Int. J. Chem. Sci.: 11(1), 2013, 639-648
ISSN 0972-768X
www.sadgurupublications.com

MOLECULAR DOCKING, SYNTHESIS, *IN VITRO* ASSAY AND KINETIC STUDY OF (*2E*)-3-(ARYL)-1-(THIOPHEN-2-YL) PROP-2-EN-1-ONE DERIVATIVES AS NEW SCAFFOLD OF ACETYLCHOLINESTERASE INHIBITORS

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ABSTRACT

Docking study of some (*2E*)-3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-ones in the binding pocket of Acetylcholinesterase (AChE), using Auto Dock 4.2 were achieved. Hits that exhibit promising binding affinity within the active site of enzyme, comparing with rivastigmine as standard were synthesized and *In Vitro* tested against human AChE using modified Ellmann's method. The kinetics of this new type of AChE inhibitors were studied in detail using one derivative as an example. Lineweaver-Burk plot described the new hits as competitive inhibitors.

Key words: (2E)-3-(Aryl)-1-(thiophen-2-yl) prop-2-en-1-ones, Acetylcholinesterase, Molecular docking.

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia among older people, defined as a neurological disorder that is characterized by a progressive loss of memory, thinking and learning behavior[1]. AD is associated with a selective decline of cholinergic neurons with decreased levels of acetylcholine (ACh) in the brain[2]. The most promising approach for the symptomatic treatment of AD is to increase the synaptic levels of ACh in the brain by inhibiting the acetylcholinesterase (AChE) enzyme, which is primarily responsible for its hydrolysis and termination of action[3]. Therefore, several AChE inhibitors (Fig. 1) are currently available as drugs for the treatment of Alzheimer's disease such as rivastigmine[4], tacrine[5], galantamine[6] and donepezil[7].

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Molecular docking of small molecule in the active site of biomacromolecule, represents one of the most effective approach for drug discovery. It explore the interactions between the candidate inhibitor and the binding site of enzyme and scoring their potential complementarity. The main application of molecular docking is the virtual screening of chemical libraries to identify potent inhibitors against target enzyme[8-10].

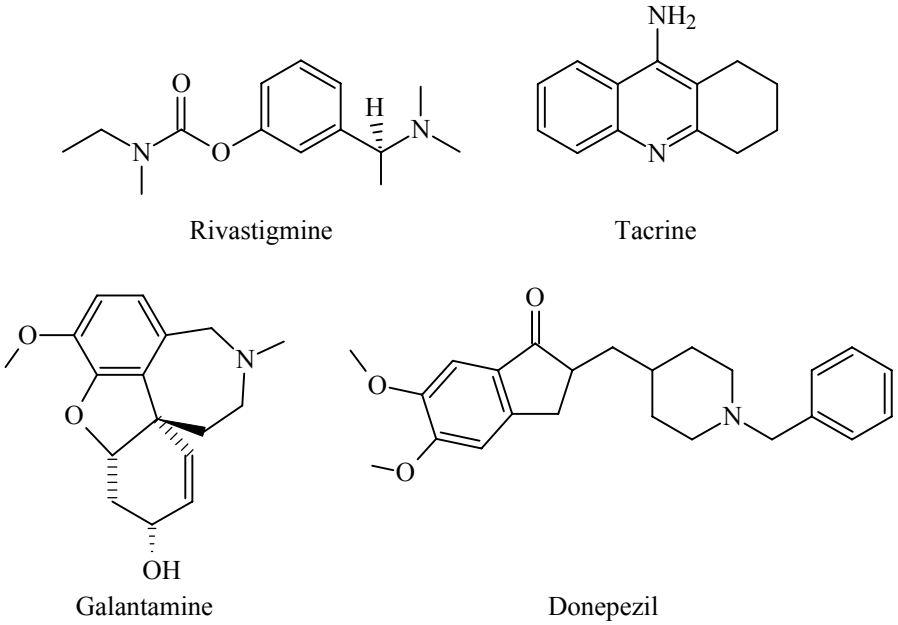


Fig. 1: Chemical structures of Acetylcholinesterase inhibitors

During the last decade the chalcone derivatives have intensive interest due to their pharmacological activities. Several chalcone derivatives were synthesized and evaluated as cholinesterases inhibitors[11,12].

Based on these concept and in our attempt to discover novel inhibitors of AChE, we study the docking of some chalcone derivatives within the binding site of AChE. The research including the ducking of (*2E*)-3-(aryl)-1-(thiophen-2-yl) prop-2-en-1-ones using AutoDock 4.2, the effective tool used for the virtual screening[13] considering rivastigmine as a positive control, so that these derivatives were docked within the same grid box employed for the standard. The hit derivatives that exhibit high binding energy compared with the rivastigmine were synthesized and *in vitro* assayed to determine the ability of docking approach to identify novel AChE inhibitors. The research was also included the kinetic study to determine the inhibition type of the novel discoverd hits.

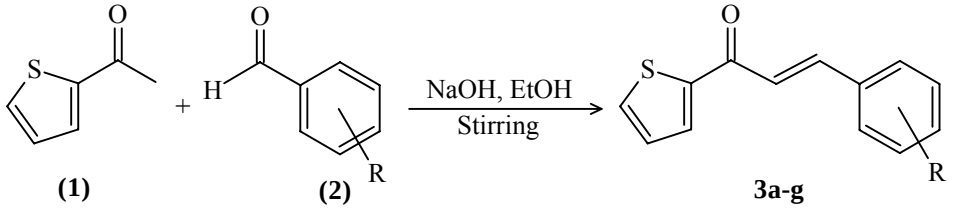
EXPERIMENTAL

Molecular docking

In this study, we used AutoDock 4.2 package software to investigate the affinity of chalcone derivatives to the binding pocket of AChE. Rivastigmine, AChE inhibitor were used as standard to determine the high ranking energies of the docked compounds. The pdb file format of AChE as receptor were obtained from the RCSB Protein Data Bank (PDB code 1B41) and used as a rigid molecule. Water molecules removed and hydrogen atoms were added to the protein amino acids. All the docked compounds were drawn using ChemDraw ultra 7.0 as mol file and converted to pdb format using open Babel 2.3.1 software. During the docking, the grid dimensions were 126 Å X 126 Å X 126 Å with points separated by 0.25 Å. The X, Y and Z coordinates specified as 90.272, 85.601 and -5.500, respectively. Lamarckian Genetic Algorithm was employed as the docking algorithm with 10 runs, 150 population size, 2500000 maximum number of energy evaluations and 27000 maximum number of generations.

Chalcone synthesis

(*2E*)-3-(Aryl)-1-(thiophen-2-yl) prop-2-en-1-ones (3a-g) were synthesized (Scheme 1) and characterized by the known methods[14-17]. A mixture of 2-acetylthiophene (1), aromatic aldehyde (2) and some pellets of solid NaOH in 20 mL of ethanol was stirred at room temperature for 6 h. The resulting solid was washed, dried and crystallized from ethanol.



R = (a) 2,5-diCH_3O, (b) 3-OH, 4-CH_3O (c) 2-Br, (d) 2-Cl, (e) 2-NO_2, (f) 3-NO_2, (g) 4-NO_2

Scheme 1: Synthesis of (*2E*)-3-(aryl)-1-(thiophen-2-yl) prop-2-en-1-one derivatives (3a-g)

AChE Assay

The inhibitory activities of synthesized compounds against human AChE were evaluated by slightly modified Ellmann's method[18]. 5,5′-Dithio-bis (2-nitrobenzoic) acid (DTNB) solution (50 µL, 0.001 M), Sodium phosphate buffer solution (2.25 mL, 0.2 M, pH = 7.3) and 10 µL of serum were mixed well, 2 mL of the mixture was transferred to a 3 mL

cuvette, then the initiated substrate, acetylthiocholine iodide (ASChI 34 µL, 0.05 M) was added. The hydrolysis of substrate was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine, at a wavelength of 430 nm for 3 min using HITACI UV-VIS-2000-Spectrophotometer. The enzyme activity were determined as µ mole/mL/3 min. Stock solutions of synthesized compounds **(3a-g)** were prepared in DMSO solution and diluted (4.5 mM), then the percentage inhibition was determined from the residual activity for each compound by comparing the enzyme activity with and without hit compound.

Kinetic study

Kinetic characterization of AChE was performed by means of Ellman's method using Lineweaver-Burk (1/V vs. 1/[S]) plot. Enzyme kinetic characterization studies were performed under same incubation conditions as described above using acetylthiocholine iodide as substrate and DTNB as chromophoric reagent. Two concentrations 4.5×10^{-5} and 4.5×10^{-3} M of compound 3 g and a parallel control with no inhibitor in the mixture were used for comparison[19].

RESULTS AND DISCUSSION

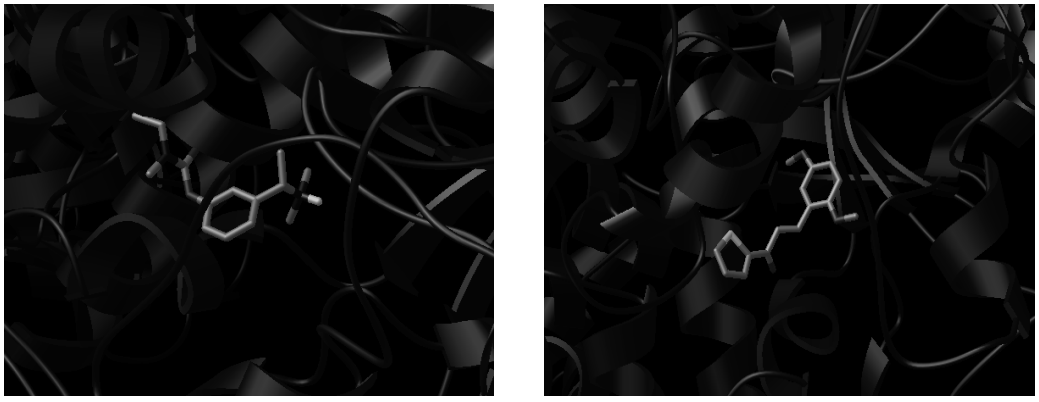
Docking studies are computational techniques for the exploration of the possible binding modes of a ligand to a given receptor, enzyme or other binding site. X-ray study of AChE with divergent inhibitors shows that the binding pocket of the enzyme include the following residues ASP74, TRP86, ASN87, GLY120, GLY121, GLY122, TYR124, SER125, GLY126, LEU130, GLU202, PHE297, TYR337, PHE338, TYR341, HIS447, GLY448 and ILE451[20]. Therefore this research included the docking of rivastigmine as a standard as well as (*2E*)-3-(aryl)-1-(thiophen-2-yl) prop-2-en-1-one derivatives within the active side of enzyme using AutoDock 4.2.

Auto Dock software consist of two main programs, *autogrid* that pre-calculates grid maps of interaction energies for various atom types of ligand with a macromolecule and *autodock*, which performs the docking of the ligand to specified grids[21]. In this study, docking is carried out using a Lamarckian Genetic Algorithm (LGA). For the typical systems, AutoDock is run several times to give several docked conformations (ten conformers by default) ranking according to their binding and intermolecular energies as well as inhibition constant. Table 1 illustrates the docking energies of different conformers generated by AutoDock.

Table 1: Binding energies of compounds based on the ranking of clustering conformers

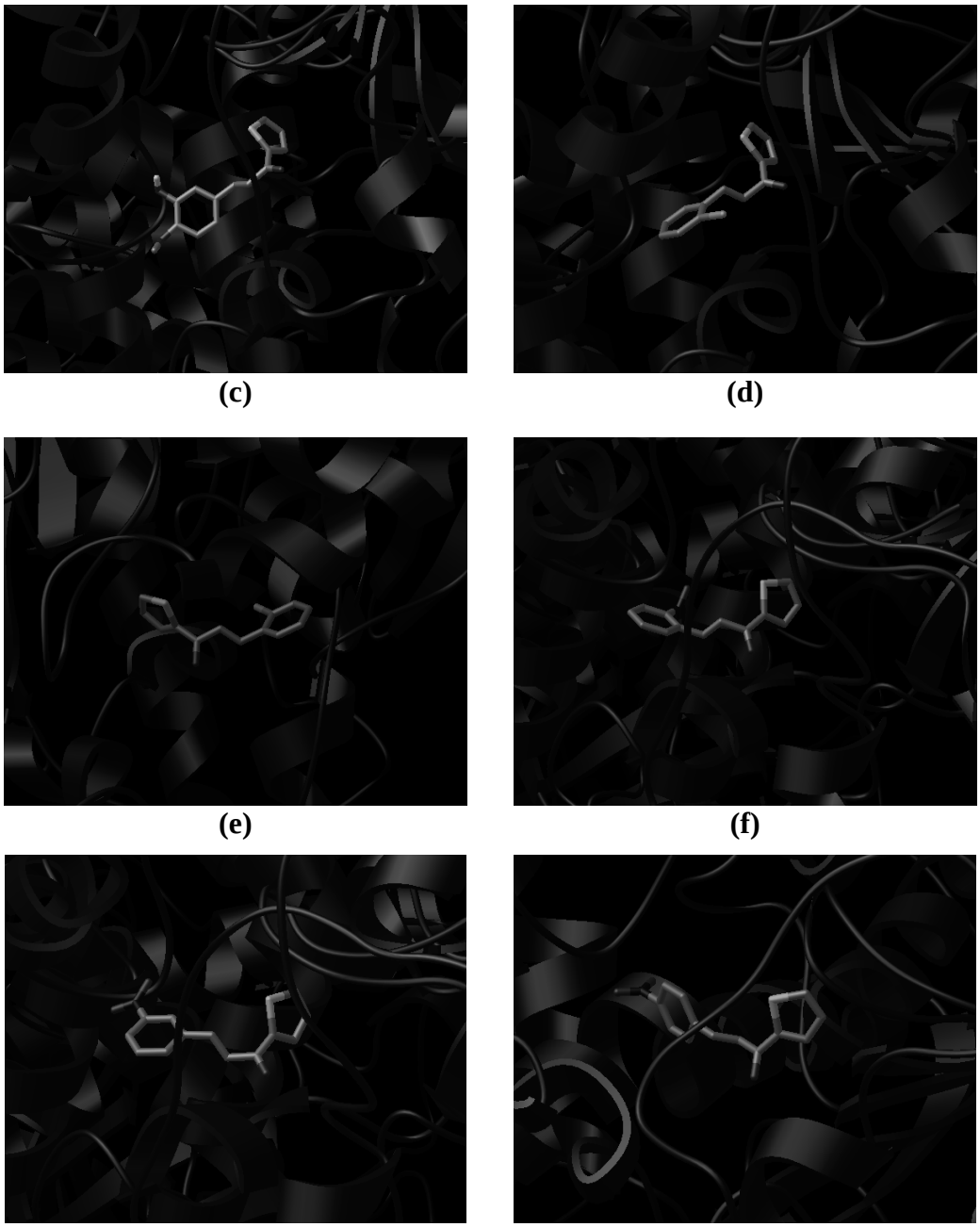
Compounds	Binding energy (Kcal mole^{-1})									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Rivastigmine	-9.29	-9.00	-8.90	-8.88	-8.55	-8.33	-8.33	-8.16	-7.84	-7.70
3a	-8.42	-8.40	-8.39	-8.34	-8.25	-8.23	-8.22	-8.19	-8.10	-7.75
3b	-8.41	-8.31	-8.25	-7.83	-7.22	-7.81	-7.57	-7.50	-7.49	-7.42
3c	-8.13	-8.13	-8.12	-8.10	-8.10	-8.08	-7.99	-7.98	-7.86	-7.76
3d	-7.95	-7.94	-7.93	-7.93	-7.91	-7.86	-7.84	-7.70	-7.65	-7.65
3e	-7.08	-7.08	-7.07	-7.07	-6.97	-6.92	-6.92	-6.84	-6.82	-6.60
3f	-7.38	-7.28	-7.24	-7.23	-7.21	-6.68	-6.67	-6.67	-6.66	-6.65
3g	-6.98	-6.98	-6.97	-6.91	-6.71	-6.70	-6.67	-6.65	-6.63	-6.49

The high ranking binding energies of docked compounds **(3a-g)** were between -8.42 to -6.98 Kcal mol^{-1} compared to -9.29 Kcal mol^{-1} for rivastigmine. The binding energies prove that the chalcone derivatives bind to active sit of enzyme in similar way to that of the reference compound. The binding of rivastigmine and the high ranking conformers of the synthesized compounds in the activepocket of AChE showed in Fig. 2.



(a) **(b)**

Cont…



(c) **(d)**

(e) **(f)**

(g) **(h)**

Fig. 2: Molecular docking of high ranking conformers of (a) rivastigmine, (b - h) compounds 3a-g within the binding site of AChE

Intermolecular energies (Table 2) and inhibition constants (Table 3) of the docked derivatives were also predicted to determine the validation of generated conformers.

Table 2: Intermolecular energies of the compounds based on the ranking of generated conformers

Compounds	Intermolecular energies (Kcal mole^{-1})									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Rivastigmine	-10.78	-10.49	-10.39	-10.37	-10.04	-9.82	-9.64	-9.65	-9.33	-9.19
3a	-9.91	-9.89	-9.88	-9.83	-9.74	-9.72	-9.72	-9.68	-9.59	-9.24
3b	-9.90	-9.80	-9.74	-9.32	-8.71	-9.30	-9.07	-8.99	-8.98	-8.91
3c	-9.03	-9.03	-9.02	-9.00	-8.99	-8.98	-8.89	-8.87	-8.76	-8.65
3d	-8.84	-8.83	-8.82	-8.82	-8.80	-8.75	-8.74	-8.60	-8.54	-8.54
3e	-8.28	-8.27	-8.27	-8.26	-8.16	-8.12	-8.11	-8.04	-8.01	-7.79
3f	-8.57	-8.47	-8.43	-8.42	-8.40	-7.87	-7.87	-7.86	-7.86	-7.84
3g	-8.17	-8.17	-8.16	-8.10	-7.90	-7.89	-7.87	-7.84	-7.83	-7.68

The intermolecular energies and the inhibition constants of the best generated conformers of chalcones **(3a-g)** were between - 9.91 to - 8.17 Kcal mol^{-1} and 672.90 nM to 7.64 μM, respectively. For the standard compound, the intermolecular energy of the high ranking hit was -10.78 Kcal mol^{-1}, while the inhibition constant was 154.68 nM. The decrease of intermolecular energy and inhibition constant simultaneously with binding energy reveals the fact that these two parameters directly proportional with the binding energy and strongly prove the expected inhibitory activity of all the docked hits. The high ranking discovered hits **(3a-g)** were prepared by Claisen-Schmidt condensation as described by the literatures. Equimolar amount of 2-acetylthiophene (1) and corresponding aldehydes **(2a-g)** in the presence of alcoholic alkali were stirred for 6 hours to give the desired chalcones as shown in Scheme 1.

The synthesized compounds were *in vitro* tested and the inhibitory activity at 4.5 mM of hits against serum AChE illustrated in Table 3. The values of inhibition ranging from 87.42% for compound 3b, the most active hit, to 43.43% for compound 3e, the lowest active one. The inhibition activity is strongly proportional to the ranking of generated hits. Results of actual activities strongly enhance the docking study of chalcone derivatives **(3a-g)** as promising AChE inhibitors.

Table 3: Calculated inhibiton constant (Ki) and actual affinity of the compounds based on the ranking of generated conformers

Compds.	Calculated inhibiton constant (Ki) (nM, µM*)										Actual affinity
	1	2	3	4	5	6	7	8	9	10	Percent inhibition at 4.5 mM
Rivastigmine	154.68	254.73	300.79	312.14	542.49	784.07	1.06*	1.05*	1.79*	2.27*	-
3a	672.90	697.79	709.07	772.97	899.55	933.17	936.63	996.50	1.15*	2.09*	73.76
3b	687.98	808.89	895.74	1.82*	5.11*	1.87*	2.81*	3.20*	3.23*	3.66*	87.42
3c	1.10*	1.10*	1.11*	1.15*	1.16*	1.18*	1.38*	1.43*	1.72*	2.06*	68.88
3d	1.49*	1.52*	1.54*	1.55*	1.60*	1.74*	1.78*	2.26*	2.48*	2.48*	62.86
3e	6.44*	6.46*	6.53*	6.58*	7.80*	8.42*	8.46*	9.63*	10.04*	14.65*	43.43
3f	3.91*	4.63*	4.97*	5.06*	5.20*	12.72*	12.87*	12.93*	13.05*	13.36*	53.75
3g	7.64*	7.71*	7.79*	8.63*	12.14*	12.37*	12.84*	13.31*	13.69*	17.47*	44.41

The kinetics of this new class of AChE inhibitors were studied in detail using compound 3 g as example. The inhibition type were determined by the determining the enzyme activity using two concentrations 4.5 x 10^{-5} and 4.5 x 10^{-3} M of inhibitor with constant substrate concentration. The nature of AChE inhibition was investigated by the graphical analysis of steady-state inhibition data (Fig. 3).

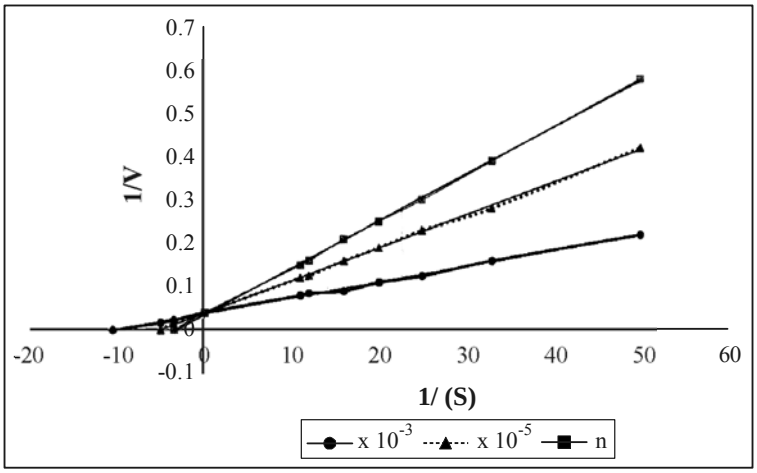


Fig. 3: Lineweaver-Burk plot of AChE in the absence —■— and presence of 4.5 x 10^{-5} M ···▲··· and 4.5 x 10^{-3} —●— inhibitor concentrations

Lineweaver-Burk plot described compound 3 g as competitive inhibitor due to change in Km values while Vmax remained constant. The values of Km and Vmax were 0.012 M and 8.33 μmol mL^{-1}/ 3 min respectively.

CONCLUSION

Chalcone derivatives as new skeleton of AChE inhibitors were discovered using AutoDock 4.2 software. (2E)-3-(Aryl)-1-(thiophen-2-yl) prop-2-en-1-ones were docked within the binding gorge of enzyme to explore their affinities against AChE. The high ranking derivatives were synthesized and *in vitro* tested as serum AChE inhibitors. The inhibitory activity of synthesized compounds **(3a-g)** strongly enhanced the potential of using docking study to identified potent inhibitors against AChE. The synthesized derivatives were evaluated as competitive inhibitors.

ACKNOWLEDGEMENT

This study was supported by Chemistry Department, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.

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Revised : 20.12.2012 *Accepted : 23.12.2012*