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Design, Synthesis, Characterization, Enzymatic Inhibition Evaluations, and Docking Study of Novel Quinazolinone Derivatives

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ABSTRACT

In this study, novel quinazolinone derivatives **7a-n** were synthesized and evaluated against metabolic enzymes α-glycosidase, acetylcholinesterase, butyrylcholinesterase, human carbonic anhydrase I, and II. These compounds exhibited high inhibitory activities in comparison to used standard inhibitors with K_i values in the range of 19.28–135.88 nM for α-glycosidase (K_i value for standard inhibitor = 187.71 nM), 0.68–23.01 nM for acetylcholinesterase (K_i value for standard inhibitor = 53.31 nM), 1.01-29.56 nM for butyrylcholinesterase (K_i value for standard inhibitor = 58.16 nM), 10.25-126.05 nM for human carbonic anhydrase I (K_i value for standard inhibitor = 248.18 nM), and 13.46-178.35 nM for human carbonic anhydrase II (K_i value for standard inhibitor = 323.72). Furthermore, the most potent compounds againt each enzyme were selected in order to evaluate interaction modes of these compounds in the active site of the target enzyme. Cytotoxicity assay of the

title compounds **7a-n** against cancer cell lines MCF-7 and LNCaP demonstrated that these compounds do not show significant cytotoxic effects.

Keywords: Quinazolinone; Metronidazole; Enzyme inhibition; Cytotoxicity; Molecular docking

1. Introduction

Quinazolinone and its derivatives have received a great deal of attention, due to their therapeutic and pharmaceutical properties, such as anti-inflammatory, antibacterial, antitumor, antifungal, and antitubercular activities [1-17]. Moreover, quinazolinone scaffold also found in the potent α-glycosidase inhibitors such as compounds **A-C** (Fig. 1) [18-20]. α-Glycosidase involved in the hydrolyzation of oligo- and disaccharides into monosaccharides and control of glucose level in blood [21]. Therefore, inhibition of this enzyme tends to slow breakdown and release of sugars into the bloodstream and can be used as a therapeutic method in the treatment of diabetes and obesity [22]. In particular, some α-glycosidase inhibitors such as acarbose, voglibose, and miglitol are used in the treatment of diabetes [23, 24].

One of the important biological effects of quinazolinone derivatives is acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition as two target enzymes used in treatment of Alzheimer's disease (AD). For example, compounds **D-F** exhibited high inhibitory activities against the latter enzymes (Fig. 1) [25-27]. One of the plenty of extensively recorded statements of AD development is the cholinergic hypothesis, which offers clear aspects of therapy strategies [28, 29]. Cholinesterase (ChE) enzymes are a family group that mainly catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) at the end of the nerve transmission in the centric neural system [30, 31]. There are two main forms of ChEs: AChE and BChE. There are documents that show while AChE activity prevails to BChE activity in a healthy brain, but in patients with AD, BChE activity increased and AChE activity decreased [32, 33].

Furthermore, several compounds containing quinazolinone core with human carbonic anhydrase I, and II (CA I and CA II) inhibitory properties were also reported (Fig. 1, compounds **G-I**) [34, 35]. CA enzymes are a family of zinc-containing metalloenzymes that composed of sixteen isoenzymes, which differ in function, kinetic properties, inhibition profiles, and tissue expression patterns [36]. The overexpression and sluggishness of several CA isoenzymes are responsible for plenty of diseases in human beings [37]. The CA I and CA II isoforms had plenty of significant normal physiological mechanisms like regulation of the acid-base homeostasis made them act as worthy drug targets in cerebral edema, glaucoma, and epilepsy [38, 39].

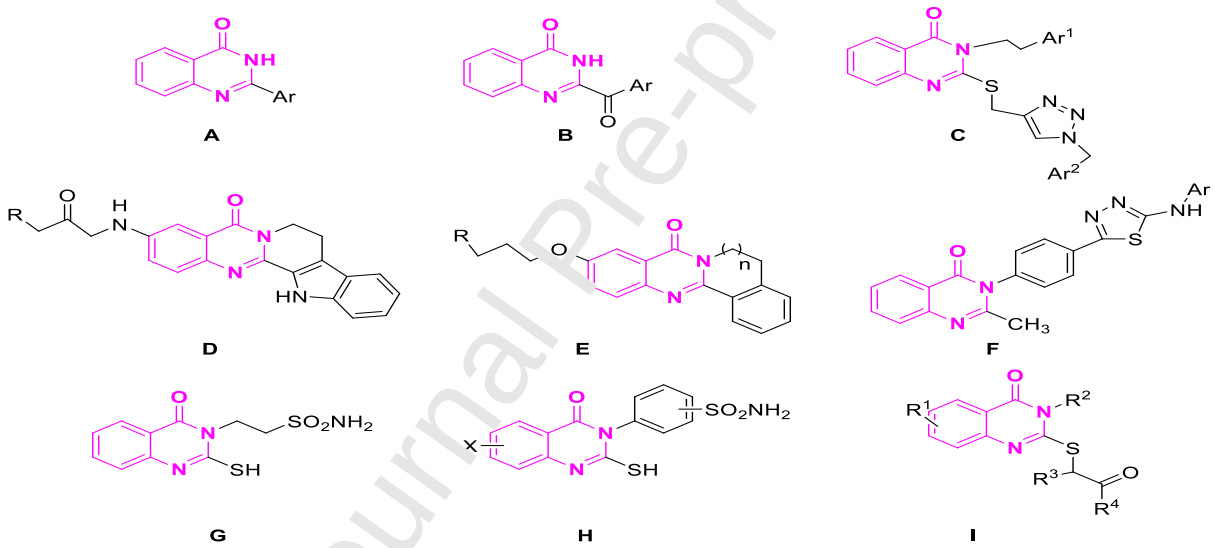


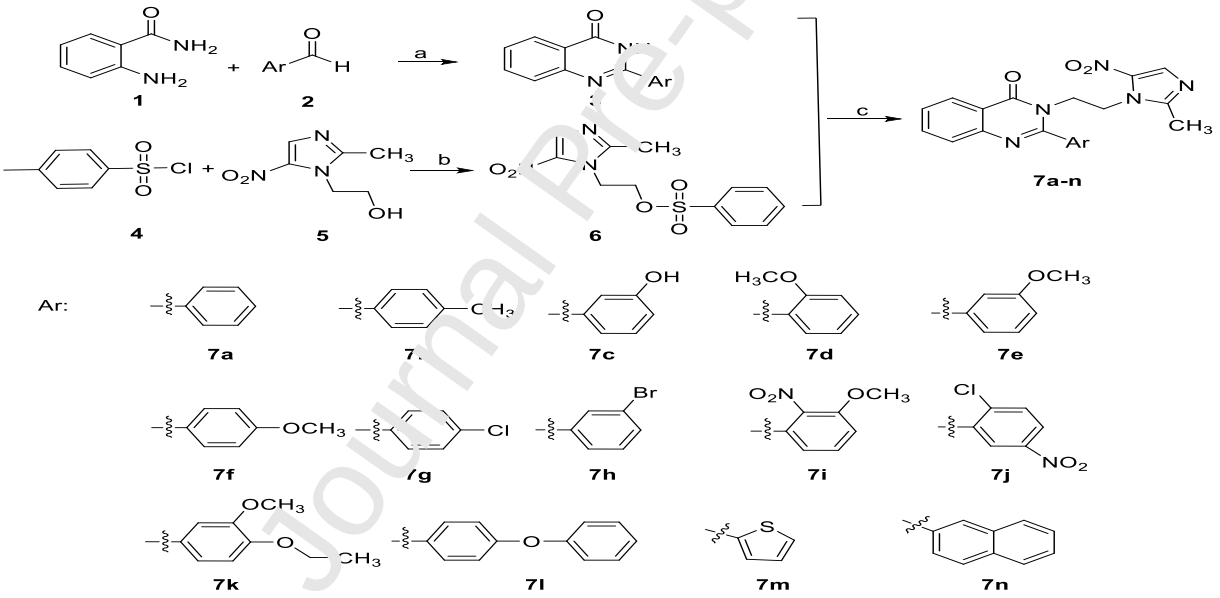
Fig. 1. α-Glycosidase inhibitors **A-C**, cholinesterase inhibitors **D-F**, and carbonic anhydrase inhibitors **G-I** bearing quinazolinone core

In light of this information, the aim of this study was to synthesis new derivatives of quinazolinone and investigates these derivatives on α-glycosidase, AChE, BChE, hCA I, and hCA II inhibitory activities. Docking studies of the most potent compounds against each enzyme were also performed. Moreover, considering the several reports of cytotoxic effects of quinazolinone derivatives, cytotoxicity of all the synthesized compounds was evaluated by the MTT assay against cancer cell lines MCF-7 and LNCaP [40, 41].

2. Result and discussion

2.1. Chemistry

The synthetic route for the synthesis of new quinazolinone derivatives **7a-n** has been depicted in Scheme 1 [42]. It was started from the reaction between 2-aminobenzamide **1** and aromatic aldehydes **2** in the presence of $Na_2S_2O_5$ in DMF at 100 °C to give quinazolinone derivatives **3**. On the other hand, by a nucleophilic substitution reaction between tosyl chloride **4** and metronidazole **5**, tosyl-metronidazole **6** was obtained. In the final step, the latter compound reacted with quinazolinone derivatives **3** in presence of K_2CO_3 in DMF at 80 °C for provided the title compounds **7a-n**.



Scheme 1. Synthesis of the new quinazolinone derivatives **7a-n**: (a) DMF, $Na_2S_2O_5$, 100 ^oC, 5 h; (b) Pyridine, 50 °C, 4h; (c) DMF, K_2CO_3, H_2O, 80 °C, 24 h

The structures of the synthesized compounds **7a-n** were determined by ^1H and ^{13}C NMR, IR, and elemental analysis. For example, ^1H NMR spectrum of compound **7a** showed a signal at 2.42 ppm corresponding to protons related to methyl group attached to imidazole ring, besides two triplets in the region of 5.14 and 4.93 ppm attributed to ethyl group between

quinazolinone and imidazole rings. In addition, aromatic hydrogen related to the imidazole ring appeared at 8.00 ppm. The number of other aromatic hydrogens of compound **7a** is fully compatible with the aromatic region picks in ^{1}H NMR spectrum.

2.2. Enzyme inhibition results

It was established that different CA isoforms are involved in numerous physiological and pathological processes associated with CO_2 hydration reaction such as bone resorption, calcification, electrolyte secretion, respiration, lipogenesis, gluconeogenesis, tumorigenicity, and many others [43]. CA Inhibitors (CAIs) for the diverse human isoforms (i.e. 16 to date) have recorded clinical applications for the handling of diseases like epilepsy, obesity, ocular hypertension in glaucoma, hypoxic cancers, and neuropathic pain [44]. The major hurdle in CAI development is relevant to the isoenzyme selectivity issue, which thrived in novel chemotypes. In this context, our research group recently reported various aromatic compounds (i.e. 1,3,5-trisubstituted-pyrazolines, 4-phenylbutenone bromophenols, novel NHC Precursors, and cyclic thioureas) as potent and novel CAIs [45-48]. Therefore, the novel quinazolinone derivatives **7a-n** were evaluated against CAs and obtained results were listed in Table 1. As can be seen in the latter table, our results demonstrated that novel quinazolinone derivatives **7a-n** had high inhibitory activity against hCA I isoform in comparison to standard CA inhibitor acetazolamide. The obtained K_i values for the newly synthesized compounds were in range of 10.25±1.26 to 126.05±25.40 nM while acetazolamide had K_i value of 248.18±23.13 nM against hCA I. As can be seen in Scheme 1, in order to better evaluation of enzymatic activities of title compounds, various aromatic groups were applied for synthesis of new derivatives of the novel quinazolinone scaffold **7**. Among the synthesized compounds, the most potent compound was compound **7j** with 2-chloro-5-nitrophenyl group with K_i value of 10.25±1.26 that was around 25-folds more potent than acetazolamide. The second potent compound among the synthesized compounds was compound **7g** with 4-chlorophenyl group.

Replacement of chloro substituent in compound **7g** with methyl or methoxy group, led to a moderate decrease in the 4-methyl derivative **7b** and a significant decrease in 4-methoxy derivative **7f** while replacement of chloro with phenoxy group, as in compound **7l**, created a negligible decrease in inhibitory activity. It is worthy to note that in addition to 4-methoxy derivative **7f**, other methoxy derivatives **7d**, **7e**, and **7k** were moderate inhibitors against CA I in comparison to chloro derivatives **7g** and **7j**. The third most potent compound was 3-hydroxyphenyl derivative **7c**. Replacement of Bromo substituent instead of hydroxyl group slightly decreased inhibitory activity (compound **7h** vs. compound **7c**). As can be seen in Table 1 and Scheme 1, 2-nitro-3-methoxy derivative **7i** had inhibitory activity similar to 3-Bromo derivative **7h**. Order of inhibitory activities of un-substituted aromatic groups demonstrated that 1-naphthalene group exhibited the best activity in comparison to phenyl and thiophene groups (compound **7n** vs. compound **7a** and **7m**, respectively).

The novel quinazolinone derivatives **7a-n** exhibited K_i values varying from 13.46±4.13 to 178.35±17.94 nM against hCA II isoform (Table 1). These results revealed that title compounds **7a-n** inhibited hCA II better than standard inhibitor acetazolamide with Ki value of 323.72±51.31. Like the effects on hCA I, chlorine derivatives of **7j** and **7g** acted better than others against hCA II. The third most potent hCA II inhibitor among the title compounds was 4-phenoxy derivative **7l** while methoxy derivatives **7d-f** and **7k** demonstrated moderate inhibitory activities in comparison to 4-phenoxy derivative **7l**. As can be seen in Table 1 and Scheme 1, replacement of phenoxy group of compound **7l** with methyl group, as in compound **7b**, led to a slight decrease in the inhibitory activity. Observed data also demonstrated that compounds with substituents 4-methyl, 3-hydroxy, 3-bromo, and or 2-nitro-3-methoxy showed approximately same inhibitory activity against hCA II. The order of inhibitory activity of unsubstituted aromatic derivatives against hCA II is similar to it against hCA I: 1-

naphthalene derivative **7n** was more potent than phenyl derivative **7a** and thiophene derivative **7m**.

BChE and AChE inhibition have been documented as critical goals for the effective management of AD. Cholinesterase inhibitors (ChEIs) have various benefits in improvement of brain ACh amounts resulting in a raised cholinergic transmission [49]. Nowadays, ChEIs represent the major therapy for behavioral dysfunctions associated with AD and ameliorate the cognitive [50]. AChE and BChE inhibition properties of novel quinazolinone derivatives **7a-n** were determined according to Ellman's procedure as previously described [51]. Novel quinazolinone derivatives **7a-n** exhibited high inhibitory activities against cholinesterase enzymes AChE (K_i values = 0.68±0.04-23.01±4.81 nM) and BChE (K_i values = 1.01±0.21-29.56±2.95 nM) when compared with standard cholinesterase inhibitor tacrine (K_i value against AChE = 53.31±11.32 nM and K_i value against BChE = 58.16±7.24 nM). Obtained results revealed that 1-naphthalene derivative **7n**, 4-phenoxyphenyl derivative **7l**, and 4-chloro derivative **7g** were the most potent compounds among the newly synthesized compounds against both AChE and BChE. On the other hand, the less potent compound against both AChE and BChE was thiophene derivative **7m**. The remaining compounds showed different activities against AChE and BChE depending on the type of substitutions on phenyl group. In this regard, in the term of AChE inhibitory activity, 3-bromo derivative **7h**, 4-methyl derivative **7b**, and 2-nitro-3-methoxy derivative **7i** with approximately same IC_{50} values showed good inhibitory activity, but remaining compounds in comparison to the compounds **7h**, **7b**, and **7i** exhibited moderate anti-AChE activity. On the other hand, in the term of BChE inhibitory activity, 3-hydroxy derivative **7c** and 3-bromo derivative **7h** with inhibitory activities approximately same, after the compounds **7n**, **7l**, and **7g** were the most active compounds. Like observed anti-AChE activity, 4-methyl derivative **7b** and 2-nitro-3-methoxy derivative **7i** also exhibited high inhibitory activities against BChE and remaining

compounds were moderate inhibitors against BChE when compared with the mentioned most potent compounds.

The α-glycosidase enzyme is located at the brush border of intestine, where it is involved in the breakdown of dietary sugars and starches to glucose [52]. Inhibition of this enzyme is an attractive target for managing blood glucose levels in type 2-diabetes [53-58]. In the final step of our *in vitro* enzymatic inhibition assays, α-glycosidase inhibitory activity of the newly synthesized compounds **7a-n** was evaluated against yeast form of this enzyme and obtained results were compared with acarbose as a standard α-glycosidase inhibitor. Our results demonstrated that all the novel quinazolinone derivatives **7a-n** (K_i values = 19.28±1.88-135.88±14.92 nM) had more inhibitory activities than acarbose (K_i value = 187.71±28.40 nM). Among the synthesized compounds, 3-bromo derivative **7h**, 2-chloro-5-nitro derivative **7j**, and 3-hydroxy derivative **7c** were the most potent compounds. Replacement of hydroxyl substituent in compound **7c** with methoxy group, as in compound **7e**, led to a significant decrease in the inhibitory activity. Moreover, changing the position of methoxy group of 3-position in compound **7e** to 2-position, as in compound **7d**, and or introduction of 2-nitro group and or 4-ethoxy group on 3-methoxy derivative **7e**, as in compounds **7i** and **7k**, respectively, led to increasing in the inhibitory activity. The fourth potent compound among the synthesized compounds was 4-chloro derivative **7g**. Replacement of chloro substituent in the latter compound with methoxy and or methyl, as in compound **7f** and **7b**, respectively, led to a dramatic decrease in the inhibitory activity while replacement of chloro of compound **7g** with phenoxy group, as in compound **7l**, led to a negligible decrease in the inhibitory activity. Inhibitory activities of un-substituted aromatic groups revealed that un-substituted phenyl derivative **7a** and 1-naphthalene derivative **7n** had good anti-α-glycosidase activity while 2-thiophene derivative **7m** was one of the weakest compounds against α-glycosidase.

Table 1. Inhibition results of novel quinazolinone derivatives (**7a-n**) on some metabolic enzymes including α-glycosidase (α-Gly), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), human carbonic anhydrase I, and II isoenzymes (hCA I, and hCA II)

Compound	IC_{50} (nM)										K_i (nM)				
	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	α-Gly	r^2	hCA I	hCA II	AChE	BChE	α-Gly
7a	67.21	0.9624	56.04	0.9725	15.47	0.9093	10.51	0.9325	101.47	0.9841	82.54±10.41	62.45±6.22	12.66±0.96	7.43±1.13	78.46±15.70
7b	26.45	0.9482	20.55	0.9511	4.06	0.9037	6.03	0.9490	105.46	0.9506	34.12±6.07	26.93±3.94	3.13±0.93	4.96±0.94	135.88±14.92
7c	15.82	0.9638	19.41	0.9876	5.83	0.9236	4.37	0.9533	22.50	0.9277	18.04±3.77	26.57±2.36	7.23±1.34	3.78±0.65	27.01±4.73
7d	91.05	0.9041	76.16	0.9889	17.32	0.9587	14.03	0.9805	67.18	0.9440	98.0[illegible]±13.56	84.01±13.73	14.70±2.52	13.01±0.97	70.47±10.03
7e	90.24	0.9581	69.08	0.9682	8.27	0.9882	11.21	0.9410	91.83	0.9583	[illegible]±13.87	78.36±8.43	5.98±0.94	9.45±1.47	99.16±15.94
7f	83.25	0.9915	63.08	0.9699	10.24	0.9581	13.95	0.9094	78.61	[illegible]	98.04±13.76	79.33±9.42	7.94±0.56	10.42±1.93	78.04±12.57
7g	12.78	0.9635	10.27	0.9827	1.87	0.9637	2.36	0.9834	26.[illegible]	0.9736	16.83±2.25	14.37±1.87	1.45±0.24	2.01±0.24	30.76±4.85
7h	33.81	0.9948	28.36	0.9633	3.78	0.9735	4.75	[illegible]	[illegible]78	0.9925	39.62±9.45	30.23±2.84	3.05±0.67	3.94±0.67	19.28±1.88
7i	30.47	0.9611	25.73	0.9947	3.87	0.9310	8.1[illegible]	[illegible]	37.38	0.9327	35.87±7.32	28.94±3.12	3.26±0.53	5.98±1.03	44.72±4.78
7j	7.93	0.9518	10.25	0.9224	8.31	0.9[illegible]	[illegible]	0.9790	18.36	0.9943	10.25±1.26	13.46±4.13	6.25±1.01	9.14±0.94	23.25±2.76
7k	46.71	0.9882	40.26	0.9424	12.47	[illegible]	16.34	0.9023	46.04	0.9167	58.02±5.47	52.94±5.47	9.27±1.21	13.66±2.78	50.01±13.88
7l	19.05	0.9506	12.05	0.9932	1.0[illegible]	0.9325	1.98	0.9912	29.36	0.9532	24.35±0.98	15.33±1.24	0.96±0.12	1.15±0.20	35.20±3.65
7m	111.58	0.9830	146.98	0.[illegible]	16.01	0.9598	34.61	0.9501	94.60	0.9701	126.05±25.40	178.35±17.94	23.01±4.81	29.56±2.95	103.04±14.90
7n	22.48	0.9889	16.58	0.9548	0.95	0.9721	1.24	0.9598	68.52	0.9290	28.55±5.62	21.76±2.56	0.68±0.04	1.01±0.21	77.95±9.90
Acetazolamide*	218.65	0.9892	281.87	0.9233	-	-	-	-	-	-	248.18±23.13	323.72±51.31	-	-	-
Tacrine*	-	-	-	-	67.21	0.9643	83.24	0.9719	-	-	-	-	53.31±11.32	58.16±7.24	-
Acarbose*	-	-	-	-	-	-	-	-	154.36	0.9983	-	-	-	-	187.71±28.40

(*They were used as control compounds for some metabolic enzymes)

2.3. Molecular modeling study

After *in vitro* enzymatic assays, the most potent compounds against studied enzymes hCA I, hCA II, AChE, BChE, and α-glucosidase were docked in the active site of these enzymes by Autodock Tools 1.5.6 software. Compounds **7j** and **7g** as the most potent compounds against hCA I were screened in the active site of this enzyme. As was mentioned in our previous works, acetazolamide as a standard inhibitor against hCA I interacted with Zn301 and His200 in hCA I active site [59]. As can be seen in Table 1, compound **7j** inhibited hCA I about 25-fold stronger than acetazolamide. Docking study of this compound in hCA I active site showed that compound **7j** interacted with Zn301 and His200 *via* π-cation interactions and Gln92 and His67 *via* hydrogen bonds (Fig. 2). Compound **7j** also formed two non-classic hydrogen bonds with Thr199 and Pro202. Furthermore, hydrophobic interactions between this compound and active site residues Val143, Ala121, His64, Leu198, His200, Ala135, Phe91, and Leu131 were observed in Fig. 2.

The second potent hCA I inhibitor **7g** formed only a π-cation interaction with His67. On the other hand, this compound established three hydrogen bonds with His64, His67, and His200 and a non-classic hydrogen bond with Gln92. Furthermore; compound **7g** also interacted with His119, His94, Leu198, Leu131, Ala135, and Val62 *via* hydrophobic interactions. Further studies showed that compound **7j** has a lower free binding energy (-7.92 kcal/mol) than compound **7g** (-7.56 kcal/mol) and therefore binds easily to hCA I than does compound **7g**.

7j **7g**

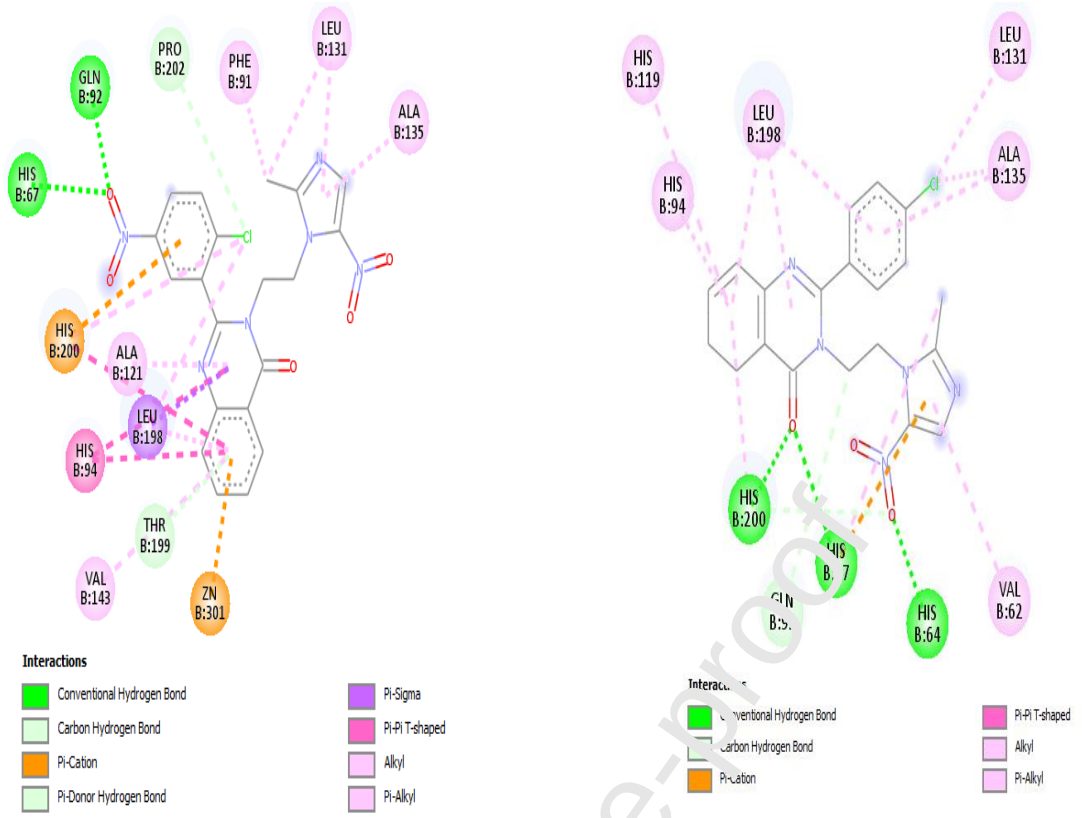


Fig. 2. Docking poses of compounds **7j** and **7g** in the active site of hCA I isoenzyme.

Compounds of **7j** and **7g** also acted as the most potent inhibitors against hCA II isoenzyme. Therefore, these compounds were also docked in the active site of this enzyme (Fig. 3). Standard hCA II inhibitor of acetazolamide interacted with Zn265, Asn67, and Thr199 in the active site hCA II. Compound **7j** formed three hydrogen bonds with Thr199 and Thr200 and several hydrophobic interactions with His94, Ala65, Val121, Leu198, Pro202, Val135, and Phe131. The compound of **7g**, with hCA II inhibitory activity approximately same with compound of **7j**, formed hydrogen bonds with Gln92 and Asn62 and hydrophobic interactions with residues His96, His94, His119, Trp209, His64, Val121, Leu198, Pro202, Val135, and Phe131. Binding energies of these compounds, like inhibitory activities against hCA II, are not significantly different (binding energy Compound of **7j** = -7.45 kcal/mol and binding energy Compound of **7j** = - 7.35 kcal/mol).

7j **7g**

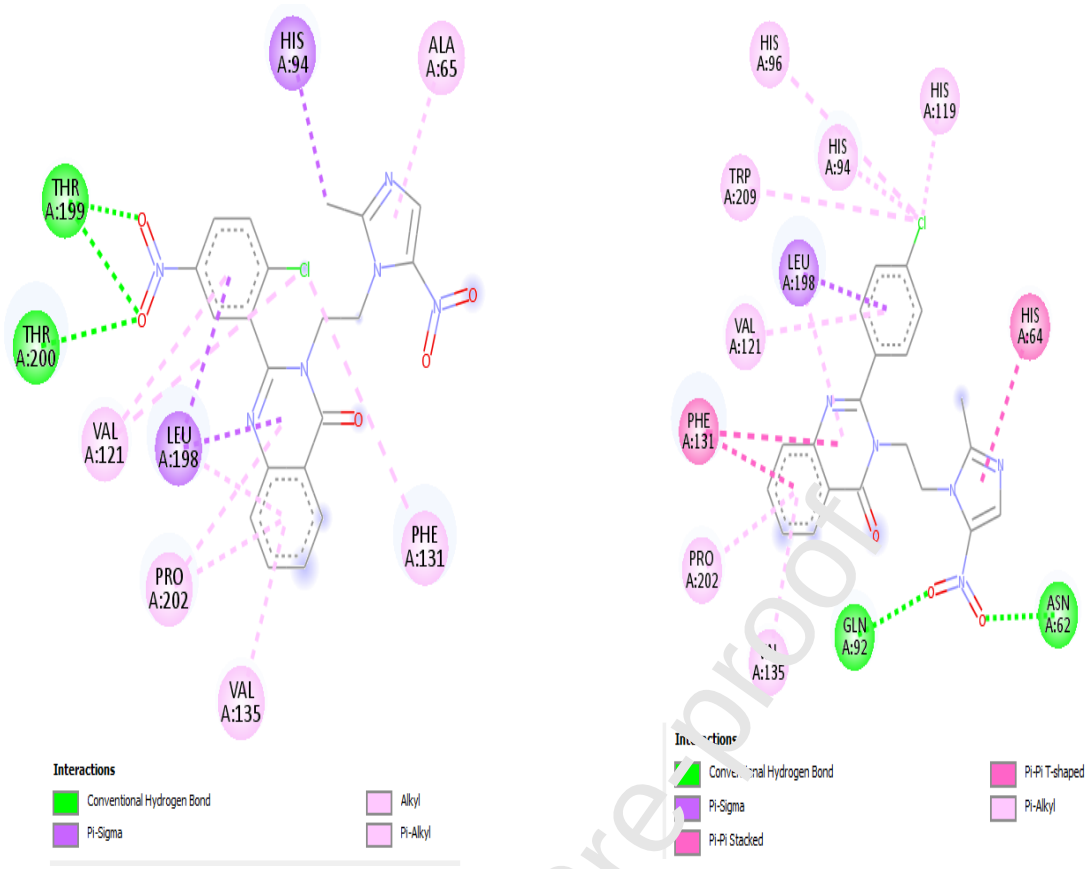


Fig. 3. Docking poses of compounds of **7j** and **7g** in the active site of hCA II isoenzyme.

The most potent compounds against cholinesterase enzymes of AChE and BChE were compounds of **7n** and **7l** that were docked into active site of these enzymes. The active site of AChE and BChE includes two important components: 1) catalytic anionic site (CAS) that itself consists of two parts, anionic site (AS) and catalytic triad (CT), and 2) peripheral anionic site (PAS) [60]. Interaction of cholinesterase inhibitors with both components CAS and PAS has an important role in therapeutic effects of these compounds [61].

Molecular modeling of the compound **7n** as most potent AChE inhibitor among the synthesized compounds showed that this compound interacted with CAS residues Trp84 (AS) and Phe330 (AS) *via* hydrophobic interactions and PAS residues Asp72 *via* π-anion interaction (Fig. 4). This compound also formed a π-cation interaction with CAS residues His440 (CT) and hydrogen bonds with other active site residues Gly119, Gly118, and Ala201.

The binding energy of this compound in the AChE active site was -10.82 kcal/mol. The second potent AChE inhibitor **7l** interacted with CAS residues Phe330 (AS), Phe331 (AS), Trp84 (AS), His440 (CT), and Ser200 (CT) and PAS residues Tyr121 and Tyr334. Furthermore, compound **7l** also formed an interaction with active site residue Glu199. The binding energy of this compound was -10.64 kcal/mol.

7n **7l**

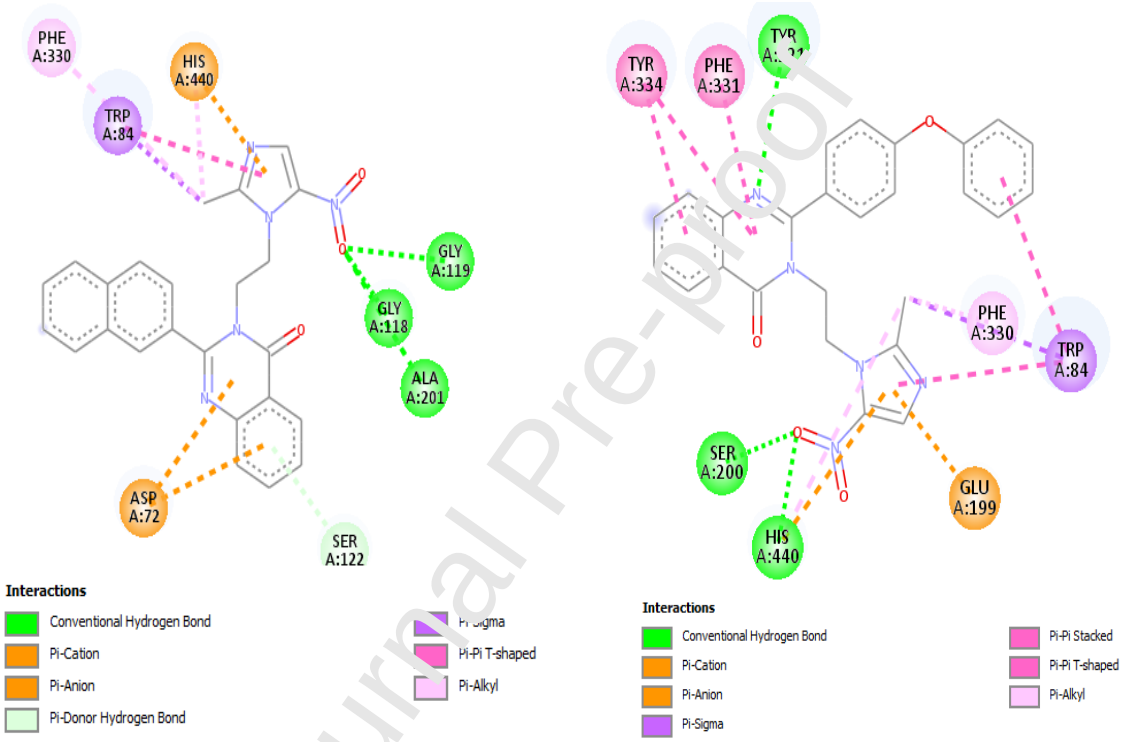


Fig. 4. Interaction modes of compounds of **7n** and **7l** in the active site of AChE

Compound **7n** established interactions with CAS residues Trp82 (AS), Phe329 (AS), and His438 (CT) in the BChE active site (Fig. 6). This compound also interacted with Gly117, Gly116, Ala199, Pro84, and Ala328 in the active site of this enzyme. Other strong BChE inhibitor **7l** established interactions with BChE active site residues Trp82 (AS), Tyr128 (AS), His438 (CT), Gly117, Gly116, Ala199, His438, Thr120, Ala328, Trp430, and Trp231 (Fig. 5). Binding energies of compounds **7n** and **7l** were -10.17 and -8.64 kcal/mol.

7n **7l**

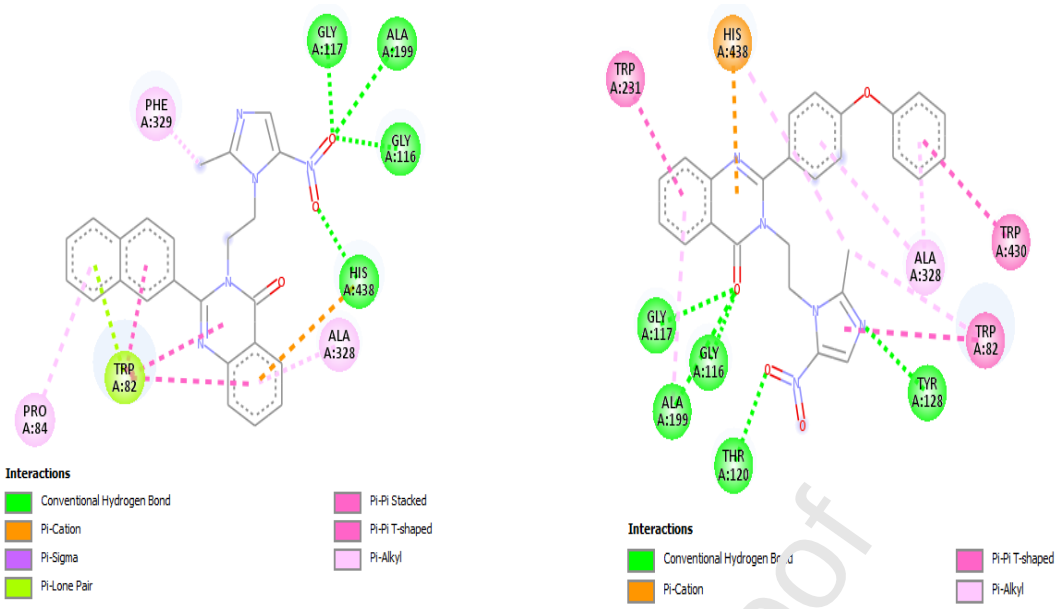


Fig. 5. Interaction modes of compounds **7n** and **7l** in the active site of BChE

The α-glycosidase inhibitory activities of synthesized compounds of **7a-n** exhibited that compounds of **7h** and **7j** acted better than others (Table 1). Docking study of compound of **7h** demonstrated that this compound established a hydrogen bond with Gln322 and two non-classical hydrogen bonds with Thr301 and Gly306 in the α-glucosidase active site. Furthermore, several hydrophobic interactions between compound of **7h** and active site residues Val305, Pro309, and His279 were also observed (Fig. 6). The second potent α-glucosidase inhibitor of **7j** formed following interactions with α-glucosidase active site: two hydrogen bonds with residues Gly306 and Thr307Gln322, two non-classical hydrogen bond with Thr301 and Glu304, a π-anion interaction with Glu304, and several hydrophobic interactions Pro309 and His279. Binding energies of compounds of **7h** and **7j** were -7.45 and -7.37 kcal/mol.

7h **7j**

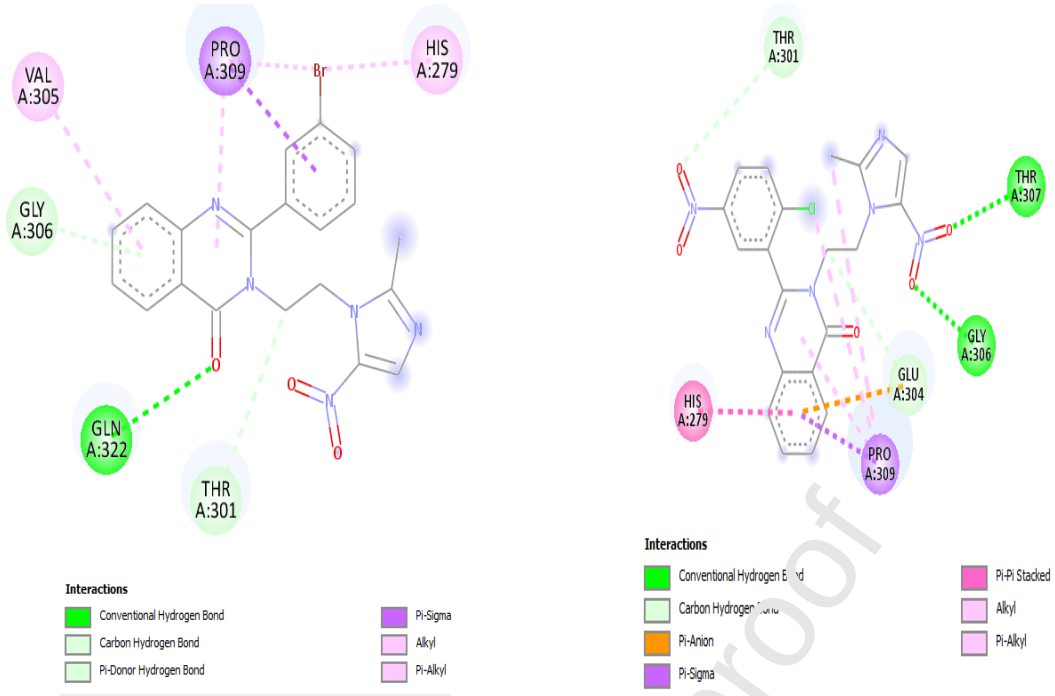


Fig. 6. Interaction modes of compounds of **7h** and **7j** in the active site of α-glucosidase enzyme

2.4. In vitro cytotoxicity assay

Given that several series of quinazoline derivatives with high cytotoxic effects were reported, all the newly synthesized compounds **7a-n** were evaluated against human cancer cell lines LNCaP and MCF-7 by MTT assay [62-64]. Obtained results revealed that title compounds do not show significant cytotoxic effects against studied cell lines (Table 2).

Table 2. In vitro cytotoxicity effects of newly synthesized compounds **7a-n**

Compound	Cytotoxicity ($LogIC_{50}$ µM)		Compound	Cytotoxicity ($LogIC_{50}$ µM)	
	LNCaP	MCF-7		LNCaP	MCF-7
7a	2.81±0.67	1.63±0.07	**7h**	3.46±0.56	1.82±0.32
7b	2.55±0.2	3.71±0.33	**7i**	2.58±0.19	2.49±0.15
7c	3.15±1.21	3.93±0.51	**7j**	3.58±1.09	2.86±0.41

7d	2.38±0.1	2.69±0.15	**7k**	2.33±0.09	3.56±0.32
7e	2.59±0.14	2.05±0.04	**7l**	2.38±0.1	1.76±0.08
7f	2.71±0.37	2.00±0.03	**7m**	5.75±2.21	2.99±0.26
7g	2.85±0.32	1.95±0.32	**7n**	3.57±0.73	3.4±0.23

3. Materials and methods

*3.1. General Procedure for the Synthesis of quinazolinone derivatives **3***

A mixture of 2-aminobenzamide **1** (1 mmol), aromatic aldehydes **2** (1 mmol), and $Na_2S_2O_5$ (1.1 mmol) in DMF (20 mL) was stirred at 100 ^oC for 5 h at the closed condition. Then, the mixture was poured in the cold water and a pure quinazolinone derivative **3** was filtered off.

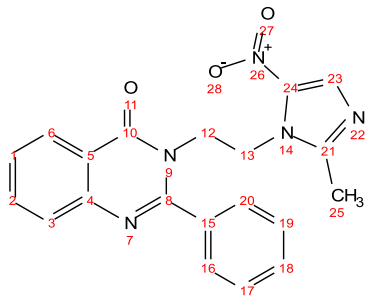
*3.2. Synthesis of tosyl-metronidazole **6***

A solution of tosyl chloride **4** (1 mmol) and metronidazole **5** (1 mmol) in pyridine (15 mL) was stirred at 50 °C for 4 h at the closed condition. Then, the mixture was poured in water and was filtered off. The obtained residue was washed with water to obtain pure tosyl-metronidazole (**6**).

*3.3. General Procedure for the Synthesis of quinazolinone derivatives linked to metronidazole **7a-n***

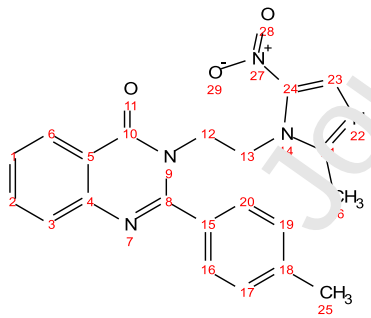
Finally, quinazolinone derivatives **3** (1 mmol), tosyl-metronidazole **6** (1 mmol), and K_2CO_3 in DMF (10 mL) were stirred at 80 °C for 24 h at the closed condition. After that, reaction mixture was poured into water and formed products were filtered off, washed with water, and purified by recrystallization (in ethylacetate) to give target compounds of **7a-n**.

*3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-phenylquinazolin-4(3H)-one **7a***



White solid (87%); mp > 250 °C; IR (KBr) ṽ [cm^{-1}] = 3073, 2925, 1641, 1299. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.36 (d, *J* = 8.2 Hz, 2H), 8.00 (s, 1H, H-C^{23}), 7.97 – 7.93 (m, 4H), 7.65 (dd, *J* = 8.5, 3.9 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 5.14 (t, *J* = 4.9 Hz, 2H, H_2C^{12}), 4.93 (t, *J* = 4.9 Hz, 2H, H_2C^{13}), 2.42 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.22 (C-carbonyl), 159.10, 151.95, 151.74, 139.16, 137.50, 134.94, 133.57, 131.36, 129.01, 128.52, 128.44, 128.22, 127.86, 123.24, 114.64, 50.52 (C^{12}), 45.49 (C^{13}), 14.49 (C^{26}) ppm. Anal. Calcd for $C_{20}H_{17}N_5O_3$ (375): C, 63.99; H, 4.56; N, 18.66. found: C, 64.02; H, 4.51; N, 18.64.

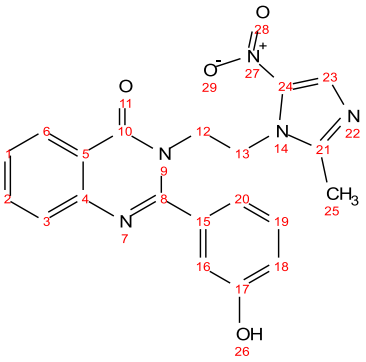
3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-(p-tolyl)quinazolin-4(3H)-one ***7b***



White solid (94%); mp 214-216 °C; IR (KBr) ṽ [cm^{-1}] = 3074, 2925, 1640, 1298. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.50 – 8.43 (m, 2H), 8.03 – 7.93 (m, 3H), 7.67 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.58 – 7.52 (m, 3H), 5.16 (t, *J* = 4.8 Hz, 2H, H_2C^{12}), 4.94 (t, *J* = 4.8 Hz, 2H, H_2C^{13}), 2.41 (s, 3H, CH_3), 2.31 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.11 (C-carbonyl), 159.12, 151.94, 151.76, 141.19, 139.16, 134.84, 133.58, 133.47, 129.60,

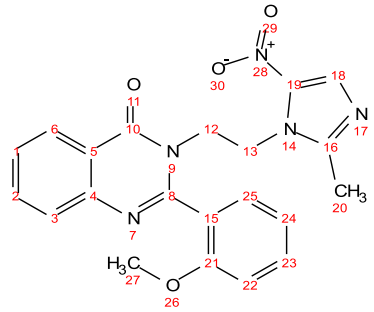
128.42, 128.42, 128.12, 127.59, 123.22, 114.51, 50.37 (C^{12}), 45.48 (C^{13}), 21.53 (C^{25}), 14.48 (C^{26}) ppm. Anal. Calcd for $C_{21}H_{19}N_5O_3$ (389): C, 64.77; H, 4.92; N, 17.98. found: C, 64.72; H, 4.93; N, 17.97.

2-(3-hydroxyphenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7c***



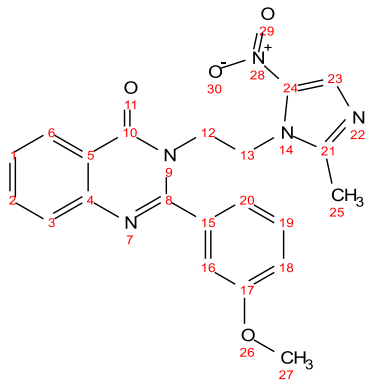
White solid (87%); mp 208-210 °C; IR (KBr) [ν /cm^{-1}] = 3073, 2937, 1643, 1239. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 9.66 (s, 1H), 7.98 (s, 1H), 7.95 – 7.93 (m, 3H), 7.69 – 7.61 (m, 1H), 7.53 (d, J = 7.9 Hz, 2H), 7.24 (t, J = 7.9 Hz, 1H), 6.96 (dd, J = 8.0, 1.7 Hz, 1H), 5.10 (m, J = 4.8 Hz, 2H, H_2C^{12}), 4.92 (t, J = 4.7 Hz, 2H, H_2C^{13}), 2.29 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.06 (C-carbonyl), 159.18, 158.05, 151.96, 151.69, 138.92, 138.56, 134.83, 133.56, 129.93, 128.68, 128.16, 127.73, 125.94, 123.17, 119.43, 114.62, 57.18 (C^{12}), 45.41 (C^{13}), 14.51 (C^{25}) ppm. Anal. Calcd for $C_{20}H_{17}N_5O_4$ (391): $C_{20}H_{17}N_5O_4$, C, 61.38; H, 4.38; N, 17.89. found: C, 61.41; H, 4.34; N, 17.88.

2-(2-methoxyphenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7d***



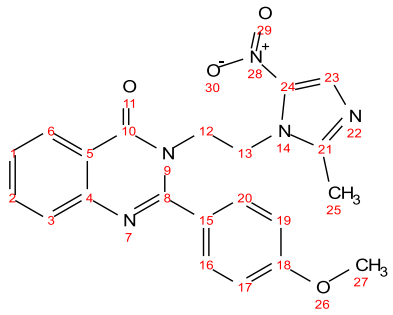
White solid (94%); mp 205-207 °C; IR (KBr) ṽ [cm^{-1}] = 3086, 2963, 1659, 1293. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.33 (d, *J* = 8.1 Hz, 2H), 7.99 – 7.89 (m, 4H), 7.67 – 7.58 (m, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 5.11 (t, *J* = 4.7 Hz, 2H, H_2C^{12}), 4.91 (t, *J* = 4.7 Hz, 2H, H_2C^{13}), 4.03 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.05 (C-carbonyl), 159.11, 151.92, 151.74, 141.14, 139.14, 134.81, 134.78, 133.57, 129.56, 129.56, 128.50, 128.40, 128.08, 127.53, 123.18, 114.51, 65.23 (C^{27}), 51.01 (C^{12}), 45.48 (C^{13}), 14.48 (C^{20}) ppm. Anal. Calcd for $C_{26}H_{21}N_5O_4$ (405): $C_{26}H_{21}N_5O_4$, C, 66.80; H, 4.53; N, 14.98. found: C, 66.83; H, 4.54; N, 14.91.

2-(3-methoxyphenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7e***



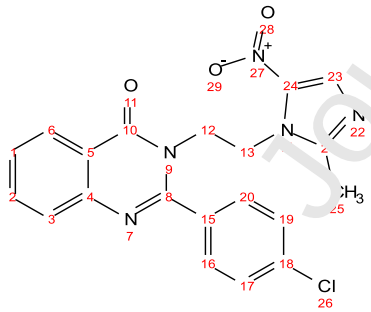
White solid (91%); mp 234-236 °C; IR (KBr) ṽ [cm^{-1}] = 2952, 1646, 1258. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.05 (d, *J* = 7.8 Hz, 1H), 8.02 – 7.89 (m, 5H), 7.70 – 7.62 (m, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.13 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.15 (t, *J* = 4.8 Hz, 2H, H_2C^{12}), 4.92 (t, *J* = 4.8 Hz, 2H, H_2C^{13}), 3.89 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.13 (C-carbonyl), 159.96, 158.84, 151.93, 151.65, 139.15, 138.98, 134.90, 133.54, 130.06, 128.23, 127.87, 123.21, 120.90, 117.10, 114.66, 113.44, 65.27 (C^{27}), 55.63 (C^{12}), 45.47 (C^{13}), 14.49 (C^{25}) ppm. Anal. Calcd for $C_{26}H_{21}N_5O_4$ (405): $C_{26}H_{21}N_5O_4$, C, 66.80; H, 4.53; N, 14.98. found: C, 66.82; H, 4.58; N, 14.96.

2-(4-methoxyphenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7f***



White solid (89%); mp 209-211 °C; IR (KBr) ṽ [cm^{-1}] = 2956, 1642, 1298. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.41 (d, *J* = 8.9 Hz, 2H), 7.97 – 7.91 (m, 3H), 7.65 – 7.58 (m, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.9 Hz, 2H), 5.13 (t, *J* = 4.8 Hz, 2H, H_2C^{12}), 4.93 (t, *J* = 4.8 Hz, 2H, H_2C^{13}), 3.87 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.02 (C-carbonyl), 162.07, 158.98, 151.86, 139.16, 134.81, 133.59, 130.16, 130.01, 128.60, 127.96, 127.30, 125.93, 123.21, 114.34, 65.20 (C^{27}), 55.82 (C^{12}), 45.50 (C^{13}), 14.50 (C^{26}) ppm. Anal. Calcd for $C_{26}H_{21}N_5O_4$ (405): $C_{26}H_{21}N_5O_4$, C, 66.80; H, 4.53; N, 14.98. found: C, 66.83; H, 4.53; N, 14.97.

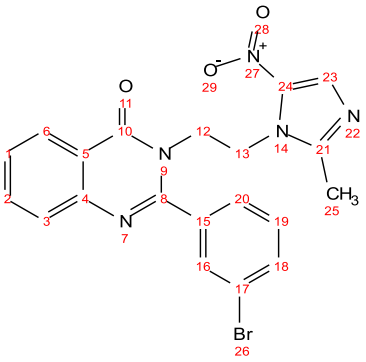
2-(4-chlorophenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7g***



White solid (95%); mp 239-241 °C; IR (KBr) ṽ [cm^{-1}] = 2983, 1647, 1291. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.42 (d, *J* = 8.5 Hz, 2H), 7.99 – 7.91 (m, 4H), 7.69 – 7.63 (m, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 5.11 (t, *J* = 4.6 Hz, 2H, H_2C^{12}), 4.92 (t, *J* = 4.5 Hz, 2H, H_2C^{13}), 2.40 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.24 (C-carbonyl), 158.03, 151.96, 151.57, 139.14, 136.32, 136.19, 134.99, 133.59, 130.10, 129.04, 128.17,

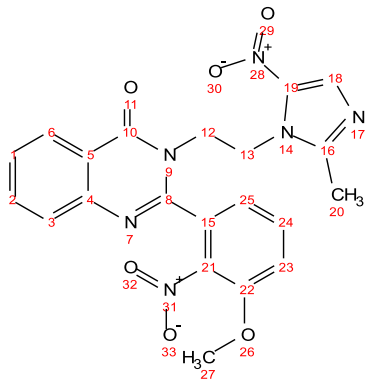
128.02, 123.23, 114.61, 55.24 (C^{12}), 45.44 (C^{13}), 14.50 (C^{25}) ppm. Anal. Calcd for $C_{20}H_{16}ClN_5O_3$ (409): C, 58.61; H, 3.94; N, 17.09. found: C, 58.57; H, 3.91; N, 17.06.

2-(3-bromophenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7h***



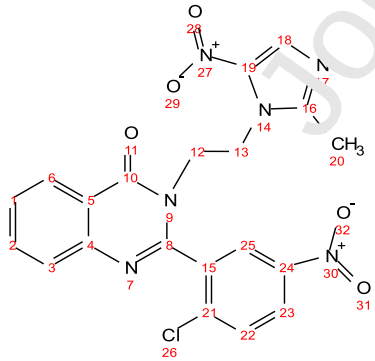
White solid (88%); mp 248-250 °C; IR (KBr) ṽ [cm^{-1}] = 2905, 1742, 1156. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.34 (d, J = 8.1 Hz, 2H), 7.97 (m, 1H), 7.94 (d, J = 3.4 Hz, 2H), 7.68 – 7.59 (m, 1H), 7.33 (d, J = 8.1 Hz, 2H), 5.12 (t, J = 4.7 Hz, 2H, H_2C^{12}), 4.92 (t, J = 4.7 Hz, 2H, H_2C^{13}), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.38 (C-carbonyl), 163.07, 157.58, 151.94, 149.04, 139.83, 134.92, 133.53, 130.80, 128.31, 127.93, 127.24, 126.33, 123.29, 122.53, 121.34, 114.80, 52.39 (C^{12}), 45.43 (C^{13}), 14.52 (C^{26}) ppm. Anal. Calcd for $C_{20}H_{16}BrN_5O_3$ (454): C, 52.88; H, 3.55; N, 15.42. found: C, 52.83; H, 3.52; N, 15.47.

2-(3-methoxy-2-nitrophenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7i***



White solid (81%); mp 174-176 °C; IR (KBr) ṽ [cm^{-1}] = 3082, 2967, 2924, 1657, 1224. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.04 – 7.99 (m, 2H), 7.97 (d, *J* = 4.8 Hz, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.76 – 7.68 (m, 2H), 7.55 (d, *J* = 7.5 Hz, 1H), 4.99 – 4.86 (m, 4H, CH_2-CH_2), 3.98 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.13 (C-carbonyl), 156.10, 151.90, 151.25, 140.47, 139.05, 135.35, 133.56, 131.67, 131.60, 130.48, 128.92, 128.29, 123.25, 122.38, 116.13, 114.56, 66.08 (C^{27}), 57.43 (C^{12}), 45.25 (C^{13}), 14.46 (C^{20}) ppm. Anal. Calcd for $C_{21}H_{18}N_6O_6$ (450): C, 56.00; H, 4.03; N, 18.66. found: C, 55.98; H, 4.01; N, 18.64.

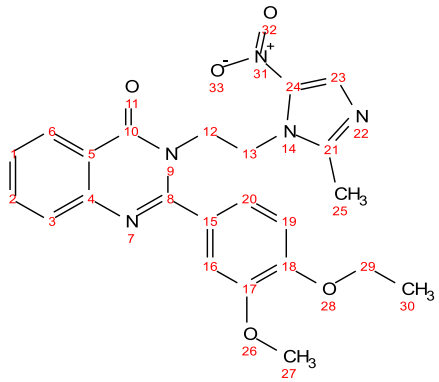
3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one ***7j***



White solid (92%); mp 185-187 °C; IR (KBr) ṽ [cm^{-1}] = 3082, 2965, 1651, 1247. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.62 (d, *J* = 2.8 Hz, 1H), 8.37 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.11 – 8.03 (m, 3H), 7.94 (d, *J* = 8.8 Hz, 1H), 7.88 (s, 1H), 7.84 – 7.77 (m, 1H), 5.08 (t, *J*

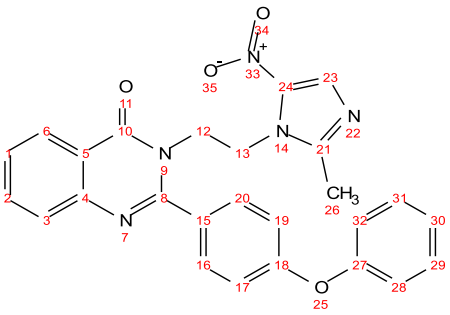
= 4.8 Hz, 2H, H_2C^{12}), 4.90 (t, *J* = 4.8 Hz, 2H, H_2C^{13}), 2.47 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.19 (C-carbonyl), 158.17, 151.86, 151.27, 146.73, 139.21, 139.17, 138.55, 135.36, 133.42, 132.59, 129.12, 128.35, 126.76, 125.74, 123.27, 114.45, 54.27 (C^{12}), 45.42 (C^{13}), 14.43 (C^{20}) ppm. Anal. Calcd for $C_{20}H_{15}ClN_6O_5$ (454): C, 52.81; H, 3.32; N, 17.48. found: C, 52.85; H, 3.31; N, 17.53.

2-(4-ethoxy-3-methoxyphenyl)-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one ***7k***



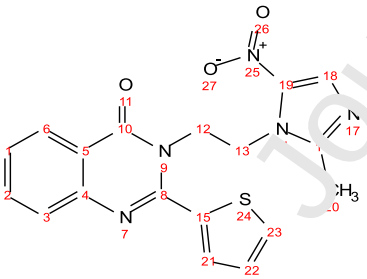
White solid (86%); mp 199-201 °C; IR (KBr) ṽ [cm^{-1}] = 2972, 2938,1634, 1252. ^1H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.04 – 7.86 (m, 6H), 7.60 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 5.12 – 5.09 (m, 2H, H_2C^{12}), 4.96 – 4.85 (m, 2H, H_2C^{13}), 4.11 (q, *J* = 6.8 Hz, 2H, H_2C^{29}), 3.91 (s, 3H, OCH_3), 2.49 (s, 3H, H_3C), 1.39 (t, *J* = 6.8 Hz, 3H, H_3C^{30}) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 165.87 (C-carbonyl), 158.94, 151.91, 151.80, 151.10, 149.10, 139.13, 134.73, 133.54, 129.93, 127.93, 127.21, 123.17, 121.98, 114.28, 112.47, 111.30, 64.94 (C^{27}), 64.21 (C^{29}), 55.82 (C^{12}), 45.55 (C^{13}), 15.16 (C^{30}), 14.47 (C^{25}) ppm. Anal. Calcd for $C_{23}H_{23}N_5O_5$ (449): C, 61.46; H, 5.16; N, 15.58. found: C, 61.43; H, 5.13; N, 15.62.

3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-(4-phenoxyphenyl)quinazolin-4(3H)-one ***7l***



White solid (84%); mp 189-191°C; IR (KBr) ṽ [cm^{-1}] =3056, 2938, 1632, 1309. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.25 (d, *J* = 7.6 Hz, 1H), 8.10 (s, 1H, H-C^{23}), 8.01 – 7.93 (m, 4H), 7.68 (s, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.49 – 7.43 (m, 2H), 7.24 – 7.18 (m, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 5.14 – 5.03 (m, 2H, H_2C^{12}), 4.98 – 4.86 (m, 2H, H_2C^{13}), 2.42 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.25 (C-carbonyl), 158.40, 157.52, 157.08, 151.93, 151.60, 143.98, 139.66, 134.99, 133.55, 130.61, 128.26, 124.12, 123.60, 123.24, 121.54, 119.25, 118.35, 114.73, 50.04 (C^{12}), 45.38 (C^{13}), 14.49 (C^{26}) ppm. Anal. Calcd for $C_{26}H_{21}N_5O_4$ (467): C, 66.80; H, 4.53; N, 14.98. found: C, 66.84; H, 4.52; N, 14.9.

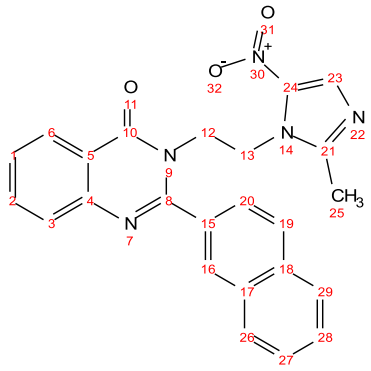
3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-(thiophen-2-yl)quinazolin-4(3H)-one ***7m***



White solid (84%); mp >250°C; IR (KBr) ṽ [cm^{-1}] = 3075, 2938, 1644, 1299. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.00 – 7.95 (m, 3H), 7.92 (dd, *J* = 6.8, 1.4 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.80 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.66 – 7.59 (m, 1H), 7.24 (dd, *J* = 5.0, 3.7 Hz, 1H), 5.12 (t, *J* = 4.9 Hz, 2H, H_2C^{12}), 4.91 (t, *J* = 4.9 Hz, 2H, H_2C^{13}), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.01 (C-carbonyl), 156.08, 151.90, 151.59, 143.31, 139.15, 135.05, 133.58, 131.42, 129.75, 128.84, 127.65, 127.51, 123.36, 114.47,

54.74 (C^{12}), 45.47 (C^{13}), 14.49 (C^{20}) ppm. Anal. Calcd for $C_{18}H_{15}N_5O_3S$ (381): C, 56.68; H, 3.96; N, 18.36. found: C, 56.74; H, 3.92; N, 18.31.

3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-2-(naphthalen-2-yl)quinazolin-4(3H)-one ***7n***



White solid (92%); mp 177-179 °C; IR (KBr) ṽ [cm^{-1}] = 3071, 2936, 1632, 1297. ^{1}H NMR (301 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.06 – 8.01 (m, 3H), 7.95 (s, 1H, H-C^{23}), 7.89 (s, 1H), 7.75 – 7.72 (m, 1H), 7.71 – 7.65 (m, 2H), 7.62 – 7.59 (m, 2H), 7.58 – 7.53 (m, 2H), 5.07 (t, J = 4.8 Hz, 2H, H_2C^{12}), 4.92 (t, J = 4.8 Hz, 2H, H_2C^{13}), 2.38 (s, 1H, CH_3) ppm. ^{13}C NMR (76 MHz, DMSO-d_6): δ = 166.00 (C-carbonyl), 161.59, 155.01, 151.95, 151.58, 135.91, 135.36, 134.96, 134.06, 133.51, 131.93, 131.08, 130.74, 129.68, 128.87, 128.22, 127.19, 126.73, 126.39, 125.63, 123.17, 121.02, 114.26, 50.63 (C^{12}), 45.33 (C^{13}), 14.49 (C^{26}) ppm. Anal. Calcd for $C_{24}H_{19}N_5O_3$ (425): C, 67.76; H, 4.50; N, 16.46. found: C, 67.72; H, 4.43; N, 16.47.

3.4. Enzymatic assays

In the present work, hCA I, and II isoenzymes were purified by Sepharose-4B-L-Tyrosine-sulfanilamide affinity column chromatography [65]. On the other hand, inhibition effects of novel quinazolinone derivatives **7a-n** on CA isoenzymes activity was determined according to the spectrophotometric method of Verpoorte et al. as described in our previous studies [66-70]. The inhibitory effect of novel quinazolinone derivatives **7a-n** on AChE and BChE

activities was performed according to spectrophotometric method of Ellman as described previously [71-74]. Butyrylcholinesterase from equine serum and acetylcholinesterase from *Electrophorus electricus* (electric eel) have been purchased from Sigma-Aldrich. α-Glycosidase inhibition effect of novel quinazolinone derivatives (**7a-n**) was evaluated according to the method of Tao et al [75-79]. The absorbance of samples was recorded at 405 nm.

3.5. Docking study

Docking studies of the compounds **7j** and **7g** as most potent compounds against hCA I (pdb code: 4WR7) and hCA II (pdb code: 5AML), compounds **7n** and **7l** as most potent compounds against AChE (pdb code: 1EVE), and BChE (pdb code: 1P0I), and compounds **7h** and **7j** as most potent compound against α-glucosidase (modeled enzyme) were performed by Autodock Tools 1.5.6 [80, 81]. The 3D structures of the selected inhibitors were constructed by MarvineSketch 5.10.4, and then the pdbqt formats of these entries were prepare by Autodock Tools 1.5.6. By using the latter software, the pdbqt structures of the target enzymes were also constructed and Autodock Tools parameters for them were set as follows: 1) hCA I: box size: 40×40×40 Å, the center of box: x = 1.8085, y = 72.66, z = 55.3515, 2) hCA II: box size: 60×60×60 Å, the center of box: x = -3.8315, y = 3.9065, z = 15.043, 3) AChE: box size: 40×40×40 Å, the center of box: x = 2.023, y = 63.295, z = 67.062, 4) BChE: box size: 56×56×56 Å, the center of box: x = 137.985, y = 122.725, z = 38.78, 5) α-glucosidase: box size: 40×40×40 Å, the center of box: x = 12.5825, y = -7.8955, z = 12.519 [82]. Each docked system was carried out by 25 runs of the AUTODOCK search (by the Lamarckian genetic algorithm). Finally, the best-docked pose of each selected compound was analyzed by Discovery Studio 2019 Client (Accelrys, Inc., San Diego, CA).

3.6. Cytotoxicity effect

3.6.1. Proliferation of cells

Human prostate and breast cancer cell lines (LNCaP and MCF-7, respectively) were used to determine the cytotoxic effects of the tested compounds. LNCaP and MCF-7 cells were cultured in RPMI-1640 medium (Sigma-Aldrich, USA) and DMEM medium (Gibco, UK), respectively. This medium supplemented with 10% fetal bovine serum (Biowest, USA) and 1% penicillin/streptomycin solution (Gibco, UK). Cell plates were maintained in 75-cm^2 culture flasks (TPP) and were placed in 5% CO_2 humidified atmosphere at 37 °C (Thermo Forma II CO_2 incubator, USA).

3.6.2. Cell Viability

The cytotoxicity of compounds was tested using 3-(4,5-dimethyl thiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) assays [82]. LNCaP and MCF-7 cells were seeded in 96-well microplate (15×10^3) and cultured for 24 h. Cells were treated with 1, 5, 25, 50 and 100 µM concentrations of test compounds (DMSO for Vehicle control) at 24 h. After incubated, 50 µL of MTT (0.5 mg/mL) solution was added to the wells and incubated further for 3 h. Then medium was removed and 100 µL of DMSO was added to each wells. A microplate reader was used for measurement of absorbance of each plate at 570 nm (Thermo Multiskan Go, USA). Absorbances from the control wells (only cells and culture medium) were measured and the mean absorbance values obtained were considered as 100% viable cells. Viability values in treated groups were calculated according to the control group. These experiments were repeated 5 times independently on different days [83].

3.7. Statistical Analysis

Statistical analysis was performed using SigmaPlot 12.0 software package. Homogeneity of variance was evaluated by Shapiro-Wilk test. In case of equal variances, ANOVA was used to determine differences between the two groups and Bonferroni test was used for multiple comparisons. In case of unequal variances (or non-normal distributions), Kruskal-Wallis H

test followed by post-hoc Tukey test was used. Quantitative data were expressed as the mean of standard deviation (Mean±SD) and were considered significant at $p<0.05$ [84].

4. Conclusion

New quinazolinone derivatives **7a-n** were synthesized and screened for their inhibitory activities on metabolic enzymes hCA I, II, AChE, BChE, and α-glycosidase. All the synthesized compounds exhibited better enzymatic inhibitory activities than acetazolamide as standard CA inhibitor, tacrine as standard ChEI, and acarbose as standard α-glycosidase inhibitor. Molecular docking analysis of the most potent derivatives against each enzyme was performed and obtained data revealed that our new potent synthesized compounds interacted with key residues in the active site of studied target enzymes. Furthermore, *in vitro* cytotoxicity study of the title compounds **7a-n** demonstrated that these compound were almost inactive against cancer cell line MCF-7 and LNCaP.

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Declaration of competing interests

■ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Novel quinazolinone derivatives **7a-n** were synthesized.
- They evaluated against some metabolic enzymes.
- Cytotoxicity study of the compounds **7a-n** demonstrated.
- Molecular docking was studied.