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Biological Evaluation of Azomethine-dihydroquinazolinone Conjugates as Cancer and Cholinesterase Inhibitors

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Abstract: In an attempt to discover novel anti-cancer agents and potent cholinesterase inhibitors, 11 azomethine-dihydroquinazolinone conjugates were evaluated against lung carcinoma cells and cholinesterases. Most of the compounds exhibited significant cytotoxicity at low micromolar concentrations and were less toxic to normal cells. After 24 h incubation period, **2i** showed maximum cytotoxicity. The 4-bromine substituted compounds showed higher acetylcholinesterase (AChE) inhibitory activity than other screened compounds. The most active compound **2c,** among the series, had an IC_{50} value 209.8 µM against AChE. The tested compounds showed less inhibition against butyrylcholinesterase. Molecular docking studies were performed in order to investigate the plausible binding modes of synthesized compounds. The compounds can be further optimized to treat cancer and Alzheimer's disease. These derivatives may open new pathways for introducing new therapies for curing cancer and senile dementia.

Keywords: Acetylcholinesterase, azomethine-dihydroquinazolinone, butyrylcholinesterase, cancer, lung carcinoma cells, senile dementia.

INTRODUCTION

Quinazolinone derivatives demonstrate a broad range of biological activities including anticancer [1-3], anticoccidial [4], 5-HT_7 receptor antagonist [5], analgesic and anti-inflammatory properties [5]. In addition, 2-[4-(aminoalkoxy) phenyl]-4(3**H**)-quinazolinone derivatives are potent human H_3 receptor inverse agonists [6] and 2-thiophen-5-yl-3H-quinazolin-4-one analogues are inhibitors of transcription factors NF-κB and AP-1 mediated transcriptional activation and thus find possible utilization as anti-inflammatory and anti-cancer agents [7]. The azomethine derivatives of quinazolinyl acetohydrazide, in which the carbonyl group being replaced by the imine or azomethine group [8], are found to exhibit antimalarial [9], antibacterial and antifungal activities [10].

Alzheimer's disease (AD) is an irreversible neurodegenerative disease characterized by loss of cholinergic neurotransmission in cerebral cortex and sub-cortical region [11]. β-Amyloid aggregation and diminished levels of acetylcholine (ACh) have been reported to cause Alzheimer's disease [12, 13]. ACh is rapidly degraded by cholinesterase (ChE) into choline and acetic acid thus terminating the cholinergic neurotransmission [14]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) share extensive sequence homology, ~ 65%, however they both differ in inhibitors sensitivity and substrate specificities [11]. AChE is most widely distributed in brain with higher selectivity for hydrolyzing ACh. On the contrary, BChE (known as pseudocholinesterase) hydrolyzes BCh more rapidly than ACh [15].

ACh released by the parasympathetic nervous system is a neurotransmitter of the cholinergic innervation, stimulating nicotinic as well as muscarinic receptors [16]. Muscarinic receptor stimulation is associated with the smooth muscles and heart, and detailed classical symptoms are previously described. Nicotinic receptors stimulate the central nervous system (CNS) and the skeletal muscles. Nicotinic receptor stimulation causes contractions in the skeletal muscles and in CNS, it primarily functions in memory and cognitive processes [17]. The cholinergic neurotransmission can be strengthened by increasing ACh levels and it can be achieved by cholinesterase inhibitors [11]. Cholinesterase inhibitors used in the treatment of AD are rivastigmine, tacrine, ensaculin [14], donepezil and galantamine [13, 14]. Compounds such as organophosphates, coumarine, carbamates, cinnamides [14] and dihydroquinazolinone [18] derivatives have been reported to inhibit cholinesterase. Dihydroquinazolinone derivatives also possess antitumor [19], antidepressant [20] and calcium channel blocking activity [21].

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Many remarkable biological functions in proteins and DNA and their profound dynamic mechanisms, such as switch between active and inactive states [22], cooperative effects [23], allosteric transition [24-26] and intercalation of drugs into DNA [27], can be revealed by studying their internal motions as summarized in a comprehensive review [28]. Likewise, to fully understand the interaction of a protein receptor with its ligand and to in-depth reveal their binding mechanism, we should release the constraints on the receptor structure allowing it flexible as well during the docking process, and we shall make efforts in this regards in our future work.

Keeping the above mentioned biological importance of quinazolinone derivatives in mind, we evaluated the azomethine-dihydroquinazolinone conjugates for their anticancer and cholinesterase inhibition studies.

MATERIALS AND METHODS

A general procedure for the synthesis of azomethine dihydroquinazolinone conjugates (**2a-k**) has been reported in our recently published paper [29].

Cell Lines and Cell Cultures

Lung carcinoma (H157) cell lines (ATCC CRL-5802) and human corneal epithelial cells (HCEC) (obtained from RIKEN Bio Resource Center, Japan) were used. Cell lines were maintained in medium and cultured at 37 ºC in a 5% CO_2 incubator using the previous method [30]. Monolayers obtained were used for experimental purpose.

Cytotoxicity Analysis by Sulforhodamine B (SRB) Assays

Cytotoxicity assays were performed using the standard SRB method of Skehan *et al.,* [31]. Cells were cultured in 96 well plates and test compounds were added to wells at final concentration of 100 µM, and incubated for 24 h. The wells containing culture media with cells being treated as control, whereas the wells with culture medium without cells were taken as blank. After 24 h incubation, cells were fixed by using 50% trichloroacetic acid (TCA) solution and incubated for 1 h at 4 °C. Plates were washed five times with phosphate-buffered saline (PBS) and left for air drying. After drying of TCA fixed cells, staining was done by 0.4% SRB solution for 30 min at room temperature. The unbound dye was removed by rinsing four times with acetic acid solution (1%) and left for air drying. The protein bound dye was solubilized, after air drying, by the addition of Tris-base solution (100 µL/well, 10 mM). The optical densities (OD) were measured at 490 nm subtracting the background measurement at 630 nm using 96-well microplate reader (Bio-Tek ELx 800^{TM}, Instruments, Inc. Winooski, VT, USA). All experiments were repeated at least three times. Results reported are mean of three independent experiments (± SEM) and expressed as percent inhibitions calculated by the formula:

Percent inhibition = 100 – (Sample absorbance / control absorbance) × 100

Blank background optical density was measured in wells incubated with growth medium without cells. Control values were obtained from H157 incubated in RPMI medium alone without test compounds. HCEC cells were used as normal cells were used as normal cell and were treated like H157 throughout the assay [32].

Determination of AChE and BChE Inhibitory Activities

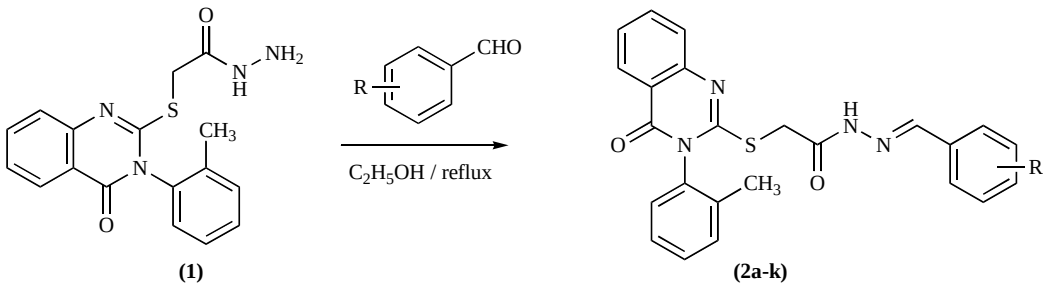
Commercially purified electric *eel* AChE and horse serum BChE was used. Cholinesterases inhibition was measured by the standard procedure by Ellman [33] with slight modification [34]. Synthesized compounds were dissolved in DMSO and were screened at 0.2 mM per well concentration for initial screening (for both AChE and BChE). Further dilutions were used for compounds having more than 50% inhibition. The initial reaction mixture consisted of assay buffer, test compound and enzyme (0.5 and 3.4 U/mg of AChE or BChE, respectively). A 10 min preincubation of the reaction mixture at 25 ^{o}C was carried out. After incubation their respective substrates (1 mM) and coloring reagent, 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (3 mM) were added. The mixtures were further incubated for 15 min at 25 °C. The absorbance was determined at 405 nm using a microplate reader (Bio-Tek ELx800^{TM}, Instruments Inc., Winooski, VT, USA). All experiments were repeated at least three times. Results reported are mean of three independent experiments (± SEM) and expressed as percent inhibitions calculated by the formula:

Percent inhibition = 100 – (Sample absorbance / control absorbance) × 100

Neostigmine and donepezil were used as reference compounds. The buffer for enzyme dilution contained 50 mM Tris-HCl and 0.1% (w/v) BSA having pH 8.0. The analysis of each concentration was done in triplicate, IC_{50} values of potential inhibitors (≥50%) were determined with the help of the Graph Pad prism 5.0 Software Inc., San Diego, California, USA [35].

Molecular Docking

The X-ray crystal structure of human AChE was downloaded from the RCSB (PDB ID: 4BDT) [36]. AutoDock 4.2 [37] was used for docking studies. Prior to docking, the enzyme or receptor was prepared by removing all water and other heteroatoms from it, hydrogen atoms and Gasteiger charges were added using MGL Tools [37]. AutoDock uses AutoGrid to calculate affinity maps for interaction energy of various atom types with the enzyme which is then used by the AutoDock docking to calculate the total interaction energy for a ligand with receptor (enzyme). In AutoDock the receptor is kept rigid, whereas the ligand is treated as flexible. The grid-box (80 x 80 x 80) was centered on the active site gorge was large enough to allow free movement of ligand. Method validation was carried out by re-docking the ligand (extracted from 4BDT) into the same enzyme. The docking methodology was able to reproduce the experimentally determined binding conformation for the ligand with an rmsd of less than 0.5 Å. For ligands, pdb files were created, Gasteiger charges were added using ANTECHAMBER [38]. Before docking the energies of the ligands were minimized using Chimera, through 100 steepest descents and 100 conjugate gradient steps using a step size of 0.02. Lamarckian Genetic Algorithm (LGA) was used for docking, the number of GA runs was set to 20 and the number of maximum evaluation was $5x10^6$ for Genetic Algorithm parameter and

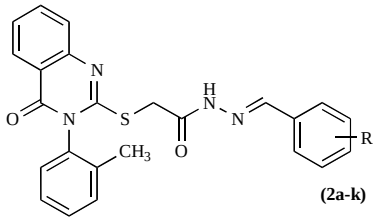


(1) **(2a-k)**

2a: R= 2-Br **2b:** R= 3-Br
2c: R= 4-Br **2d:** R= 2-Cl
2e: R= 3-Cl **2f:** R= 4-Cl
2g: R= 3-NO_2 **2h:** R= 4-NO_2
2i: R= 2-OMe **2j:** R= 3,4,5-$(OMe)_3$
2k: R= 2-OBz

Scheme 1. Synthetic pathway to azomethine dihydroquinazolinone conjugates.

Table 1. Cytotoxicity activity (%) *in vitro* of the 2a-k against lung carcinoma cells (H157) and human corneal epithelial cells (HCEC).



(2a-k)

Compounds	H157	HCEC
	% Inhibition ± SEM (100 µM)	
2a	35.2 ± 6.41	6.21 ± 0.13
2b	36.3 ± 7.46	5.13 ± 1.21
2c	38.4 ± 4.91	5.70 ± 0.12
2d	34.2 ± 4.92	7.33 ± 2.11
2e	53.0 ± 2.51	7.42 ± 2.12
2f	40.1 ± 2.58	5.81 ± 1.91
2g	52.1 ± 7.05	4.42 ± 1.42
2h	56.3 ± 5.27	7.21 ± 3.5
2i	62.3 ± 7.82	7.35 ± 2.65
2j	42.1 ± 1.44	7.51 ± 2.18
2k	53.7 ± 3.74	4.58 ± 1.12
Methotrexate	22.3 ± 1.42	6.48 ± 3.18

docking generations were 27000. For visualization of docked results, Discovery Studio Visualizer 3.5 [39] was used.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway leading to the synthesis of title compounds is shown in Scheme **1**. Thus, eleven azomethine dihydroquinazolinone conjugates **(2a-k)** were obtained by treating acetohydrazide **(1)** with suitably substituted aromatic aldehydes in ethanol [29].

Biological Assays

In vitro Evaluation of Cytotoxic Activity

Cytotoxic properties of compounds (2a-k) were evaluated *in vitro* in lung carcinoma cells H157 and human corneal epithelial cells HCEC. Cytotoxicity data is shown in Table **1**. A dose-dependent decrease of cell density in H157 cells was observed after 24 h exposure to all azomethine derivatives. The tested compounds were non toxic to normal cells, HCEC (Table **1**). At 100 µM, compounds presented the percent inhibition against H157 ranging from 62% (2i) to 34% (2d). The compound 2i was found to be the most active in the se-

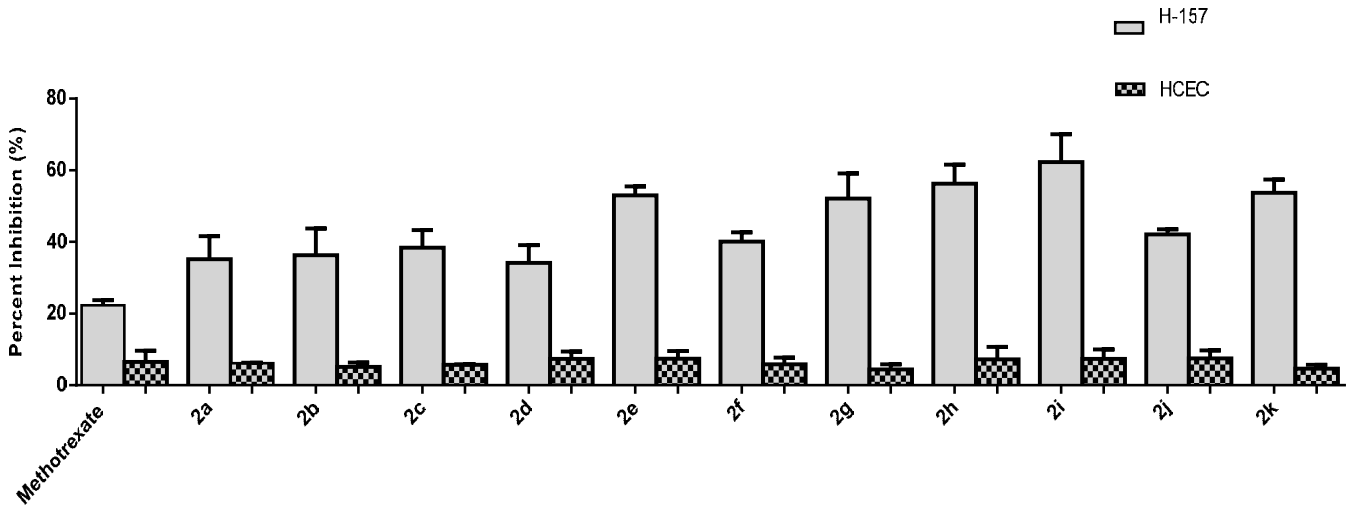


Fig. (1). Percent inhibition of derivatives and methotrexate against H157 and HCEC cell line at 100 μM.

series with 62.3% inhibition against cancer cells, whereas same compound represents 7.35% inhibition against normal cells. It was indicated that the compound has shown inhibitory potential against cancer cells, in contrast, the same compound was non-toxic to normal cells. The responses of the tested cell lines to new drugs were compared to the effect of methotrexate, a drug approved for use in lung cancer. To test the toxicity of derivatives against normal cells, HCEC cells were treated in the same way as cancer cell line, H-157. The results of cytotoxicity investigation proved azomethine derivatives as good anticancer agents having significant cytotoxic property against lung carcinoma cells. Whereas the compounds were non-toxic towards normal cell lines. The toxicity against normal cell lines was less than 10% for all the compounds and reference drug at 100 μM. The percent inhibition of screened compounds against cancer cells and normal cells has been shown in (Fig. **1**). Based on the results, further derivatives of most active compounds may be synthesized, which after *in vivo* studies may be used as a lead molecule for cancer treatment. These derivatives showed relatively low toxicity against normal cells which could be a positive aspect of this study in the design of more active and safer analogues.

Anticholinesterase Activity

The synthetic dihydroquinazolin derivatives were evaluated in vitro as inhibitors of electric eel acetylcholinesterase (AChE) and horse serum butyrylcholinesterase (BChE). The inhibitory activities of these derivatives were compared to those of neostigmine and donepezil. Inhibition potencies for compound showing greater than 50% were expressed as IC_{50} values and compounds with less than 50% were shown in percentage values (as in case of BChE) in Table **2**.

Bromine substituted derivative **2c** showed potent AChE inhibitory activity because of the presence of electron withdrawing bromine at *para*-position, substituting the same group at *ortho*-position (**2a**) decreases potency and substituting the same group at *meta*-position (**2b**) further declines the potency. However, adding electron donating groups such as nitro (**2g**) or methoxy group (**2i**) at *meta*-position results in potencies comparable to **2a**. Substituting the nitrite group from *meta* to *para* position as in **2h** decreases the potency. A further decrease in potency was observed when chlorine was added at either of the three positions (i.e. *o*-, *m*- or *p*-) in **2d**, **2e** and **2f**, respectively, and decreased activity was observed for **2j** containing tri-substituted methoxy groups. **2k** showed least activity owing to the presence of the electron donating benzyloxy group at *ortho*-position.

For the determination of potential interest of synthesized compounds in the treatment of AD, their AChE and BChE inhibitory activities were assayed. Compounds displayed inhibitory abilities in micromolar range for AChE, but they were not active against BChE. Recent studies have shown that in AD patients with severe pathology, BChE increases while AChE is reduced in specific brain regions [40], while another recent study shows a decrease of BChE activity *in vivo* [41]. The results presented herein showed that these derivatives may be used as an important starting point for further investigation of the possible use of these compounds in the therapy of neurodegenerative diseases.

Docking Studies

Molecular docking studies were carried out in order to gain insight into important binding site interactions that could aid in the design of more potent inhibitors. Compounds **2a**-**k**, were found to have similar binding modes. All compounds occupied the same position inside the active site (Fig. **2**). AutoDock calculated binding free energies of compounds **2a**-**k** are given in Table **3**. In compound **2c**, the aromatic dihydroquinazlinone ring was found to be involved in a π-stacking interaction with the hydrophobic residue Tyr337. The NH of azomethine group is making a hydrogen bond with oxygen atom of Tyr124 (2.05 Å). The amino acid residues Phe338 and Phe297 are in close contact with the azomethine group (Fig. **3**). Predicted binding site interactions of compound **2g** are given in (Fig. **4**). The dihydroquinazlinone ring occupies the same position as compound **2c**. The NH of azomethine group is making a hydrogen bond with oxygen atom of Tyr124 (1.82 Å). This hydrogen bond interaction was also found for compound **2c**, and seems to be an important contact. An additional hydrogen bond interac-

Table 2. Inhibitory activities of azomethine derivatives (2a-k) against cholinesterase enzymes (AChE and BChE).

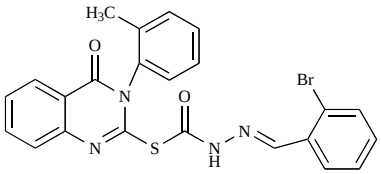
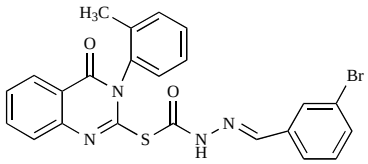
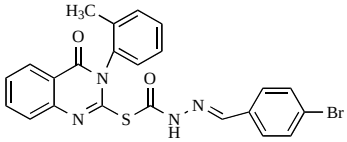
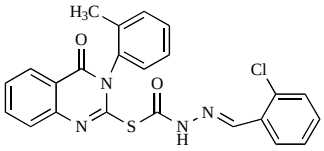
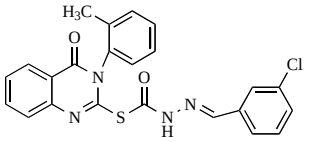
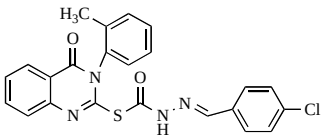
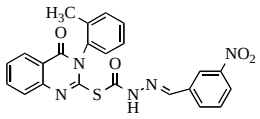
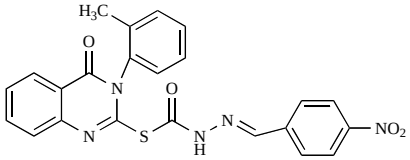
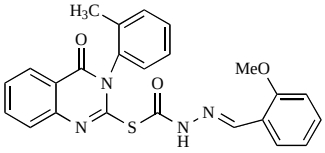
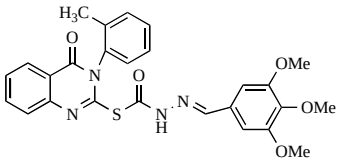
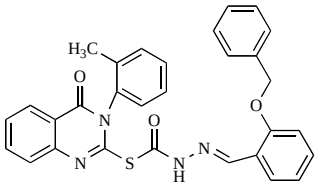
Codes	Compounds	AChE IC_{50} (μM ± SEM)	BChE % Inhibition
2a		281.1 ± 1.08	23.2
2b		642.9 ± 1.97	24.6
2c		209.8 ± 1.11	6.8
2d		690.5 ± 1.58	33.3
2e		582.3 ± 1.28	26.5
2f		627.2 ± 1.24	36.5
2g		276.1 ± 2.31	25.9
2h		765.7 ± 2.86	26.6
2i		281.5 ± 2.13	21.7
2j		538.6 ± 1.12	27.5

Table 2. contd…

Codes	Compounds	AChE IC_{50} (μM ± SEM)	BChE % Inhibition
2k		926.1 ± 2.08	34.9
Standard	**Neostigmine**	24.3 ± 4.11	IC_{50} = 37.1 ± 7.23 μM
Standard	**Donepezil**	0.02 ± 0.01	IC_{50} = 6.72 ± 0.48 μM

IC_{50} = Concentration of inhibitor required for half-maximal enzyme inhibition and are reported as means of three independent experiments, each performed in triplicate (SEM, standard error of mean).

Table 3. AutoDock 4.2 calculated binding free energies for docked inhibitors.

Codes	Binding Free Energy (ΔG) kcal/mol
2a	-12.2
2b	-11.76
2c	-12.89
2d	-11.5
2e	-11.55
2f	-11.72
2g	-11.93
2h	-11.55
2i	-11.69
2j	-11.45
2k	-11.44

tion was found to exist between the oxygen of NO_2 group and NH of Arg296 (1.74 Å).

The information of a binding pocket of a receptor for its ligand is very important for drug design, particularly for conducting mutagenesis studies [42]. In the literature, the binding pocket of a protein receptor to a ligand is usually defined by those residues that have at least one heavy atom (i.e., an atom other than hydrogen) within a distance of 5Å from a heavy atom of the ligand. Such a criterion was originally used to define the binding pocket of ATP in the Cdk5-Nck5a* complex [43] that has later proved quite useful in identifying functional domains and stimulating the relevant truncation experiments. The similar approach has also been used to define the binding pockets of many other receptor-ligand interactions important for drug design [25, 44-49].

CONCLUSION

Azomethine-dihydroquinazolinone derivatives (**2a-k**) were evaluated for cytotoxicity and cholinesterase inhibitory activities. The investigated compounds possess anticancer activity. Compounds **2i** and **2h** were the most active compounds against lung carcinoma cells H157 and were less toxic to normal cells. The compounds were further tested against cholinesterases such as acetylcholinesterase and bu-

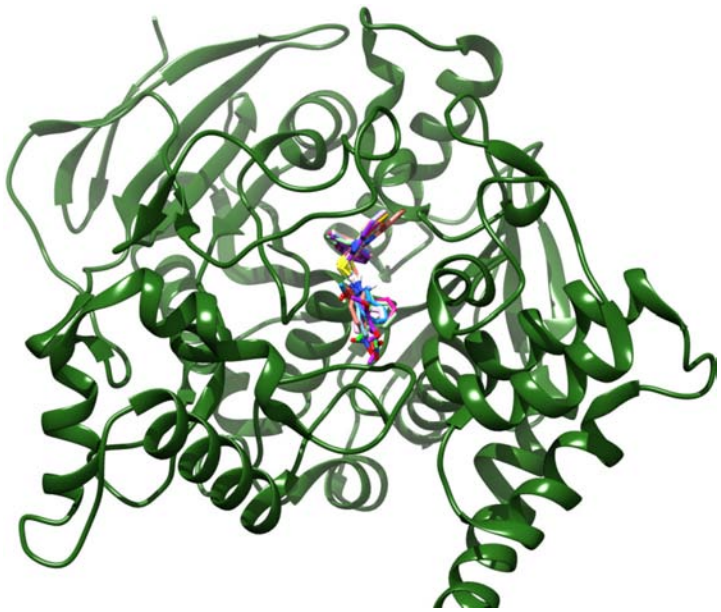


Fig. (2). Overlap of compounds **2a-k** into the active site gorge of AChE. All inhibitors were found to have similar binding orientations.

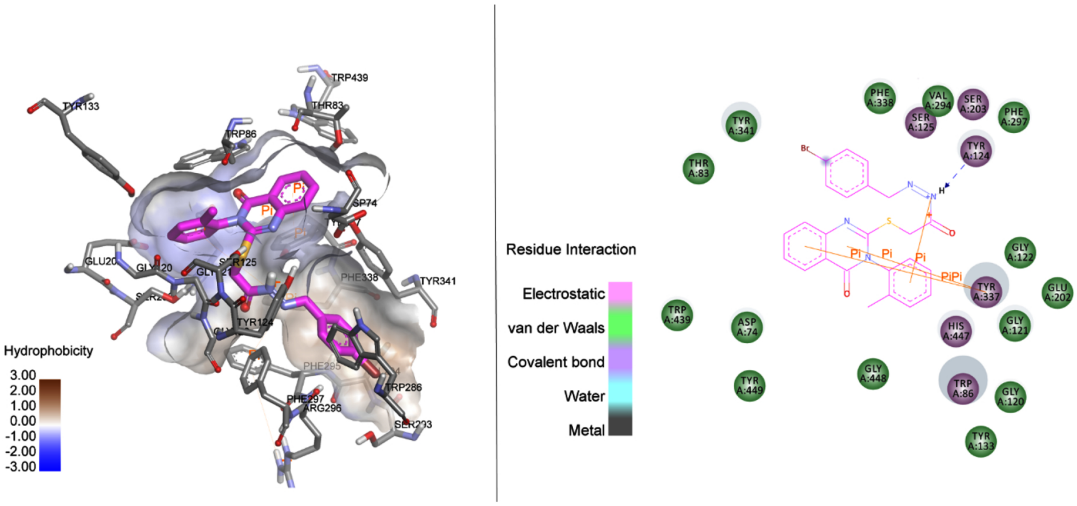


Fig. (3). Binding site interacions of compound **2c** (pink) inside active site gorge of AChE and its 2D interaction diagram. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

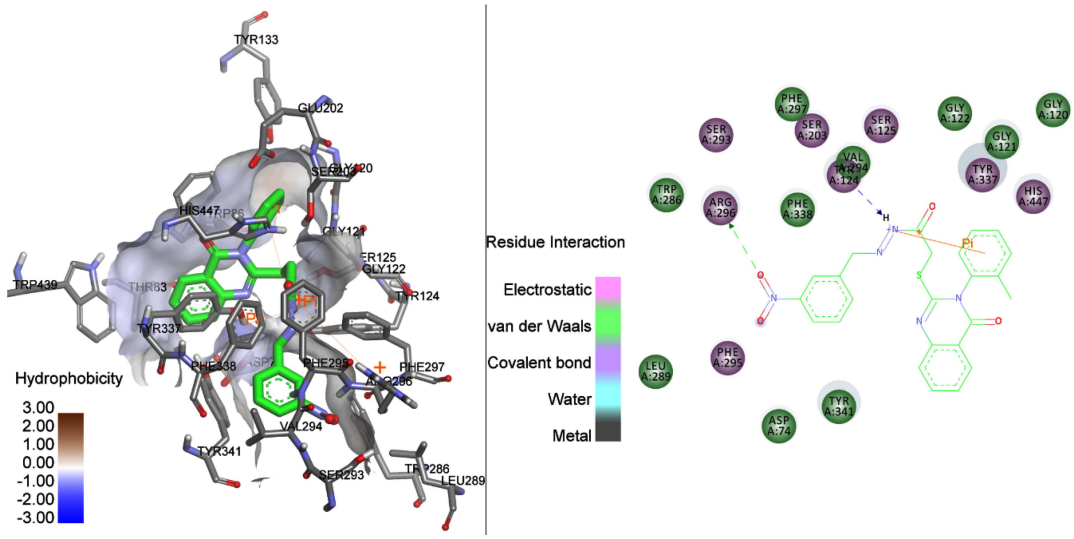


Fig. (4). Binding site interacions of compound **2g** (green) inside active site gorge of AChE and its 2D interaction diagram. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

tyrylcholinestrease. Most of the evaluated compounds were significant inhibitors of the AChE. The azomethine derivatives can be considered as anti-acetylcholinesterase and therefore, can be used in the search of new therapies for Alzheimer's disease. Furthermore, the results suggest that these derivatives may be used as preventive and chemotherapeutic agents for cancer. Hence it is recommended to find the correlation of AChE inhibitors and their role in the therapy of cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Hamel, E.; Lin, C. M.; Plowman, J.; Wang, H. K.; Lee, K. H.; Paull, K. D. Antitumor 2,3-dihydro-2-(aryl)-4(1H)-quinazolinone derivatives: Interactions with tubulin. *Biochem. Pharmacol*., **1996**, *51*(1), 53-9.

[2] Kamal, A.; Vijaya Bharathi, E.; Janaki Ramaiah, M.; Dastagiri, D.; Surendranadha Reddy, J.; Viswanath, A.; Sultana, F.; Pushpavalli, S. N.; Pal-Bhadra, M.; Srivastava, H. K.; Narahari Sastry, G.; Juvekar, A.; Sen, S.; Zingde, S. Quinazolinone linked pyrrolo[2,1-c][1,4]benzodiazepine (PBD) conjugates: Design, synthesis and biological evaluation as potential anticancer agents. *Bioorg. Med. Chem*., **2010**, *18*(2), 526-42.

[3] Ye, C.; You, J.; Li, X. F.; You, R.; Weng, Y.; Li, J.; Wang, Y. Design, synthesis and anticoccidial activity of a series of 3-(2-(2-methoxyphenyl)-2-oxoethyl) quinazolinone derivatives. *Pestic. Biochem. Phys*., **2010**, *97*(3), 194-8.

[4] Abdel Gawad, N. M.; Georgey, H. H.; Youssef, R. M.; El-Sayed, N. A. Synthesis and antitumor activity of some 2, 3-disubstituted quinazolin-4(3H)-ones and 4, 6- disubstituted- 1, 2, 3, 4-tetrahydroquinazolin-2H-ones. *Eur. J. Med. Chem*., **2010**, *45*(12), 6058-67.

[5] Hoonur, R. S.; Patil, B. R.; Badiger, D. S.; Vadavi, R. S.; Gudasi, K. B.; Dandawate, P. R.; Ghaisas, M. M.; Padhye, S. B.; Nethaji,

M. Transition metal complexes of 3-aryl-2-substituted 1,2-dihydroquinazolin-4(3H)-one derivatives: New class of analgesic and anti-inflammatory agents. *Eur. J. Med. Chem.*, **2010**, *45*(6), 2277-82.

[6] Mizutani, T.; Nagase, T.; Ito, S.; Miyamoto, Y.; Tanaka, T.; Takenaga, N.; Tokita, S.; Sato, N. Development of novel 2-[4-(aminoalkoxy)phenyl]-4(3H)-quinazolinone derivatives as potent and selective histamine H3 receptor inverse agonists. *Bioorg. Med. Chem. Lett.*, **2008**, *18*(23), 6041-5.

[7] Giri, R. S.; Thaker, H. M.; Giordano, T.; Williams, J.; Rogers, D.; Vasu, K. K.; Sudarsanam, V. Design, synthesis and evaluation of novel 2-thiophen-5-yl-3H-quinazolin-4-one analogues as inhibitors of transcription factors NF-κB and AP-1 mediated transcriptional activation: Their possible utilization as anti-inflammatory and anti-cancer agents. *Bioorg. Med. Chem.*, **2010**, *18*(7), 2796-808.

[8] Saeed, A.; Shams-ul-Mahmood, Flörke, U. Synthesis and characterization of new (E) -N ' - (substituted benzylidene) -2- (3- (2-methyl) -4-oxo-3 , 4-dihydroquinazolin-2-ylthio) acetohydrazides. *Turk. J. Chem.*, **2014**. *38(2)*, 275-87

[9] Gemma, S.; Kukreja, G.; Fattorusso, C.; Persico, M.; Romano, M. P.; Altarelli, M.; Savini, L.; Campiani, G.; Fattorusso, E.; Basilico, N.; Taramelli, D.; Yardley, V.; Butini, S. Synthesis of N1-arylidene-N2-quinolyl- and N2-acrydinylhydrazones as potent antimalarial agents active against CQ-resistant P. falciparum strains. *Bioorg. Med. Chem. Lett.*, **2006**, *16*(20), 5384-8.

[10] Mohamed, M. S.; Kamel, M. M.; Kassem, E. M. M.; Abotaleb, N.; Abd El-moez, S. I.; Ahmed, M. F. Novel 6,8-dibromo-4(3H)quinazolinone derivatives of anti-bacterial and anti-fungal activities. *Eur. J. Med. Chem.*, **2010**, *45*(8), 3311-9.

[11] Ul-haq, Z.; Khan, W.; Kalsoom, S.; Ansari, F. L. In silico modeling of the specific inhibitory potential of against BChE in the formation of β-amyloid plaques associated with Alzheimer ' s disease. *Theor. Biol. Med. Model.*, **2010**, *7*, 22-44.

[12] Belluti, A. F.; Bartolini, M.; Bottegoni, G.; Bisi, A.; Cavalli, A.; Andrisano, V.; Rampa, A. Benzophenone-Based Derivatives: A Novel Series of Potent and Selective Dual Inhibitors of Acetylcholinesterase and Acetylcholinesterase-Induced Beta-Amyloid Aggregation. *Eur. J. Med. Chem.*, **2011**, *46*(5), 1682-93.

[13] Shinada, M.; Narumi, F.; Osada, Y.; Matsumoto, K.; Yoshida, T.; Higuchi, K.; Kawasaki, T.; Tanaka, H.; Satoh, M. Synthesis of phenserine analogues and evaluation of their cholinesterase inhibitory activities. *Bioorg. Med. Chem.*, **2012**, *20*(16), 4901-14.

[14] Saeed, A.; Mahesar, P. A.; Zaib, S.; Khan, M. S.; Matin, A.; Shahid, M.; Iqbal, J. Synthesis, cytotoxicity and molecular modelling studies of new phenylcinnamide derivatives as potent inhibitors of cholinesterases. *Eur. J. Med. Chem.*, **2014**, *78*, 43-53.

[15] Krsti, D. Z.; Lazarevi, T. D; Bond, A. M.; Vasi, V.M. Acetylcholinesterase Inhibitors : Pharmacology and Toxicology. *Curr. Neuropharmacol.*, **2013**, *11*(3), 315-35

[16] Kolár, D.; Wimmerová, L.; Kádek, R. Acetylcholinesterase and Butyrylcholinesterase inhibitory activities of *Berberis vulgaris*. *Phytopharmacol.*, **2010**, *1*(1), 7-11.

[17] Houghton, P. J.; Ren, Y.; Howes, M. J. Acetylcholinesterase inhibitors from plants and fungi. *Nat Prod. Rep.*, **2006**, *23*(2), 181-99.

[18] Jaén, J. C.; Gregor, V. E.; Lee, C.; Davis, R.; Emmerling, M. Acetylcholinesterase inhibition by fused dihydroquinazoline compounds. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 737-42.

[19] Mikiciuk-Olasik, E.; Baszczak-Swiatkiewiz, K.; Zurek, E.; Krajewska, U.; Rózalski, M.; Kruszyński, R.; Bartczak, T. J. New derivatives of quinazoline and 1, 2-dihydroquinazoline n3-oxide with expected antitumor activity. *Arch. Pharm.*, **2004**, *337*, 239-46.

[20] Dukat, M.; Alix, K.; Worsham, J.; Khatri, S.; Schulte, M. K. 2-Amino-6-chloro-3,4-dihydroquinazoline: A novel 5-HT3 receptor antagonist with antidepressant character. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 5945-8.

[21] Lee, Y. S.; Lee, B. H.; Park, S. J.; Kang, S. B.; Rhim, H.; Park, J. Y.; Lee, J. H.; Jeong, S. W.; Lee, J. Y. 3,4-Dihydroquinazoline derivatives as novel selective T-type Ca^{2+} channel blockers. *Bioorg. Med. Chem. Lett.*, **2004**, *14*(13), 3379-84.

[22] Wang, J. F.; Gong, K.; Wei, D. Q.; Li, Y. X.; Chou, K. C. Molecular dynamics studies on the interactions of PTP1B with inhibitors: from the first phosphate-binding site to the second one. *Protein Eng. Des. Sel.*, **2009**, *22*(6), 349-55.

[23] Chou, K. C. Low-frequency resonance and cooperativity of hemoglobin. *Trends Biochem. Sci.*, **1989**, *14*(6), 212-213.

[24] Schnell, J. R.; Chou, J. J. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature*, **2008**, *451*(7178), 591-595.

[25] Pielak, R. M.; Jason R.; Schnell, J. R. Mechanism of drug inhibition and drug resistance of influenza A M2 channel. *Proc. Natl. Acad. Sci. USA.*, **2009**, *106*(18), 7379-7384.

[26] Wang, J. F. Insights from studying the mutation-induced allostery in the M2 proton channel by molecular dynamics. *Protein Eng. Des. Sel., (PEDS)*, **2010**, *23*(8), 663-666.

[27] Mao, B. Collective motion in DNA and its role in drug intercalation. *Biopolymers*, **1988**, *27*(11), 1795-1815.

[28] Chou, K. C. Review: Low-frequency collective motion in biomacromolecules and its biological functions. *Biophys. Chem.*, **1988**, *30*(1), 3-48.

[29] Saeed, A.; Shams-ul Mahmood, Flörke, U. Synthesis and characterization of new (E)-N'-(substituted benzylidene)-2-(3-(2-methyl)-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetohydrazides. *Turk. J. Chem.*, **2014**, *38*, 275-287.

[30] Erben, F.; Michalik, D.; Feist, H.; Kleeblatt, D.; Hein, M.; Matin, A.; Iqbal, J.; Langer, P. Synthesis and Antiproliferative Activity of (Z)-1-Glycosyl-3-(5-oxo-2-thioxoimidazolidin-4-ylidene)indolin-2-ones and (Z)-3-(2-Glycosylsulfanyl-4-oxo-4,5-dihydro-thiazol-5-ylidene)indolin-2-ones. *RSC Adv.*, **2014**, *4*, 10879-10893.

[31] Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, **1990**, *82*(13), 1107-12.

[32] Raza, R.; Matin, A.; Sarwar, S.; Barsukova-Stuckart, M.; Ibrahim, M.; Kortz, U.; Iqbal, J. Polyoxometalates as Potent and Selective Inhibitors of Alkaline Phosphatases with Profound Anticancer and Amoebicidal Activities. *Dalton Trans.*, **2012**, *41(47)*, 14329-36.

[33] Ellman, G. L.; Courtney, K. D.; Andres Jr, V.; Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **1961**, *7*, 88–95.

[34] Aslam, S.; Zaib, S.; Ahmad, M.; Gardiner, J. M.; Ahmad, A.; Hameed, A.; Furtmann, N.; Gütschow, M.; Bajorath, J.; Iqbal, J. Novel structural hybrids of pyrazolobenzothiazines with benzimidazoles as cholinesterase inhibitors. *Eur. J. Med. Chem.*, **2014**, *78*, 106-117.

[35] Khan, I.; Bakht, S. M.; Ibrar, A.; Abbas, S.; Hameed, S.; White, J. M.; Rana, U. A.; Khan, S. U.; Zaib, S.; Shahid, M.; Iqbal, J. Exploration of a library of triazolothiadiazole and triazolothiadiazine compounds as a highly potent and selective family of cholinesterase and monoamine oxidase inhibitors: Design, synthesis, X-ray diffraction analysis and molecular docking studies. *RSC Adv.*, **2015**, *5*, 21249-21267.

[36] Nachon, F.; Carletti, E.; Ronco, C.; Trovaslet, M.; Nicolet, Y.; Jean, L.; Renard, P. Y. Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl- and butyryl-cholinesterase. *Biochem. J.*, **2013**, *453(*3), 393-9.

[37] Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.*, **2009**, *30*(16), 2785-2791.

[38] Wang, J.; Wang, W.; Kollman, P. A.; Case, D. A. Automatic atom type and bond type perception in molecular mechanical calculations. *J. Mol. Graph. Model.*, **2006**, *25*(2), 247–60.

[39] Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, San Diego, CA: Accelrys Software Inc.; **2013**.

[40] Giacobini, E. Cholinesterases: New roles in brain function and in Alzheimer's disease. *Neurochem. Res.*, **2003**, *28*(3-4), 515-522.

[41] Kuhl, D. E.; Koeppe, R. A.; Snyder, S. E.; Minoshima, S.; Frey, K. A.; Kilbourn, M. R. In vivo butyrylcholinesterase activity is not increased in Alzheimer's disease synapses. *Ann. Neurol.*, **2006**, *59*(1), 13-20.

[42] Chou, K. C. Review: Structural bioinformatics and its impact to biomedical science. *Curr. Med. Chem.*, **2004**, *11*(16), 2105-2134.

[43] Heinrikson, R. L. A Model of the complex between cyclin-dependent kinase 5 (Cdk5) and the activation domain of neuronal Cdk5 activator. *Biochem. Biophys. Res. Commun.*, **1999**, *259*(2), 420-428.

[44] Wei, D. Q.; Zhong, W. Z. Binding mechanism of coronavirus main proteinase with ligands and its implication to drug design against SARS. (Erratum: ibid., 2003, Vol. 310, 675). *Biochem. Biophys. Res. Commun.*, **2003**, *308*(1), 148-151.

[45] Tomasselli, A. G.; Heinrikson, R. L. Prediction of the Tertiary Structure of a Caspase-9/Inhibitor Complex. *FEBS Lett.*, **2000**, *470*(3), 249-256.

[46] Wang, S. Q.; Du, Q. S. Study of drug resistance of chicken influenza A virus (H5N1) from homology-modeled 3D structures of neuraminidases. *Biochem. Biophys. Res. Commun.*, **2007**, *354*(3), 634-640.

[47] Chou, K. C. Molecular therapeutic target for type-2 diabetes. *J. Proteome Res.*, **2004**, *3*(6), 1284-1288.

[48] Li, X. B.; Wang, S. Q.; Xu, W. R. Novel Inhibitor Design for Hemagglutinin against H1N1 Influenza Virus by Core Hopping Method. *PLoS One.*, **2011**, *6*(11), e28111.

[49] Wang, J. F. Insights into the Mutation-Induced HHH Syndrome from Modeling Human Mitochondrial Ornithine Transporter-1. *PLoS One.*, **2012**, *7*(1), e31048.

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