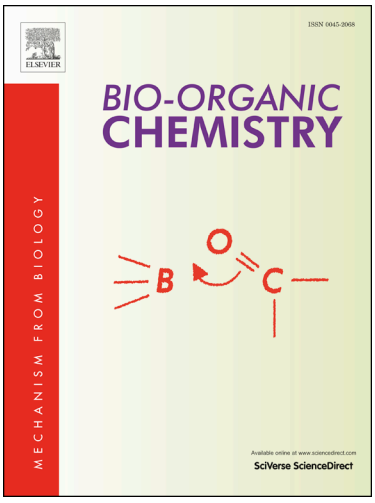

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Conventional and Microwave Prompted Synthesis, Antioxidant, Anticholinesterase Activity Screening and Molecular Docking Studies of New Quinolone-Triazole Hybrids

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Abstract. The synthesis of ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylates (**4, 5**) was performed via the reaction of corresponding anilines with diethyl ethoxymethylenemalonate under conventional and also microwave promoted conditions. The treatment of **4** and **5** afforded the corresponding hydrazides (**6** and **7**). These hydrazides were converted to the corresponding carbo(thio)amides (**9a-f** and **10a-e**) which were then subjected to an intramolecular cyclisation leading to the formation of quinolone-triazole hybrids (**11a-f** and **12a-e**). The newly synthesized compounds were screened for their biological activities such as antioxidant capacity (AC) and acetylcholinesterase Activity. Inhibition of cholinesterases is an effective method to curb Alzheimer's disease, a progressive and fatal neurological disorder. A series of some novel quinolone derivatives were designed, synthesized, and their inhibitory effects on AChE were evaluated. We obtained our compounds and determined their anticholinesterase activities according to the Ellman's method. **9b** and **10c** showed the best AChE inhibition with 0.48±0.02 and 0.52±0.07, respectively. Docking studies were performed for the most active compounds (**9b**, **10c**) and interaction modes with enzyme active sites were determined. As a result of these studies, a strong interaction between these compounds and the active sites of AChE enzyme was revealed.

Keywords. Quinolone, 1,2,4-triazole, molecular docking, antioxidant capacity, anticholinesterase, microwave irradiation.

1. INTRODUCTION

Quinolones are one of most important kind of synthetic antibacterial agents due to their well tolerability with excellent safety profile, favorable pharmacokinetic characteristics, broad antibacterial spectrum and good treatment effectiveness [1-3]. The quinolone structures include motifs exhibiting a wide variety of biological activities and they have been shown to possess various pharmacological activities such as anticonvulsant [4], antitumor [5] and

antiviral [6]. These compounds have also been extensively used to treat genitourinary infections, prostatitis, respiratory diseases, gastroenteritis, sexually transmitted diseases, as well as skin and soft tissue infections [7, 8]. Another biological activity of quinolones is ROS scavenging ability [9] and they have already been efficiently used for design of dual AChE inhibitor [10]. Acetylcholinesterase (AChE) is known as serine hydrolase enzyme managing on the hydrolysis of acetylcholine (ACh), which is a significant neurotransmitter for arrangement of cognition in animals [11-14].Inhibition of AChE leads to the rise of ACh levels in cholinergic synapses [15]. Thus, cholinesterase inhibitors are used in the treatment of various neuromuscular disorders which occur as a result of reduced cortical and hippocampal levels of ACh such as Alzheimer's disease (AD) which is a complex neurodegenerative disorder characterized by synapse dysfunction, neuronal death, and loss of memory and learning ability [16-18]. Current treatment approaches for AD continue to be principally symptomatic, with the major therapeutic AD, known as the cholinergic hypothesis, and specifically on cholinesterase inhibition [19, 20]. Various AChE inhibitors such as tacrine, donepezil, rivastigmine, and galantamine (**Figure 1**) have been used contemporarily for the symptomatic treatment of AD [21-24]. In recent years, novel cholinesterase inhibitors from natural resources or synthetic ways, which have coumarin, benzofuran, berberine, β-carboline, benzophenone, ferulic acid, naphthyridine, triazine, and quinolone scaffolds as the main pharmacophoric groups, have been reported [25-29].

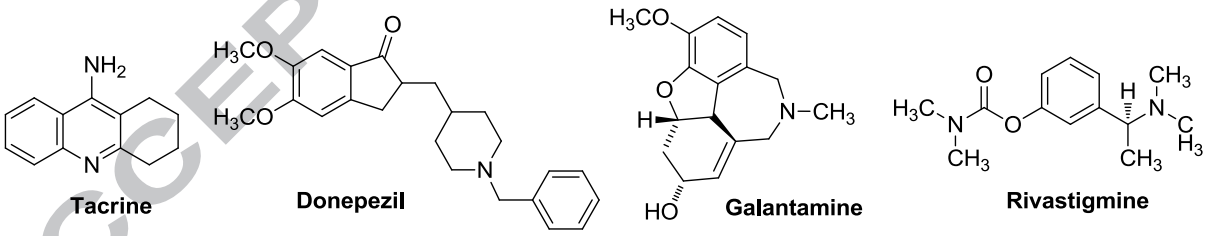


Figure 1. Molecular structure of tacrine, donepezil, rivastigmine, and galantamine

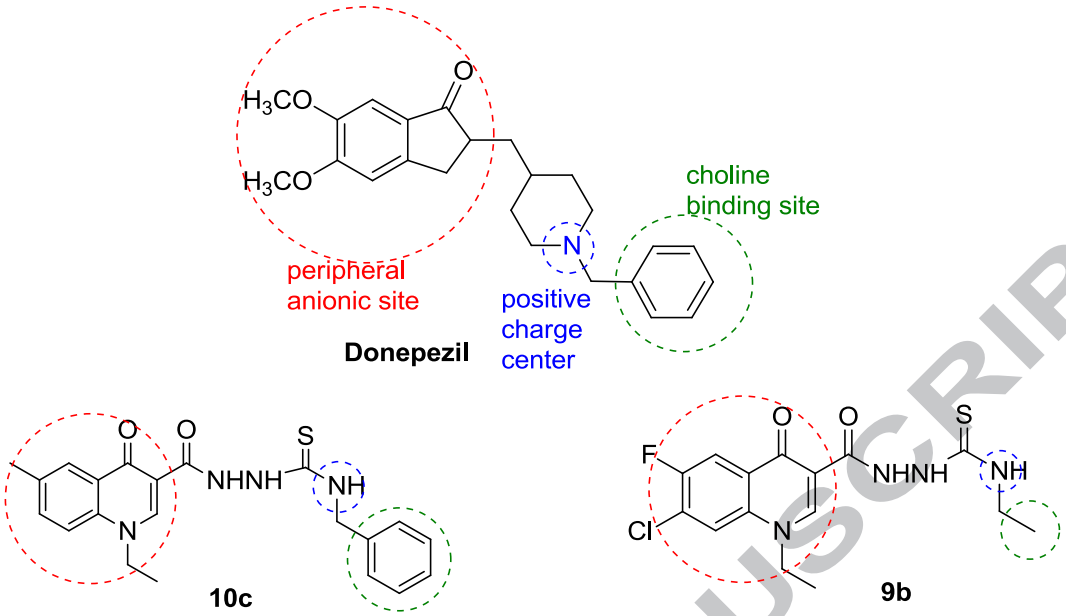


Figure 2. Structural hypothesis for AChE inhibitors.

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 Trp86, Tyr130, Tyr133, Ser203, Glu334, Tyr337, Phe338, His447 and the peripheral anionic site (PAS) consisting of Tyr72, Asp74, Tyr124, Trp286, Tyr341 [30, 31]. It has been reported that DNP interacts with both PAS and CAS and thus it is situated in the active site concordantly owing to the feature of dual binding site (DBS) [32, 33]. Analyses of binding modes of the DNP indicate that the benzyl moiety is in a pi-pi 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 pi-pi 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 [34, 35]. Docking studies were performed for the most active compounds **9b and 10c** 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 these compounds. Ideally, a cholinesterase inhibitor is expected to effectively interact with these sites (**Figure 2**). Using molecular docking approach, we tried to get insights into binding interactions of our derivatives in comparison with **9b, 10c** in the active site of enzyme and understand the facts that underlie the relationships between structural modifications on these ligands and their efficacy.

Due to its multifactorial pathogenesis, the current strategy for the development of new drugs for AD is focusing on multipotent molecules acting in a complementary manner, in different neural and biochemical targets, which could be more efficacious for AD patients [36]. Many quinolone derivatives have been studied for their biological activity in AD. They are used as radical scavengers, such as vitamin E (a tocopherol), as copper or iron chelators such as clioquinol, or as inhibitors of AChE such as tacrine. Following our studies on the synthesis of quinolone carried out in our laboratory [37], we now report the synthesis of 4-oxo-1,4-dihydroquinoline-3-carbonyl)hydrazine carbothioamide, 4-oxo-1,4-dihydroquinoline-3-carbonyl)hydrazine carboxamide 3-(5-mercapto-4H-1,2,4-triazol-3-yl)quinolin-4(1*H*)-one and 3-(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)quinolin-4(1*H*)-one. These new compounds were tested as AChE inhibitors and investigated antioxidant capacity. Finally, and thanks to molecular docking, we have identified the interactions with AChE.

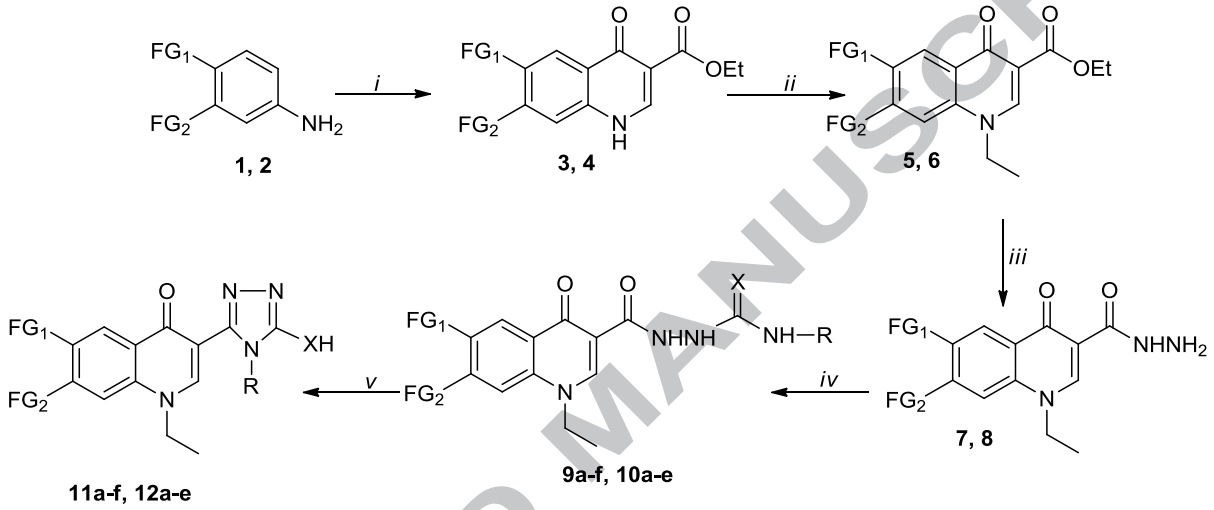
2. Results and discussion

2.1. Chemistry

In the present study, the ecofriendly synthesis, acetylcholinenzyme inhibition and antioxidant activity screening, and molecular docking studies of new hybrid molecules was contemplated. The structures of newly synthesized compounds were established on the basis of physicochemical, elemental analysis and spectral data (FT IR, ^{1}H NMR, ^{13}C NMR and EI-MS).

Compounds **3**, **5** and **7** were synthesized in the following procedure [38]. The complete conversion of hydrazides **8** was observed after microwave irradiation at 100 W for 25 min. Synthesis of corresponding carbo(thio)amides (**9a-f**) and (**10a-e**) was accomplished by nucleophilic attack of the **7** and **8** hydrazide compounds to the alkyliso(thio) cyanates (Scheme 1). The reaction conditions were investigated in ethanol under reflux and also microwave irradiation conditions to maximize the yield of the product. To optimize reaction conditions, the synthesis of compound **9a** was chosen as model reaction and microwave (MW) irradiation was implemented at different power values of 50, 100, 150, 200 and 250 W (the progress of reaction was monitored by TLC) (Table 1). When compared to conventional method, microwave irradiation reduced the reaction time from 5 h to 10 min. It is notable to underline that shorter reaction time, lower microwave energy or very high microwave energy power give rise to lower conversion rate, while increasing reaction time or MW power resulted in fragmentation of the target product as revealed by TLC analysis. In the FT IR

spectra, the signal originated from –C=S function of compounds **9a-d** and **10a-d**appeared between 1223-1263 cm^{-1}. In the ^{13}C NMR spectra of these compounds (**9a–f**, **10a-e**), –C=S or –C=O function resonated between 153.05–175.37 ppm. Further evidence for the formation of carbo(thio)amides was the presence of three –NH- signals (exchangeable with D_2O) at 7.04–12.75 ppm in the ^1H NMR spectra. The elemental analysis data of carbon, hydrogen and nitrogen were formed to be satisfactory and within the permissible limit error.



1, 3, 5, 7, 9, 11: FG_1= -Cl, FG_2= -F; **2, 4, 6, 8, 10, 12**: FG_1= $-CH_3$, FG_2= -H

9a-12a: X=S, R= $-C_6H_5$; **9b-12b**: X=S, R= $-CH_2C_6H_5$; **9c-12c**: X=S, R= $-C_2H_5$; **9d-12d**: X=S, R= $-C_6H_4F$(*-4*); **9e-12e**: X=O, R= $-C_6H_5$; **9f, 11f**: X=O, R= $-CH_2C_6H_5$

Scheme 1. Synthetic pathway for the preapation of new compounds. *i*: diethyl ethoxymethylenemalonate, Ph_2O; *ii*: K_2CO_3, C_2H_5Br; *iii*: H_2NNH_2; *iv*: RNCX; *v*: NaOH, H_2O-EtOH

The synthesis of triazoles (**11a–f**, **12a-e**) was achieved by the treatment of NaOH in ethanol-water under reflux condition as well as under microwave conditions in order to introduce the 1,2,4-triazole nucleus to piperazine skeleton, because, according to molecular hybridization concept, more efficacious bioactive compounds can be designed by combining two or more biologically active heterocyclic systems together in a single molecular framework [39, 40]. In order to maximize product yield in the microwave irradiation method, the optimization operation at power values of 50, 100, 150, 200, 250 was carried out by selecting compound **11a** while keeping the temperature at 150 ^oC (Table 1). The best product yield was obtained at 200 Watt. The conversion of carbo(thio)amides to triazoles did not take place completely at low temperature and low power values, while high temperature and power values were applied, compounds started to decompose and product yields decreased. Furthermore, compared with conventional thermal heating, microwave irradiation decreased

the reaction time from 6–12 h to 20 min and increased the yields from 71%–82% to 79%–90% (Table 2).

Table 1. Optimization conditions for **9a** and **11a** in microwave irradiation

Power (W)		**Time (min)**		**Yield (%)**	
9a	**11a**	**9a**	**11a**	**9a**	**11a**
50	50	5	5	70	50
100	100	10	10	**84**	57
150	150	20	20	78	68
200	200	30	30	75	**81**
250	250	40	40	60	74

Table 2. Time, power and yield for the synthesized compounds.

Compd.	**Microwave irradiation method**			**Conventional method**		**Melting point** ^{o}C	**Crystallization Solvent**
	Time (min)	**Power (W)**	**Yield (%)**	**Time (h)**	**Yield (%)**		
4	5	225	86	1	76	143-144	DMF:H_2O (1:3)
6	20	50	84	10	79	>300	Ethyl acetate
8	25	100	79	10	71	260	Ethanol
9a	20	100	84	3	76	223-224	Ethyl acetate
9b	20	100	86	4	80	231-232	Ethyl acetate
9c	20	100	80	6	72	244	Ethyl acetate
9d	20	100	87	3	79	216-217	Ethyl acetate
9e	25	100	84	5	81	245-246	Methanol
9f	25	100	79	6	69	241-242	Methanol
10a	20	100	82	4	74	286	Acetone
10b	20	100	79	5	69	236-237	Ethanol
10c	20	100	86	5	78	236	Ethanol
10d	20	100	88	4	80	209-210	Methanol

10e	25	100	80	6	66	211-212	Methanol
11a	30	200	81	8	70	>300	Ethanol
11b	30	200	80	9	75	280-281	Methanol
11c	30	200	89	7	82	254	Methanol
11d	30	200	86	6	80	>300	Ethanol
11e	35	200	82	7	76	>300	Acetone
11f	35	200	78	10	71	>300	Methanol
12a	30	200	83	7	76	>300	Ethyl acetate
12b	30	200	81	9	72	228	Ethanol
12c	30	200	90	9	78	253-254	Ethyl acetate
12d	35	200	82	11	81	223-224	Ethyl acetate
12e	35	200	79	12	73	227	Methanol

It is well known that 1,2,4-triazol-3-(thi)ol derivatives can exist as mercapto-thioxo (or enol-keto) tautomeric forms. The –SH proton due to mercapto form resonates at about 13–14 ppm, while NH signal originated from thioxo tautomeric form appears between 9–12 ppm as D_2O exchangeable signals [41-45]. In the FT-IR spectra of compounds **11a-d** and **12a-d**, the presence of stretching bands ranging from 2830 cm^{-1} to 2853 cm^{-1} originated from -SH function supported the mercapto form for these compounds. The -SH proton of these compounds resonated between 13.85 and 14.13 ppm in the ^1H NMR spectra. On the other hand, our previous studies showed that the compounds having 5-oxo-4-alkyl-4,5-dihydro-1*H*-1,2,4-triazoles exist almost in keto form [37,41-45]. Another evidence supporting the formation of the targeted compounds, **11a-f** and **12a-e** were obtained by the appearance of [M+1], [M+2], [M+Na] and/or [M+K] ion peaks at corresponding *m/z* values confirming their molecular masses; and these compounds have given elemental analysis results consistent with the proposed structures.

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 Trp86, Tyr130, Tyr133, Ser203, Glu334, Tyr337, Phe338, His447 and the peripheral anionic site (PAS) consisting of Tyr72, Asp74, Tyr124, Trp286, Tyr341, [45, 46]. It has been reported that DNP interacts with both PAS and CAS and thus it is situated in the active site concordantly owing

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2.2. Biological Activity

2.2.1. Antioxidant Activity

To determine antioxidant capacity (AC, µmol TE/g) value of the newly synthesized compounds, DPPH, FRAP and CUPRAC assays were assayed. Compounds **10c** (3912.33±4.71 µmol TE/g), followed **9** and **10e**, **12e** (3460.55±8.35 µmol TE/g), followed **9e** (3379.89±13.00 µmol TE/g), **11e** (3336.03±16.00 µmol TE/g) and **9b** (3219.07±3.00 µmol

TE/g) for FRAP, **10e** (7241.13±28.53µmol TE/g), followed **10c** and **11a, 9c** (6266.43±50.12 µmol TE/g), followed **8** (6185.61±21.01 µmol TE/g), **11e** (5194.36±15.66 µmol TE/g) and **9b** (4392.85±11.01 µmol TE/g) for CUPRAC. Trolox was used as standard for DPPH radical scavenging method and the results are given SC_{50} value. According to this method, **9b**, **11e**, **10c**, **9e**, **10e**, **8 and 9c** had the SC50 values, respectively. On the other hand, **12b** (123.17±1.18 µmol TE/g) and **10b** (949.83±3.54 µmol TE/g) for FRAP, **9d** (491.86±1.01 µmol TE/g) and **9d** (3.86±0.02) had the lowest AC values among the synthesized compounds (Table 5). In the table, for **12c** no AC value and acetylcholinesterase activity was determined. Also, while **10b** had no activity for AChE and CUPRAC, **12b** had no activity for CUPRAC, DPPH and AChE.

Table 5. Antioxidant capacity (AC) values and acetylcholinesterase activity of 23 synthesized novel compound.

Compound	FRAP (µmol TE/g)	DPPH SC_{50}	CUPRAC (µmol TE/g)	EeAChE Activity IC_{50} (mg/mL)
8	**3711.50±1.18**	**0.12±0.01**	**6185.61±21.01**	**0.75±0.05**
9a	1606.16±7.00	3.12±0.07	1860.79±3.54	5.26±0.01
9b	**3219.07±3.00**	**0.07±0.00**	**4392.85±11.01**	**0.48±0.02**
9c	1396.42±2.00	**0.24±0.00**	**6266.43±50.12**	**0.81±0.01**
9d	1378.99±5.00	3.86±0.02	491.86±1.01	-
9e	**3379.89±13.00**	**0.10±0.00**	**3272.21±18.69**	**0.98±0.02**
9f	1436.38±4.00	**0.36±0.00**	**4247.32±29.12**	**0.76±0.01**
10a	1535.67±2.36	1.24±0.03	3842.64±10.85	5.51±0.08
10b	949.83±3.54	2.21±0.07	-	-
10c	**3912.33±4.71**	**0.09±0.01**	**6987.45±11.14**	**0.52±0.07**
10d	1370.67±7.07	1.39±0.04	2896.12±22.14	6.85±0.07
10e	**3770.67±9.43**	**0.11±0.00**	**7241.13±28.53**	**0.68±0.02**
11a	1042.74±6.00	**0.39±0.00**	**6723.57±5.05**	1.86±0.03

11b	1448.16±4.00	1.13±0.01	1444.57±10.30	1.54±0.03
11c	987.07±4.00	2.18±0.03	2709.29±5.09	3.43±0.01
11d	1219.30±3.00	**0.98±0.00**	**3165.43±8.08**	2.47±0.01
11e	**3336.03±16.00**	**0.09±0.00**	**5194.36±15.66**	**0.57±0.02**
11f	1187.12±4.00	3.15±0.02	2234.17±5.34	2.25±0.01
12a	1233.46±3.67	2.01±0.02	2348.24±9.65	3.29±0.05
12b	123.17±1.18	-	-	-
12c	-	-	-	-
12d	1454.00±9.43	1.19±0.03	2965.13±32.12	4.18±0.01
12e	**3460.55±8.35**	**0.54±0.00**	**4278.15±22.75**	**0.64±0.02**
Trolox		0.04±0.00		
Donepezil				0.03±0.00

2.2.2. Anticholinesterase activity

*In vit*ro AChE inhibition of synthesized compounds was determined by the method of Ellman et al. [48] using donepezil as reference and the activity data of the compounds were summarized as IC_{50} value ± standard deviation (SD) values at 100 µg/ml concentration in Table 3. None of the test compounds showed higher AChE inhibition than donepezil. All compounds showed inhibitory activity against AChE. **9b** and **10c** showed the best AChE inhibition with 0.48±0.02 and 0.52±0.07, respectively. In the structure of the 4-member group compound we have synthesized, there are groups that can interact with the choline binding site (CAS) and peripheral anionic site (PAS) and nitrogen atoms that can be protonated. This allows the compounds to interact with the AChE enzyme and thus to show activity. It is also evidenced by molecular modeling studies in which the compounds are linked to the CAS and PAS regions of the enzyme. The compounds with the highest activity are in group **9**. This indicates that the hydrazide structure, including the protonatable nitrogen atom between the quinolone ring attached to the PAS and the group bound to the CAS, contributes to the activity more than the triazole ring. The electron attracting halogen substituents added to the quinolone ring did not change significant activity in the molecule.

Table 3. AChE inhibition values (IC_{50}) of the title compounds at 100 µg/mL

Compound	AChE Inhibition IC_{50} (mg/mL) ± SD*
8	0.75±0.05
9a	5.26±0.01
9b	0.48±0.02
9c	0.81±0.01
9d	**
9e	0.98±0.02
9f	0.76±0.01
10a	5.51±0.08
10b	**
10c	0.52±0.07
10d	6.85±0.07
10e	0.68±0.02
11a	1.86±0.03
11b	1.54±0.03
11c	3.43±0.01
11d	2.47±0.01
11e	0.57±0.02
11f	2.25±0.01
12a	3.29±0.05

12b	**
12c	**
12d	4.18±0.01
12e	0.64±0.02
Donepezil	0.03±0.00

*Standart deviation, **IC_{50} value not detected

The fluorine atom in the 4th position of the phenyl ring, which is thought to be located in the CAS, decreases activity markedly (**9d**, **11d**, **10d**, **12d**). Compounds which are attached to the phenyl ring of the nitrogen atom which may be protonated are generally more active than ethyl chain-bearing derivatives. This suggests that the phenyl ring enhances the interaction of van der Waals interactively with this region. It has been observed that activity is generally higher in the nitrogen atom bearing benzyl structures bound by a methyl bridge instead of the compounds directly attached by the phenyl ring. This suggests that the unpaired electrons of the nitrogen atom bound to the phenyl ring are introduced into the ring to resonance and that the nitrogen is less bound to the enzyme by reducing the probability of protonation. Molecular modelling studies also demonstrate this decrease in the phenyl ring relative to the benzyl.

2.2.3. Molecular docking studies

Glide predicted good fit for **9b, 10c** in the active site of human AChE (PDB ID: 4EY7) along the gorge with some characteristic interactions previously defined for some known inhibitors (Figure 3).

10c **9b**

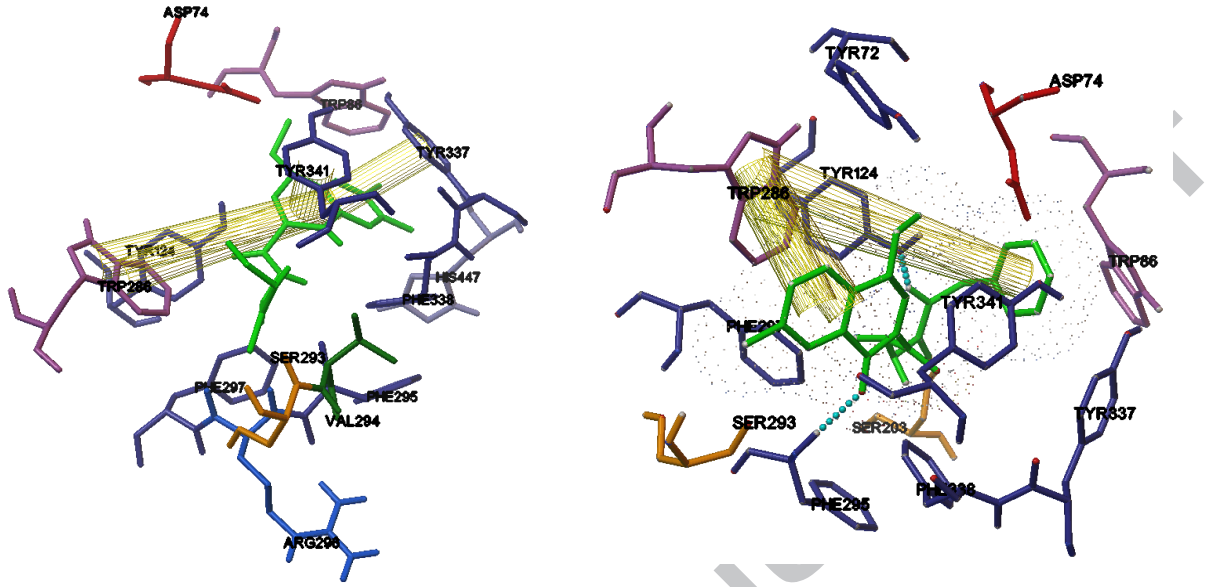

Figure 3. Binding mode of compound **9b** and **10c** into the AChE (PDB ID: 4EY7) binding cavity. For clarity, only interacting residues are displayed. Ligand (green), interacting key residues (RasMol amino color) are represented as stick models. H-bonds are shown as spheres (turquoise) lines and π- π interactions are shown as cylinders (yellow).

The 1-ethylquinolin-4(1*H*)-one-3-yl moiety of **9b** and **10c** occupied the choline-binding pocket and engaged in strong pi-pi interactions against Trp286 aromatic side chain furthermore weak close van der Waals interactions with Tyr72, Asp74, Tyr124, Tyr341, Trp286 in the PAS region and Trp86, Ser203, Tyr337, Phe338in the CAS region. These residues are reportedly involved in ligand-receptor complexes of tacrine, galantamaine, huperzine A, and donepezil [47, 49]. Donepezil was reported to form water-mediated H-bonds with Tyr337 and Tyr341 via its quaternary N of the piperidine ring. Instead, via its carbonyl oxygen of the quinolin-4(1*H*)-one ring, **10c** formed a direct H-bond to Phe295 backbone NH. The latter interaction was reportedly observed with the carbonyl oxygen of the indanone moiety of donepezil [47]. The quinolin-4(1*H*)-one ring of **10c** occupied the lower PAS and π-π interactions with Tyr341 aromatic side chain (Table 4).

Table 4. Molecular docking binding scores and binding interactions of compounds **9b** and **10c,** and within the AChE (PDB ID: 4EY7) active site. Residues participating in hydrogen bonds and close van der Waals contacts (<3,75 Å) with the inhibitors are shown and distances detected by selecting Closer Than VDW algorithms.

			H-bonds		**Close van der Waals contacts**		
Comp.	**Estimated Free Energy of Binding (kcal/mol)**	**Estimated Inhibition Constant, Ki, Temp. = 298.15 K**	**Residue**	**Distance (Å)**	**Residue**	**Distance (Å)**	**Number of Interaction**
9b	-10.25	30.74 nM	Tyr124 (C=O--) Phe295 (N-H--)	1,766 2,12	Tyr72 Asp74 Trp86 Tyr124 Ser203 Trp286^{+} Ser293 Phe297 Tyr337 Phe338 Tyr341	3,318-3,638 in 3,094-3,598 range 3,677-3,7 in 1,776-3,234 range 2,827 in 3,18-3,602 range 2,863-3,657 in 2,214-3,387 range in 3,232-3,434 range in 2,847-3,452 range in 2,914-3,592 range	2 3 2 5 1 7 2 4 3 6 9
10c	-8.38	716.31 nM	-		Asp74 Trp86 Tyr124 Trp286^{+}	2,929-3,021 3,671 in 2,622-3,094 range 3,027	2 1 6 1

				Ser293	3,664	1
				Val294	in 3,17-3,568 range	3
				Phe295	2,767	1
				Arg296	2,509-3,249	2
				Phe297	2,766	1
				Tyr337[+]	in 3,086-3,678 range	6
				Phe338	in 2,941-3,676 range	7
				Tyr341[+]	in 2,784-3,603 range	9
				His447	in 2,85-3,169 range	3

[+] : π- π interactions; nM: nanomolar

Binding modes of **9b, 10c** and co-crystal ligand Donepezil superimposed with the rest in AChE active side that shown **9b**'s and **10c**'s binding mode and interactions in human AChE active side very similar to E20 (Figure 4).

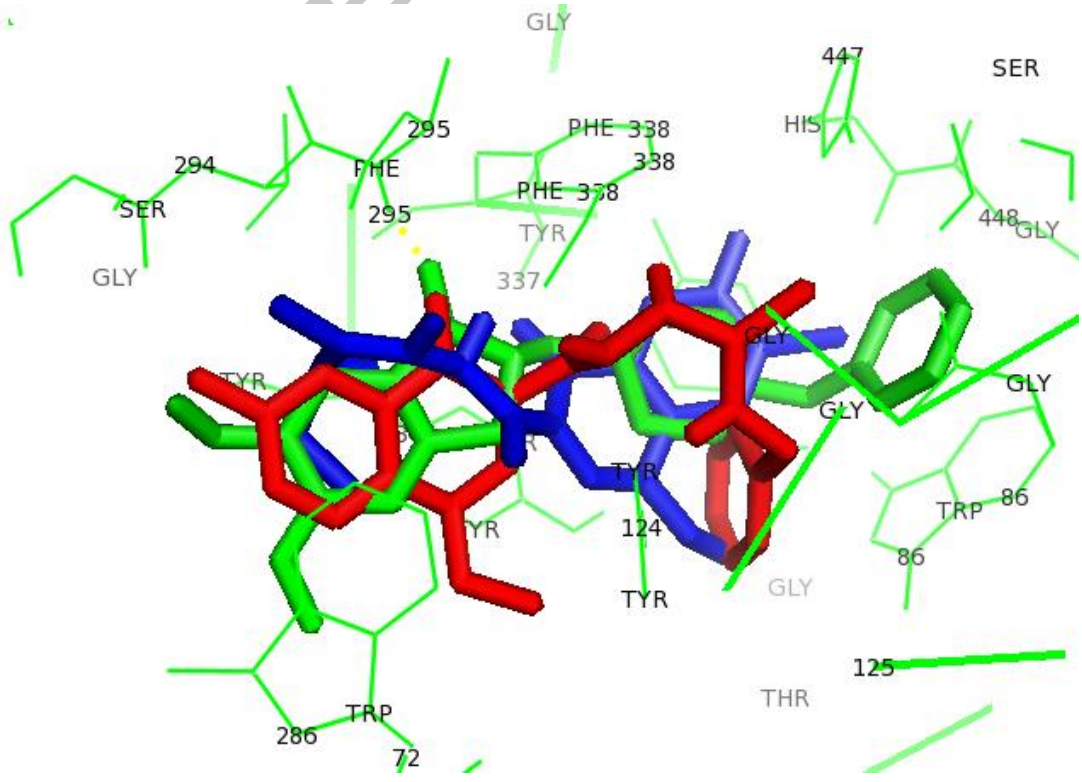

Figure 4. Binding modes of **9b**, **10c**, and co-crystal ligand Donepezil (Pdb ID: E20) superimposed with the rest in AChE active site. View of the active site show ligand E20 and binding-site interacting residues as green, **9b** as red, **10c** as blue by PyMOL (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

3. Experimental

3.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, *J* values are given in Hz. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within -0.4 % of the theoretical values. The mass spectra were obtained on a Quattro EI-MS (70 eV) Instrument. Microwave irradiated reactions were performed in a CEM Discovery mono-mode synthesis reactor.

3.1.1. General Method for the Preparation of Compounds 3 and 4

Method 1. The mixture of the corresponding aniline (10 mmol) and diethyl ethoxymethylene malonate (10 mmol) in diphenyl ether (10 mL) was heated at 250 ^{o}C in an oil bath for 1 h. On cooling the mixture to room temperature, a white solid formed. This crude product was filtered off, washed with hexane and purified through recrystallization from DMF to give corresponding compound.

Method 2. The mixture of the corresponding aniline (10 mmol) and diethyl ethoxymethylene malonate (10 mmol) in diphenyl ether (4 mL) was irradiated in monomod microwave reactor in closed vessel at 225 ^{o}C, 200 W, for 5 min. The white solid formed was filtered off, washed with hexane and purified through recrystallization from DMF to give corresponding compound.

Ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (3) [38]: FT-IR(υ_{max}, cm^{-1}): 3389 (NH), 3065 (ar-CH), 1687 (C=O). Mp. 138 ^{o}C.

3.1.1.1. Ethyl 6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (4)

Elemental analysis for $C_{13}H_{13}NO_3$, calculated (%), C, 67.52; H, 5.67; N, 6.06, found (%), C, 67,12; H, 5,28; N, 5,99. FT-IR (υ_{max}, cm^{-1}): 3152 (NH), 3094 (ar-CH), 1690 (C=O), 1581 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.28 (t, 3H, *J*= 4 Hz, CH_3), 2.43 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.00 (s, 1H, ArH), 7.39 (s, 1H, ArH), 7.96 (s, 1H, CH), 8.49 (s, 1H, CH), 12.19 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 14.80 (CH_3), 21.26 (CH_3), 59.94 (CH_2), arC: [109.98 (C), 119.07 (CH), 123.89 (CH), 127.67 (C), 130.50 (CH), 134.59 (C), 137.42 (C)], 144.84 (CH), 165.39 (C=O), 173.76 (C=O). EI MS *m/z* (%): 186.11 (25), 232.23 ($[M+1]^+$, 58), 254.19 ($[M+Na]^+$, 100).

3.1.2. General Method for The Synthesis of Compounds 5 and 6

Method 1. A mixture of compound **3** or **4** (10 mmol), K_2CO_3 (50 mmol) and ethyl bromide (50 mmol) in 20 mL DMF was heated at 90 ^{o}C for 10 h. After evaporating the solvent under reduced pressure to dryness an oily mass appeared. This was treated with water and extracted with 10 mL of chloroform three times. The combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was recrystallized from ethyl acetate to afford the corresponding compound.

Method 2. A mixture of compound **3** or **4** (10 mmol), K_2CO_3 (50 mmol) and ethyl bromide (50 mmol) in 5 mL of DMF was irradiated in monomod microwave reactor in closed vessel at 80 ^{o}C, 50 W for 20 min. After evaporating the solvent under reduced pressure to dryness an oily mass formed. This was dissolved in water and extracted with 10 mL of chloroform three times. The combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was recrystallized from ethyl acetate to afford the corresponding compound.

Ethyl 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (5) [38]: FT-IR (υ_{max}, cm^{-1}): 3048 (ar-CH), 1678 (C=O). Mp. >300^{o}C.

3.1.2.1. Ethyl 1-ethyl-6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (6)

Elemental analysis for $C_{15}H_{17}NO_3$, calculated (%), C, 69.48; H, 6.61; N, 5.40, found (%), C, 69,18; H, 6,42; N, 5,11. FT-IR (υ_{max}, cm^{-1}): 3050 (ar-CH), 1675 (C=O), 1595 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.22 (t, 3H, *J*= 12 Hz, CH_3), 1.38 (t, 3H, *J*= 8 Hz, CH_3), 2.38 (s,

3H, CH_3), 4.10 (q, 2H, *J*=16 Hz, CH_2), 4.23 (q, 2H, *J*=12 Hz, CH_2), 6.70 (d, 1H, *J*=8 Hz, ArH), 7.27 (dd, 1H, *J*=8 Hz, ArH), 7.71 (d, 1H, *J*=5 Hz, ArH), 8.33 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 12.40 (CH_3), 14.69 (CH_3), 21.20 (CH_3), 48.85 (CH_2), 61.50 (CH_2), arC: [109.25 (C), 118.80 (CH), 127.78 (C), 129.04 (CH), 132.59 (CH), 133.87 (C), 140.82 (C)], 147.60 (CH), 164.77 (C=O) , 174.70 (C=O). EI MS *m/z* (%): 282.33 ($[M+Na]^+$, 31), 298.30 ($[M+K]^+$, 100).

3.1.3. General method for the synthesis of compounds 7 and 8

Method 1. NH_2NH_2 (25 mmol) was added the solution of the corresponding compound **5** or **6** (10 mmol) in ethanol and the mixture was refluxed for 10 h. The product obtained upon evaporating the solvent under reduced pressure was washed with water and recrystallized from ethanol to afford the desired product.

Method 2. NH_2NH_2 (25 mmol) was added the solution of the corresponding compound **5**or **6** (10 mmol) in ethanol and the mixture was irradiated in monomod microwave reactor in closed vessel at 100 ^{o}C, 80 W for 25 min. The product obtained upon evaporating the solvent under reduced pressure was washed with water and recrystallized from ethanol to afford the desired product.

7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (7) [38]: FT-IR (υ_{max}, cm^{-1}): 3323 (NH), 3248 and 3212 (NH_2), 3013 (ar-CH). Mp. 265^{o}C.

3.1.3.1. 1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (8)

Elemental analysis for $C_{15}H_{15}N_3O_2$, calculated (%), C, 63.66; H, 6.16; N, 17.13; found (%), C, 63,06; H, 6,09; N, 6,07. FT-IR (υ_{max}, cm^{-1}): 3319 (NH), 3198-3170 NH_2), 3047 (ar-CH), 1650 (C=O), 1590 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.31 (t, 3H, *J*= 16 Hz, CH_3), 2.0 (bs, 2H, NH_2), 2.34 (s, 3H, CH_3), 4.62 (q, 2H, *J*=16 Hz, CH_2), 6.68 (d, 1H, *J*=8 Hz, ArH), 7.29 (d, 1H, *J*=8 Hz, ArH), 7.38 (s, 1H, ArH), 8.0 (s, 1H, NH), 8.96 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 14.37 (CH_3), 20.70 (CH_3), 48.21 (CH_2), arC: [113.50 (CH), 117.51 (C), 125.73 (C), 126.63 (CH), 131.47 (C), 134.255 (CH), 138.59 (C), 181.80 (C=O)], 141.95 (CH), 162.14 (C=O). EI MS *m/z* (%): 214.15 (90), 246.16 ($[M+1]^+$, 88), 286.29 (100).

3.1.4. General method for the synthesis of compounds 9a-f and 10a-e

Method 1. A mixture of compound **7** or **8** (10 mmol) and the corresponding iso(thio)cyanate (15 mmol) in ethanol was refluxed for 3-6 h (Table 2). After evaporating the solvent under

reduced pressure, a solid obtained. The crude product was purified by recrystallisation from an appropriate solvent.

Method 2. A mixture of compound **7** or **8** (10 mmol) and the corresponding iso(thio)cyanate (15 mmol) was irradiated in monomod microwave reactor in closed vessel at 100 ^{o}C, 100 Watt for 20-25 min. The solid obtained upon the evaporation of solvent under reduced pressure was purified by crystallization from an appropriate solvent.

3.1.4.1. *N*-Phenyl-2-(7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl) hydrazinecarbothioamide (9a)

Elemental analysis for $C_{19}H_{16}ClFN_4O_2S$, calculated (%), C, 54.48; H, 3.85; N, 13.38; found (%), C, 54,16; H, 3,55; N, 13,15. FT-IR (υ_{max}, cm^{-1}): 3277 (NH), 3070 (ar-CH), 1645 (C=O), 1552 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.22 (t, 3H, *J*=8 Hz, CH_3), 4.74 (s, 2H, CH_2), 7.23 (s, 1H, *J*=7 Hz, ArH), 7.31 (s, 3H, ArH), 7.61 (s, 2H, ArH), 8.06 (s, 1H, ArH), 8.58 (bs, 1H, NH), 8.73 (s, 1H, CH), 9.51 (bs, 1H, NH), 11.58 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm**):** 15.73 (CH_3), 48.47 (CH_2), arC: [117.78 (CH), 126.09 (CH), 127.54 (C), 128.86 (2CH), 131.55 (CH), 134.89 (2CH), 135.43 (C), 137.38 (C), 139.76 (C), 142.34 (C), 153.85-156.88 (d_{C-F}, *J*=303 Hz, C)], 147.39 (CH), 157.31 (C=O), 171.73 (C=S), 175.38 (C=O). EI MS *m/z* (%): 441.34 ($[M+Na]^+$, 100).

3.1.4.2. *N*-Benzyl-2-(7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl) hydrazinecarbothioamide (9b)

Elemental analysis for $C_{20}H_{18}ClFN_4O_2S$, calculated (%), C, 55.49; H, 4.19; N, 12.94; found (%), C, 55,21; H, 4,02; N, 12,76. FT-IR (υ_{max}, cm^{-1}): 3255 and 3146 (NH), 3030 (ar-CH), 1668 (C=O), 1544 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.37 (t, 3H, *J*=16 Hz, CH_3), 4.57 (d, 2H, *J*=8 Hz, CH_2), 4.73 (d, 2H, *J*=4 Hz, CH_2), 7.23 (d, 1H, *J*=7 Hz, ArH), 7.30 (d, 4H, *J*=7 Hz, ArH), 8.16 (d, 1H, *J*=8 Hz, ArH), 8.31 (d, 1H, *J*=7 Hz, ArH), 8.60 (bs, 1H, NH), 8.94 (s, 1H, CH), 9.59 (bs, 1H, NH), 11.17 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm):15.08 (CH_3), 47.31 (CH_2), 49.22 (CH_2), arC: [110.94 (C), 112.83 (d, *J*=22 Hz, CH), 120.87 (CH), 121.81 (CH), 127.11 (CH), 127.58 (CH), 127.90 (d, *J*=6 Hz, C), 128.55 (CH), 136.19 (C), 139.70 (C), 145.34 (C), 153.75-156.21 (d_{C-F}, *J*=246 Hz, C)], 148.92 (CH), 160.64 (C=O), 174.18 (C=S), 179.26 (C=O). EI MS *m/z* (%): 395.39 (32), 433.89 ($[M+1]^+$, 100).

3.1.4.3. 2-(7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl)-*N*-ethyl hydrazinecarbothioamide (9c)

Elemental analysis for $C_{15}H_{16}ClFN_4O_2S$, calculated (%), C, 48.58; H, 4.35; N, 15.11; found (%), C, 48,20; H, 4,19; N, 14,98. FT-IR (υ_{max}, cm^{-1}): 3267 and 3202 (NH), 3029 (ar-CH), 1677 (C=O), 1545 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.08 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.29 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 7.99 (s, 1H, ArH), 8.17 (d, 1H, *J*=8 Hz, ArH), 8.30 (s, 1H, CH), 8.91 (s, 1H, NH), 9.47 (bs, 1H, NH), 11.33 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 14.85 (CH_3), 15.107 (CH_3), 39.03 (CH_2), 49.24 (CH_2), arC: [110.92 (C), 112.82 (d, *J*=22 Hz, CH), 120.86 (CH), 126.80 (d, *J*=20 Hz, C), 127.90 (d, *J*=6 Hz, C), 136.20 (C), 153.75-156.21 (d_{C-F}, *J*=246 Hz, C)], 148.91 (CH), 162.31 (C=O), 174.17 (C=S), 181.10 (C=O).EI MS *m/z* (%): 403.46 ($[M+Na]^+$, 100), 404.40 ($[M+1+Na]^+$, 26).

3.1.4.4. 2-(7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl)-*N*-(4-fluoro phenyl) hydrazinecarbothioamide (9d)

Elemental analysis for $C_{19}H_{15}ClF_2N_4O_2S$, calculated (%), C, 52.24; H, 3.46; N, 12.82; found (%), C, 52,08; H, 3,21; N, 12,69. FT-IR (υ_{max}, cm^{-1}): 3282 and 3158 (NH), 3039 (ar-CH), 1686 (C=O), 1542 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.21 (t, 3H, *J*=8 Hz, CH_3), 4.10-4.13 (m, 2H, CH_2), 6.75 (d, 1H, *J*=4 Hz, ArH), 7.04 (s, 1H, ArH), 7.08 (d, 1H, *J*=7.1 Hz, ArH), 7.27 (t, 2H, *J*=7.2 Hz, ArH), 7.45 (d, 1H, *J*=8 Hz, ArH), 8.44 (s, 1H, CH), 8.65 (bs, 1H, NH), 9.14 (bs, 1H, NH), 9.84 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm**):** 14.51 (CH_3), 49.40 (CH_2), arC: [114.17 (CH), 116.39 (C), 117.88 (2CH), 119.17 (2CH), 121.18 (d, *J*=21.0 Hz, C), 127.26 (C), 128.09 (C), 137.16 (C), 152.22-154.55 (d_{C-F}, *J*=233 Hz, C), 155.58-157.91 (d_{C-F}, *J*=233 Hz, C)], 147.56 (CH), 162.06 (C=O), 175.29 (C=S), 179.94 (C=O). EI MS *m/z* (%): 196.36 (42), 205.37 (100), 459.40 ($[M+Na]^+$, 78).

3.1.4.5. 2-(7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl)-*N*-phenyl hydrazinecarboxamide (9e)

Elemental analysis for $C_{19}H_{16}ClFN_4O_3$, calculated (%), C, 56.65; H, 4.00; N, 13.91; found (%), C, 56,31; H, 3,89; N, 13,76. FT-IR (υ_{max}, cm^{-1}): 3301 (NH), 3048 (ar-CH), 1677 (C=O), 1537 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.41 (s, 3H, CH_3), 4.57 (d, 2H, *J*=8 Hz, CH_2), 6.96 (s, 1H, ArH), 7.26 (s, 1H, ArH), 7.46 (d, 1H, *J*=8 Hz, ArH), 8.16 (d, 2H, *J*=8 Hz, ArH), 8.28 (s, 1H, CH), 8.84 (s, 1H, NH), 8.96 (s, 1H, NH), 12.53 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6,

δppm): 15.04 (CH_3), 49.36 (CH_2), arC: [111.24 (C), 112.79 (d, *J*=22 Hz, CH), 118.82 (CH), 120.80 (2CH), 122.33 (CH), 126.75 (d, *J*=20 Hz, 2C), 129.12 (2CH), 136.19 (C), 140.08 (C), 153.69-156.15 (d_{C-F}, *J*=246 Hz, C)], 149.14 (CH), 154.11 (C=O), 160.30 (C=O), 174.62 (C=O).EI MS *m/z* (%): 102 (41), 314 (100), 316 (46), 402 ($[M]^+$, 26).

3.1.4.6. *N*-Benzyl-2-(7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl) hydrazinecarboxamide (9f)

Elemental analysis for $C_{19}H_{16}ClFN_4O_3$, calculated (%), C, 56.65; H, 4.00; N, 13.91; found (%), C, 56,31; H, 3,89; N, 13,76. FT-IR (υ_{max}, cm^{-1}): 3319 and 3203 (NH), 3033 (ar-CH), 1675 (C=O), 1547 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.37 (s, 3H, CH_3), 4.26 (d, 2H, *J*=6 Hz, CH_2), 4.55 (d, 2H, *J*=8 Hz, CH_2), 7.04 (s, 1H, NH), 7.23 (d, 1H, *J*=8 Hz, ArH), 7.30 (s, 4H, ArH), 8.13 (d, 1H, *J*=8 Hz, ArH), 8.26 (s, 1H, CH), 8.28 (d, 1H, *J*=7 Hz, ArH), 8.91 (s, 1H, NH), 11.06 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 15.04 (CH_3), 43.19 (CH_2), 49.21 (CH_2), arC: [110.92 (C), 112.77 (d, *J*=22 Hz, CH), 120.80 (CH), 126.62 (C), 127.01 (CH), 127.44 (2CH), 127.90 (C), 128.61 (2CH), 136.20 (C), 140.92 (C), 153.70-156.16 (d_{C-F}, *J*=246 Hz, C)], 148.86 (CH), 158.26 (C=O), 164.01 (C=O), 174.19 (C=O).EI MS *m/z* (%): 371 (48), 417 ($[M+1]^+$, 100).

3.1.4.7. 2-[(1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinolin-3-yl)carbonyl]-*N*-phenyl hydrazinecarbothioamide (10a)

Elemental analysis for $C_{20}H_{20}N_4O_2S$, calculated (%), C, 63.14; H, 5.30; N, 14.73; found (%), C, 63,02; H, 5,18; N, 14,49. FT-IR (υ_{max}, cm^{-1}): 3273-3198 (NH), 3060 (ar-CH), 1647 (C=O).^{1}H NMR (DMSO-d_6, δppm): 1.40 (t, 3H, *J*=16 Hz, CH_3), 2.49 (s, 3H, CH_3), 4.54 (d, 2H, *J*=4 Hz, CH_2), 7.14 (t, 1H, *J*=16 Hz, ArH), 7.33 (t, 2H, *J*=16 Hz, ArH), 7.71 (d, 2H, *J*=8 Hz, ArH), 7.86 (d, 1H, *J*=8 Hz, ArH), 8.20 (s, 1H, ArH), 8.90 (s, 1H, CH), 10.58 (bs, 1H, NH), 11.48 (bs, 1H, NH), 12.75 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 15.09 (CH_3), 21.05 (CH_3), 48.81 (CH_2), arC: [117.88 (CH), 126.17 (2CH), 127.54 (C), 128.65 (CH), 131.25 (CH), 135.03 (2CH), 135.50 (C), 137.16 (C), 139.76 (C), 143.99 (C)], 147.70 (CH), 161.74 (C=O), 170.74 (C=S), 175.26 (C=O). EI MS *m/z* (%): 106 (63), 135 (38), 149 (100), 213 (47), 409 (25).

3.1.4.8. 2-[(1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinolin-3-yl)carbonyl]-*N*-benzyl-hydrazinecarbothioamide (10b)

Elemental analysis for $C_{21}H_{22}N_4O_2S$, calculated (%), C, 63.94; H, 5.62; N, 14.20; found (%), C, 63,77; H, 5,45; N, 14,04. FT-IR (υ_{max}, cm^{-1}): 3291-3246 (NH), 3036 (ar-CH), 1663 (C=O). ^{1}H NMR (DMSO-d_6, δppm): 1.38 (t, 3H, *J*=16 Hz, CH_3), 2.48 (d, 3H, *J*=8 Hz, CH_3), 4.55 (q, 2H, *J*=20 Hz, CH_2), 4.73 (d, 2H, *J*=4 Hz, CH_2), 7.23 (d, 1H, *J*=4 Hz, ArH), 7.31 (s, 3H, ArH), 7.65 (s, 1H, ArH), 7.72 (t, 1H, *J*=8 Hz, ArH), 7.86 (d, 1H, *J*=8 Hz, ArH), 8.18 (s, 1H, ArH), 8.65 (s, 1H, NH), 8.91 (s, 1H, CH), 9.56 (bs, 1H, NH), 11.44 (bs, 1H, NH).^{13}C NMR (DMSO-d_6, δppm): 15.11 (CH_3), 21.04 (CH_3), 47.27 (CH_2), 48.75 (CH_2), arC: [117.87 (CH), 119.46 (CH), 125.22 (CH), 126.16 (CH), 127.57 (CH), 128.54 (CH), 134.73 (CH), 133.05 (CH), 135.51 (C), 137.14 (C), 137.60 (C), 140.07 (C), 153.49 (C)], 147.82 (CH), 161.24 (C=O), 175.37 (C=S), 176.44 (C=O). EI MS *m/z* (%): 102 (92), 395 ($[M+1]^+$, 100), 396 ($[M+2]^+$, 90).

3.1.4.9. 2-[(1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinolin-3-yl)carbonyl]-*N*-ethyl-hydrazinecarbothioamide (10c)

Elemental analysis for $C_{16}H_{20}N_4O_2S$, calculated (%), C, 57.81; H, 6.06; N, 16.85; found (%), C, 57,64; H, 5,93; N, 16,62. FT-IR (υ_{max}, cm^{-1}): 3302-3237 (NH), 3033 (ar-CH), 1664 (C=O). ^{1}H NMR (DMSO-d_6, δppm): 1.06 (t, 3H, *J*=12 Hz, CH_3), 1.39 (t, 3H, *J*=16 Hz, CH_3), 2.49 (s, 3H, CH_3), 3.46 (t, 2H, *J*=12 Hz, CH_2), 4.55 (q, 2H, *J*=20 Hz, CH_2), 7.72 (d, 1H, *J*=8 Hz, ArH), 7.86 (d, 1H, *J*=8 Hz, ArH), 8.06 (s, 1H, NH), 8.19 (s, 1H, ArH), 8.89 (s, 1H, CH), 9.45 (bs, 1H, NH), 11.49 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 13.88 (CH_3), 14.97 (CH_3), 20.95 (CH_3), 40.57 (CH_2), 48.13 (CH_2), arC: [107.56 (C), 117.61 (CH), 125.93 (CH), 126.92 (C), 134.78 (CH), 134.84 (C), 137.67 (C)], 146.19 (CH), 160.25 (C=O), 166.94 (C=S), 174.19 (C=O). EI MS *m/z* (%): 117 (38), 135 (100), 149 (69), 333 ($[M+1]^+$, 10).

3.1.4.10. 2-[(1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinolin-3-yl)carbonyl]-*N*-(4-fluoro phenyl) hydrazinecarbothioamide (10d)

Elemental analysis for $C_{20}H_{19}FN_4O_2S$, calculated (%), C, 60.29; H, 4.81; N, 14.06, found (%), C, 60,10; H, 4,56; N, 13,88. FT-IR (υ_{max}, cm^{-1}): 3280-3173 (NH), 3049 (ar-CH), 1646 (C=O).^{1}H NMR (DMSO-d_6, δppm): 1.39 (t, 3H, *J*=16 Hz, CH_3), 2.48 (s, 3H, CH_3), 4.54 (d, 2H, *J*=8 Hz, CH_2), 7.16 (d, 1H, *J*=8 Hz, ArH), 7.19 (s, 1H, ArH), 7.50 (s, 2H, ArH), 7.71 (d, 2H, *J*=12 Hz, ArH), 7.84 (d, 1H, *J*=12 Hz, ArH), 8.18 (s, 1H, ArH), 8.89 (s, 1H, CH), 9.85 (bs, 1H, NH), 11.55 (bs, 1H, NH), 12.68 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 15.09 (CH_3), 21.05 (CH_3), 48.80 (CH_2), arC:[117.88 (2CH), 119.33 (CH), 125.09 (CH), 126.17

(2CH), 127.54 (C), 129.25 (C), 131.54 (C), 135.04 (CH), 135.78 (d, J=55 Hz, C), 137.16 (C), 158.64-160.23 (d_{C-F}, *J*=159 Hz, C)], 147.74 (CH), 153.05 (C=O), 170.49 (C=S), 175.29 (C=O). EI MS *m/z* (%): 349 (28), 365 (63), 409 (100), 437 ($[M+K]^+$, 35).

3.1.4.11. 2-[(1-Ethyl-6-methyl-4-oxo-1,4-dihydroquinolin-3-yl)carbonyl]-*N*-phenyl hydrazinecarboxamide (10e)

Elemental analysis for $C_{20}H_{20}N_4O_3$, calculated (%), C, 65.92; H, 5.53; N, 15.38; found (%), C, 65,78; H, 5,38; N, 15,12. FT-IR (υ_{max}, cm^{-1}): 3320-3272 (NH), 3042 (ar-CH), 1675 (C=O).^{1}H NMR (DMSO-d_6, δppm): 1.39 (t, 3H, *J*=12 Hz, CH_3), 2.50 (d, 3H, *J*=8 Hz, CH_3), 4.54 (q, 2H, *J*=16 Hz, CH_2), 6.96 (t, 1H, *J*=16 Hz, ArH), 7.26 (d, 1H, *J*=8 Hz, ArH), 7.28 (s, 1H, ArH), 7.47 (d, 2H, *J*=8 Hz, ArH), 7.72 (dd, 1H, *J*=12 Hz, ArH), 7.86 (d, 1H, J=8 Hz, ArH), 8.19 (s, 1H, ArH), 8.74 (bs, 2H, 2NH), 8.90 (d, 1H, *J*=8 Hz, CH), 11.44 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 15.07 (CH_3), 21.06 (CH_3), 48.79 (CH_2), arC: [110.42 (C), 117.87 (CH), 118.78 (CH), 119.46 (CH), 122.30 (CH), 125.14 (CH), 126.14 (CH), 127.60 (C), 129.14 (CH), 135.10 (CH), 135.44 (C), 137.20 (C), 140.13 (C)], 147.80 (CH), 164.44 (C=O), 175.37 (C=O), 176.01 (C=O). EI MS *m/z* (%): 117 (100), 321 (36), 365 ($[M+1]^+$, 85).

3.1.5. General method for the synthesis of compounds 11a-f and 12a-e

Method 1. A solution of corresponding carbo(thio)amide **9a-f** or **10a-e** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2N NaOH (20 mmol) 6-12 h (Table 2). Then, the resulting solution was cooled to room temperature and acidified to pH 5 with 37 % HCl. The precipitate formed was filtered off, washed with water, and recrystallized from an appropriate solvent to give the target compound.

Method 2. The mixture of compound **9a-f** or **10a-e** (10mmol) and 2N NaOH (20mmol) in ethanol (10 mL) was irradiated in monomod microwave reactor in closed vessel at 150 ^{o}C, 200 Watt for 30-35 min (the progress of reaction was monitored by TLC). Then the resulting solution was cooled to room temperature and acidified to pH 5 with 37 % HCl. The precipitate formed was filtered off, wash with water, and recrystallized from an appropriate solvent to give the target compounds.

3.1.5.1. 7-Chloro-1-ethyl-6-fluoro-3-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)quinolin-4(1H)-one (11a)

Elemental analysis for $C_{19}H_{14}ClFN_4OS$, calculated (%), C, 56.93; H, 3.52; N, 13.98; found (%), C, 56,74; H, 3,29; N, 13,69. FT-IR(υ_{max}, cm^{-1}): 3060 (ar-CH), 1690 (C=O), 1595 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.22 (t, 3H, *J*=8 Hz, CH_3), 4.09-4.13 (m, 2H, CH_2), 6.75 (d, 2H, *J*=7.2 Hz, ArH), 7.27 (t, 3H, *J*=8 Hz, ArH), 7.51 (d, 1H, *J*=8 Hz, ArH), 7.61 (dd, 2H, *J*=7.1 Hz, ArH), 8.11 (s, 1H, ArH), 8.11 (s, 1H, CH), 14.13 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm):14.95 (CH_3), 47.77 (CH_2), arC: [113.51 (C), 116.64 (CH), 118.25 (CH), 123.67 (CH), 125.93 (2CH), 126.95 (C), 131.07 (2CH), 134.49 (C), 135.12 (C), 136.15 (C), 153.36-155.66 (d_{C-F}, *J*=233 Hz, C)], 142.66 (triazole C-3), 145.53 (CH), 166.42 (triazole C-5), 174.03 (C=O). EI MS *m/z* (%): 112.95 (32), 417.36 (44), 439.99 ($[M+K]^+$, 100).

3.1.5.2. 7-chloro-1-ethyl-6-fluoro-3-(4-Benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)quinolin-4(1H)-one (11b)

Elemental analysis for $C_{20}H_{16}ClFN_4OS$, calculated (%), C, 57.90; H, 3.89; N, 13.50; found (%), C, 57,58; H, 3,68; N, 13,33. FT-IR (υ_{max}, cm^{-1}): 3116 (NH), 3062 (ar-CH), 1632 (C=O), 1546 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.42 (t, 3H, *J*=8 Hz, CH_3), 4.29 (d, 2H, *J*=8 Hz, CH_2), 5.28 (d, 2H, *J*=8 Hz, CH_2), 6.96 (d, 2H, *J*=8 Hz, ArH), 7.14 (d, 3H, *J*=8 Hz, ArH), 8.11 (s, 1H, ArH), 8.18-8.21(m, 1H, ArH), 8.27 (s, 1H, CH), 14.03 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm):19.60 (CH_3), 52.01 (CH_2), 52.78 (CH_2), arC: [112.23 (C), 112.50 (CH), 124.16 (CH), 131.73 (2C), 132.30 (d, *J*=3 Hz, 2CH), 132.66 (d, *J*=3 Hz, 2CH), 133.52 (CH), 138.41 (C), 141.30 (C), 154.00-156.46 (d_{C-F}, *J*=246 Hz, C)], 133.67 (triazole C-3), 150.48 (CH), 172.80 (triazole C-5), 177.80 (C=O). EI MS *m/z* (%): 205.11 (82), 307.28 (39), 414.32 ($[M]^+$, 100).

3.1.5.3. 7-Chloro-1-ethyl-3-(4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl)-6-fluoroquinolin-4(1H)-one (11c)

Elemental analysis for $C_{15}H_{14}ClFN_4OS$, calculated (%), C, 51.06; H, 4.00; N, 15.88; found (%), C, 50,94; H, 3,87; N, 15,62. FT-IR (υ_{max}, cm^{-1}): 3079 (ar-CH), 1545 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.15 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 8.09 (d, 1H, *J*=7 Hz, ArH), 8.26 (d, 1H, *J*=7 Hz, ArH), 8.52 (s, 1H, CH), 13.87 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm):13.85 (CH_3), 14.91 (CH_3), 48.65 (CH_2), 65.76 (CH_2), arC: [107.67 (C), 112.61 (d, *J*=22 Hz, CH), 120.58 (CH), 127.19 (d, *J*=6 Hz, 2C), 148.37 (C), 153.50-155.96 (d_{C-F}, *J*=246 Hz, C)], 136.81 (triazole C-3), 147.31 (CH), 167.12 (triazole C-5), 173.15 (C=O). EI MS *m/z* (%): 205.16 (80), 266.35 (41), 307.30 (40), 352.85 ($[M]^+$, 100).

3.1.5.4. 7-Chloro-1-ethyl-6-fluoro-3-(4-(4-fluorophenyl)-5-mercapto-4H-1,2,4-triazol-3-yl)quinolin-4(1H)-one (11d)

Elemental analysis for $C_{19}H_{13}ClF_2N_4OS$, calculated (%), C, 54.48; H, 3.13; N, 13.38; found (%), C, 54,22; H, 3,02; N, 13,10. FT-IR (υ_{max}, cm^{-1}): 3007 (ar-CH), 1672 (C=O), 1511 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.22 (t, 3H, *J*=8 Hz, CH_3), 4.14 (q, 2H, *J*=12 Hz, CH_2), 6.76 (s, 1H, ArH), 7.14 (t, 2H, *J*=12 Hz, ArH), 7.50 (d, 1H, *J*=8 Hz, ArH), 7.60 (dd, 2H, *J*=8 Hz, ArH), 8.11 (s, 1H, ArH), 8.12 (s, 1H, CH), 13.87 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm):14.85 (CH_3), 49.22 (CH_2), arC: [107.10 (C), 115.11 (CH), 117.47 (CH), 118.77 (2CH), 125.69 (2CH), 128.12 (C), 129.58 (C), 131.73 (C), 135.90 (C), 152.55-154.60 (d_{C-F}, *J*=205 Hz, C), 156.34-158.85 (d_{C-F}, *J*=251 Hz, C)], 141.66 (triazole C-3), 147.09 (CH), 168.51 (triazole C-5), 177.94 (C=O). EI MS *m/z* (%): 394.94 (70), 441.64 ($[M+Na]^+$, 100).

3.1.5.5. 7-Chloro-1-ethyl-6-fluoro-3-(5-oxo-4-phenyl-4,5-dihydro-1H-1,2,4-triazol-3-yl)quinolin-4(1H)-one (11e)

Elemental analysis for $C_{19}H_{14}ClFN_4O_2$, calculated (%), C, 59.31; H, 3.67; N, 14.56; found (%), C, 59,08; H, 3,32; N, 14,28. FT-IR (υ_{max}, cm^{-1}): 3070 (ar-CH), 1673 (C=O), 1541 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.39 (t, 3H, *J*=16 Hz, CH_3), 4.43 (t, 2H, *J*=12 Hz, CH_2), 6.96 (t, 1H, *J*=16 Hz, ArH), 7.26 (t, 1H, *J*=16 Hz, ArH), 7.46 (d, 1H, *J*=8 Hz, ArH), 7.99 (d, 2H, *J*=8 Hz, ArH), 8.13 (d, 1H, *J*=7.1 Hz, ArH), 8.68 (s, 1H, CH), 14.78 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm):15.44 (CH_3), 48.19 (CH_2), arC: [108.17 (C), 117.22 (CH), 119.32 (CH), 125.90 (CH), 126.71 (C), 127.28 (2CH), 128.76 (2CH), 129.84 (2C), 135.02 (C), 152.10-155.10 (d_{C-F}, *J*=300 Hz, C)], 143.49 (triazole C-3), 148.18 (CH), 160.61 (triazole C-5), 174.91 (C=O). EI MS *m/z* (%): 134.98 (44), 208.18 (55), 384.41 ($[M]^+$, 100).

3.1.5.6. 7-Chloro-1-ethyl-6-fluoro-3-(5-oxo-4-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)quinolin-4(1H)-one (11f)

Elemental analysis for $C_{20}H_{16}ClFN_4O_2$, calculated (%), 60.23; H, 4.04; N, 14.05; found (%), C, 60,04; H, 3,91; N, 14,01. FT-IR (υ_{max}, cm^{-1}): 3177 (OH), 3060 (ar-CH), 1691 (C=O), 1548 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.23 (s, 3H, CH_3), 4.31 (s, 2H,CH_2), 4.82 (s, 2H,CH_2), 6.99 (s, 2H, ArH), 7.15 (s, 2H, ArH), 7.30 (s, 1H, ArH), 8.09-8.15 (m, 2H, ArH), 8.22 (s, 1H, CH), 11.89 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, δppm):14.87 (CH_3), 45.54 (CH_2), 48.80 (CH_2), arC: [110.47 (C), 117.70 (CH), 118.51 (CH), 122.29 (CH), 126.01 (2CH), 127.52 (C),

129.13 (2CH), 135.56 (C), 137.20 (C), 139.89 (C), 150.06-152.44 (d_{C-F}, *J*=238 Hz, C)], 147.92 (CH), 155.21 (triazole C-3), 159.33 (triazole C-5), 175.95 (C=O). EI MS *m/z* (%): 415.41 (84), 437.63 ($[M+K]^+$, 100).

3.1.5.7. 1-Ethyl-3-(5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-6-methylquinolin-4(1*H*)-one (12a)

Elemental analysis for $C_{20}H_{18}N_4OS$, calculated (%), C, 66.28; H, 5.01; N, 15.46; found (%), C, 66,11; H, 4,91; N, 15,20. FT-IR (υ_{max}, cm^{-1}): 3085 (ar-CH), 1710 (C=O), 1567 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.31 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 7.35 (s, 5H, ArH), 7.58 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.83 (s, 1H, ArH), 8.42 (s, 1H, CH), 14.08 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm): 14.84 (CH_3), 20.83 (CH_3), 47.98 (CH_2), arC: [107.39 (C), 117.41 (CH), 125.71 (CH), 126.66 (C), 128.30 (2CH), 128.94 (2CH), 129.14 (CH), 134.52 (CH), 134.55 (C), 134.93 (C), 137.52 (C)], 146.07 (CH), 148.99 (triazole C-3), 168.36 (C=S), 173.58 (C=O). EI MS *m/z* (%): 135 (100), 246 (39), 320 (38), 363 ($[M+1]^+$, 40).

3.1.5.8. 1-Ethyl-3-(5-mercapto-4-benzyl-4*H*-1,2,4-triazol-3-yl)-6-methylquinolin-4(1*H*)-one (12b)

Elemental analysis for $C_{21}H_{20}N_4OS$, calculated (%), C, 67.00; H, 5.35; N, 14.88; found (%), C, 66,87; H, 5,17; N, 14,49. FT-IR (υ_{max}, cm^{-1}): 3084 (ar-CH), 1551 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.18 (t, 3H, *J*=12 Hz, CH_3), 2.47 (s, 3H, CH_3), 4.27 (d, 2H, *J*=8 Hz, CH_2), 5.29 (s, 2H, CH_2), 6.95 (d, 2H, *J*=4 Hz, ArH), 7.15 (d, 3H, *J*=4 Hz, ArH), 7.67 (d, 1H, *J*=8 Hz, ArH), 7.74 (d, 1H, *J*=8 Hz, ArH), 8.12 (s, 1H, ArH), 8.19 (s, 1H, CH), 14.03 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm): 14.87 (CH_3), 20.98 (CH_3), 47.23 (CH_2), 47.82 (CH_2), arC: [107.77 (C), 117.57 (CH), 125.90 (CH), 126.89 (C), 127.48 (2CH), 127.88 (CH), 128.76 (2CH), 134.83 (CH), 134.93 (C), 136.60 (C), 137.42 (C)], 145.93 (CH), 149.46 (triazole C-3), 168.01 (C=S), 174.02 (C=O). EI MS *m/z* (%): 135 (100), 149 (39), 213 (37), 377 ($[M+1]^+$, 32).

3.1.5.9. 1-Ethyl-3-(5-mercapto-4-ethyl-4*H*-1,2,4-triazol-3-yl)-6-methylquinolin-4(1*H*)-one (12c)

Elemental analysis for $C_{16}H_{18}N_4OS$, calculated (%), C, 61.12; H, 5.77; N, 17.82; found (%), C, 60,95; H, 5,32; N, 17,48. FT-IR (υ_{max}, cm^{-1}): 3112 (NH), 3088 (ar-CH), 1602 (C=N).^{1}H NMR (DMSO-d_6, δppm): 1.14 (t, 3H, *J*=16 Hz, CH_3), 1.38 (t, 3H, *J*=12 Hz, CH_3), 2.47 (s,

3H, CH_3), 3.94 (q, 2H, *J*=20 Hz, CH_2), 4.40 (q, 2H, *J*=16 Hz, CH_2), 7.69 (d, 1H, *J*=8 Hz, ArH), 7.80 (d, 1H, *J*=8 Hz, ArH) 8.10 (s, 1H, ArH), 8.45 (s, 1H, CH), 13.85 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm): 13.86 (CH_3), 14.95 (CH_3), 20.95 (CH_3), 40.62 (CH_2), 48.12 (CH_2), arC: [107.39 (C), 117.60 (CH), 125.93 (CH), 126.95 (C), 134.77 (CH), 134.83 (C), 137.70 (C)], 146.16 (CH), 149.06 (triazole C-3), 166.99 (C=S), 174.20 (C=O). EI MS *m/z* (%): 135 (100), 246 (22), 320 (25).

3.1.5.10. 1-Ethyl-3-[4-(4-fluorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-yl]-6-methyl quinolin-4(1*H*)-one (12d)

Elemental analysis for $C_{20}H_{17}FN_4OS$, calculated (%), C, 63.14; H, 4.50; N, 14.73; found (%), C, 63,01; H, 4,29; N, 14,56. FT-IR (υ_{max}, cm^{-1}): 3121 (NH), 3046 (ar-CH), 1541 (C=N).^{1}H NMR (DMSO-d_6, δppm): 1.33 (t, 3H, *J*=16 Hz, CH_3), 2.38 (s, 3H, CH_3), 4.33 (t, 2H, *J*=16 Hz, CH_2), 7.21 (d, 1H, *J*=8 Hz, ArH), 7.24 (s, 1H, ArH), 7.39 (dd, 2H, *J*=16 Hz, ArH), 7.59 (t, 1H, *J*=8 Hz, ArH), 7.71 (d, 1H, *J*=8 Hz, ArH), 7.83 (s, 1H, ArH), 8.43 (s, 1H, CH), 14.09 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δppm): 14.85 (CH_3), 20.83 (CH_3), 48.06 (CH_2), arC: [107.18 (C), 115.88 (d, *J*=13 Hz, 2CH), 117.47 (CH), 125.69 (2CH), 126.67 (C), 130.50 (CH), 130.59 (C), 134.59 (CH), 137.55 (2C), 162.06 (d_{C-F}, *J*=244 Hz, C)], 146.17 (CH), 149.13 (triazole C-3), 168.46 (C=S), 173.47 (C=O). EI MS *m/z* (%): 135 (100), 381 ($[M+1]^+$, 30), 408 (21).

3.1.5.11. 1-Ethyl-3-(5-hydroxy-4-phenyl-4*H*-1,2,4-triazol-3-yl)-6-methylquinolin-4(1*H*)-one (12e)

Elemental analysis for $C_{20}H_{18}N_4O_2$, calculated (%), C, 69.35; H, 5.24; N, 16.17; found (%), C, 69,19; H, 5,15; N, 16,02. FT-IR (υ_{max}, cm^{-1}): 3273 (OH), 3042 (ar-CH), 1679 (C=O), 1553 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 1.39 (t, 3H, *J*=16 Hz, CH_3), 2.51 (s, 3H, CH_3), 4.54 (q, 2H, *J*=20 Hz, CH_2), 6.95 (t, 1H, *J*=16 Hz, ArH), 7.26 (t, 2H, *J*=16 Hz, ArH), 7.47 (d, 2H, *J*=8 Hz, ArH), 7.71 (d, 1H, *J*=8 Hz, ArH), 7.86 (d, 1H, *J*=8 Hz, ArH), 8.19 (s, 1H, ArH), 8.56 (s,1H, ArH), 8.88 (s, 1H, CH), 11.42 (s, 1H, OH).^{13}C NMR (DMSO-d_6, δppm): 15.07 (CH_3), 21.06 (CH_3), 48.79 (CH_2), arC: [110.42 (C), 117.87 (CH), 118.78 (CH), 119.46 (CH), 122.30 (CH), 125.14 (CH), 126.14 (CH), 127.60 (C), 129.14 (CH), 135.00 (CH), 135.43 (C), 137.20 (C), 140.21 (C)], 147.81 (CH), 155.16 (triazole C-3), 164.37 (C=O), 175.37 (C=O). EI MS *m/z* (%): 346 ($[M+1]^+$, 30).

3.2. Antioxidant activity

3.2.1. Antioxidant activity studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity: The scavenging activity of different chemicals was determined using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by Blois [50]. A 100 mL chemical solution was mixed with 1 mL of freshly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. The graph showing the concentrations against the absorbances is drawn, and the the concentration (mg/mL) of the sample that removes 50% of the total amount of the DPPH radical is accepted as SC_{50}.

FRAP (the ferric reducing ability of plasma): FRAP was measured using the method described by Benzie & Strain [51] with some modification. To 100 mL of each sample was added 2.9 mL freshly prepared FRAP reagent containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyle-s-triazine) and 20 mmol/L $FeCl.6H_2O$ in proportions of 10:1:1 (v/v/v). The mixture was incubated for 30 min at 37 ^{o}C and measured at 593 nm. The values were expressed as mmol of Trolox/g.

CUPRAC (cupric ion reducing antioxidant capacity): CUPRAC was measured following the procedure described by Apak et al. [52] with some modification. Briefly, 100 mL of each chemical solution was mixed with 900 mL bi-distilled water, 1 mL acetate buffer solution (1 mmol/L, pH: 7.0), 1 mL $CuCl_2$ (10 mmol/L) and 1 mL 7.5 mmol/L neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 450 nm against a water blank. Trolox was used as the standard calibration curves, and the results were expressed as mmol Trolox equivalent per g.

3.2.2. Measurement of AChE activity

The AChE inhibitory activity of the compounds was determined using Ellman's method [48], using AChE from E. electricus (Sigma) and acetylthiocholine iodide (0.35 mM) as a substrate. The reaction took place in the final volume of 3 mL of a phosphate-buffered solution at pH 8, containing 0.035 U/mL of EeAChE and 0.35 mM of 5,50-dithiobis-2-nitrobenzoic acid (DTNB), which produced yellow anion 5-thio-2-nitrobenzoic acid. Inhibition curves were made by incubating with the different compounds for 15 min; a sample without any compound was always used to determine the 100% of enzymatic activity. After

the 15 min incubation period, the production of color, as an indicator of enzymatic activity, was evaluated by measuring absorbance at 412 nm in a spectrophotometer plate reader.

3.2.4. Molecular docking

Ligands were energy-minimized using GAMESS (M.W. Schmidt) module for ChemOffice version Ultra 8.0.3 (PerkinElmer Inc.) on an Intel® (Core™ i7-3632QM CPU @ 2.20GHz 2.20GHz) using Windows 8.1 operating system. These modified ligand pdb files saved as pdbqt files. Appropriate grid box points were determined by centering on ligand separately for each compound. Ligand-centred grid box for all ligands, defined with a size of 126*126*126 $Å^3$ and a regular space of 0.375 Å, was considered for docking.

"Crystal Structure of Recombinant Human acetylcholinesterase in complex with Donepezil" [47] pdb file (PDB ID: 4EY7) was get (www.rcsb.org) and was modified using the ADT package version 1.5.6rc3. All water molecules were deleted and polar hydrogens were added. Subsequently, Gasteiger charges were calculated and the generated pdbqt files were saved. To validate the docking program, the co-crystallized ligand (PDB ID: E20) was redocked on the target enzyme and RMSD value of 0.709 was found for donepzil-bound acetylcholinesterase. RMSD value were obtained using Lamarckian Genetic Algorithm and scoring function of AutoDock 4.2 release 4.2.5.1 [53] software.

4. Conclusions

We have designed the synthesis, antioxidant and anti-acetylcholinesterase screening studies of new quinolone-triazole hybrids. Also, the intermediate products which are quinolones-carbo(thio)amides have investigated of their antioxidant capacity and AChE inhibition activity. All compounds except **9d**, **10b**, **12b** and **12c** show promising AChE inhibition activity. Among the active compounds against AChE, 9b and 10c are the most potent. Surprisingly, while the quinolone-triazole compounds were expected to be more active, the inhibition results showed that the most active compounds were quinolone-carbothioamide derivatives. In antioxidant capacity, **10c** is for FRAP, **10e** is for CUPRAC and **9b** is for DPPH showed the best results. Our work established to synthesize new multipotent hybrid molecules which are quinolone-triazole for the treatment of Alzheimer's Disease.

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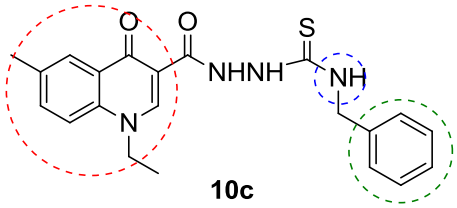
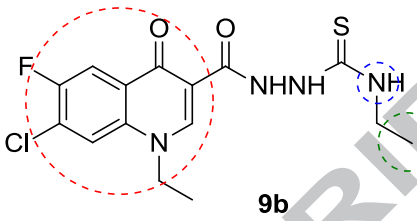
Conventional and Microwave Prompted Synthesis, Antioxidant, Anticholinesterase Activity Screening and Molecular Docking Studies of New Quinolone-Triazole Hybrids



10c

IC_{50}= 0.52±0.07



9b

IC_{50}= 0.48±0.02

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Highlights

- Conventional and microwave prompted synthesis of new quinolone-triazole hybrids.
- Inhibition of cholinesterases is an effective method to curb Alzheimer's disease, a progressive and fatal neurological disorder.
- Screening antioxidant capacity of novel drug-like compounds.
- Docking studies were performed for the most active compounds and interaction modes with enzyme active sites were determined.