.84; N, 9.36.

4.1.1.4.16. 2-(4-(4-Phenylpiperazin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (***30***). Yield: 90%. Mp: 147.9 °C. IR (ATR) V_{max} (cm^{-1}): 1680 (C=O stretching), 1593-1496 (C=C stretching), 1192 (C-N stretching), 1076 (C-O stretching), 808 (1,4-disubstitued benzene out of plane bending), 759-692 (Monosubstitued benzen out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 3.29–3.30 (4H, m, $H_{2''}$+$H_{6''}$), 3.46–3.47 (2H, m, $H_{3''}$), 3.55–3.57 (2H, m, $H_{5''}$),3.90 (3H, s, -OCH_3), 4.02 (2H, s, H_3), 6.99–7.04 (4H, m, =CH-+$H_{2'''}$+$H_{4'''}$+$H_{6'''}$), 7.10 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.19–7.20 (2H, m, $H_{3'''}$+$H_{5'''}$), 7.22–7.26 (2H, m, H_4+H_6), 7.66 (2H, d, J = 8.96 Hz, $H_{2'}$+$H_{6'}$).7.75 (1H, d, J = 8.90 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.63 (1C, C_3), 46.80 (2C, $C_{3''}$+$C_{5''}$), 48.58 (2C, $C_{2''}$+$C_{6''}$), 56.23 (1C, C_5-OCH_3), 110.67 (1C, C_6), 113.86 (1C, $C_{4'''}$), 114.53 (2C, $C_{3'}$+$C_{5'}$), 115.26 (1C, C_4), 119.67 (2C, $C_{2'''}$+$C_{6'''}$), 125.18 (1C, $C_{1'}$), 125.59 (1C, C_7), 129.34 (2C, $C_{3'''}$+$C_{5'''}$), 129.48 (1C, C_{7a}), 132.06 (1C, $C_{4'}$), 132.53 (1C, =CH-), 132.71 (2C, $C_{2'}$+$C_{6'}$), 151.84 (1C, $C_{1'''}$), 152.104 (1C, C_2), 152.95 (1C, C_{3a}), 166.26 (1C, C_5), 190.76 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{27}H_{26}N_2O_2$ Calculated: 411.2067. Found: 411.2063. Elemental analyses: $C_{27}H_{26}N_2O_2$, Calculated (%): C, 79.00; H, 6.38; N, 6.82. Found (%): C, 79.01; H, 6.39; N, 6.81.

4.1.1.4.17. 2-(4-(4-Phenylpiperazin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (***31***). Yield: 92%. Mp: 182.4 °C. IR (ATR) V_{max} (cm^{-1}): 2987 (Aliphatic C-H stretching), 1689 (C=O stretching), 1595-1489 (C=C stretching), 1105 (C-N stretching), 1056 (C-O stretching), 821 (1,4-disubstitued benzene out of plane bending), 765-754 (Monosubstitued benzen out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 3.29–3.31 (4H, m, $H_{2''}$+$H_{6''}$), 3.47–3.49 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, -OCH_3), 3.99 (2H, s, H_3), 7.01 (2H, d, J = 7.95 Hz, $H_{3'}$+$H_{5'}$), 7.10–7.12 (2H, m, $H_{2'''}$+H6‴), 7.23–7.29 (5H, m, =CH-+H_6+$H_{3'''}$+$H_{4'''}$+$H_{5'''}$), 7.48 (1H, s, H_4), 7.59 (1H, d, J = 8.30 Hz, H_7), 7.68 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 31.86 (1C, C_3), 47.37 (2C, $C_{3''}$+$C_{5''}$), 48.56 (2C, $C_{2''}$+$C_{6''}$), 55.97 (1C, C_6-OCH_3), 106.00 (1C, C_7), 115.10 (2C, $C_{3'}$+$C_{5'}$), 116.14 (2C, $C_{2'''}$+$C_{6'''}$), 119.66 (1C, $C_{4'''}$), 123.21 (1C, C_5), 125.22 (1C, $C_{1'}$), 127.87 (1C, C_4), 129.47 (2C, $C_{3'''}$+$C_{5'''}$), 132.23 (1C, $C_{4'}$), 132.98 (2C, $C_{2'}$+$C_{6'}$), 133.86 (1C, =CH-), 142.63 (1C, C_{3a}), 149.34 (1C, C_2), 151.29 (1C, $C_{1'''}$), 152.01 (1C, C_{7a}), 159.60 (1C, C_6), 192.15 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{27}H_{26}N_2O_2$ Calculated: 411.2067. Found: 411.2065. Elemental analyses: $C_{27}H_{26}N_2O_2$, Calculated (%): C, 79.00; H, 6.38; N, 6.82. Found (%): C, 79.02; H, 6.37; N, 6.80.

4.1.1.4.18. 2-(4-(4-Phenylpiperazin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (***32***). Yield: 93%. Mp: 190.1 °C. IR (ATR) V_{max} (cm^{-1}): 1674 (C=O stretching), 1595-1494 (C=C stretching), 1190 (C-N stretching), 1056 (C-O stretching), 759-690 (Monosubstitued benzen out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 3.29–3.33 (4H, m, $H_{2''}$+$H_{6''}$), 3.46–3.48 (4H, m, $H_{3''}$+$H_{5''}$), 3.84 (3H, s, C_5-OCH_3), 3.91 (3H, s, C_6-OCH_3), 3.96 (2H, s, H_3), 7.01–7.02 (3H, m, $H_{2'''}$+$H_{4'''}$+$H_{6'''}$), 7.11 (2H, d, J = 8.90 Hz, $H_{3'}$+$H_{5'}$), 7.22–7.26 (4H, m, H_4+H_6+$H_{3'''}$+$H_{5'''}$), 7.38 (1H, s, =CH-), 7.65 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 32.23 (1C, C_3), 47.49 (2C, $C_{3''}$+$C_{5''}$), 48.59 (2C, $C_{2''}$+$C_{6''}$), 56.44 (1C, C_6-OCH_3), 105.02 (1C, C_7), 108.62 (1C, C_4), 115.21 (2C, $C_{3'}$+$C_{5'}$), 116.15 (2C, $C_{2'''}$+$C_{6'''}$), 119.69 (1C, $C_{4'''}$), 125.52 (1C, $C_{1'}$), 129.48 (2C, $C_{3'''}$+$C_{5'''}$), 132.07 (1C, C_2), 130.92 (1C, $C_{4'}$), 132.31 (1C, =CH-), 132.61 (2C, $C_{2'}$+$C_{6'}$), 145.02 (1C, C_{3a}), 149.73 (1C, C_{7a}), 151.31 (1C, C_6), 151.44 (1C, $C_{1'''}$), 155.40 (1C, C_5), 192.27 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{28}H_{28}N_2O_3$ Calculated: 441.2173. Found: 441.2178. Elemental analyses: $C_{28}H_{28}N_2O_3$, Calculated (%): C, 76.34; H, 6.41; N, 6.36. Found (%): C, 76.33; H, 6.40; N, 6.35.

4.1.1.4.19. 2-(4-(Pyrrolidin-1-yl)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (***33***). Yield: 89%. Mp: 188 °C. IR (ATR) V_{max} (cm^{-1}): 1676 (C=O stretching), 1575-1521 (C=C stretching), 1182 (C-N stretching), 1056 (C-O stretching), 808 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.97–2.00(4H, m, $H_{3''}$+$H_{4''}$), 3.32–3.34 (4H, m, $H_{2''}$+$H_{5''}$),3.89 (3H, s, -OCH_3), 3.98 (2H, s, H_3), 6.65 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.01 (1H, dd, J = 2.20 Hz and J = 8.45 Hz, H_4), 7.18 (1H, d, J = 2.10 Hz, H_6), 7.37 (1H, s, =CH-), 7.59 (2H, d, J = 8.75 Hz, $H_{2'}$+$H_{6'}$), 7.68 (1H, d, J = 8.40 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 25.41 (2C, $C_{3''}$+$C_{4''}$), 32.74 (1C, C_3), 47.72 (2C, $C_{2''}$+$C_{5''}$), 56.18 (1C, C_5-OCH_3), 110.63 (1C, C_6), 112.42 (2C, $C_{3'}$+$C_{5'}$), 115.48 (1C, C_4), 122.35 (1C, $C_{1'}$), 125.39 (1C, C_7), 129.98 (1C, C_{7a}), 131.31 (1C, $C_{4'}$), 133.05 (2C, $C_{2'}$+$C_{6'}$), 133.45 (1C, =CH-), 148.96 (1C, C_2), 152.75 (1C, C_{3a}), 164.80 (1C, C_5), 191.81 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{21}H_{21}NO_2$ Calculated: 320.1645. Found: 320.1646. Elemental analyses: $C_{21}H_{21}NO_2$, Calculated (%): C, 78.97; H, 6.63; N, 4.39. Found (%): C, 78.98; H, 6.62; N, 4.38.

4.1.1.4.20. 2-(4-(Pyrrolidin-1-yl)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (***34***). Yield: 88%. Mp: 178.7 °C. IR (ATR) V_{max} (cm^{-1}): 1676 (C=O stretching), 1587-1521 (C=C stretching), 1186 (C-N stretching), 1095 (C-O stretching), 812 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.98–199 (4H, m, $H_{3''}$+$H_{4''}$), 3.29–3.33 (4H, m, $H_{2''}$+$H_{5''}$),3.84 (3H, s, -OCH_3), 3.93 (2H, s, H_3), 6.64 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.22 (1H, d, J = 2.40 Hz, H_5), 7.24–7.26 (1H, dd, J = 2.55 Hz and J = 7.75 Hz, H_7), 7.45 (1H, s, =CH-), 7.56 (1H, d, J = 8.30 Hz, H_4), 7.61 (2H, d, J = 8.75 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 25.40 (2C, $C_{3''}$+$C_{4''}$), 31.97 (1C, C_3), 47.73 (2C, $C_{2''}$+$C_{5''}$), 55.94 (1C, C_6-OCH_3), 105.92 (1C, C_7), 112.45 (2C, $C_{3'}$+$C_{5'}$), 122.21 (1C, $C_{1'}$), 122.79 (1C, C_5), 127.74 (1C, C_4), 130.20 (1C, $C_{4'}$), 133.33 (2C, $C_{2'}$+$C_{6'}$), 134.87 (1C, =CH-), 139.86 (1C, C_2), 142.37 (1C, C_{3a}), 129.98 (1C, C_{7a}), 149.15 (1C, C_6), 193.11 (1C, C_1). HRMS (ESI) $(M+H)^+$ (*m*/*z*): For $C_{21}H_{21}NO_2$ Calculated: 320.1645. Found: 320.1644. Elemental analyses: $C_{21}H_{21}NO_2$, Calculated (%): C, 78.97; H, 6.63; N, 4.39. Found (%): C, 78.99; H, 6.64; N, 4.40.

4.1.1.4.21. 2-(4-(Pyrrolidin-1-yl)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (***35***). Yield: 84%. Mp: 209.8 °C. IR (ATR)

V_{max} (cm^{-1}): 1672 (C=O stretching), 1573-1521 (C=C stretching), 1184 (C-N stretching), 1091 (C-O stretching), 812 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.97–2.00 (4H, m, $H_{3''}$+$H_{4''}$), 3.31–3.32 (4H, m, $H_{2''}$+$H_{5''}$), 3.84 (3H, s, C_5-OCH_3), 3.91 (5H, s, H_3 + C_6-OCH_3), 6.64 (2H, d, J = 8.80 Hz, $H_{3'}$+$H_{5'}$), 7.20 (2H, d, J = 4.85 Hz, H_4+H_7), 7.35 (1H, s, =CH-), 7.58 (2H, d, J = 8.75 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 25.41 (2C, $C_{3''}$+$C_{4''}$), 32.33 (1C, C_3), 47.72 (2C, $C_{2''}$+$C_{5''}$), 56.11 (1C, C_5-OCH_3), 6.40 (1C, C_6-OCH_3), 104.97 (1C, C_7), 108.59 (1C, C_4), 112.42 (2C, $C_{3'}$+$C_{5'}$), 122.40 (1C, $C_{1'}$), 130.32 (1C, $C_{4'}$), 131.18 (1C, C_2), 132.91 (2C, $C_{2'}$+$C_{6'}$), 132.95 (1C, =CH-), 144.70 (1C, C_{3a}), 148.90 (1C, C_{7a}), 151.04 (1C, C_6), 155.10 (1C, C_5), 192.15 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{22}H_{23}NO_3$ Calculated: 350.1751. Found: 350.1748. Elemental analyses: $C_{22}H_{23}NO_3$, Calculated (%): C, 75.62; H, 6.63; N, 4.01. Found (%): C, 75.61; H, 6.62; N, 4.00.

*4.1.1.4.22. 2-(4-(Diethylamino)benzylidene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**36**).* Yield: 87%. Mp: 270 °C. IR (ATR) V_{max} (cm^{-1}): 1680 (C=O stretching), 1593-1519 (C=C stretching), 1249 (C-N stretching), 1188 (C-O stretching), 812 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.14 (6H, t, J = 7.00 Hz, 2x -N-CH_2-CH_3), 3.41–3.45 (4H, m, 2x -N-CH_2-CH_3), 3.83 (3H, s, -OCH_3),3.98 (2H, s, H_3), 6.77 (2H, d, J = 8.90 Hz, $H_{3'}$+$H_{5'}$), 7.02 (1H, d, J = 10.60 Hz, H_6), 7.18 (1H, s, =CH-), 7.36 (1H, s, H_4), 7.58 (2H, d, J = 8.85 Hz, $H_{2'}$+$H_{6'}$), 7.68 (1H, d, J = 8.45 Hz, H_7). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.94 (2C, 2x-CH_2-CH_3), 32.62 (1C, C_3), 47.44 (2C, 2x-CH_2-CH_3), 56.11 (1C, C_5-OCH_3), 110.32 (1C, C_6), 114.89 (2C, $C_{3'}$+$C_{5'}$), 115.46 (1C, C_4), 122.22 (1C, $C_{1'}$), 125.92 (1C, C_7), 131.13 (1C, C_{7a}), 131.84 (1C, $C_{4'}$), 132.53 (2C, $C_{2'}$+$C_{6'}$), 132.65 (1C, =CH-), 152.25 (1C, C_2), 152.91 (1C, C_{3a}), 165.02 (1C, C_5), 191.95 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{21}H_{23}NO_2$ Calculated: 322.1802. Found: 322.1798. Elemental analyses: $C_{21}H_{23}NO_2$, Calculated (%): C, 78.47; H, 7.21; N, 4.36. Found (%): C, 78.46; H, 7.20; N, 4.35.

*4.1.1.4.23. 2-(4-(Diethylamino)benzylidene)-6-methoxy-2,3-dihydro-1H-inden-1-one (**37**).* Yield: 83%. Mp: 137.4 °C. IR (ATR) V_{max} (cm^{-1}): 1678 (C=O stretching), 1589-1485 (C=C stretching), 1186 (C-O stretching), 1095 (C-N stretching), 810 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.14 (6H, t, J = 7.00 Hz, 2x -N-CH_2-CH_3), 3.41–3.45 (4H, m, 2x -N-CH_2-CH_3), 3.84 (3H, s, -OCH_3),3.94 (2H, s, H_3), 6.77 (2H, d, J = 8.95 Hz, $H_{3'}$+$H_{5'}$), 7.22 (1H, d, J = 2.45 Hz, H_5), 7.25 (1H, dd, J = 2.55 Hz and J = 8.30 Hz, H_7), 7.44 (1H, s, =CH-), 7.56 (1H, d, J = 8.30 Hz, H_4), 7.60 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.94 (2C, 2x-CH_2-CH_3), 31.94 (1C, C_3), 44.27 (2C, 2x-CH_2-CH_3), 55.94 (1C, C_6-OCH_3), 105.93 (1C, C_7), 111.90 (2C, $C_{3'}$+$C_{5'}$), 121.95 (1C, $C_{1'}$), 122.80 (1C, C_5), 127.75 (1C, C_4), 129.54 (1C, $C_{4'}$), 133.34 (2C, $C_{2'}$+$C_{6'}$), 133.84 (1C, =CH-), 139.87 (1C, C_2), 142.36 (1C, C_{3a}), 149.19 (1C, C_{7a}), 159.55 (1C, C_6), 193.13 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z):For $C_{21}H_{23}NO_2$ Calculated: 322.1802. Found: 322.1797. Elemental analyses: $C_{21}H_{23}NO_2$, Calculated (%): C, 78.47; H, 7.21; N, 4.36. Found (%): C, 78.48; H, 7.22; N, 4.37.

*4.1.1.4.24. 2-(4-(Diethylamino)benzylidene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**38**).* Yield: 89%. Mp: 179.3 °C. IR (ATR) V_{max} (cm^{-1}): 1668 (C=O stretching), 1573-1460 (C=C stretching), 1120 (C-O stretching), 1076 (C-N stretching), 813 (1,4-disubstitued benzene out of plane bending). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 1.14 (6H, t, J = 7.00 Hz, 2x -N-CH_2-CH_3), 3.40–3.44 (4H, m, 2x -N-CH_2-CH_3), 3.84 (3H, s, C_5-OCH_3), 3.90 (5H, s, C_6-OCH_3+H_3), 6.76 (2H, d, J = 8.90 Hz, $H_{3'}$+$H_{5'}$), 7.20 (2H, d, J = 1.75 Hz, =CH- + H_4), 7.34 (1H, s, H_7), 7.56 (2H, d, J = 8.90 Hz, $H_{2'}$+$H_{6'}$). ^{13}C-APT NMR (125 MHz) (DMSO-d_6) δ (ppm): 12.94 (2C, 2x-CH_2-CH_3), 32.29 (1C, C_3), 44.22 (2C, 2x-CH_2-CH_3), 56.11 (1C, C_5-OCH_3), 56.40 (1C, C_6-OCH_3), 104.97 (1C, C_7), 108.59 (1C, C_4), 111.88 (2C, $C_{3'}$+$C_{5'}$), 122.13 (1C, $C_{1'}$), 130.35 (1C, $C_{4'}$), 131.20 (1C, C_2), 132.76 (1C, =CH-), 133.15 (2C, $C_{2'}$+$C_{6'}$), 144.68 (1C, C_{3a}), 148.42 (1C, C_{7a}), 148.89 (1C, C_6), 155.12 (1C, C_5), 192.17 (1C, C_1). HRMS (ESI) $(M+H)^+$ (m/z): For $C_{22}H_{25}NO_3$ Calculated: 352.1907. Found: 352.1911. Elemental analyses: $C_{22}H_{25}NO_3$, Calculated (%): C, 75.19; H, 7.17; N, 3.99. Found (%): C, 75.20; H, 7.16; N, 4.00.

4.2. Anticholinesterase activity assay

Acetylcholinesterase (AChE, E.C.3.1.1.7, from electric eel), butyrylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), 5,5'-dithiobis-(2-nitrobenzoic acide) (DTNB), tacrine and DNP hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Acetylthiocholine iodide (ATC) and butyrylthiocholine iodide (BTC) were obtained from Fluka (Germany). All pipetting processes were performed using a Biotek Precision XS robotic system (USA). Measurements of the percentage inhibition were carried out at 412 nm by using a BioTek-Synergy H1 microplate reader (USA). The inhibitory activities of the compounds against AChE and BChE were determined in 96-well plates by modified Ellman's method [51] using DNP and tacrine as reference drugs. Initially, the synthesized compounds (**1**–**38**) were prepared at two concentrations (10^{-3} and 10^{-4} M) using 2% DMSO and inhibition potencies were measured. Then the selected compounds (**26**–**29**) displaying more than 50% inhibition were tested at further concentrations (10^{-5}-10^{-9} M).

The final volume of a well was 210 μL consisting of 140 μL phosphate buffer (0.1 M, pH = 8), 20 μL inhibitor solution, 20 μL enzyme solution (2.5 U/mL), 20 μL DTNB (0.01 M) and 10 μL substrate solution (0.075 M ATC or BTC). First of all, the solutions of inhibitor, enzyme and DTNB were added to phosphate buffer and incubated at 25 °C for 15 min. After the incubation, the substrate (ATC or BTC) was added to the enzyme-inhibitor mixture. The production of the yellow anion (5-thio-2-nitrobenzoic acid) was recorded for 5 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor was processed. Control and inhibitor readings were corrected with blank-readings. Each concentration was analyzed in quadruplicate. The IC_{50} values were calculated from a dose-response curve obtained by plotting the percentage inhibition versus the log concentration with the use of GraphPad 'PRISM' software (version 5.0). The results were displayed as mean ± standard deviation (SD).

4.3. Kinetic studies of enzyme inhibition

Kinetic studies were performed by using Ellman's method. The compounds **26** were tested at their IC_{50} and 2x IC_{50} concentrations. The solution of phosphate buffer (0.1 M, pH = 8, 140 μL/well), inhibitor (20 μL/well), AChE (2.5 U/mL, 20 μL/well) and DTNB (10 μM, 20 μL/well) was added to the wells and incubated at 25 °C for 15 min. After incubation period, the solutions, including various concentrations (150, 75, 37.5, 18.75, 9.375, 4.6875, 2.3437, 1.1718, 0.5859 and 0.2929 μM) of substrate (ATCI) (10 μL/well) were added. The increase of the absorbance was recorded for 5 min at 412 nm. A parallel experiment was carried out without inhibitor. All processes were assayed in quadruplicate. The results were analyzed as Lineweaver-Burk plots using Microsoft Office Excel 2013.

4.4. Cytotoxicity test

NIH/3T3 mouse embryonic fibroblast cell lines (ATCC CRL 1658) was used for cytotoxicity study. Cells were replicated into Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% 100 IU/mL penicillin/100 mg/mL streptomycin, at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The proliferation of the NIH/3T3 cell line was assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide) assay.

Briefly, cells were inoculated into 96-well culture plates at densities of 10,000 cells per well. After 24 h, they were treated with synthesized compounds (concentrations of 10^{-2} M – 3.16×10^{-6} M) for 24 h. After the incubation period, MTT solution (5 mg/mL) was added to each well and incubated for 3 h at 37 °C. At the end of the incubation, the purple MTT–formazan crystals were dissolved by adding 100 μg/ml of DMSO to each well. The plates were then read on a Microplate reader at 540 nm wavelength [64,65]. The IC_{50} value was calculated from the plots of cell proliferations against concentrations by applying regression analyses on GraphPad Prism Version 5.

4.5. Theoretical calculation of ADME parameters

Some physicochemical parameters (molecular weight, log P, topological polar surface area (tPSA), number of hydrogen donors, number of hydrogen acceptors, number of rotatable bonds and volume) of the compounds (**1**–**38**) were analyzed by online Molinspiration property calculation program [57].

4.6. Molecular docking

A structure based *in silico* procedure was applied to discover the binding modes of the compounds **26**–**29** to AChE enzyme active site. Interaction analysis between ligand and protein was performed using AChE X-ray crystal structure, retrieved from Protein Data Bank server (PDB ID: 4EY7) (www.pdb.org). Because of including DNP as a ligand, comprising two pockets of enzyme active region and being very similar to *Electrophorus electricus, Homo sapiens* protein structure was selected for docking studies [25–27].

The X-ray crystal structure used in this study was submitted to the *Protein Preparation Wizard* protocol of the *Schrödinger Suite 2015 Update 2* [66] to follow similar procedures described previously [26–28]. Ligand preparation was applied by the *LigPrep 3.4* [67] to correctly assign the protonation states and atom types of a molecule. An additional conformational search was needed to obtain proper binding pose. *Macromodell 10.8* [68] was used to regulate these conformations. The grid generation was formed using *Glide 6.7* [69] program and docking runs were performed with single precision docking mode (SP). The binding modes were visualized by *VMD 1.9.2* [70].

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.10.042.

References

[1] L. Wang, G. Esteban, M. Ojima, O.M. Bautista-Aguilera, T. Inokuchi, I. Moraledo, I. Iriepa, A. Samadi, M.B.H. Youdim, A. Romero, E. Soriano, R. Herrero, A.P. Fernandez, R.M. Murillo, J. Marco-Contelles, M. Unzeta, Eur. J. Med. Chem. 80 (2014) 543–561.
[2] R. Yaari, J. Corey-Bloom, Semin. Neurol. 27 (1) (2007) 32–41.
[3] A.A. Sadun, M. Borchert, E. DeVita, D.R. Hinton, C.J. Bass, Am. J. Ophthalmol. 104 (2) (1987) 113–120.
[4] G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Neurology 34 (1984) 939–944.
[5] R. Beeri, N. Le Novere, R. Mervis, T. Huberman, E. Grauer, J.P. Changeux, H. Soreq, J. Neurochem. 69 (1997) 2441–2451.
[6] H.A. De Silva, S.B. Gunatilake, A.D. Smith, Int. J. Geriatr. Psychiatry 18 (8) (2003) 711–715.
[7] A. Castro, A. Martinez, Curr. Pharm. Des. 12 (2006) 4377–4387.
[8] J. Poirier, J. Davignon, D. Bouthillier, S. Kogan, P. Bertrand, S. Gauthier, Lancet 342 (1993) 697–699.
[9] D.W. Dickson, Neurobio. Aging 18 (1997) 21–26.
[10] C. Carvalho, S.C. Correia, G. Perry, R.J. Castellani, P.I. Moreira, J. Neural Transm. 123 (2016) 107–111.
[11] A. Gella, N. Durany, Cell Adhes. Migr. 3 (2009) 88–93.
[12] M. Kivilpelto, E.L. Helkala, M.P. Laakso, BMJ 322 (7300) (2001) 1447–1451.
[13] C. Geula, M.M. Mesulam, Neuroscience 33 (1989) 469–481.
[14] C.T. Sadashiva, J.N. Narendra Sharath Chandra, K.C. Ponnappa, T. Veerabasappa Gowdab, K.S. Rangappaa, Bioorg. Med. Chem. Lett. 16 (2006) 3932–3936.
[15] Ü. Demir Özkay, Ö. Devrim Can, Y. Özkay, Y. Öztürk, Pharmacol. Rep. 64 (2012) 834–847.
[16] M. Mohammadi-Khanaposhtani, M. Saeedi, N.S. Zafarghandi, M. Mahdavi, R. Sabourian, E.K. Razkenari, H. Alinezhad, M. Khanavi, A. Foroumadi, A. Shafiee, T. Akbarzadeh, Eur. J. Med. Chem. 92 (2015) 799–806.
[17] D. Wilkinson, J. Murray, Int. J. Geriatr. Psychiatr. 16 (2001) 852–857.
[18] P. Davies, A.J. Maloney, Lancet 2 (1976) 1403.
[19] R.T. Bartus, R.L. Dean, B. Beer, A.S. Lippa, Science 217 (1982) 408–414.
[20] S. Darvesh, D.L. Grantham, D.A. Hopkins, J. Comp, Neurol 393 (1998) 374–390.
[21] M.M. Mesulam, C. Geula, Ann. Neurol. 36 (1994) 722–727.
[22] S.S. Xie, J.S. Lan, X.B. Wang, N. Jiang, G. Dong, Z.R. Li, K.D.G. Wang, P.P. Guo, L.Y. Kong, Eur. J. Med. Chem. 93 (2015) 42–50.
[23] M.B. Colovic, D. Krstic, T. Lazarevic-Pactil, A.M. Bondzic, V. Vasic, Pharmacol. Toxicol. Cur. Neuropharmacol. 11 (2013) 315–335.
[24] M. Atanasova, G. Stavrakov, L. Philipova, D. Zheleva, N. Yordanov, I. Doytchinova, Bioorg. Med. Chem. 23 (2015) 5382–5389.
[25] M.Y. Wu, G. Esteban, S. Brogi, M. Shionoya, I. Wang, G. Campiani, M. Unzeta, T. Inokuchi, S. Butini, J. Marco-Contelles, Eur. J. Med. Chem. (2015). http://dx.doi.org/10.1016/j.ejmech.2015.10.001.
[26] D. Genest, C. Rochais, C. Lecoutey, S. Jana Sopkova-de Oliveira, C. Ballandonne, S. Butt-Gueulle, R. Legay, M. Since, P. Dallemagne, Med. Chem. Commun. 4 (2013) 925–931.
[27] Z.F. Al-Rashid, R.P. Hsung, Bioorg. Med. Chem. 25 (2015) 4848–4853.
[28] M. Alipour, M. Khoobi, A. Foroumadi, H. Nadri, A. Moradi, A. Sakhteman, M. Ghandi, A. Shafiee, Bioorg. Med. Chem. 20 (2012) 7214–7222.
[29] J. Cheung, M.J. Rudolph, F. Burshteyn, M.S. Cassidy, E.N. Gary, J. Love, C.F. Matthew, J.J. Height, J. Med. Chem. 55 (2012) 10282–10286.
[30] C.S. Huang, W.T. Tu, M. Luo, J.G. Shi, Chin. J. Struct. Chem. 35 (6) (2016) 839–848.
[31] M.D. Vitorovic-Todorovic, C. Koukoulitsa, I.O. Juranic, L.M. Mandic, B.J. Drakulic, Eur. J. Med. Chem. 81 (2014) 158–175.
[32] A. Samadi, M. Estrada, C. Pérez, M.L. Rodríguez-Franco, I. Iriepa, L. Moraleda, M. Chioua, J. Marco-Contelles, Eur. J. Med. Chem. 57 (2012) 296–301.
[33] A. Asadipour, M. Alipour, M. Jafari, M. Khoobi, S. Emami, H. Nadri, H. Sakhteman, A. Moradi, V. Sheibani, H. Moghadam, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 70 (2013) 623–630.
[34] M. Pudlo, V. Luzet, L. Ismaïli, I. Tomassoli, A. Iutzeler, B. Refouvelet, Bioorg. Med. Chem. 22 (2014) 2496–2507.
[35] P. Meena, V. Nemaysh, M. Khatri, A. Manral, P.M. Luthra, M. Tiwari, Bioorg. Med. Chem. 23 (2015) 1135–1148.
[36] M.A. Ali, R. Ismail, T.S. Choon, R.S. Kumar, H. Osman, N. Arumugam, A.I. Almansour, K. Elumalai, A. Singh, Bioorg. Med. Chem. Lett. 22 (2012) 508–511.
[37] Q. Jin-Ping, G. Chang-Sheng, W. Chen-Wei, G. Jin-Fang, N. Dou-Dou, P. Jian, Z. Jiang-Ning, Chem. Bio. Chem. 13 (2012) 1652–1662.
[38] F.C. Meng, F. Mao, W.J. Shan, F. Qin, L. Huang, X.S. Li, Bioorg. Med. Chem. Lett. 22 (2012) 4462–4466.
[39] E. Zurek, P. Szymański, E. Mikiciuk-Olasik, Drug Res. 63 (2013) 137–144.
[40] A. Rampa, F. Mancini, A. De Simone, F. Falchi, F. Belluti, R.M. Concetta Di Martino, S. Gobbi, V. Andrisano, A. Tarozzi, M. Bartolini, A. Cavalli, A. Bisi, Bioorg. Med. Chem. Lett. 25 (2015) 2804–2808.
[41] R. Modh, S.P. Kumar, Y.T. Jasrai, K.H. Chikhalia, Arch. Pharm. Chem. Life Sci. 346 (2013) 793–804.
[42] L. Yurttaş, Z.A. Kaplancıklı, Y. Özkay, J. Enz, Inh. Med. Chem. 28 (5) (2013) 1040–1047.
[43] N. Gundogdu-Karaburun, Lett. Drug Des. Discov. 11 (6) (2014) 814–823.
[44] S. Hamulakovaa, J. Imricha, L. Janoveca, P. Kristiana, L. Danihela, O. Holasb, M. Pohankac, S. Böhmd, M. Kozurkovaa, K. Kucac, Int. J. Bio. Macro 70 (2014) 435–439.
[45] U.A. Mohsen, Z.A. Kaplancıklı, Y. Özkay, L. Yurttaş, Drug Res. 65 (2015) 176–183.
[46] M.A. Ali, R. Ismail, T.S. Choon, Y.K. Yoon, A.C. Wei, S. Pandian, R.S. Kumar, H. Osman, E. Manogaran, Bisi Bioorg. Med. Chem. Lett. 20 (2010) 7064–7066.
[47] Z. Sang, X. Qiang, Y. Li, W. Yuan, Q. Liu, Y. Shi, W. Ang, Y. Luo, Z. Tan, Y. Deng, Eur. J. Med. Chem. 94 (2015) 348–366.
[48] W. Huang, H. Yu, R. Sheng, J. Li, Y. Hu, Bioorg. Med. Chem. 16 (2008) 10190–10197.
[49] R. Sheng, X. Lin, J. Li, Y. Jiang, Z. Shang, Y. Hu, Bioorg. Med. Chem. Lett. 15 (2005) 3834–3837.
[50] R. Leurs, R.A. Bakker, H. Timmerman, I. de Esch, J. Nat. Rev. Drug Discov. 4 (2005) 107–120.
[51] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Feather-Stone, Biochem. Pharmacol. 7 (1961) 88–95.
[52] M.D. Altıntop, A.S. Gurkan-Alp, Y. Özkay, Z.A. Kaplancıklı, Arch. Pharm. Chem. Life Sci. 346 (2013) 571–576.
[53] M. Pozzolini, M.S. Scarfì, U. Benatti, M. Giovine, Anal. Biochem. 313 (2003) 338–341.
[54] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[55] International Organization for Standardization, Biological Evaluation of Medical Devices-part 5: Tests for *in Vitro* Cytotoxicity ISO-10993-5, third ed., International Organization for Standardization, 2009.
[56] H.V. de Waterbeemd, E. Gifford, Nat. Rev. Drug Discov. 2 (2013) 192–204.

[57] M. Cheminformatics, Bratislava, Slovak Republic, http://www.molinspiration.com/services/properties.html (accessed June 2016).
[58] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 19 (2001) 3–26.
[59] Ü. Demir Özkay, Y. Özkay, Ö. Devrim Can, Med. Chem. Res. 20 (2) (2011) 152–157.
[60] G. Turan- Zitouni, Y. Özkay, A. Özdemir, Z.A. Kaplancıklı, M.D. Altıntop, Lett. Drug Des. Discov. 8 (9) (2011) 830–837.
[61] Y. Özkay, Y. Tunalı, H. Karaca, İ. Işıkdağ, Arch. Pharm. Chem. Life Sci. 344 (4) (2011) 264–271.
[62] A.S. Gürkan-Alp, H. Göker, M. Akp, T. Özkan, A. Sunguroğlu, Arch. Pharm. Res. 38 (5) (2015) 650–658.
[63] Ş. Demirayak, L. Yurttaş, N. Gündoğdu-Karaburun, A.Ç. Karaburun, İ. Kayagül, J. Enzyme Inhib. Med. Chem. 30 (5) (2015) 816–825.
[64] M.D. Altıntop, A. Özdemir, G. Turan-Zitouni, S. Ilgın, Ö. Atlı, F. Demirci, Z.A. Kaplancıklı, Molecules 19 (9) (2014) 14809–14820.
[65] L. Yurttaş, Y. Özkay, G. Akalın-Çiftçi, Ş. Ulusoylar-Yıldırım, J. Enzyme Inhib. Med. Chem. 29 (2014) 175–184.
[66] Schrödinger, LLC, New York, NY (2015).
[67] LigPrep, Version 3.4, Schrödinger, LLC, New York, NY, 2015.
[68] MacroModel, Version 10.8, Schrödinger, LLC, New York, NY, 2015.
[69] Glide, Version 6.7, Schrödinger, LLC, New York, NY, 2015.
[70] W. Humphrey, A. Dalke, K. Schulten, J. Mole. Graph. 14 (1996) 33–38.