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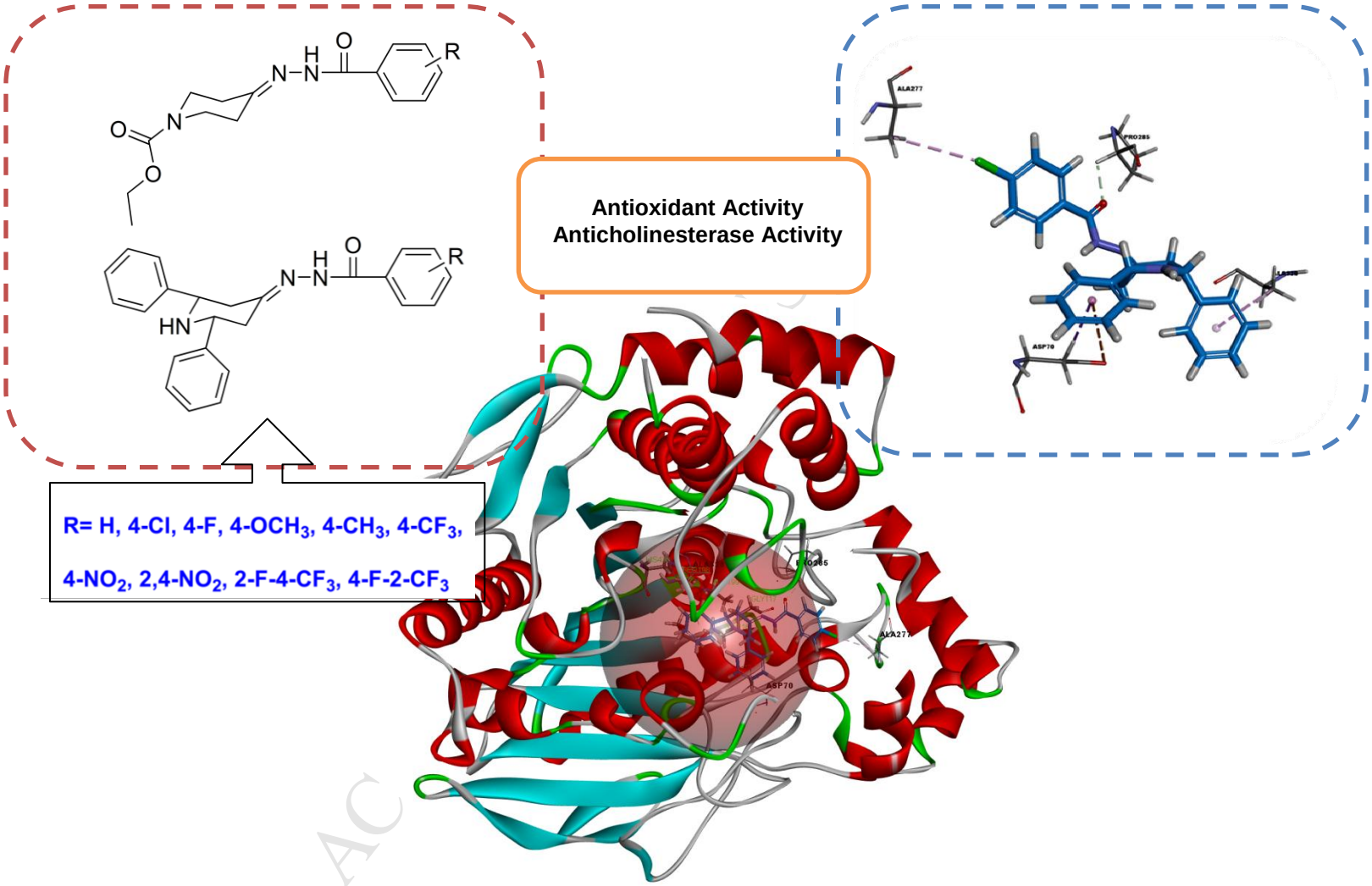
Antioxidant Activity
Anticholinesterase Activity
R= H, 4-Cl, 4-F, 4-OCH3, 4-CH3, 4-CF3,
4-NO2, 2,4-NO2, 2-F-4-CF3, 4-F-2-CF3

Title: New Piperidine-Hydrazone Derivatives: Synthesis, Biological Evaluations and Molecular Docking Studies as AChE and BChE Inhibitors

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Keywords: Piperidine, Hydrazone, Anticholinesterase activity, Antioxidant activity, Molecular docking.

Abstract:

Hydrazones and the piperidine ring containing compounds were considered as beneficial substrates in drug design. Therefore, this study was aimed at the synthesis of new benzoyl hydrazones derived from ethyl 4-oxopiperidine-1-carboxylate and 2,6-diphenylpiperidin-4-one. The synthesized compounds (**1–19**) were screened for their antioxidant, anticholinesterase and anticancer activities. The antioxidant capacity of the compounds was evaluated by using four complementary tests. The results showed that compound **7** and **17** have the higher lipid peroxidation inhibitory activity than the other compounds. In $DPPH^{\cdot}$ scavenging assay, compounds **5, 6, 10, 14, 17** demonstrated better activity than that of standard BHT, while in $ABTS^{+\cdot}$ scavenging assay compound **6** and **17** exhibited better activity among the other compounds. The CUPRAC assay disclosed that compound **2** displayed better activity than *α*-tocopherol. The anticholinesterase activity was performed against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Compound **11** (IC_{50}: 35.30±1.11 μM) inhibited BChE better than galantamine (IC_{50}: 46.03±0.14 μM). We conclude that the compound **11** can be considered as a candidate for BChE inhibitor. Moreover docking method was applied to elucidate the AChE and BChE inhibitory mechanism of the compound **11**. Molecular docking analysis revealed that compound **11** bound to BChE enzyme more efficiently when compared to the AChE due to its orientations and different types of interactions. In addition, the non-cytotoxic properties of the compounds brought them into prominence, although they did not show significant anticancer properties.

1. Introduction

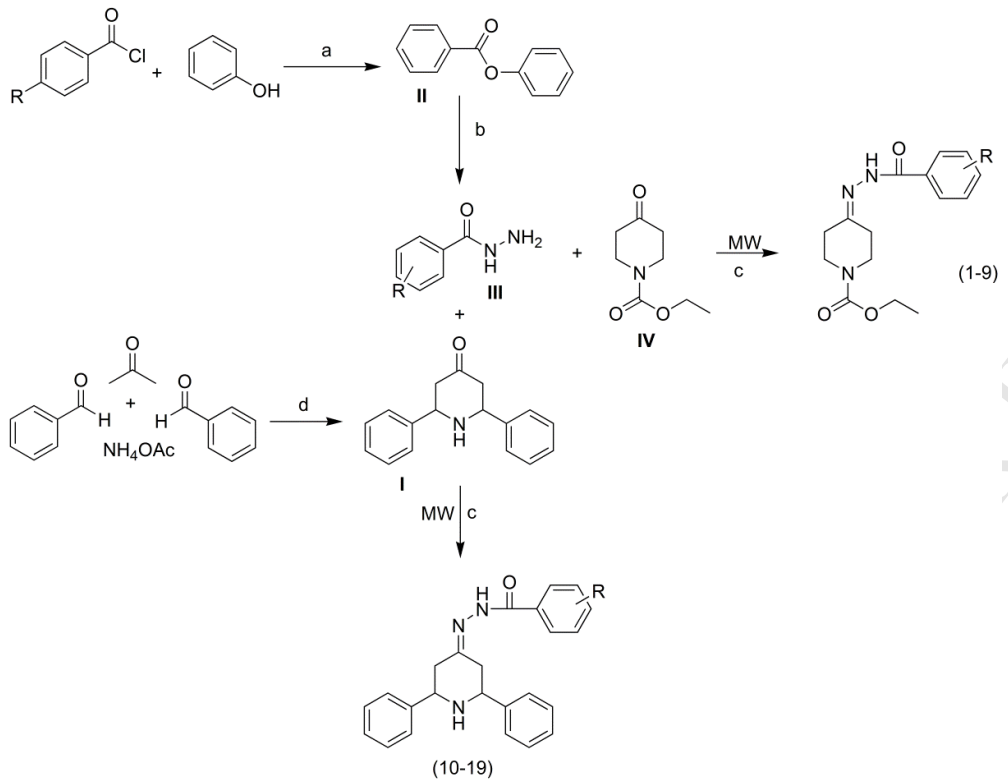
Reactive oxygen species (ROS) are reactive oxygen containing molecules including free radicals. ROS have important roles in biological systems such as cell signaling, apoptosis, gene expression and ion transportation. In case of excessive generation of reactive oxygen species, a chain reaction starts by the free radicals and then oxidative stress occurs in the cells. This situation initiates the cell or genetic material damages. Such cell damages can cause disorders in physiology of tissues and organs which can be resulted in many diseases such as cancer, heart diseases, decline in brain function and immune system. Natural and synthetic antioxidants inhibit the oxidation by stabilizing or deactivating free radicals thus they became valuable components to protect or support the body against oxidative stress and found use as therapeutics [1-5]. Antioxidant molecules can protect or treat the human body for many chronic diseases. In some researches this relationship between the antioxidant properties and anticancer, anticholinesterase properties of the compounds were mentioned [1, 4, 6].

Hydrazones are potential substrates for drug design. They exhibited various biological activities such as antioxidant, anticonvulsant, antidepressant, antimicrobial, antitumoral, anticholinesterase, analgesic and anti-inflammatory activities [6-14]. Wide range of biological activities was also reported for the molecules containing piperidine ring [1, 10, 15, 16]. With the motivation of obtaining more potent antioxidant, anti-Alzheimer and anticancer agents for lung cancer and as a continuation of our previous work [17], we designed hydrazone derivatives of ethyl 4-(2-benzoylhydrazono)piperidine-1-carboxylate and *N'*-(2,6-diphenylpiperidin-4-ylidene)benzohydrazide. Different substituted phenyl groups were used in the synthesis of the compounds to determine the effects of the substituents on activities. In this work, new hydrazones were synthesized, characterized and then screened for their pharmacological activities. The structure of the molecules was also elucidated by using the molecular docking application of Discovery Studio (DS) 2016 software to investigate the AChE and BChE inhibitory effects. This approach was applied to model the interactions between the studied compounds and AChE and BChE at the atomic level. Molecular docking enables to characterize the behavior of small molecules in the active site of AChE and BChE and explain the fundamental biochemical processes.

2. Results and Discussion

2.1. Chemistry

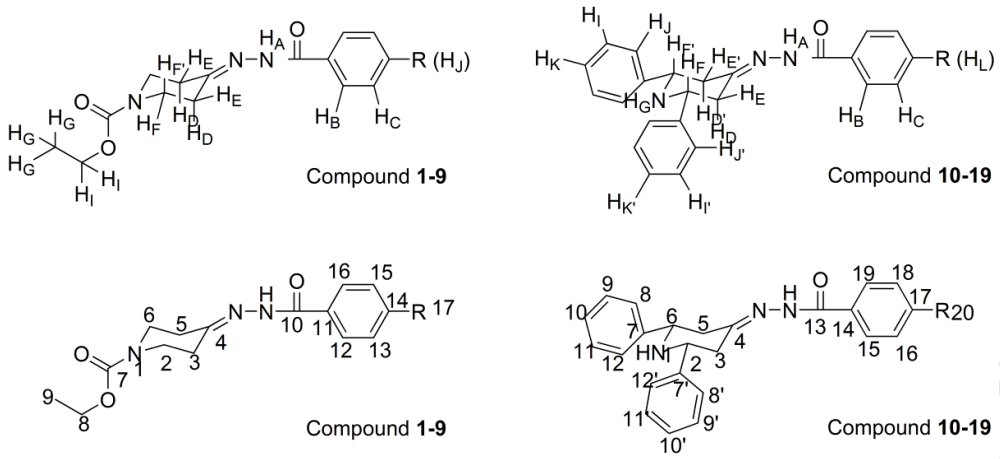
In this study, we synthesized nineteen benzoyl hydrazones bearing piperidine ring and screened for their antioxidant activity, anticholinesterase activity against AChE and BChE and cytotoxic activity against lung cancer cell lines (A549) and fibroblast (BJ) cell lines. The overall synthetic representation of target compounds is outlined in Scheme 1. 2,6-diaryl piperidine 4-one (**I**) was obtained with low yield using the procedure mentioned in the literature [18, 19]. In order to increase this yield, several different procedures and catalysts were tried. The results of the trials were reported in our previous work and with this modified method the yield was improved to 75% by using *L*-proline catalyst [17]. The benzoyl hydrazides (**III**) were obtained according to the literature [12, 20] and then reacted with ethyl 4-oxo-piperidine-1-carboxylate and 2,6-diarylpiperidine-4-one to obtain hydrazone derivatives (compound **1–19**). For the synthesis of benzoyl hydrazones, *MW*-assisted method was tried as an alternative to the conventional method in order to increase the yield. As a consequence, approximately twofold increase was achieved in the yields. The compounds were characterized by FTIR, NMR, mass spectra and elemental analysis.



Scheme 1. Synthetic pathway of 4-substitutedphenylbenzoyl hydrazones (**1–19**). Reagents and conditions: (a) NaOH; (b) NH_2NH_2, CH_3OH (c) EtOH, reflux; (d) EtOH, *L*-proline, rt.

In the IR spectra of all compounds, the C=N stretching vibration bands were observed at 1630–1667 cm^{-1} except for the compounds **7** and **17** that they were observed at 1615 and 1613 cm^{-1}, respectively. The stretching vibrations of C=O due to ester and hydrazones were observed at 1679–1693 cm^{-1} and 1643–1667 cm^{-1}, respectively. Broad peaks at 3150–3370 cm^{-1} indicated N–H stretching vibrations of hydrazone; where sharp peaks at 3285–3319 cm^{-1} indicated N–H stretching vibrations of piperidine (for compound **10–19**). N–H stretching vibrations of hydrazone of compound **7** and **17** were observed at 3313 and 3323 cm^{-1}, respectively.

Piperidine ring generally prefer chair conformation [17, 21, 22]. To elucidate the conformation and correlate the peaks of the hydrogen with the carbons, NOESY, ROESY, HMBC, HSQC spectra were used (Supplementary Data). All discussions were performed according to the numbering of the atoms in Scheme 2.



Scheme 2. Atom numbering for ^{1}H NMR and ^{13}C NMR.

In the ^{1}H NMR spectra, H_A protons of the compounds appeared at 10.59–11.05 ppm as a singlet. As methyl and methoxy groups were donating electrons to the ring and chlorine and fluorine atoms sharing the lone pairs; H_A was less deshielded and displayed resonance signals having relatively high field chemical shift for those substituents, whereas with the electron withdrawing character trifluoromethyl and nitro groups shifted to the downfield region. Halogens and oxygen atoms strongly withdraw the electrons from the ring but due to the effect of lone pairs H_B shifted more to the downfield then H_C. This was seen also in the NOESY and ROESY spectra of compound **1** and **11** (Fig. 1 and 2) that far interaction between H_B and H_A was observed. Due to the effect of strongly withdrawing groups; nitro and trifluoromethyl for the compounds **5, 6, 7, 15, 16** and **17**, H_C was deshielded to the downfield. This effect was observed clearly for the compounds **7** and **17**. When the coupling constants were analyzed, for compound **7**, H_B was resonated as doublet by H_C; where the H_C was affected by both H_B and H_J and gives doublet of doublet at relatively low field region than the H_B. Except from the fluorine atom containing compounds H_B and H_C were resonated as doublets. For the fluorine containing compounds H_C splitted both by the H_B and the F atom and also H_C observed as triplet because of the spin number of fluorine atom (1/2). Similarly, H_B also affected by the F atom but by the far interaction peak was unable to splitted into triplet. It was observed as multiplet. Among the compounds **1–9**, H_I for compounds **8** and **9** resonated at 4.02–4.10 ppm as multiplet because of the fluorine atom interactions and for the other compounds resonated at 4.08 ppm as quartet. According to NOESY and ROESY spectra of compound **1**, H_A had far interaction with H_D or H_E and also with H_G. These interactions described by the structure of the molecule that hydrazone group twisted towards one side of the piperidine ring. So, H_G resonates as multiplet at 1.17–1.22 ppm for compounds **8** and **9** because of the far interactions of F atoms; where H_G resonated at 1.21 as triplet for the other compounds. H_D and H_E were not identical because of the chair confirmation and generally resonated at 2.47–2.57 ppm as multiplet; where for compounds **5, 7, 8** and **9** they were observed as two or three triplets in the same region. H_F and $H_{F'}$ also resonated as multiplet at 3.38–3.57 ppm; but for compound **5** $H_{F'}$ gave triplet at 3.53 ppm. For compound **7**, H_F and $H_{F'}$ resonated as triplets at 3.63 and 3.58 ppm, respectively. The NOESY and ROESY spectra of the compound **11** verified that the hydrazone group was twisted towards the one side of the piperidine ring that; there were far interactions between H_A and H_G; H_A and H_D; H_A and H_B. H_D and $H_{D'}$ affected by the H_E and $H_{E'}$ and resonated as doublets at 3.16–3.20 ppm and 2.56–2.69 ppm where H_E and $H_{E'}$ splitted into triplets by $H_{D,D'}$ and $H_{F,F'}$ hydrogens at 2.06–2.37 ppm and 2.38–2.45 ppm, respectively. Similarly, H_F and $H_{F'}$ splitted into doublets by

H_E and $H_{E'}$ at 3.85–3.89 ppm and 3.94–3.97 ppm, respectively. By the far interactions observed between H_F and H_J, $H_{F'}$ and $H_{J'}$; the chemical shifts of the H_J and $H_{J'}$ were appeared as a doublet or multiplet at 7.49–7.58 ppm. In addition, in the spectrum of the compound **17** the peaks of H_I, $H_{I'}$, H_J, $H_{J'}$, were clearly separated and identified. Chemical shifts of H_I, $H_{I'}$ and H_K, $H_{K'}$ were observed at 7.33–7.45 ppm and 7.24–7.34 ppm, respectively, as multiplets.

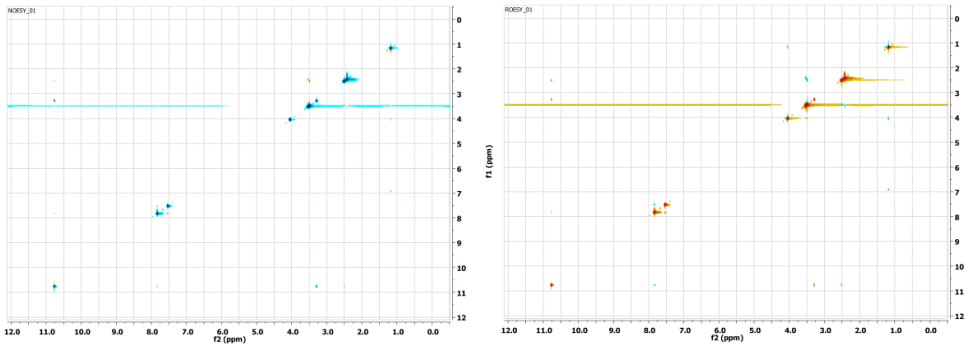


Fig. 1 NOESY and ROESY spectrum of compound **1**

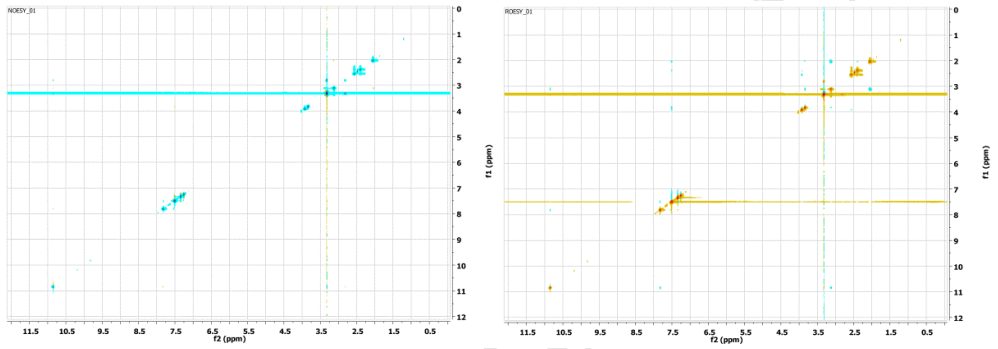


Fig. 2 NOESY and ROESY spectrum of compound **11**

In ^{13}C NMR spectra for compounds **1–9**, C9 carbons were observed at 15.02–15.06 ppm, where C8 at 61.32–61.37 ppm; C3 and C5 carbons resonated as two separate peaks at 27.62–28.75 ppm and 33.49–34.15 ppm; similarly, C2 and C6 was observed as two peaks at 40.62–42.15 ppm and 43.42–43.95 ppm. For compounds **10–19**, aliphatic carbons appeared as four separate peaks; C2 and C6 at 60.07–60.53 ppm and 61.26–61.46 ppm and C3 and C5 at 36.57–37.39 ppm and 43.64–43.95 ppm. In order to define the aromatic carbon peaks, HSQC spectrum of compound **4** (Fig. 3) and HSQC and HMBC spectra of compound **18** (Fig. 4) were used. According to HSQC of compound **4** the peaks of H_B correlated with 128.12 ppm belonged to C12 and C16; similarly, H_C interacted with 129.20 ppm C13 and C15. When the 2D NMR spectra of compound **18** were analyzed, many interactions between the carbons with hydrogens were observed. The hydrogen peaks of the benzene ring attached to the hydazone correlated with the peaks (114.00, 114.14, 121.84, 131.84 ppm); H_I , $H_{I'}$ correlated with the peaks (128.74, 128.67, 128.62, 128.60 ppm) belonged to C9 and C11; H_K , $H_{K'}$ correlated with the peaks (127.65, 127.59, 127.24 ppm) belonging to C10; H_J , $H_{J'}$ correlated with the peaks (127.18, 127.05 ppm) belonging to C8 and C12. The remaining carbon peaks belonged to the ipso and the carbonyl carbons. C7 and C7' peaks were observed at 144.34 and 144.19 ppm in the spectrum of compound **18**, similarly, for the other compounds two peaks were observed at 144.16–144.60 ppm. In the spectra of the compounds **1–9**, peaks observed at 155.03–155.13 ppm were attributed to

C7. Except from fluorine atom substituted compounds, C4 and carbonyl carbon of the hydrazones (C10 for compounds **1–9**, C13 for compounds **10–19**) were observed at the paramagnetic region with a largest chemical shift. For F containing compounds (**2**, **8**, **9**, **12**, **18**, **19**) the ipso carbon attached to the fluorine atom had a chemical shift at 165.67–166.91 ppm when the F atom at 4 position of the benzene ring [23]; where when the F atom at 2 position by the effect of both carbonyl and the F, ipso carbons more deshielded and observed at 169.42 and 169.45 ppm.

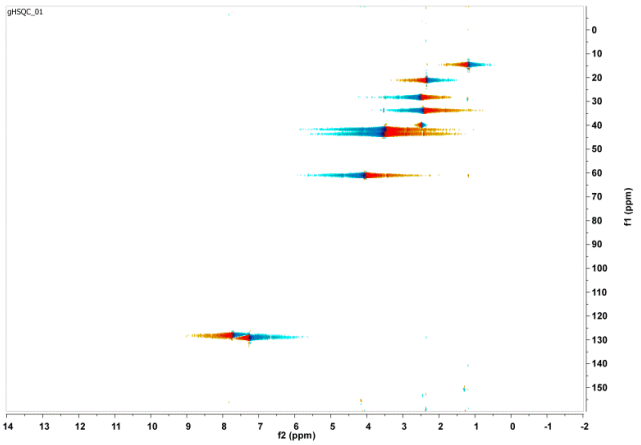


Fig. 3 HSQC spectrum of compound **4**

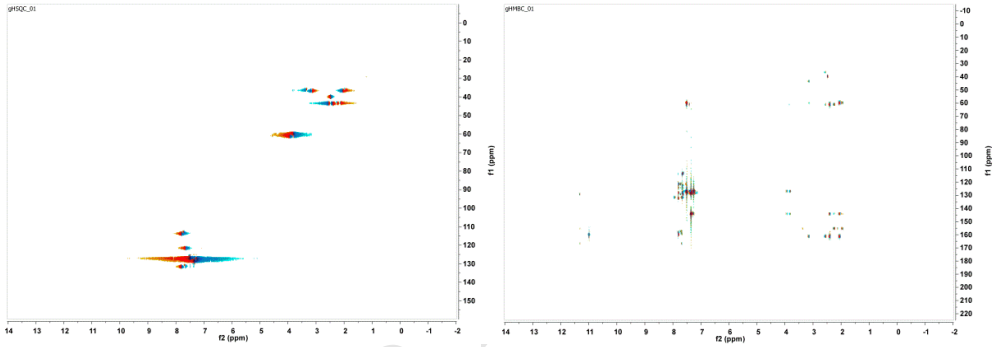


Fig. 4 HSQC and HMBC spectrum of compound **18**

The structures of the benzoyl hydrazones were also confirmed by the mass spectra. The cleavage of N–N, C–N and C=N bond of hydrazone and the separation of benzene groups were specific fragments of these compounds. The other peaks were due to the specific fragmentations of piperidine ring seen in the Fig. 5.

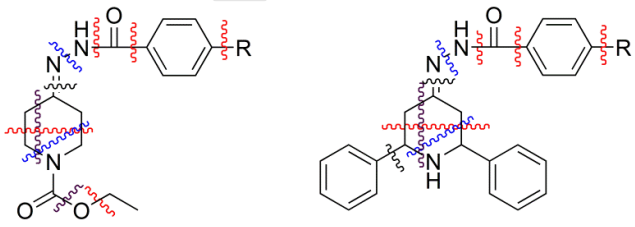


Fig. 5 Mass fragmentation pattern of the compounds.

2.2. Pharmacology

2.2.1. Antioxidant activity of synthesized compounds

The synthesized compounds were evaluated for their antioxidant activity using four different methods (Table 1), where *α*-.Tocopherol and butylated hydroxyl toluene (BHT) was used as positive control. Among the tested compounds, better antioxidant activities were observed generally with compounds having electron-withdrawing substituent NO_2. The effect of the fluorine atom can be analyzed by comparing compounds **2, 5, 8, 9** and compounds **12, 15, 18, 19**. F containing compounds at 4 position of the phenyl ring had better activity than the compounds having CF_3 group at 4 positions. In the CUPRAC assay, $A_{0.5}$ values of the compounds were found lower than 100 µM. Compound **2** indicated the best reducing effect among the compounds (**1–19**). At the same time, it was better than the antioxidant standard *α*-tocopherol and close activity to that of BHT. In addition, compounds **4, 6, 16, 1, 9, 8, 17** and **3** exhibited better activity than the *α*-tocopherol, respectively. In β-carotene-linoleic acid assay, compounds **7** and **17** exhibited the higher lipid peroxidation inhibitory activity, followed by **13, 6, 18, 10** and **11,** respectively. In DPPH assay, the IC_{50} values of the compounds (**1–19**) were lower than 100 µM. Compounds**, 5, 6, 10**, **14** and **17** exhibited better DPPH free radical scavenging activity than BHT (IC_{50}:54.97±0.99 µM). In the ABTS assay, among the tested compounds, **6**, **17** showed the best ABTS cation radical scavenging activity with IC_{50} values of 10.86±1.80 and 19.25±1.70, respectively. Except compounds **7, 10, 15, 16** this series showed good IC_{50} values that were lower than 100 µM.

Table 1 Antioxidant activity results of compounds (**1–19)**, BHT and *α*-tocopherol by the *β*-carotene-linoleic acid, DPPH, ABTS and CUPRAC assay.

Compounds	R	β-carotene/linoleic acid assay IC_{50} (µM)[a]	DPPH assay IC_{50} (µM) [a]	ABTS [+] assay IC_{50} (µM) [a]	CUPRAC assay $A_{0.50}$(µM) [a]
1	**4-Cl**	72.27±1.95	58.95±0.24	63.87±1.64	23.52±0.08
2	**4-F**	65.55±0.95	74.83±0.46	39.21±0.71	4.16±0.04
3	**4-OCH_3**	-	85.41±0.41	38.27±1.05	36.94±0.00
4	**4-CH_3**	52.54±0.53	75.50±0.21	28.08±1.40	11.11±0.02
5	**4-CF_3**	49.33±0.70	33.84±0.48	23.50±1.60	51.38±0.09
6	**4-NO_2**	40.24±1.09	51.70±1.39	10.86±1.80	13.32±0.02
7	**2,4- NO_2**	27.83±0.95	59.95±0.20	117.16±0.55	83.13±0.01
8	**2-F-4-CF_3**	77.44±0.78	86.01±0.15	59.45±1.02	27.27±0.01
9	**4-F-2-CF_3**	68.23±0.72	86.81±0.20	41.85±0.39	25.63±0.02
10	**H**	41.85±0.11	41.99±0.32	238.01±1.06	45.42±0.00
11	**4-Cl**	43.56±0.23	96.71±0.28	67.87±1.90	49.76±0.01
12	**4-F**	56.61±0.80	66.04±0.08	83.67±0.41	59.57±0.04
13	**4-OCH_3**	34.46±0.65	75.81±0.18	27.92±0.73	77.73±0.01
14	**4-CH_3**	69.03±0.69	53.29±0.57	64.14±1.78	50.59±0.01
15	**4-CF_3**	55.28±0.95	64.95±0.19	152.81±1.33	92.13±0.00
16	**4-NO_2**	45.16±1.10	88.14±0.56	169.60±0.51	17.83±0.13
17	**2,4- NO_2**	28.15±1.26	19.99±1.03	19.25±1.70	29.20±0.02
18	**2-F-4-CF_3**	41.08±0.39	70.17±0.65	63.48±2.24	85.18±0.03
19	**4-F-2-CF_3**	114.87±0.51	88.28±0.16	88.48±1.64	88.20±0.01
BHT [b]		2.34 ± 0.09	54.97±0.99	2.91±0.55	3.80±0.02
α-Tocopherol[b]		4.50 ± 0.09	12.26±0.07	4.87±0.45	40.48±1.87

[a]Values expressed are mean ± SEM of three parallel measurements ($p< 0.05$).

[b]Reference compounds.

2.2.2. Acetyl- and butyryl- cholinesterase inhibitory activities of synthesized compounds

The compounds (**1-19**) were further screened for *in vitro* inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are the chief enzymes of Alzheimer' disease (Table 2). Galantamine, used for mild Alzheimer' disease, was used as positive control. BChE inhibitory effects of the newly synthesized compounds were better than the AChE inhibitory effect. In BChE assay, compound **11** (IC_{50}: 35.30±1.11 µM) was a leader compound among the others as it exhibited better activity than galantamine. In addition, in the same assay, compounds **14, 19, 10** and **1** competed with the galantamine. Compounds **3, 4, 5, 8, 9, 12, 15, 16,** and **17** have also considerable inhibitory values since their IC_{50} values were lower than 100 µM. In AChE assay, only the IC_{50} values of compounds **11** and **19** were lower than 50 µM. The compounds **1, 4, 12, 13, 14, 15, 16, 17, 18** and **19** had also considerable results since they exhibited IC_{50} values between 50 and 100 µM.

Table 2 Acetyl- and butyryl-cholinesterase inhibitory activities of compounds (**1–19**).

	Anticholinesterase Assay			Anticholinesterase Assay	
Compounds	**AChE Assay IC_{50} (µM) [a]**	**BChE Assay IC_{50} (µM) [a]**	**Compounds**	**AChE Assay IC_{50} (µM) [a]**	**BChE Assay IC_{50} (µM) [a]**
1	81.76±0.63	69.49±1.11	**11**	**41.19±0.40**	**35.30±1.11**
2	128.18±0.66	113.44±0.74	**12**	91.06±0.70	72.81±0.39
3	100.60±0.08	82.23±1.47	**13**	92.23±0.02	110.07±1.02
4	94.82±0.42	76.93±0.87	**14**	74.94±0.20	50.10±1.76
5	135.60±0.46	91.59±0.93	**15**	84.38±0.30	99.14±1.34
6	125.34±0.27	155.57±0.41	**16**	63.93±0.17	86.82±1.62
7	102.04±0.47	103.15±1.93	**17**	70.64±1.02	93.90±1.77
8	122.54±0.23	95.06±1.56	**18**	90.06±1.42	117.29±0.78
9	124.43±0.44	99.61±0.35	**19**	49.54±1.87	52.38±1.40
10	99.74±0.30	65.96±1.42	**Galantamine[b]**	4.48±0.78	46.03±0.14

[a]Values expressed are mean ± SEM of three parallel measurements ($p< 0.05$).

[b]Reference compounds.

2.2.3. Anticancer activity of synthesized compounds

At the side of the antioxidant, acetyl- and butyryl- cholinesterase inhibitor activities of the compounds, we investigated their anticancer activities. There was no significant decrease on viability of A549 and BJ cell lines according to the MTT assay results (supplementary material). Based on the positive control cisplatin (IC_{50} 64.77 µM), only compound **7** slightly inhibited viability of the A549 lung carcinoma cells. On the other hand the viability of BJ fibroblast cells was not affected. Due to the non-cytotoxic nature of these compounds, they may hold promise for other diseases treatment.

2.3. Computational Studies

Biological activity tests on ethyl 4-(2-benzoylhydrazono)piperidine-1-carboxylate and *N'*-(2,6-diphenylpiperidin-4-ylidene)benzohydrazide derivatives have been realized by using *in vitro* method. It was known that galantamine which was used in the treatment of mild Alzheimer' disease was a positive control in this research. So that, we performed molecular docking for galantamine by using DS 2016. Then the compound **11** was docked with the mentioned enzymes. The detailed information about galantamine with the enzymes was given in supplementary data. In this study, the aim of the docking was to give a prediction of the most active compound **11**- AChE and BChE complex structures using computation methods. Based on the mentioned before, it performed to explain why compound **11** demonstrated more BChE efficient inhibition capacity than AChE's. It is known that the active sites of AChE includes an anionic binding site, Trp84,

Glu199, and Phe330; an esteratic binding site that contains the catalytic triad Ser200, His440, and Glu327; an acyl binding site, Phe288, and Phe299, which bound to the acetyl group of AChE. In addition, peripheral anionic sites (PAS) of AChE also contained Trp279, Tyr70, Tyr121, Asp72, Glu199 and Phe290 residues of the enzyme [24-28]. As a result of the docking, the compound **11** displayed multiple binding patterns in catalytic active sites (CAS) and peripheral active sites (PAS) of AChE, and BChE. In the 1ACJ-**11** complex, oxygen atoms from Ser286 (2.2 Å) and Tyr334 (2.6 Å) into the CAS formed two hydrogen bonds with nitrogen moieties of the compound **11**. The compound **11** formed hydrophobic interactions (π–π and π-alkyl) withTrp279 (4.7 Å) and Leu282 (5.2 Å) residues of the peripheral anionic sites (PAS), (Fig. 6). In addition, molecular docking was performed with 4EY6 model which include galantamine for the compound **11**. Figure 6 showed that there were three hydrogen bonds (Phe295, Tyr124), two electrostatic (His447 and Asp74), two hydrophobic (Trp286) interactions between compound **11** and AChE. The most potent BChE inhibitor, compound **11** showed interactions with the CAS amino acid residues such as Ala328 and Asp70, Ala277, Pro285 in the PAS of human butyryl cholinesterase (Fig. 7). In addition, compound **11** had hydrophobic interactions (π–sigma, alkyl, π–alkyl) with the C-H, alkyl and C-H groups of Asp70 (2.7 Å), Ala277 (4.1 Å), and Ala328 (4.6 Å) in the enzyme. The carbonyl moiety of the compound **11** interacted with Pro285 (2.2 Å). In addition, the phenyl moiety of the compound **11** interacted with electrostatic interaction with residue oxygen atom of the Asp70 (4.9 Å). The possible interactions of compound **11** with the related enzymes were summarized in Table 3.

The generated conformations (117 confs) of the compound **11** were presented in Table S3 (supplementary material). The obtained docking data (the bottom ten binding energy values of the generated conformations of the compound **11** and the related enzyme complexes) were shown in Table S4 (supplementary material). It was mentioned that compound **11** formed more strong interactions with BChE (BE: -56.978 kcal/mol) than AChE (BE: -0.91680 kcal/mol). These numerical values revealed why compound **11** showed more BChE efficient inhibition capacity than AChE's. Furthermore, the compound **11** was compared with galantamine for the enzymes (AChE and BChE) in Figure 8. The results of the docking exhibited that the compound **11** in BChE was more appropriate than the compound **11** in AChE based on the superimposed the galantamine. The molecular docking results showed that hydrogen bond, electrostatic interaction and hydrophobic interaction of chloride atom of the compound **11** were mainly responsible for more BChE efficient inhibition capacity than AChE's with CAS and PAS of the related enzyme, respectively, given in Fig. 7.

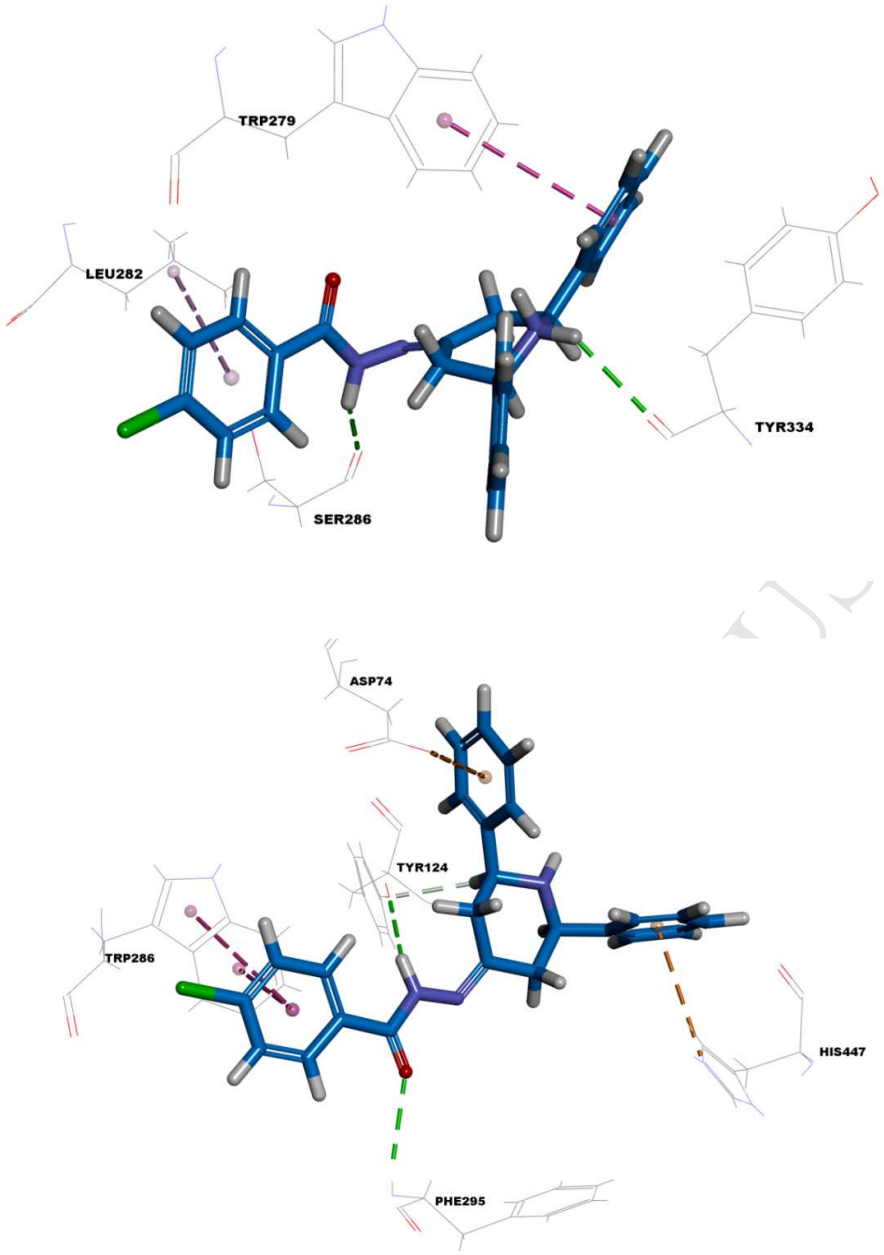


Fig. 6. Global minimum energy binding mode of the compound **11** at the active site of AChE (1ACJ and 4EY6, respectively).

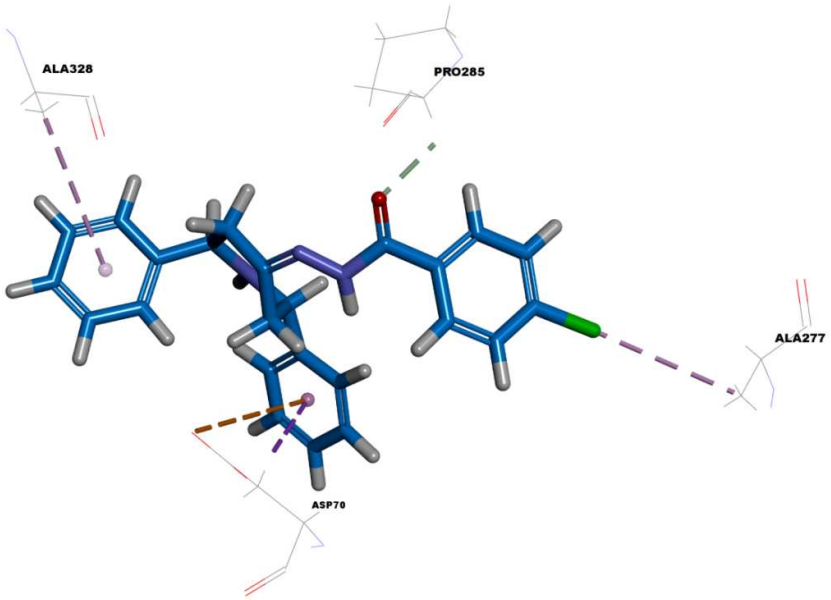


Fig. 7. Global minimum energy binding mode of the compound **11** at the active site of BChE.

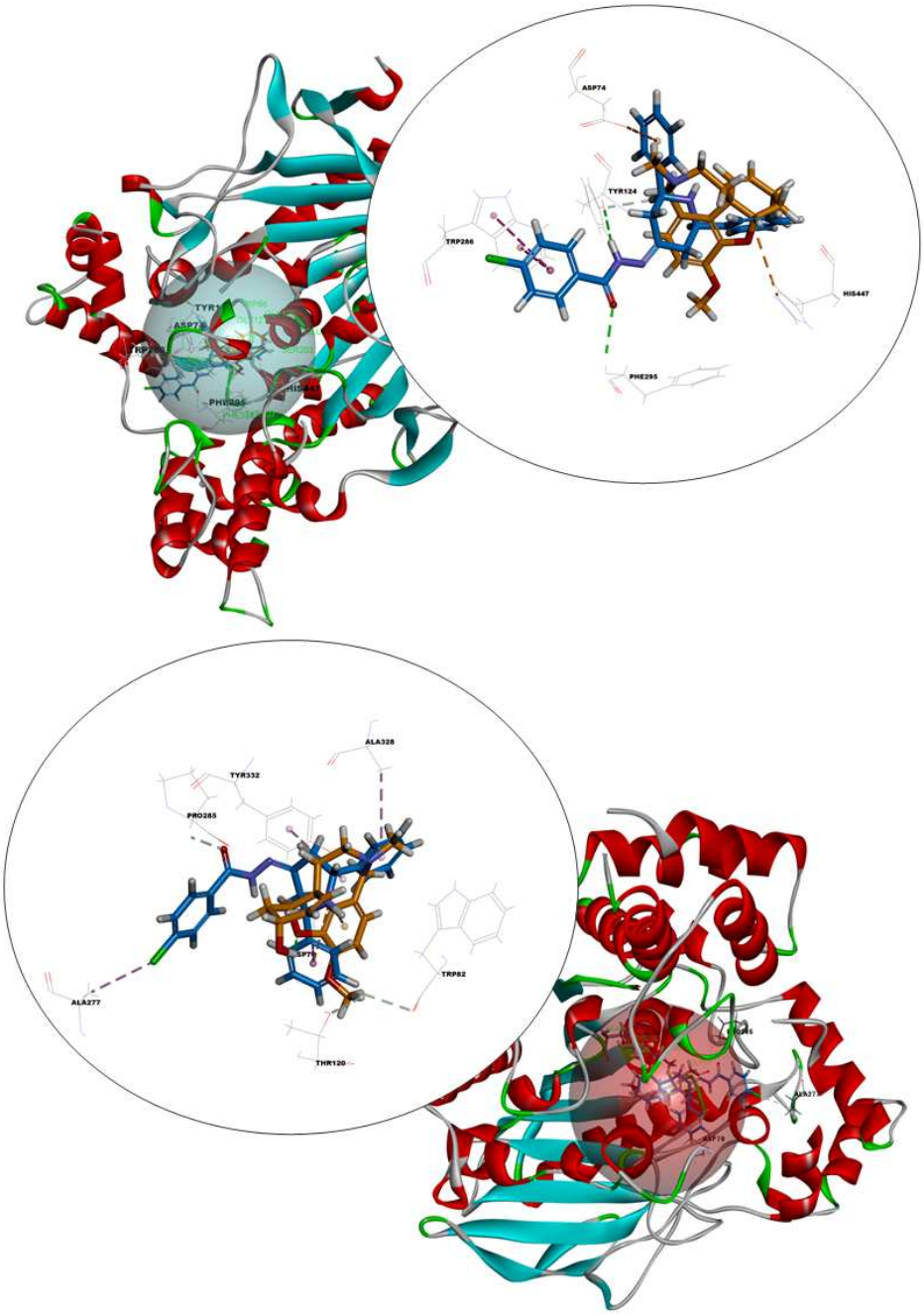


Fig. 8. Global minimum energy binding modes of the compound **11** (blue sticks) and GNT(orange sticks) at the active site of AChE (light blue sphere) and BChE (red sphere). The binding sites residues of the enzymes, (1ACJ and 1POI) were displayed.

Table 3 The possible interactions of the compound **11** for AChE and BChE at docking study.

Name-AChE -11	Distance	Category	Types	From	From Chem.	To	To Chem.
B11:H50 - A:SER286:O	2.210	Hydrogen Bond	Conventional Hydrogen Bond	B12:H50	H-Donor	A:SER286:O	H-Acceptor
B11:H51 - A:TYR334:O	2.655	Hydrogen Bond	Conventional Hydrogen Bond	B12:H51	H-Donor	A:TYR334:O	H-Acceptor
A:TRP279 - B11	4.795	Hydrophobic	Pi-Pi T-shaped	A:TRP279	Pi-Orbitals	B11	Pi-Orbitals
B11 - A:LEU282	5.258	Hydrophobic	Pi-Alkyl	B12	Pi-Orbitals	A:LEU282	Alkyl
Name-AChE (4EY6) -11	**Distance**	**Category**	**Types**	**From**	**From Chem.**	**To**	**To Chem.**
A:PHE295:HN - B11:O14	2.64444	Hydrogen Bond	Conventional Hydrogen Bond	A:PHE295:HN	H-Donor	B11:O14	H-Acceptor
B11:H50 - A:TYR124:OH	2.57366	Hydrogen Bond	Conventional Hydrogen Bond	B11:H50	H-Donor	A:TYR114:OH	H-Acceptor
B11:H10 - A:TYR124:OH	2.54344	Hydrogen Bond	Carbon Hydrogen Bond	B11:H10	H-Donor	A:TYR114:OH	H-Acceptor
A:HIS447:NE2 - B11	4.14455	Electrostatic	Pi-Cation	A:HIS447:NE2	Positive	B11	Pi-Orbitals
A:ASP74:OD2 - B11	4.36531	Electrostatic	Pi-Anion	A:ASP74:OD2	Negative	B11	Pi-Orbitals
A:TRP286 - B11	5.41047	Hydrophobic	Pi-Pi Stacked	A:TRP286	Pi-Orbitals	B11	Pi-Orbitals
A:TRP286 - B11	4.10229	Hydrophobic	Pi-Pi Stacked	A:TRP286	Pi-Orbitals	B11	Pi-Orbitals
Name-BChE -11	**Distance**	**Category**	**Types**	**From**	**From Chem.**	**To**	**To Chem.**
A:PRO285:HA - B11:O14	2.159	Hydrogen Bond	Carbon Hydrogen Bond	A:PRO285:HA	H-Donor	B11:O14	H-Acceptor
A:ASP70:OD2 - B11	4.856	Electrostatic	Pi-Anion	A:ASP70:OD2	Negative	B11	Pi-Orbitals
A:ASP70:HB2 - B11	2.729	Hydrophobic	Pi-Sigma	A:ASP70:HB2	C-H	B11	Pi-Orbitals
A:ALA277 - B11:Cl49	4.137	Hydrophobic	Alkyl	A:ALA277	Alkyl	B11:Cl49	Alkyl
B11 - A:ALA328	4.646	Hydrophobic	Pi-Alkyl	B11	Pi-Orbitals	A:ALA328	Alkyl

3. Conclusion

In this research, ethyl 4-(2-benzoylhydrazono)piperidine-1-carboxylate and N'-(2,6-diphenylpiperidin-4-ylidene)benzohydrazide derivatives were synthesized.

- The antioxidant activity was studied, most of the compounds (**1, 2, 3, 4, 5, 6, 8, 9, 10, 14, 16, 17**) exhibited more activity than positive controls α-Tocopherol and BHT.
- The anticholinesterase assay results demonstrate that, BChE assay results were better than the AChE assay results for the studied compounds. The compound **11** became the most prominent one among the other compounds. It was found more active than the galantamine as a BChE inhibitor.
- According to the molecular docking results, global minimum energy binding mode of compound **11** showed efficient BChE inhibition capacity due to multiple binding patterns in CAS and PAS of BChE.
- For anticancer studies; the compounds did not show significant effect on the viability of A549 cells.
- Compounds did not decrease the viability of the healthy BJ fibroblast cells.

As a conclusion, because of the non-cytotoxic properties and the good activity results, some of these compounds can be improved for using as antioxidant agents and potent butyrylcholinesterase inhibitors.

4. Experimental

4.1. General

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reactions were carried on Milestone Start Synth Microwave Synthesis Labstation (Sorisole, İtaly). The reactions and the purities of the compounds were monitored by thin layer chromatography (TLC) on silica gel 60 F_{254} aluminum sheets purchased from Merck (Darmstadt, Germany). Melting points were recorded by open capillaries on EzMelt melting point apparatus. C, H, N, S percent of the compounds were detected by Thermo Scientific Flash 2000 (Finnegan MAT, USA) elemental analyzer. FTIR spectra were recorded on Perkin Elmer Frontier spectrometer by attenuated total reflectance (ATR) apparatus (Waltham, Massachusetts, USA). Mass spectra were recorded on Ab-SciEx 3200 Q-Trap MSMS detector with electronspray ionization probe (Framingham, MA, USA). NMR spectra were recorded on Brucker Avance-400 MHz spectrometer (Billerica, MA, USA) by using DMSO-d_6 or $CDCl_3$ as a solvent and TMS as an internal standard. The absorbance was measured for MTT assay by using a Bio-Tek Cytation 3 Cell Imaging Multi-Mode Reader (Winooski, VT, USA). The other activity tests were done by using Molecular Devices Spectra Max PC340 microplate reader (Sunnyvale, CA, USA). The NMR and MS spectra of the compounds **1–19** were given in the supplementary data.

4.2. Synthesis

*4.2.1. Synthesis of 2,6-diphenylpiperidine-4-one **(I)***

Acetone, benzaldehyde and ammonium acetate were reacted by using *L*-proline catalyst according to the previously reported procedure [17].

4.2.2. Synthesis of substitutedphenyl benzoate **(II)**

Substituted benzoyl chloride (0,1 mol) was reacted with phenol (0.1 mol in 100 mL of 10% sodium hydroxide solution). The crude product was washed with water and recrystallized from ethanol [12, 20].

*4.2.3. Synthesis of substitutedbenzoyl hydrazides **(III)***

The substituted phenyl benzoate (II) (0.01 mol) was reacted with hydrazine hydrate (0.02 mol) in methanol. The mixture was refluxed and monitored by TLC. The crude product was washed with water and recrystallized from ethanol [12, 20].

*4.2.4. Synthesis of substitutedbenzoyl hydrazones **(1–19)***

0.01 mol ketone (I or IV) and 0.01 mol substitutedbenzoyl hydrazide in 30 mL ethanol were heated inside a microwave for 15 minute at 400 W and the product was obtained as reported previously [11, 12, 17].

*4.2.4.1. Ethyl 4-(2-(4-chlorobenzoyl)hydrazono)piperidine-1-carboxylate **(1)***

Yield: 82%; white solid, m.p. 105–107℃; IR (υ, cm^{-1}): 3200 (N–H); 3031 (aromatic C–H); 2984, 2908 (aliphatic C–H); 1690 (ester C=O); 1651 (hydrazone C=O); 1637 (C=N); 1091 (C–Cl); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.21 (t, 3H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, H_G); 2.45–2.55 (m, 4H, H_D, H_E); 3.50–3.56 (m, 4H, $H_{F, F'}$); 4.08 (q, 2H, J = 7.2 Hz, H_I); 7.56 (d, 2H, J = 8.0 Hz, H_C); 7.86 (d, 2H, J = 7.6 Hz, H_B); 10.81 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 162.96, 162.31 (C4, C10); 155.11 (C7); 136.65, 133,10 (C11, C14); 130.08, 128.8 (C12, C13, C15, C16); 61.37 (C8); 43.93, 42.13 (C2, C6); 34.1, 28.64 (C3, C5); 15.04 (C9); MS (*m/z*) (%): 324.8 $[M+H]^+$ (8.57); 265.0 (5.71); 247.2 (21.43); 236.2 (11.43); 203.4 (100); 189.4 (41.43); 187.2 (10.00); 175.2 (12.86); 171 (10.00); 169.4 (22.86); 167 (5.71); 165.2 (5.71); 143 (10.00); 140.4 (14.29); 139 (10.00); 137.2 (5.71); 125.4 (85.71); 111.4 (60.00); 101.21 (10.00); 97.4 (27.14); 89.2

(5.71); 85.2 (5.71); 79.2 (10.00); 71.4 (12.86); 70.6 (8.57); 61 (7.14); 57.4 (22.86); 56.4 (7.14); Anal. calcd. for $C_{15}H_{18}ClN_3O_3$: C 55.64; H 5.60; N 12.98%; found: C 54.26; H 5.53; N 12.93%.

4.2.4.2. Ethyl 4-(2-(4-fluorobenzoyl)hydrazono)piperidine-1-carboxylate ***(2)***

Yield: 60%; Cream solid, m.p. 120–122℃; IR (υ, cm^{-1}): 3200 (N–H); 3061 (aromatic C–H); 2985, 2935, 2909, 2869 (aliphatic C–H); 1707, 1694 (ester C=O); 1650 (hydrazone C=O); 1636 (C=N); 1158 (C–F); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.21 (t, 3H, J = 7.2 Hz, H_G); 2.45–2.55 (m, 4H, H_D, H_E); 3.50–3.56 (m, 4H, H_F); 4.08 (q, 2H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, H_I); 7.32 (t, 2H, J = 8.8 Hz, H_C); 7.91 (s, 2H, H_B); 10.75 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 165.67 (C14); 162.95, 161.98 (C4, C10); 155.10 (C7); 130.87 (C11); 115.75, 115.53 (C12, C13, C15, C16); 61.35 (C8); 43.95, 42.14 (C2, C6); 34.10, 28.63 (C3, C5); 15.04 (C9); MS (*m/z*) (%): 308.8 $[M+H]^+$ (1.51); 231.4 (27.78); 217.4 (8.33); 199.4 (8.08); 171.4 (6.57); 153.4 (55.55); 151.4 (6.06); 139.4 (12.62); 121.4 (23.23); 119.4 (5.30); 105.6 (5.81); 93.4 (7.83); 76.6 (17.68); 75.4 (100); 72.6 (7.07); 71.6 (20.45); 61.4 (18.18); 57.4 (6.31); Anal. calcd. for $C_{15}H_{18}FN_3O_3$: C 58.62; H 5.90; N 13.67%; found: C 58.24; H 5.90; N 13.32%.

4.2.4.3. Ethyl 4-(2-(4-methoxybenzoyl)hydrazono)piperidine-1-carboxylate ***(3)***

Yield: 61 %; White solid, m.p. 117–119 ℃; IR (υ, cm^{-1}): 3211 (N–H); 3060 (aromatic C–H); 2984, 2936, 2905 (aliphatic C–H); 1695 (ester C=O); 1638 (C=N); 1662 (hydrazone C=O); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.21 (t, 3H, J = 7.2 Hz, H_G); 2.44–2.54 (m, 4H, H_D, H_E); 3.50–3.57 (m, 4H, H_F); 4.08 (q, 2H, J = 7.2 Hz, H_I); 7.01 (d, 2H, J = 8.8 Hz, H_C); 7.83 (d, 2H, J = 8.4 Hz, H_B); 10.59 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 162.93, 158.33 (C4, C10, C14); 155.03 (C7); 131.21, 130.14, 114.60 (C11, C12, C13, C15, C16); 61.32 (C8); 56.10 (C17); 43.68, 41.76 (C2, C6); 33.49, 28.02 (C3, C5); 15.02 (C9); MS (*m/z*) (%): 318.4 $[M-H]^-$ (100); 272.4 (42.45); 229.4 (20.76); 217.6 (42.45); 213.0 (5.66); 201.4 (22.64); 174 (7.55); 173.6 (10.38); 159.2 (5.66); 159.0 (5.66); 120.0 (7.55); 108.2 (10.38); 95.0 (5.66); 93.4 (5.66); 92.4 (22.64); 81.4 (14.15); 42.4 (81.14); Anal. calcd. For $C_{16}H_{21}FN_3O_4$: C 60.17; H 6.63; N 13.16 %; found: C 59.89; H 6.48; N 12.96 %.

4.2.4.4. Ethyl 4-(2-(4-methylbenzoyl)hydrazono)piperidine-1-carboxylate ***(4)***

Yield: 68%; Cream solid, m.p. 120–122℃; IR (υ, cm^{-1}): 3199 (N–H); 3059 (aromatic C–H); 2976, 2907, 2868 (aliphatic C–H); 1698 (ester C=O); 1634 (C=N, hydrazone C=O); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.21 (t, 3H, J = 7.2 Hz, H_G); 2.37 (s, 3H, H_J); 2.45–2.55 (m, 4H, H_D, H_E); 3.50–3.56 (m, 4H, H_F); 4.08 (q, 2H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, H_I); 7.29 (d, 2H, J = 7.6 Hz, H_C); 7.75 (d, 2H, J = 7.6 Hz, H_B); 10.66 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 163.76, 161.50 (C4, C10); 155.06 (C7); 141.75, 131.49 (C11, C14); 129.20, 128.12 (C12, C13, C15, C16); 61.32 (C8); 43.94, 42.13 (C2, C6); 34.08, 28.55 (C3, C5); 21.43 (C17); 15.03 (C9); MS (*m/z*) (%): 304.8 $[M+H]^+$ 228.8 (22.22); 213.4 (12.22); 195.4 (13.11); 167.4 (8.88); 149.6 (46.86); 135.4 (28.47); 117.8 (38.41); 101.8 (9.60); 89.4 (12.53); 72.6 (31.32); 71.6 (100); 57.6 (25.49); Anal. calcd. for $C_{16}H_{21}N_3O_3$: C 63.35; H 6.98; N 13.85%; found: C 63.01; H 6.85; N 13.67%.

4.2.4.5. Ethyl 4-(2-(4-(trifluoromethyl)benzoyl)hydrazono)piperidine-1-carboxylate ***(5)***

Yield: 55 %; White solid, m.p. 116–118 ℃; IR (υ, cm^{-1}): 3166 (N–H); 3063 (aromatic C–H); 2987, 2914, 2871 (aliphatic C–H); 1683 (ester C=O); 1658 (hydrazone C=O); 1645 (C=N); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.21 (t, 3H, J = 7.2 Hz, H_G); 2.47 (t, 2H, J_1 = 6.0 Hz, J_2 = 5.6 Hz, H_E); 2.55 (t, 2H, J = 5.6 Hz, H_D); 3.53 (t, 2H, J = 6.0 Hz, H_F); 3.57 (t, 2H, J = 5.6 Hz, H_F); 4.08 (q, 2H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, H_I); 7.87 (d, 2H, J = 8.0 Hz, H_B); 8.03 (d, 2H, J = 8.0 Hz, H_C); 10.95 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-

d_6/TMS) δ (ppm): 162.94, 162.65 (C4, C10); 155.08 (C7);138.29, 131.84, 129.81, 129.09, 125.74, 123.03, 115.67 (C11, C12, C13, C14, C15, C16, C17); 61.36 (C8); 43.92, 42.14 (C2, C6); 34.15, 28.70 (C3, C5); 15.04 (C9); MS (*m/z*) (%): 356.4 [M-H] $^-$ (100); 310.4 (23.81); 267.4 (26.10); 255.4 (45.91); 237.4 (15.05); 145.4 (24.76); 109.4 (5.52); 93.4 (6.86); 81.4 (8.38); 42.4 (59.43); Anal. calcd. For $C_{16}H_{18}F_3N_3O_3$: C 53.78; H 5.08; N 11.76 %; found: C 53.37; H 5.34; N 11.39 %.

4.2.4.6. Ethyl 4-(2-(4-nitrobenzoyl)hydrazono)piperidine-1-carboxylate ***(6)***

Yield: 56 %; Light yellow solid, m.p. 112–114℃; IR (υ, cm^{-1}): 3198 (N–H); 3040 (aromatic C–H); 2987, 2918, 2872, 2829 (aliphatic C–H); 1679 (ester C=O); 1645 (C=N); 1663 (hydrazone C=O); 1516, 1343 (NO_2); ^{1}H-NMR (400 MHz) (DMSO-*d_6*/TMS) δ (ppm): 1.21 (t, 3H, H_G); 2.47–2.57 (m, 4H, H_D, H_E); 3.51–3.57 (m, 4H, H_I, H_J); 4.08 (q, 2H, H_F); 8.06 (d, 2H, H_B); 8.33 (d, 2H, H_C); 11.05 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d_6*/TMS) δ (ppm): 163.21, 162.40 (C4, C10); 155.09 (C7); 149.51, 140.18 (C11, C14); 129.70, 123.87 (C12, C13, C15, C16); 61.37 (C8); 43.91, 42.15 (C2, C6); 34.15, 28.75 (C3, C5); 15.05 (C9); MS (*m/z*) (%): 333.4 [M-H] $^-$ (100); 287.4 (19.13); 244.4 (25.65); 232.4 (46.52); 214.4 (9.13); 122.4 (38.26); 109.4 (4.78); 93.4 (8.26); 81.4 (6.52); 46.4 (29.57); 42.6 (36.96); Anal. calcd. for $C_{15}H_{18}N_4O_5$: C 53.89; H 5.43; N 16.76%; found: C 53.75; H 5.74; N 16.69%.

4.2.4.7. Ethyl 4-(2-(2,4-dinitrophenyl)hydrazono)piperidine-1-carboxylate ***(7)***

Yield: 62%; yellow solid, m.p. 196–198℃; IR (υ, cm^{-1}): 3313(N–H); 3089 (aromatic C–H); 3000, 2978, 2931, 2880 (aliphatic C–H); 1686 (ester C=O); 1615 (C=N); 1517, 1335 (NO_2); ^{1}H-NMR (400 MHz) (DMSO-*d_6*/TMS) δ (ppm): 1.22 (t, 3H, *J* = 7.2 Hz, H_G); 2.59 (t, 2H, *J* = 6.0 Hz, H_E); 2.64 (t, 2H, *J* = 6.0 Hz, H_D); 3.58 (t, 2H, *J* = 5.6 Hz, $H_{F'}$); 3.63 (t, 2H, *J* = 6.0 Hz, H_F); 4.08 (q, 2H, *J* = 7.2 Hz, H_I); 7.86 (d, 1H, *J* = 9.6 Hz, H_B); 8.38 (dd, 1H, J_1 = 2.8 Hz, J_2 = 9.6 Hz, H_C,); 8.86 (d, 1H, *J* = 2.8 Hz, H_J); 10.92 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d_6*/TMS) δ (ppm): 159.32 (C4); 155.09 (C7); 145.18, 137.32, 130.57, 129.65, 123.53, 116.41 (C11, C12, C13, C14, C15, C16); 61.37 (C8); 43.91, 42.15 (C2, C6); 34.15, 28.75 (C3, C5); 15.05 (C9); MS (*m/z*) (%): 352.2 [M+H] $^+$ 309.0 (8.33); 275.4 (13.33); 231.4 (90.00); 230.2 (30.00); 229.2 (21.67); 228.4 (8.33); 228.0 (13.33); 217.2 (20.00); 215.4 (10.00); 214.2 (8.33); 199.4 (26.67); 197.6 (45.00); 171.2 (10.00); 170.0 (5.00); 153.4 (53.30); 152.2 (11.67); 151.4 (23.33); 150.4 (13.33); 149.4 (25.00); 139.4 (28.3); 137.4 (16.67); 135.6 (11.67); 121.4 (43.3); 119.6 (46.7); 105.4 (10.00); 104.2 (10.00); 93.4 (10.00); 91.4 (10.00); 89.6 (10.00); 76.6 (18.30);75.6 (100); 73.4 (21.67); 72.4 (40.00); 71.6 (90.00); 61.4 (23.30); 60.6 (10.00);57.4 (16.7); Anal. calcd. for $C_{14}H_{17}N_5O_6$: C 47.86; H 4.88; N 19.93%; found: C 48.05; H 4.84; N 19.59%.

4.2.4.8. Ethyl 4-(2-(2-fluoro-4-(trifluoromethyl)benzoyl)hydrazono)piperidine-1-carboxylate ***(8)***

Yield: 69 %; White solid, m.p. 148–150 ℃; IR (υ, cm^{-1}): 3264(N–H); 3102 (aromatic C–H); 2983, 2911, 2870 (aliphatic C–H); 1693 (ester C=O); 1634 (C=N); 1652 (hydrazone C=O); 1151 (C–F); ^{1}H-NMR (400 MHz) (DMSO-*d_6*/TMS) δ (ppm): 1.17–1.22 (m, 3H, H_G); 2.16 (t, 1H, H_E); 2.44–2.57 (m, 3H, H_D, $H_{E'}$); 3.47–3.56 (m, 4H, $H_{F,\ F'}$); 4.04–4.10 (m, 2H, H_I); 7.64–7.86 (m, 3H, $H_{B,\ C,\ J}$); 10.98 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d_6*/TMS) δ (ppm): 166.91 (C12); 160.67, 160.01 (C4, C10); 155.08 (C7); 131.87, 130.94, 128.35, 128.18, 121.88, 114.29, 114.03 (C11, C13, C14, C15, C16, C17); 61.37 (C8); 43.80, 41.92 (C2, C6); 33.86, 28.40 (C3, C5); 15.04 (C9); MS (*m/z*) (%): 374.4 [M-H] $^-$ (64.81); 354.2 (10); 328.2 (5.56); 265.4 (19.91); 253.4 (99.54); 251.2 (6.48); 201.4 (51.39); 200.2 (83.8); 163.4 (22.69); 145.4 (14.82); 143.4 (60.65); 123.4 (7.87); 117.2 (10.65); 93.4 (25.46); 73.4 (12.04); 42.4 (46.3); Anal. calcd. for $C_{16}H_{17}F_4N_3O_3$: C 51.20; H 4.57; N 11.20 %; found: C 51.16; H 5.08; N 11.08 %.

4.2.4.9. Ethyl 4-(2-(4-fluoro-2-(trifluoromethyl)benzoyl)hydrazono)piperidine-1-carboxylate ***(9)***

Yield: 52 %; White solid, m.p. 132–134 ℃; IR (υ, cm^{-1}): 3204(N–H); 3054 (aromatic C–H); 2994, 2975, 2920, 2856 (aliphatic C–H); 1705 (ester C=O); 1631 (C=N); 1654 (hydrazone C=O); 1150 (C–F); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.17–1.22 (m, 3H, H_G); 2.11 (t, 1H, J = 6.0 Hz, H_E); 2.43–2.55 (m, 3H, $H_{D, E'}$); 3.38–3.57 (m, 4H, $H_{F, F'}$); 4.02–4.10 (m, 2H, H_I); 7.50–7.62 (m, 1H, H_C); 7.63–7.72 (m, 1H, H_J); 7.74–7.77 (dd, 1H, H_B); 10.94 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 169.42 (C14); 162.99, 160.40 (C4, C10); 155.09 (C7); 132.43, 132.34, 132.05, 131.56, 131.47, 119.87, 119.63 (C11, C12, C13, C15, C16, C17); 61.34 (C8); 43.79, 41.89 (C2, C6); 33.76, 28.30 (C3, C5); 15.03 (C9); MS (*m/z*) (%): 374.2 $[M-H]^-$ (100); 328.2 (21.2); 285.2 (15.89); 273.4 (24.8); 253.2 (18.36); 163.4 (65.21); 143.4 (7.95); 123.4 (13.56); 93.4 (6.71); 73.4 (6.57); 69.4 (14.93); 42.4 (54.25); Anal. calcd. for $C_{16}H_{17}F_4N_3O_3$: C 51.20; H 4.57; N 11.20 %; found: C 50.97; H 4.64; N 11.12 %.

4.2.4.10. N'-(2,6-diphenylpiperidin-4-ylidene)benzohydrazide ***(10)[29]***

Yield: 51%; Light yellow solid, m.p. 216–218℃; IR (υ, cm^{-1}): 3308 (seconder amine N–H); 3188 (hydrazone N–H); 3064, 3032 (aromatic C–H); 2965, 2900, 2845, 2803 (aliphatic C–H); 1646 (hydrazone C=O, C=N); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.08 (t, 1H, J_1 = 12.4 Hz, J_2 = 13.2 Hz, H_E); 2.43 (t, 1H, J_1 = 12.0 Hz, J_2 = 12.8 Hz, $H_{E'}$); 2.61 (d, 1H, J = 12.8 Hz, $H_{D'}$); 2.88 (s, 1H, H_G); 3.18 (d, 1H, J = 13.2 Hz, H_D);); 3.88 (d, 1H, J = 10.8 Hz, H_F); 3.96 (d, 1H, J = 10.8 Hz, $H_{F'}$); 7.26–7.30 (m, 2H, $H_{K, K'}$); 7.34–7.39 (m, 4H, $H_{I,I'}$); 7.46–7.55 (m, 7H, H_C, $H_{J, J'}$, H_L); 7.83 (d, 2H, J = 7.2 Hz, H_B); 10.86 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 164.21, 162.49 (C4, C13); 144.57, 144.51 (C7); 134.51, 131.75, 128.78, 128.69, 128.19, 127.61, 127.31, 127.10 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19, C20); 61.46, 60.20 (C2, C6); 43.94, 37.37 (C3, C5); MS (*m/z*) (%): 368.4 $[M-H]^-$ (100); 263.6 (59.92); 245.4 (12.06); 235.2 (7.78); 232.4 (15.95); 185.4 (8.56); 157.4 (5.06); 144.4 (5.45); 135.4 (5.45); 120.4 (8.17); 42.4 (21.4); Anal. calcd. for $C_{24}H_{23}N_3O$: C 78.02; H 6.27; N 11.37%; found: C 77.67; H 6.28; N 11.34%.

4.2.4.11. N'-(2,6-diphenylpiperidin-4-ylidene)-4-chlorobenzohydrazide ***(11)***

Yield: 64%; Yellow solid, m.p. 226–228℃; IR (υ, cm^{-1}): 3291 (seconder amine N–H); 3174 (hydrazone N–H); 3065, 3030 (aromatic C–H); 2961, 2899, 2795, 2737 (aliphatic C–H); 1646 (hydrazone C=O, C=N); 1084 (C–Cl); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.08 (t, 1H, J_1 = 12.0 Hz, J_2 = 13.6 Hz, H_E); 2.43 (t, 1H, J_1 = 12.0 Hz, J_2 = 13.2 Hz, $H_{E'}$); 2.60 (d, 1H, J = 12.0 Hz $H_{D'}$); 2.88 (s, 1H, H_G); 3.18 (d, 1H, J = 12.0 Hz, H_D); 3.87 (d, 1H, J = 10.8 Hz, H_F); 3.95 (d, 1H, J = 10.8 Hz, $H_{F'}$); 7.28–7.30 (m, 2H, $H_{K, K'}$); 7.35–7.38 (m, 4H, $H_{I,I'}$); 7.53–7.56 (m, 6H, H_C, $H_{J, J'}$); 7.86 (d, 2H, J = 8.0 Hz, H_B); 10.89 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 163.16, 162.86 (C4, C13); 144.53, 144.48 (C7); 130.15, 129.33, 128.77, 128.68, 127.62, 127.31, 127.10 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19); 61.44, 60.21 (C2, C6); 43.89, 37.38 (C3, C5); MS (*m/z*) (%): 402.4 $[M-H]^-$ (100); 297.4 (65.09); 279.2 (16.57); 232.4 (14.2); 218.8 (6.51); 178.2 (7.1); 169.2 (8.28); 154.4 (8.28); 111.2 (7.69); 42.4 (20.12); Anal. calcd. for $C_{24}H_{22}ClN_3O$: C 71.37; H 5.49; N 10.4%; found: C 71.5; H 5.18; N 9.96%.

4.2.4.12. N'-(2,6-diphenylpiperidin-4-ylidene)-4-fluorobenzohydrazide ***(12)***

Yield: 50%; Light yellow solid, m.p. 159–160℃; IR (υ, cm^{-1}): 3309 (seconder amine N–H); 3179 (hydrazone N–H); 3064, 3032 (aromatic C–H); 2970, 2902, 2842 (aliphatic C–H); (); 1646 (hydrazone C=O, C=N); 1155 (C–F); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.08 (t, 1H, J = 12.0 Hz, H_E); 2.43 (t, 1H, J_1 = 12.0 Hz, J_2 = 12.4 Hz, $H_{E'}$); 2.58–2.61 (m, 1H, $H_{D'}$); 2.88 (s, 1H, H_G); 3.16 (d, 1H, J = 13.2 Hz, H_D); 3.87 (d, 1H, J =

11.2 Hz, H_F); 3.96 (d, 1H, *J* = 11.2 Hz, $H_{F'}$); 7.28–7.39 (m, 8H, $H_{I,\ I'\ K,\ K',\ C}$); 7.49–7.54 (m, 4H, H_J, $H_{J'}$); 7.88–7.92 (m, 2H, H_B); 10.83 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 163.11, 162.68 (C4, C13); 144.53, 144.47 (C7); 130.90, 128.79, 128.77, 128.69, 127.68, 127.61, 127.30, 127.10, 115.74, 115.51 (C8, C9, C10, C11, C12, C14, C15, C16, C18, C19); 61.44, 60.53 (C2, C6); 43.87, 37.35 (C3, C5); MS (*m/z*) (%): 388.6 $[M+H]^+$ (15.77); 283.6 (26.00); 194.8 (100); 167.6 (21.48); 165.6 (13.93); 152.6 (12.42); 144.6 (7.05); 123.4 (68.46); 116.6 (20.47); 103.6 (17.45); 95.4 (23.49); 91.4 (10.24); 89.6 (8.73); 77.6 (10.07); 75.6 (18.46); 71.6 (5.54); 65.6 (10.74); ; Anal. calcd. for $C_{24}H_{22}FN_3O$: C 78.4; H 5.72; N 10.85%; found: C 78.17; H 5.6; N 10.53%.

4.2.4.13. N'-(2,6-diphenylpiperidin-4-ylidene)-4-methoxybenzohydrazide ***(13)***

Yield: 50 %; White solid, m.p. 151–153 ℃; IR (υ, cm^{-1}): 3319 (seconder amine N–H); 3371 (hydrazone N–H); 3059, 3026 (aromatic C–H); 2978, 2911, 2841, 2819 (aliphatic C–H); 1629 (C=N); 1643(hydrazone C=O); ^{1}H-NMR (400 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 2.06 (t, 1H, J_1 = 13.2 Hz, J_2 = 12.4 Hz, H_E); 2.41 (t, 1H, *J* = 12.4 Hz $H_{E'}$); 2.59 (d, 1H, *J* = 12.8 Hz, $H_{D'}$); 2.88 (s, 1H, H_G); 3.16 (d, 1H, *J* = 13.2 Hz, H_D); 3.81 (s, 3H, H_L); 3.86 (d, 1H, *J* = 11.6 Hz, H_F); 3.95 (d, 1H, *J* = 10.8 Hz, $H_{F'}$); 7.01 (d, 2H, *J* = 8.8 Hz, H_C); 7.28–7.30 (m, 2H, $H_{K,\ K'}$); 7.34–7.39 (m, 4H, $H_{I,I'}$); 7.52–7.55 (m, 4H, $H_{J,\ J'}$); 7.83 (d, 2H, *J* = 8.8 Hz, H_B); 10.71 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 163.62, 162.14, 161.89 (C4, C13, C17); 144.59 (C7); 130.08, 128.78, 128.69, 127.60, 127.30, 127.10, 126.53, 113.92 (C8, C9, C10, C11, C12, C14, C15, C16, C18, C19); 61.46, 60.20 (C2, C6); 55.83 (C20); 43.95, 37.39 (C3, C5); MS (*m/z*) (%): 398.4 $[M-H]^-$ (100); 293.4 (54.9); 232.4 (18.63); 231.4 (7.84); 174.2 (5.88); 150.4 (8.82); 120.2 (10.78); 119.8 (6.86); 108.4 (15.69); 97.2 (8.82); 96.6 (6.86); 96.2 (5.88); 95.2 (8.82); 93.4 (7.84); 92.4 (13.73); 81.2 (6.86); 42.4 (14.71); Anal. calcd. for $C_{25}H_{25}N_3O_2$: C 75.16; H 6.31; N 10.52 %; found: C 74.85; H 6.47; N 10.19 %.

4.2.4.14. N'-(2,6-diphenylpiperidin-4-ylidene)-4-methylbenzohydrazide ***(14)***

Yield: 54%; Light yellow solid, m.p. 190–193 ℃; IR (υ, cm^{-1}): 3285 (seconder amine N–H); 3171 (hydrazone N–H); 3059, 3032 (aromatic C–H); 2963, 2899, 2851 (aliphatic C–H); 1644 (hydrazone C=O, C=N); ^{1}H-NMR (400 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 2.07 (t, 1H, J_1 = 12.4 Hz, J_2 = 13.2 Hz, H_E); 2.36 (s, 3H, H_L); 2.42 (t, 1H, J_1 = 12.4 Hz, J_2 = 13.2 Hz, $H_{E'}$); 2.59 (d, 1H, *J* =12.4 Hz, $H_{D'}$); 2.88 (s, 1H, H_G); 3.16 (d, 1H, *J* = 13.6 Hz, H_D); 3.87 (d, 1H, *J* = 11.2 Hz, H_F); 3.95 (d, 1H, *J* = 10.8 Hz, $H_{F'}$); 7.27–7.30 (m, 4H, $H_{C,\ K,\ K'}$); 7.34–7.39 (m, 4H, $H_{I,I'}$); 7.54 (d, 4H, $H_{J,\ J'}$); 7.74 (d, 2H, *J* = 8.0 Hz, H_B); 10.77 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 163.99, 162.28 (C4, C13); 144.60 (C7); 141.70, 131.64, 129.21, 128.77, 128.68, 128.20, 127.60, 127.30, 127.10 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19); 61.46, 60.20 (C2, C6); 43.93, 37.37 (C3, C5); 21.46 (C20); MS (*m/z*) (%): 382.6 $[M-H]^-$ (100); 277.4 (63.42); 259.4 (14.63); 249.2 (8.54); 232.4 (24.39); 199.4 (6.1); 194.2 (6.1); 158.2 (7.32); 149.4 (10.98); 134.4 (8.54); 42.4 (15.85); Anal. calcd. for $C_{25}H_{25}N_3O$: C 78.3; H 6.57; N 10.96%; found: C 77.97; H 6.52; N 10.75%.

4.2.4.15. N'-(2,6-diphenylpiperidin-4-ylidene)-4-(trifluoromethyl)benzohydrazide ***(15)***

Yield: 63 %; White solid, m.p. 188–190 ℃; IR (υ, cm^{-1}): 3305 (seconder amine N–H); 3185 (hydrazone N–H); 3065, 3033 (aromatic C–H); 2959, 2911, 2845, 2823 (aliphatic C–H); 1659 (hydrazone C=O, C=N); ^{1}H-NMR (400 MHz) (DMSO-*d*$_6$/TMS) δ (ppm): 2.10 (t, 1H, J_1 = 12.4 Hz, J_2 = 13.2 Hz, H_E); 2.45 (t, 1H, J_1 = 12.0 Hz, J_2 = 12.8 Hz, $H_{E'}$); 2.61 (d, 1H, *J* = 13.4 Hz, $H_{D'}$); 2.89 (s, 1H, H_G); 3.19 (d, 1H, *J* = 13.6 Hz, H_D); 3.88 (d, 1H, *J* = 11.12 Hz H_F); 3.96 (d, 1H, *J* = 10.8 Hz $H_{F'}$); 7.26–7.30(m, 2H, $H_{K,\ K'}$); 7.34–7.39(m, 4H, $H_{I,\ I'}$); 7.54 (d, 4H, *J* = 7.2 Hz, $H_{J,\ J'}$); 7.85 (d, 2H, *J* = 8.0 Hz, H_B); 8.02 (d, 2H, *J* = 8.0 Hz, H_C); 11.03 (s, 1H, H_A); ^{13}C-NMR (100

MHz) (DMSO-d_6/TMS) δ (ppm): 163.16 (C4, C13); 144.50 (C7); 129.15, 128.78, 128.69, 127.63, 127.33, 127.11, 125.71 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19, C20); 61.43, 60.21 (C2, C6); 43.92, 37.38 (C3, C5); MS (*m/z*) (%): 436.6 $[M-H]^-$ (100); 331.4 (56.71); 313.4 (16.71); 303.4 (5.75); 253.4 (6.85); 203.4 (7.40); 185.4 (5.75); 145.4 (21.92); 42.4 (17.81); Anal. calcd. for $C_{25}H_{22}F_3N_3O$: C 68.64; H 5.07; N 9.61 %; found: C 68.29; H 5.31; N 9.21 %.

4.2.4.16. N'-(2,6-diphenylpiperidin-4-ylidene)-4-nitrobenzohydrazide ***(16)***

Yield: 65%; Orange solid, m.p. 208–210℃; IR (υ, cm^{-1}): 3294 (seconder amine N–H); 3184 (hydrazone N–H); 3085, 3032 (aromatic C–H); 2959, 2911, 2849, 2826 (aliphatic C–H); 1652 (hydrazone C=O, C=N); 1522, 1342 (NO_2); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.12 (t, 1H, J_1 = 12.4 Hz, J_2 = 13.6 Hz, H_E); 2.46 (t, 1H, J_1 = 12.0 Hz, J_2 = 12.8 Hz, $H_{E'}$); 2.63 (d, 1H, J = 12.8 Hz, $H_{D'}$); 2.89 (s, 1H, H_G); 3.20 (d, 1H, J = 13.6 Hz, H_D); 3.88 (d, 1H, J = 10.4 Hz, H_F); 3.97 (d, 1H, J = 10.4 Hz, $H_{F'}$); 7.28–7.38(m, 6H, $H_{K, K', I, I'}$); 7.54 (d, 4H, J = 7.2 Hz, $H_{J, J'}$); 8.07 (d, 2H, J = 8.4 Hz, H_B); 8.31 (d, 2H, J = 8.8 Hz, H_C); 9.90 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 163.83 (C4, C13); 149.45 (C17); 144.33 (C7); 143.83, 129.72, 129.64, 129.37, 129.18, 128.91, 128.80, 128.71, 127.69, 127.32, 127.09, 124.12, 124.00, 123.86 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19); 61.42, 60.36 (C2, C6); 43.76, 37.33 (C3, C5); MS (*m/z*) (%): 413.6 $[M-H]^-$ (100); 308.4 (51.22); 290.4 (12.20); 185.4 (6.23); 180.4 (7.05); 122.4 (33.6); 46.4 (33.6); 42.4 (13.55); Anal. calcd. for $C_{24}H_{22}N_4O$: C 69.55; H 5.35; N 13.52%; found: C 69.17; H 5.04; N 13.16%.

4.2.4.17. 1-(2,4-Dinitrophenyl)-2-(2,6-diphenylpiperidin-4-ylidene)hydrazine ***(17)***

Yield: 49%; Yellow solid, m.p. 194–196℃; IR (υ, cm^{-1}): 3302 (piperidine N–H); 3324 (hydrazone N–H); 3096 (aromatic C–H); 2969, 2906, 2821 (aliphatic C–H); 1613 (C=N); 1519, 1332 (NO_2); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.33–2.39 (m, 1H, H_E); 2.54–2.57 (m, 1H, $H_{E'}$); 2.69 (d, 1H, J = 12.4 Hz, $H_{D'}$); 2.97–3.00 (m, 2H, H_D, H_G); 3.95–.01 (m, 2H, $H_{F, F'}$); 7.31–7.34 (m, 2H, $H_{K, K'}$); 7.36–7.42 (m, 4H, $H_{I,I'}$); 7.54–7.57 (m, 4H, $H_{J, J'}$); 7.90 (d, 1H, J = 9.6 Hz, H_B); 8.39 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.8 Hz H_C); 8.86 (d, 1H, J = 2.8 Hz, H_L); 11.05 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 160.37 (C4); 145.21, 144.16 (C7); 137.17, 130.51, 129.59, 128.86, 128.80, 127.87, 127.71, 127.23, 127.12, 123.53, 116.39 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19); 61.26, 60.07 (C2, C6); 43.64, 36.57 (C3, C5); MS (*m/z*) (%): 432.6 $[M+H]^+$ (13.85); 232.6 (6.33); 154.4 (5.67); 129.6 (5.13); 128.6 (6.76); 115.6 (8.18); 106.8 (100); 105.2 (6.87); 104.6 (12.54); 103.6 (30.75); 91.6 (12.98); 79.6 (22.79); 78.2 (7.63); 77.6 (24.3); Anal. calcd. for $C_{23}H_{21}N_5O_4$: C 64.03; H 4.91; N 16.23%; found: C 64.01; H 4.88; N 15.97%.

4.2.4.18. N'-(2,6-diphenylpiperidin-4-ylidene)-2-fluoro-4-(trifluoromethyl)benzohydrazide ***(18)***

Yield: 45 %; White solid, m.p. 205–207 ℃; IR (υ, cm^{-1}): 3300 (seconder amine N–H); 3178 (hydrazone N–H); 3085, 3063 (aromatic C–H); 2958, 2898, 2804 (aliphatic C–H); 1659 (hydrazone C=O); 1632 (C=N); 1153 (C–F); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 2.08 (t, 1H, J_1 = 12.8 Hz, J_2 = 13.2 Hz, H_E); 2.43 (t, 1H, J_1 = 12.4 Hz, J_2 = 12.8 Hz, $H_{E'}$); 2.58 (d, 1H, J = 12.8 Hz, $H_{D'}$); 2.91 (s, 1H, H_G); 3.18(d, 1H, J = 13.6 Hz, H_D); 3.85 (d, 1H, J = 12.0 Hz, H_F); 3.96(d, 1H, J = 11.6 Hz, $H_{F'}$); 7.24–7.31 (m, 2H, $H_{K, K'}$); 7.33–7.45 (m, 4H, $H_{I, I'}$); 7.52–7.56 (m, 4H, $H_{J, J'}$); 7.65–7.85 (m, 3H, $H_{B, C, L}$); 11.04 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 166.83 (C15); 161.44 (C4); 160.05 (C13); 144.34, 144.19 (C7); 158.48, 155.50, 131.84, 128.74, 128.67, 128.62, 128.60, 127.65, 127.59, 127.24, 127.18, 127.05, 121.84, 114.14, 114.00 (C8, C9, C10, C11, C12, C14, C16, C17,C18, C19, C20); 61.42, 60.20 (C2, C6); 43.81, 36.98 (C3, C5); MS (*m/z*) (%): 456.4 $[M+H]^+$ (7.88); 352.0 (15.56); 247.4 (9.93); 204.6 (6.24); 194.8 (100); 191.6 (34.08); 167.8 (20.16); 165.6

(12.28); 163.4 (24.56); 152.6 (14.12); 144.6 (9.83); 143.2 (6.35); 129.6 (8.60); 116.6 (21.6); 106.6 (6.45); 103.6 (33.5); 91.6 (12.39); 89.6 (7.27); 77.6(12.79); 65.6 (9.62); Anal. calcd. for $C_{25}H_{21}F_4N_3O$: C 65.93; H 4.65; N 9.23 %; found: C 65.57; H 5.03; N 9.22 %.

4.2.4.19. N'-(2,6-diphenylpiperidin-4-ylidene)-4-fluoro-2-(trifluoromethyl)benzohydrazide ***(19)***

Yield: 47 %; White solid, m.p. 177–178 ℃; IR (υ, cm^{-1}): 3295 (seconder amine N–H); 3150 (hydrazone N–H); 3061, 3033 (aromatic C–H); 2975, 2904, 2841, 2819 (aliphatic C–H); 1667 (hydrazone C=O, C=N); 1155 (C–F); ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ (ppm): 1.99–2.09 (m, 1H, H_E); 2.41 (t, 1H, J_1 = 12.0 Hz, J_2 = 12.8 Hz, $H_{E'}$); 2.56 (d, 1H, J = 13.2 Hz, $H_{D'}$); 2.90 (s, 1H, H_G); 3.17(d, 1H, J = 13.6 Hz H_D); 3.11–3.84 (m, 1H, H_F); 3.95(d, 1H, J = 10.8 Hz, $H_{F'}$); 7.23–7.42 (m, 6H, $H_{K,\ K',\ I,\ I'}$); 7.50–7.58 (m, 4H, $H_{J,\ J'}$); 7.60–7.65 (m, 1H, H_C); 7.69–7.78 (m, 2H, $H_{B,L}$); 10.99 (s, 1H, H_A); ^{13}C-NMR (100 MHz) (DMSO-d_6/TMS) δ (ppm): 169.45 (C17); 163.04, 161.07 (C4, C13); 144.49, 144.40 (C7); 154.85, 144.24, 132.44, 132.36, 132.20, 128.78, 128.67, 127.65, 127.61, 127.25, 127.09, 127.04, 119.92, 119.71, 114.57 (C8, C9, C10, C11, C12, C14, C15, C16, C17, C18, C19, C20); 61.45, 60.22 (C2, C6); 43.83, 36.85 (C3, C5); MS (*m/z*) (%): 456.6 $[M+H]^+$ (8.83); 351.8 (14.50); 227.4 (6.88); 195.4 (100); 191.4 (43.03); 186.4 (9.76); 167.6 (22.77); 165.6 (13.38); 163.4 (33.64); 152.6 (10.41); 144.6 (7.90); 129.4 (5.58); 116.6 (20.35); 113.4 (8.08); 103.6 (21.38); 91.6 (11.25); 89.6 (7.25); Anal. calcd. for $C_{25}H_{21}F_4N_3O$: C 65.93; H 4.65; N 9.23 %; found: C 65.80; H 5.01; N 9.14 %.

4.3. Biological Studies

β-Carotene-linoleic acid assay.

The total antioxidant activity was evaluated using the *β*-carotene-linoleic acid model test system [30, 31]. DMSO was used as negative control while BHT and *α*-tocopherol were used as positive controls.

DPPH free radical scavenging assay.

The free radical scavenging activity was determined spectrophotometrically using the DPPH radical commercially available [32, 33]. DMSO was used as negative control while BHT and *α*-tocopherol were used as positive controls.

ABTS cation radical scavenging assay.

The spectrophotometric analysis of $ABTS^{\cdot+}$ scavenging activity was determined according to the previously described method [34] with slight modifications [32]. DMSO was used as negative control, while BHT and *α*-tocopherol were used as positive controls.

Cupric reducing antioxidant capacity (CUPRAC).

The cupric reducing antioxidant capacity was determined according to the previous method [32, 35]. DMSO was used as negative control, while BHT and *α*-tocopherol were used as positive controls.

Determination of acetylcholinesterase- (AChE) and butyrylcholinesterase- (BChE) inhibitory activity.

AChE and BChE inhibitory activities were measured by the spectrophotometric method using Ellman method. AChE from electric eel and BChE from horse serum were used as enzymes [36].

Cell culture

The A549 (ATCC® CCL-185™) human lung carcinoma and BJ (ATCC® CRL-2522™) human fibroblast cell lines were gained from American Type Culture Collection (ATCC®) and stored in 10% fetal

bovine serum and 1% penicillin/streptomycin containing RPMI (A549) or EMEM (BJ) medium at 37℃ in a humidified incubator with 5% CO_2.

Cell viability assay

Cell viability was evaluated by MTT (3, 4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay [37, 38] which was based on the reduction of the yellow MTT by the mitochondrial dehydrogenase of intact viable or living cells to a purple formazan product. This reduction happened only when mitochondrial reductase enzymes were active. Therefore, increased purple color was directly related to the number of viable cells. The amount of purple formazan produced by cells treated with compounds was compared with the amount of formazan produced by untreated control cells.

Cellular viability was detected by MTT assay as described previously [39]. In short, cells were grown in 96-well plates at a density of $3x10^3$ cells per well and subjected to different concentrations of the compounds (**1–19** and cisplatin) (1000, 500, 250, 125, 62,5, 31,2, 15,6 7,8, 3,9, 1,9 µM). After 24 hours incubation, MTT solution was added to reach a final concentration of 0.5 mg/mL. The cells were incubated for another 3 h. Then current medium was removed and 100 µl of DMSO solution was added. The absorbance was measured at 540 nm. Cell survival rates were meant as the percentage of the DMSO (0.1%) solvent control. The IC_{50} values of the compounds were calculated according to these rates.

4.4. Computational Studies

Molecular docking method was performed using CDOCKER in Discovery Studio 2016 by BIOVIA to provide an insight into the mentioned compounds. Firstly, compounds (**1–19**) and enzymes were prepared using Gaussian 09 (G09) [40] and Discovery Studio (DS) 2016 [41] softwares. The compounds were drawn and optimized at DFT/B3LYP/6-31G* level by using G09. In the meantime, their conformational analysis was performed using DS 2016. The protein crystal structures of AChE and BChE (PDB codes: 1ACJ and 1P0I) were selected for this study. Hydrogen and missing heavy atoms were added to the protein structure, and atom types and partial charges were assigned. The enzymes were optimized using CHARMm forcefield and the adopted-basis Newton-Raphson (ABNR) method [42] in the DS 2016 until the root mean square deviation (RMSD) gradient was < 0.05 kcal/mol Å2. The active sites were determined by using define and edit binding site tool of the DS software and literatures.

In docking analysis, the compounds were flexible and enzymes were held rigid. Dock Ligands (CDOCKER) was performed using the default settings. The docking parameters were default form. The best orientation of the compounds was determined based on the global minimum energy binding, CDOCKER energy, CDOCKER interaction energy and RMSD values [43].

5. Acknowledgements

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6. Conflict of Interest

The Authors declare that they have no conflicts of interest to disclose.

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- A series of benzoyl hydrazones bearing piperidine ring were synthesized.
- Antioxidant, anticholinesterase and anticancer activities were studied.
- Most of the compounds were found more active than the antioxidant reference standards.
- The compound **11** was found more active than the galantamine as a BChE inhibitor.
- Molecular docking studies confirmed the anticholinesterase assay results.

Compound	InChI
1	1S/C15H18ClN3O3/c1-2-22-15(21)19-9-7-13(8-10-19)17-18-14(20)11-3-5-12(16)6-4-11/h3-6H,2,7-10H2,1H3,(H,18,20)
2	1S/C15H18FN3O3/c1-2-22-15(21)19-9-7-13(8-10-19)17-18-14(20)11-3-5-12(16)6-4-11/h3-6H,2,7-10H2,1H3,(H,18,20)
3	1S/C16H21N3O4/c1-3-23-16(21)19-10-8-13(9-11-19)17-18-15(20)12-4-6-14(22-2)7-5-12/h4-7H,3,8-11H2,1-2H3,(H,18,20)
4	1S/C16H21N3O3/c1-3-22-16(21)19-10-8-14(9-11-19)17-18-15(20)13-6-4-12(2)5-7-13/h4-7H,3,8-11H2,1-2H3,(H,18,20)
5	1S/C16H18F3N3O3/c1-2-25-15(24)22-9-7-13(8-10-22)20-21-14(23)11-3-5-12(6-4-11)16(17,18)19/h3-6H,2,7-10H2,1H3,(H,21,23)
6	1S/C15H18N4O5/c1-2-24-15(21)18-9-7-12(8-10-18)16-17-14(20)11-3-5-13(6-4-11)19(22)23/h3-6H,2,7-10H2,1H3,(H,17,20)
7	1S/C14H17N5O6/c1-2-25-14(20)17-7-5-10(6-8-17)15-16-12-4-3-11(18(21)22)9-13(12)19(23)24/h3-4,9,16H,2,5-8H2,1H3
8	1S/C16H17F4N3O3/c1-2-26-15(25)23-7-5-11(6-8-23)21-22-14(24)12-4-3-10(9-13(12)17)16(18,19)20/h3-4,9H,2,5-8H2,1H3,(H,22,24)
9	1S/C16H17F4N3O3/c1-2-26-15(25)23-7-5-11(6-8-23)21-22-14(24)12-4-3-10(9-13(12)17)16(18,19)20/h3-4,9H,2,5-8H2,1H3,(H,22,24)
10	1S/C24H23N3O/c28-24(20-14-8-3-9-15-20)27-26-21-16-22(18-10-4-1-5-11-18)25-23(17-21)19-12-6-2-7-13-19/h1-15,22-23,25H,16-17H2,(H,27,28)
11	1S/C24H22ClN3O/c25-20-13-11-19(12-14-20)24(29)28-27-21-15-22(17-7-3-1-4-8-17)26-23(16-21)18-9-5-2-6-10-18/h1-14,22-23,26H,15-16H2,(H,28,29)
12	1S/C24H22FN3O/c25-20-13-11-19(12-14-20)24(29)28-27-21-15-22(17-7-3-1-4-8-17)26-23(16-21)18-9-5-2-6-10-18/h1-14,22-23,26H,15-16H2,(H,28,29)
13	1S/C25H25N3O2/c1-30-22-14-12-20(13-15-22)25(29)28-27-21-16-23(18-8-4-2-5-9-18)26-24(17-21)19-10-6-3-7-11-19/h2-15,23-24,26H,16-17H2,1H3,(H,28,29)
14	1S/C25H25N3O/c1-18-12-14-21(15-13-18)25(29)28-27-22-16-23(19-8-4-2-5-9-19)26-24(17-22)20-10-6-3-7-11-20/h2-15,23-24,26H,16-17H2,1H3,(H,28,29)
15	1S/C25H22F3N3O/c26-25(27,28)20-13-11-19(12-14-20)24(32)31-30-21-15-22(17-7-3-1-4-8-17)29-23(16-21)18-9-5-2-6-10-18/h1-14,22-23,29H,15-16H2,(H,31,32)
16	1S/C24H22N4O3/c29-24(19-11-13-21(14-12-19)28(30)31)27-26-20-15-22(17-7-3-1-4-8-17)25-23(16-20)18-9-5-2-6-10-18/h1-14,22-23,25H,15-16H2,(H,27,29)
17	1S/C23H21N5O4/c29-27(30)19-11-12-20(23(15-19)28(31)32)26-25-18-13-21(16-7-3-1-4-8-16)24-22(14-18)17-9-5-2-6-10-17/h1-12,15,21-22,24,26H,13-14H2
18	1S/C25H21F4N3O/c26-21-13-18(25(27,28)29)11-12-20(21)24(33)32-31-19-14-22(16-7-3-1-4-8-16)30-23(15-19)17-9-5-2-6-10-17/h1-13,22-23,30H,14-15H2,(H,32,33)
19	1S/C25H21F4N3O/c26-18-11-12-20(21(13-18)25(27,28)29)24(33)32-31-19-14-22(16-7-3-1-4-8-16)30-23(15-19)17-9-5-2-6-10-17/h1-13,22-23,30H,14-15H2,(H,32,33)