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Research Article

Design, Synthesis, Biological Evaluation, and Docking Study of Acetylcholinesterase Inhibitors: New Acridone-1,2,4-oxadiazole-1,2,3-triazole Hybrids

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In this study, novel acridone-1,2,4-oxadiazole-1,2,3-triazole hybrids were designed, synthesized, and evaluated for their acetylcholinesterase and butyrylcholinesterase inhibitory activity. Among various synthesized compounds, 10-((1-((3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl) acridin-9(10*H*)-one 10b showed the most potent anti-acetylcholinesterase activity (IC_{50} = 11.55 μM) being as potent as rivastigmine. Also docking outcomes were in good agreement with in vitro results confirming the dual binding inhibitory activity of compound 10b.

Key words: acetylcholinesterase, acridone-1,2,4-oxadiazole-1,2,3-triazole, Alzheimer's disease, docking study

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Alzheimer's disease (AD) has emerged as the main cause of dementia in elderly people affecting cognitive characteristics including intelligence, memory, language, and speech (1). It is known that two cholinesterases (ChEs), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are responsible for the regulation of cholinergic neurotransmission, the decline of which leads to AD (2). In spite of the fact that physiological function of AChE at cholinergic synapses is well known, the function of BChE has not been fully recognized (3). Koelle demonstrated that contrary to AChE, BChE could be more active at high concentrations of ACh (4). In another study, Li *et al.* (5) found that it may act as backup to AChE when the AChE activity has been decreased.

There is no definite cure for Alzheimer's disease and current investigations continue to treat symptoms (2,6). At present, the neuroprotective effect is one of the most important aspects of the currently marketed anti-Alzheimer agents (7–9)

Two main binding sites are involved in the structure of AChE: the catalytic binding site (CS) and the peripheral anionic binding site (PAS) (10,11). Recently, dual binding site inhibitors (binding to both CS and PAS) have attracted lots of attention and in this field, heterocyclic compounds play crucial role. They can interact with both sites efficiently and in this regard, indolinones (12), huperzine A-tacrine (13), quinolone–benzylpiperidine (14), thiazolo-1,2,4-triazinones (15), and oxoisoaporphines (16) have shown remarkable activities.

As an extension of our continuous investigation on the design and synthesis of anti-AChE agents (12,17–19), herein, we decided to investigate AChEI activity of novel acridone-1,2,4-oxadiazole-1,2,3-triazole hybrids (Scheme 1). Latterly, in view of the efficiency of 1,2,3-triazoles in the inhibition of AChE (20), we reported synthesis and *in vitro* evaluation of acridone linked to 1,2,3-triazoles (21). Most of the synthesized derivatives showed good activity in comparison with rivastigmine as the reference drug and among them, 10-((1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methoxyacridin-9(10*H*)-one **A** was found to be more potent than rivastigmine (IC_{50} = 7.31 μM) (Figure 1A). Considering the fact that 1,2,4-oxadiazoles exhibited a wide variety of biological properties (22) and have not been extensively considered in anti-AChE research (23), we decided to insert 1,2,4-oxadiazole moiety to acridone-1,2,3-triazole moiety and investigate AChEI activity (Figure 1).

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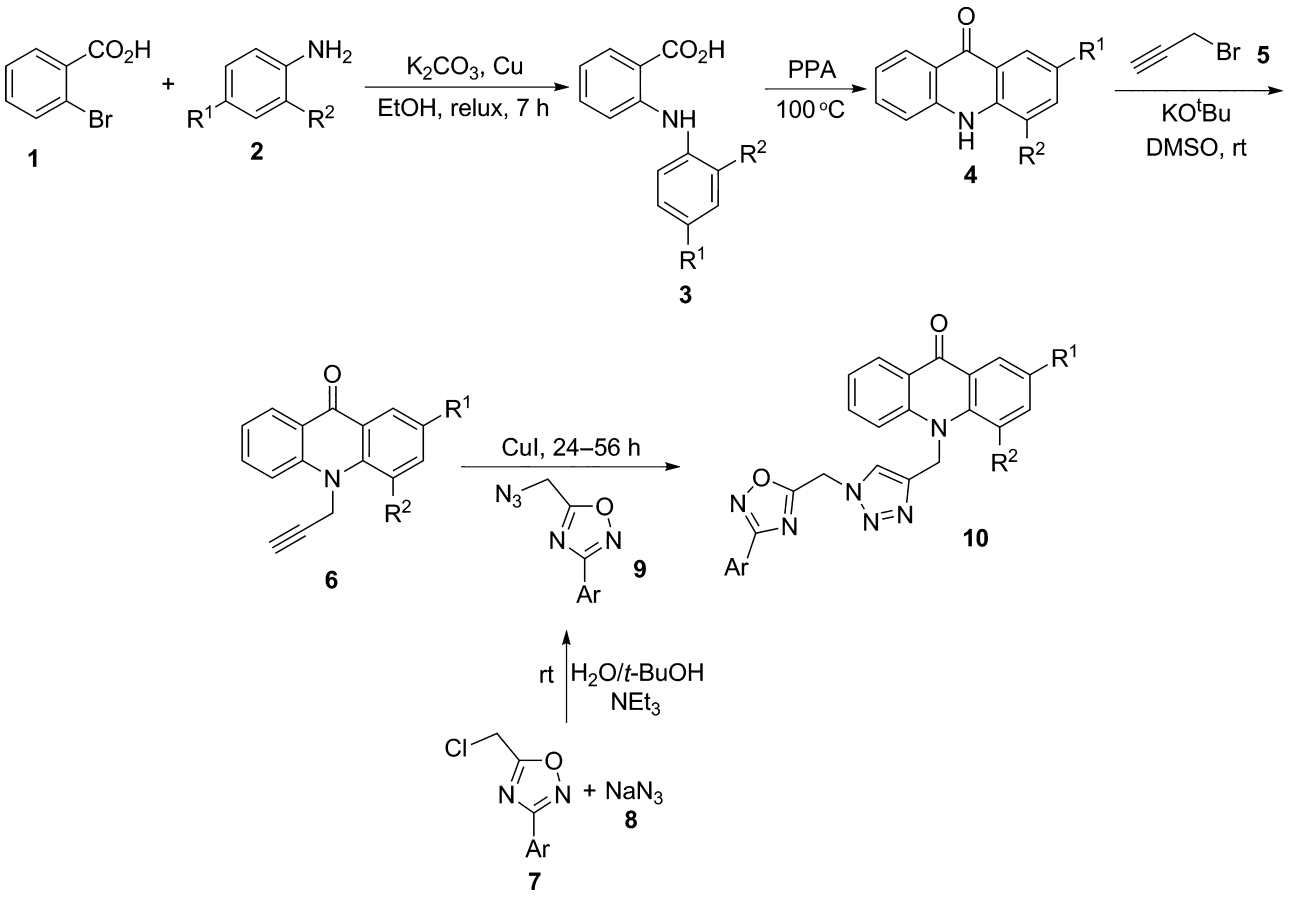


Scheme 1: Synthesis of acridone-1,2,4-oxadiazole-1,2,3-triazole derivatives **10**.

Methods and Materials

General chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-500, using TMS as an internal standard. IR spectra were obtained with a Nicolet Magna FTIR 550 spectrophotometer (KBr disks). MS values were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode.

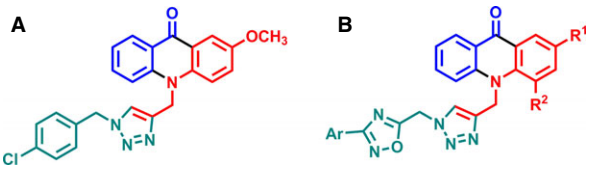


Figure 1: The structure of 10-((1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methoxyacridin-9(10*H*)-one as a potent anti-AChE (IC_{50} = 7.31 μM) (A) and acridone-1,2,4-oxadiazole-1,2,3-triazole hybrids (B).

General procedure for the synthesis of acridone-1,2,4-oxadiazole-1,2,3-triazole derivatives 10

In the beginning, azide derivatives **9** were prepared *in situ*. For this purpose, a solution of 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivative **7** (1.1 mmol), sodium azide **8** (0.9 mmol), and Et_3N (1.3 mmol) in the mixture of water (4 mL)/*t*-BuOH (4 mL) was stirred at room temperature for 1 h. Subsequently, mixture of 10-(prop-2-yn-1-yl) acridin-9-one derivative **6** (1 mmol) and CuI (7 mol %) was added to the freshly prepared azide derivative **9** and the reaction mixture was stirred at room temperature for 24–56 h. Upon completion of the reaction, monitored by TLC, the reaction mixture was diluted with water, poured into crushed ice, and the precipitated product was filtered off, washed with cold water, and purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (4:1).

AChE and BChE inhibition assay

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit), butylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide (ATCI), and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich.

Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, and sodium hydrogen carbonate were purchased from Fluka. The acridone-1,2,4-oxadiazole-1,2,3-triazole derivatives **10a-p** were dissolved in a mixture of DMSO (5 mL) and methanol (5 mL) and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to obtain final assay concentrations. All experiments were achieved at 25 °C, and four different concentrations were tested for each compound in triplicate to obtain the range of 20–80% inhibition for AChE.

To evaluate *in vitro* AChE activity, modified Ellman's method was performed (24) using a 96-well plate reader (BioTek ELx808, Highland Park, IL, USA). Each well contained 50 μL potassium phosphate buffer (KH_2PO_4/K_2HPO_4, 0.1 M, pH 8), 25 μL sample dissolved in 50% methanol and 50% DMSO, and 25 μL enzyme (final concentration 0.22 U/mL in buffer). They were preincubated for 20 min at room temperature, and then, 125 μL DTNB (3 mM in buffer) was added. Characterization of the hydrolysis of ATCI catalyzed by AChE was performed spectrometrically at 405 nm followed by the addition of substrate (ATCI 3 mM in water). The change in absorbance was measured at 405 nm after 20 min. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration versus percent of inhibition). A control experiment was performed under the same conditions without inhibitor and the blank contained buffer, DMSO, DTNB, and substrate. The described method was also used for BChE inhibition assay.

DPPH radical scavenging activity (DPPH)

Antioxidant activity of compounds **10a-d**, **10k**, and **10n** were assessed using DPPH (1,1-diphenyl-2-picrylhydrazyl) (25). Several concentrations of the above-mentioned compounds in DMSO were prepared. The compound solution (0.5 mL) was added to the methanolic DPPH solution (1.0 mL, 0.1 mM), and the mixture was kept in the dark for 30 min. Then, the absorbance at 517 nm was measured by an UV/visible spectrophotometer. The percent scavenging activity was calculated using the following formula: inhibition (%) = $100-[100 \times (Abs_{sample} - Abs_{control})/Abs_{blank}]$.

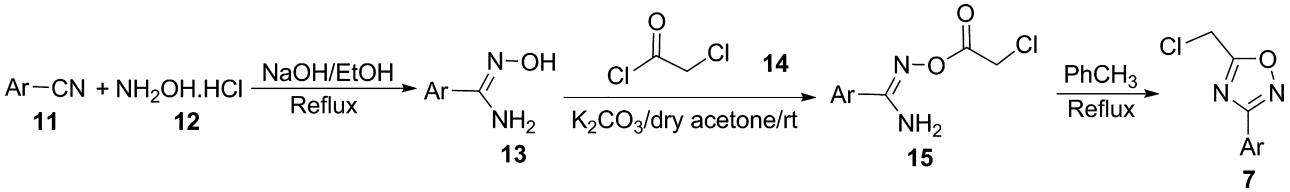
Molecular docking study

Docking studies were carried out using the AUTODOCK 4.2 program (La Jolla, CA, USA). For this purpose, the pdb structure of 1EVE was taken from the Brookhaven protein database (http://www.rcsb.org) as a complex bound with inhibitor E2020 (donepezil). Subsequently, the water molecules and the original inhibitors were removed from the protein structure. The 3D structure of the compounds **10b**, **10c**, **10m**, and **10n** were provided using MARVINESKETCH 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and converted to pdbqt co-ordinate by AUTODOCK 4.2 program. Also, the autodock format of protein was provided using the same software. Polar hydrogen atoms were added to amino acid residues using AUTODOCK TOOLS (ADT; version 1.5.6), Koullman charges were assigned to all atoms of the enzyme, and the obtained enzyme structure was used as an input for the AUTOGRID program. AUTOGRID performed a precalculated atomic affinity grid maps for each atom type in the ligand, plus an electrostatics map and a separate desolation map presented in the substrate molecule. All maps were calculated with 0.375 A° spacing between grid points. The center of the grid box was placed at the center of donepezil with co-ordinates x = 2.023, y = 63.295, and z = 67.062. The dimensions of the active site box were set at 40 × 40 × 40 × A. Flexible ligand docking was accomplished for the compounds **10b**, **10c**, **10m**, and **10n**. Each docked system was carried out by 100 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). Other than the above-mentioned parameters, the other parameters were accepted as default. The lowest energy conformation of ligand–enzyme complex was considered for analyzing the interactions between AChE and the inhibitor. The results were visualized using DISCOVERY STUDIO 4.0 CLIENT (Figures 1 and 2).

Results and Discussion

Chemistry

The synthetic pathways for the synthesis of novel acridone-1,2,4-oxadiazole-1,2,3-triazoles **10** have been shown in Scheme 1. Our approach was started from the Ullmann condensation reaction of 2-bromobenzoic acid **1** and different anilines **2** (26) using potassium carbonate and copper in refluxing EtOH leading to the formation of 2-arylamino benzoic acids **3**. Compound **3** easily participated in the cyclization reaction in the presence of PPA at 100 °C and afforded acridones **4** (27). Acridone derivative **4** reacted with propargyl bromide **5** using potassium



Scheme 2: Synthesis of 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivatives **7**.

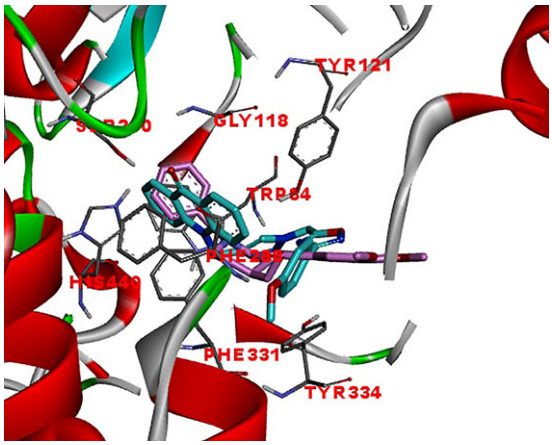


Figure 2: Superimposition of the most potent compound **10b** (cyan) and donepezil (pink) in the active site of AChE.

tert-butoxide in DMSO at room temperature. The resulting 10-(prop-2-yn-1-yl) acridin-9-one derivative **6** tolerated click reaction through the method described by Sharpless *et al.* to construct 1,2,3-triazole ring (28). For this purpose, different 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivatives **7** were prepared as approached in Scheme 2 (29). Accordingly, benzonitrile derivative **11** and hydroxylamine hydrochloride **12** reacted in refluxing EtOH in the presence of sodium hydroxide to obtain **13**. The reaction of compound **13** and chloroacetyl chloride **14** in the presence of potassium carbonate in dry acetone gave N'-(2-chloroacetoxy)-4-substituted benzimidamide **15** which underwent the further cyclization reaction in refluxing toluene to give 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivative **7**. Then, compound **7** and sodium azide **8** reacted in the presence of Et_3N in the mixture of H_2O/*t*-BuOH at room temperature for 1 h. After that, mixture of 10-(prop-2-yn-1-yl) acridin-9-one derivative **6** and CuI was added to the freshly prepared azide derivative **8** and the reaction was continued at room temperature for 24–56 h to give the corresponding product **10** in good yields (68–86%).

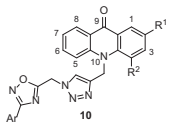
Pharmacology

The anti-AChE activity of acridone-1,2,4-oxadiazole-1,2,3-triazole derivatives

In vitro AChE and BChE inhibition assays were accompanied according to the Ellman's method (24), and all results were compared to rivastigmine as the reference drug (Table 1). Among sixteen newly synthesized acridone-1,2,4-oxadiazole-1,2,3-triazoles **10a-p**, compounds **10a-d**, **10k**, and **10n** (IC_{50} = 11.55–77.79 μM) showed anti-AChE activity and **10b** (IC_{50} = 11.55 μM) was found as potent as rivastigmine (IC_{50} = 11.07 μM).

According to our results, compound **10b** possessing unsubstituted acridone and 4-methoxyphenyl-1,2,4-

Table 1: The IC_{50} values of the compounds **10** against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)[a]



10

Entry	Compound **10**	R^1	R^2	Ar	AChE inhibition IC_{50} (μM)	BChE inhibition IC_{50} (μM)
1	**10a**	H	H	4-Me-C_6H_4	49 ± 0.04	>100
2	**10b**	H	H	4-MeO-C_6H_4	11.55 ± 0.08	>100
3	**10c**	H	H	4-Cl-C_6H_4	24.72 ± 0.53	88.86 ± 0.24
4	**10d**	H	H	4-Br-C_6H_4	67.44 ± 0.17	>100
5	**10e**	H	OMe	4-Me-C_6H_4	>100	>100
6	**10f**	H	OMe	4-MeO-C_6H_4	>100	>100
7	**10g**	H	Cl	4-MeO-C_6H_4	>100	>100
8	**10h**	Me	H	4-Cl-C_6H_4	>100	>100
9	**10i**	OMe	H	4-Me-C_6H_4	>100	>100
10	**10j**	OMe	H	4-MeO-C_6H_4	>100	>100
11	**10k**	OMe	H	4-Cl-C_6H_4	77.79 ± 0.01	>100
12	**10l**	Cl	H	4-Me-C_6H_4	>100	>100
13	**10m**	Cl	H	4-MeO-C_6H_4	>100	>100
14	**10n**	Cl	H	4-Cl-C_6H_4	44.93 ± 0.06	>100
15	**10o**	Br	H	4-MeO-C_6H_4	>100	>100
16	**10p**	Br	H	4-Cl-C_6H_4	>100	>100
17	**Rivastigmine**				11.07 ± 0.01	N.D

[a]Data are expressed as mean ± SE (three independent experiments).

oxadiazole moieties showed the most potent activity (IC_{50} = 11.55 μM). However, AChE inhibition disappeared when the acridone moiety was substituted at C-2 or C-4 position by methoxy, chlorine, and bromine in compounds **10f**, **10g**, **10j**, **10l**, and **10o**. In the series of compounds **10**, compound **10c** was found as the second active derivative (IC_{50} = 24.72 μM). It consisted of unsubstituted acridone and 4-chlorophenyl-1,2,4-oxadiazole moieties. Introduction of chlorine or methoxy group to the C-2 position of acridone moiety led to the reduction of AChE activity in compounds **10n** and **10k** (IC_{50} = 44.93 and77.79 μM, respectively), and AChE inhibition activity was canceled out when the hydrogen at C-2 was replaced by methyl or bromine (**10h** and **10p**). Our results showed that compounds **10a** and **10d** having 4-methyphenyl/4-bromophenyl-1,2,4-oxadiazole moiety were less active (IC_{50} = 49.46 and 67.44 μM, respectively) than compounds **10b** and **10c**. However, compound **10a** presented more potency in comparison with its counterparts **10e**, **10i**, and **10l**.

Other instructive results were obtained from derivatives having substituted acridone moieties (**10e-p**). In this series of compounds, only **10k** and **10n** (IC_{50} = 77.79 and 44.93 μM, respectively) possessing 4-chlorophenyl-1,2,4-oxadiazole moiety exhibited relatively good anti-AChE effects. It should be noted that **10k** and **10n** had methoxy and chlorine at C-2 position of acridone moiety, respectively.

To sum up, the electronic properties of substituents on all three heterocyclic moieties play crucial role in anti-AChE activity. As can be seen in Table 1, the satisfactory results with anti-AChE effects were related to derivatives possessing unsubstituted acridone moiety and it is strongly confirmed by compounds **10b** and **10c**. At this juncture, substituted 1,2,4-oxadiazole ring was perceived to be important and the AChEI activity in the unsubstituted acridone series was observed in the order of **10b**>**10c**>**10a**>**10d**. It seems that methoxy group imparted a higher activity in comparison with chlorine, methyl, and bromine substituents (OMe>Cl>Me>Br). It should be note that the anti-AChEI activity of compounds **10** was in good agreement with those reported in the literature as the efficiency of methoxy and chlorine groups to establish suitable interactions has been well documented, the ability of formation of a hydrogen bond between methoxy oxygen and amino acids NH protons (30) as well as interaction between chlorine and backbone carbonyl group (31).

To determine selectivity toward AChE and BChE enzymes, all compounds **10** were evaluated for their BChEI activity, and there were no significant activities.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The total antioxidant activity of the six AChEI active compounds **10a–d**, **10k**, and **10n** were evaluated using 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) assay based on the method described in the literature (25). In this colorimetric method, 1,1-diphenyl-2-picrylhydrazyl radical has an absorption band at 515 nm which is disappeared upon reduction by an antioxidant agent. The inhibition percentage values were determined by comparison with butylated hydroxyanisole (BHA) as a standard antioxidant. As can be seen in Table 2, among tested compounds, **10d** was found to be more potent antioxidant agent (inhibition percentage: 84.16) in comparison with BHA (inhibition percentage: 95.70).

Docking studies

We also performed docking studies parallel to the synthesis and *in vitro* evaluation of acridone-1,2,4-oxadiazole-1,2,3-triazoles to gain a more exhaustive perception of the interactions and binding to the active site of AChE. They were obtained using AUTODOCK TOOLS (1.5.6) and DISCOVERY STUDIO 4.0 CLIENT. Several ligand-bounded crystallographic structures of AChE were obtained from the RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do). In this study, PDB structure of 1EVE was retrieved for docking purpose.

As it is clear in Figure 2, the orientation of the most potent compound **10b** in the active site of AChE was the same as donepezil. Our results related to the most active compound **10b**, relatively good active compounds including **10c** and **10n**, and inactive compound **10m** are shown in Figure 3. The most active compound **10b** is located in the binding site of AChE as shown in Figure 3. It confirms the dual binding site activity. It is clear that the acridone moiety is linked to the bottom of active site through π–π interaction with Trp84 in the catalytic anionic site (CAS). Also there is another interaction between acridone and Phe331. Indeed, compound **10b** depicted a hydrogen-bond interaction between carbonyl group of acridone and hydroxyl group of Ser200 in catalytic triad site. In addition, 1,2,4-oxadiazole moiety exhibited π–π interaction with a phenyl group of Tyr121 in the peripheral anionic site (PAS). It should be noted that there was an interaction *via* hydro-

Table 2: DPPH antioxidant activities of the compounds **10a–d**, **10k**, and **10n**[a]

Entry	Compound **10**	Inhibition (%) (700 μg/mL)	EC_{50} (μg/mL)
1	**10a**	30.68 ± 0.92	>700
2	**10b**	38.06 ± 0.94	>700
3	**10c**	43.44 ± 0.27	>700
4	**10d**	84.16 ± 0.53	299
5	**10k**	35.37 ± 0.65	>700
6	**10n**	67.19 ± 0.20	400
7	**BHA**	95.70 ± 0.50	1.27

[a]Data are expressed as mean ± SE (three independent experiments).

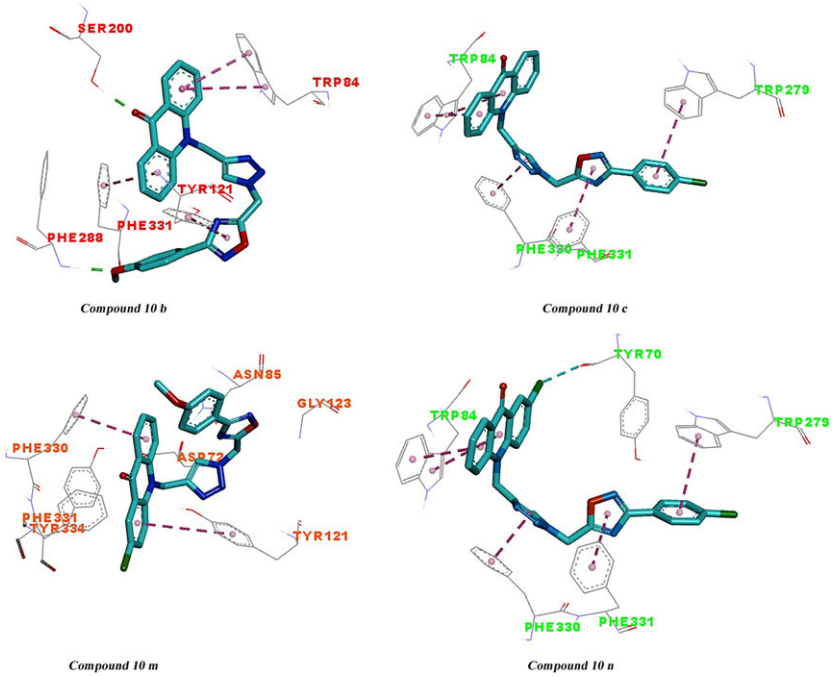


Figure 3: Docking study of compounds **10b**, **10c**, **10m**, and **10n**. π–π interactions and hydrogen bonds are represented as dashed lines and green lines, respectively.

gen bond between methoxy group of aryl and backbone NH atom. The docking study was also performed for two other active compounds **10c** and **10n** (Figure 3). Both compounds exhibited π–π interaction of acridone moiety with Trp84 and lacked the interaction between acridone carbonyl group and Ser200 as well as the interaction of 1,2,4-oxadiazole ring with Tyr121 in PAS. It suggested that these interactions play vital role in anti-AChE activity. Docking study of compounds **10c** and **10n** depicted two extra interactions including π–π interaction of 1,2,3-triazole with Phe330 and the π–π interaction of aryl group with Trp279, which were not observed in the docking study of compound **10b**. Also π–π interaction between 1,2,4-oxadiazole ring and Phe331 was observed for compounds **10c** and **10n**. Docking study of inactive AChEI compound **10m** showed significant difference lacking desirable interactions with active sites of enzyme. It seems that the interaction of acridone moiety with Phe330 and Tyr121 did not lead to the AChEI activity.

Consequently, the high activity of compound **10b** is indebted to multilateral interactions with the active site of AChE including catalytic site CS (both CAS and catalytic triad) and peripheral anionic site (PAS).

Conclusion

In conclusion, we have designed, synthesized, and evaluated novel acridone-1,2,4-oxadiazole-1,2,3-triazole hybrids as efficient anti-AChE agents. Among sixteen synthesized compounds, 10-((1-((3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl) acridin-9(10*H*)-one **10b** showed the highest activity for the inhibition of AChE (IC_{50} = 11.55 μM) being as potent as reference drug, rivastigmine. Comparing with our recent report on the anti-AChE activity of acridone linked to 1,2,3-triazoles [18], insertion of 1,2,4-oxadiazole moiety to acridone-1,2,3-triazole system did not lead to higher potency but presented dual binding site AChE inhibitors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. It includes all required procedures as well as ^{1}H NMR and ^{13}C NMR full characterization and spectra.