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# SYNTHESIS OF FOUR CHALCONE DERIVATIVES BEARING HETEROCYLIC MOIETIES AS NEW AChE INHIBITORS BY DOCKING SIMULATION

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## ABSTRACT

In our attempt to discover new skeleton of Acetylcholinesterase (AChE) inhibitors, docking study of our chalcone data base against the binding pocket of enzyme was achieved. Auto Dock 4.2 program, the effective tool for docking simulation was used to exploring the binding affinity of docked derivatives towards AChE active site. Four hits that exhibit high scoring affinity within the binding pocket of enzyme, comparing with rivastigmine as standard were synthesized and *in vitro* tested against human AChE using modified Ellmann's method.

**Key words**: Chalcones, Acetylcholinesterase, Molecular docking.

## INTRODUCTION

Like all types of dementia, Alzheimer's is caused by brain cell death. It's defined as a neurological disorder in which the death of brain cells causes memory loss and cognitive decline[1]. The decline of cholinergic neurons and the decreased levels of acetylcholine (ACh) in the brain are commonly associated with the Alzheimer's disease[2]. The most effective strategy for the AD treatment is to increase the synaptic levels of ACh in the brain by inhibiting the acetylcholinesterase enzyme (AChE), which isprimarily responsible for its hydrolysis and termination of action[3]. Therefore, several AChE inhibitors[4-7]such as rivastigmine (Fig. 1), are commercially available as drugs for Alzheimer's treatment.

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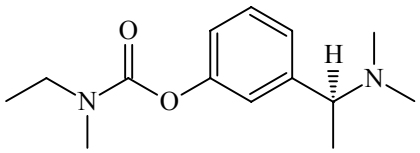


**Fig. 1: Chemical structures of rivastigmine**

During the last decade, chalcone derivatives have intensive interest and several derivatives of 1,3-diaryl-2-propene-1-one were reported as new scaffold of ACh Einhibitors[8]. These facts promoted us to searching our data base to evaluate the most effective chalcones against AChE. Docking study of chalcone derivatives containing heterocyclic moieties within the binding site of AChE were achieved. Auto Dock, the effective tool for the virtual screening[9] was used to explore the promising AChE inhibitors considering rivastigmine as a positive control, so that chalcone derivatives from our data base as well as the control were docked within the active site of enzyme. The hits that exhibit high binding energy compared with the rivastigmine were synthesized and the *in vitro* inhibition was determined.

## EXPERIMENTAL

### Docking study

AutoDock 4.2 package software was used to explore the affinity of chalcone derivatives to the binding pocket of AChE. Rivastigmine, AChE inhibitor was used as positive control to determine the high ranking energies of the docked derivatives. 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 eliminated 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 study, the grid dimensions were 80 Å X 80 Å X 80 Å with points separated by 0.375 Å. The X, Y and Z coordinates specified as 122, 106 and -135, 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[10].

### Synthetic part

### Materials and method

All starting materials and solvents were purchased from Sigma-Aldrich and Fluka and used without further purification. Melting points were determined on electro-thermal

capillary apparatus and are uncorrected. FT-IR measurements were recorded on Shimadzu model FT-IR-8400S. Mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra apparatus.

## Chalcone synthesis

Chalcone derivatives **(3a-d)** were synthesized **(Scheme 1)** and characterized by the known methods[11-14]. A mixture of 2-acetylfuran or 3-acetylpyridine **(1)** (0.004 mol), aromatic aldehyde **(2)** (0.004 mol) and some pellets of solid NaOH in 20 mL of ethanol were stirred at room temperature for 6 hr. The resulting solid was washed, dried and crystallized from ethanol.

### (2E)-1-Furan-2-yl-3-(1H-pyrrol-2-yl)-propenone (3a)

Color: Yellow powder. Yield: 88%. M.p.: 168-170°C. FT-IR (KBr, ν, $cm^{-1}$): 3215 (pyrrole N-H), 3080 (aromatic C-H), 2968, 2931 (aliphatic C-H), 1653 (C=O), 1541, 1548 (C=C). GC-MS (EI, m/z): 187 ($M^+$), 158, 130, 92.

### (2E)-1-Furan-2-yl-3-thiophen-2-yl-propenone (3b)

Color: Brown powder. Yield: 61%. M.p.: 188-190°C. FT-IR (KBr, ν, $cm^{-1}$): 3117 (aromatic C-H), 2968, 2933 (aliphatic C-H), 1651 (C=O), 1560, 1462 (C=C). GC-MS (EI, m/z): 204 ($M^+$), 175, 147, 137, 109, 95.

### (2E)-1-Pyridin-3-yl-3-(1H-pyrrol-2-yl)-propenone (3c)

Color: Brown. Yield: 63%. M.p.: 102-104°C. FT-IR (KBr, ν, $cm^{-1}$): 3215 (pyrrole N-H), 3097 (aromatic C-H), 2970, 2929 (aliphatic C-H), 1683 (C=O), 1585, 1460 (C=C). GC-MS (EI, m/z): 198 (M + 1), 185, 171, 157, 129.

### (2E)-1-Pyridin-3-yl-3-thiophen-2-yl-propenone (3d)

Color: Yellow powder. Yield: 58%. M.p.: 228-230°C. FT-IR (KBr, ν, $cm^{-1}$): 3080 (aromatic C-H), 2968, 2933 (aliphatic C-H), 1676 (C=O), 1558, 1417 (C=C). GC-MS (EI, m/z): 215 ($M^+$), 186, 137, 109.

## AChE assay part

The inhibitory activities of chalcone derivatives **(3a-d)** against human AChE were evaluated by slightly modified Ellmann's method[15]. 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 ofacetylthiocholine, 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 tested compounds 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.

## RESULTS AND DISCUSSION

### Docking study

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 ILE45120[16]. Therefore, this research included the docking of rivastigmine as a standard as well as the chalcone derivatives within the active side of enzyme using AutoDock 4.2. Auto Docksoftware 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[17]. 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 for the rivastigmine and the high ranking chalcone derivatives **(3a-d)** generated by Auto Dock.

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

| | **Binding energy (Kcal mole$^{-1}$)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Compounds** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| Rivastigmine | -7.49 | -7.37 | -7.25 | -7.16 | -7.14 | -7.08 | -6.97 | -7.33 | -7.08 | -6.91 |
| **3a** | -7.36 | -6.70 | -6.10 | -6.21 | -6.18 | -6.18 | -6.16 | -5.85 | -5.85 | -5.70 |
| **3b** | -6.93 | -6.86 | -6.85 | -6.52 | -6.51 | -6.28 | -6.76 | -6.66 | -6.60 | -6.24 |
| **3c** | -7.11 | -7.06 | -6.99 | -6.89 | -6.88 | -6.85 | -6.84 | -6.80 | -6.73 | -6.63 |
| **3d** | -7.16 | -7.08 | -7.06 | -6.91 | -6.85 | -7.10 | -7.08 | -6.82 | -6.79 | -6.77 |

The high ranking binding energies of docked compounds **(3a-d)** were between -7.36 to -5.70 Kcal $mol^{-1}$ compared to -7.49 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 active pocket of AChE showed in Fig. 2.

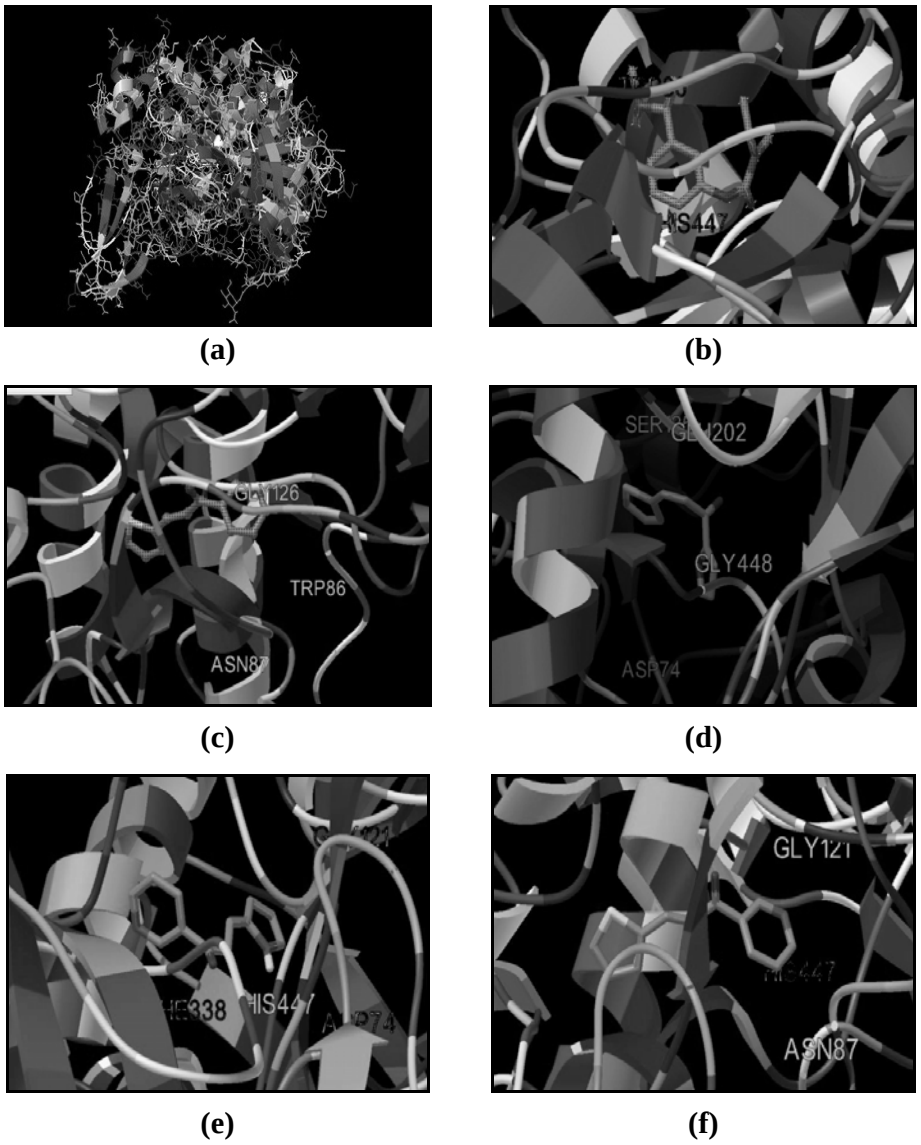



**Fig. 2: (a) Structure of AChE, (b-f) Molecular docking of rivastigmine and chalcone compounds (3a-d), respectively within the binding site of AChE**

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

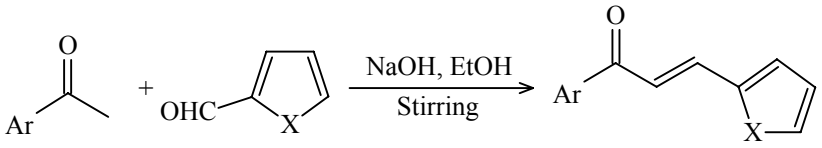
**Table 2: Intermolecular energies of the compounds based on the ranking of generated conformers**

| | Compounds Intermolecular energies (Kcal mole$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Compounds** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| Rivastigmine | -8.98 | -8.86 | -8.74 | -8.65 | -8.63 | -8.57 | -8.46 | -8.82 | -8.57 | -8.40 |
| **3a** | -8.26 | -7.60 | -6.99 | -7.11 | -7.08 | -7.07 | -7.06 | -6.74 | -6.74 | -6.59 |
| **3b** | -7.82 | -7.76 | -7.74 | -7.42 | -7.4 | -7.17 | -7.66 | -7.55 | -7.49 | -7.13 |
| **3c** | -8.01 | -7.95 | -7.88 | -7.78 | -7.78 | -7.74 | -7.73 | -7.7 | -7.63 | -7.53 |
| **3d** | -8.06 | -7.97 | -7.96 | -7.81 | -7.74 | -7.99 | -7.98 | -7.71 | -7.69 | -7.66 |

The intermolecular energies and the inhibition constants of the best generated conformers of chalcones **(3a-d)** were between -8.26 to -8.06 Kcal mol$^{-1}$ and 4.02 to 5.63 μM, respectively. For the standard compound, the intermolecular energy of the high ranking hit was -8.98 Kcal mol$^{-1}$, while the inhibition constant was 3.22 μM. 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-d)** were prepared by Claisen-Schmidt condensation as described by the literatures.

## Synthesis of chalcone derivatives

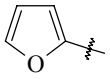
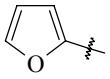
Equimolar amount of 2-acetylfuran or 3-acetylpyridine **(1)** and corresponding aldehydes **(2a-d)** in the presence of alcoholic alkali were stirred for 6 hrs to give the desired chalcones as shown in **Scheme 1**.




**(1)** **(2)** **(3)**

**Scheme 1: Synthesis of chalcone derivatives (3a-d)**

Cont…

| Compound | Ar | X |
|---|---|---|
| **3a** |  | NH |
| **3b** |  | S |
| **3c** |  | NH |
| **3d** |  | S |

The structures of prepared compounds were confirmed by infrared spectroscopy and mass spectroscopy. The IR spectrum of compound **3a** (Fig. 3) showed absorption at 3215 $cm^{-1}$ due to the pyrrole N-H stretching, while the aromatic C-H stretching frequency absorption appear at 3080 $cm^{-1}$. The aliphatic C-H stretching appear at 2968, 2931 $cm^{-1}$. The carbonyl group C=O frequency band appeared at 1653 $cm^{-1}$. Bands at 1541 and 1548 $cm^{-1}$ related to **C=C** absorption further confirm the structure of compound.

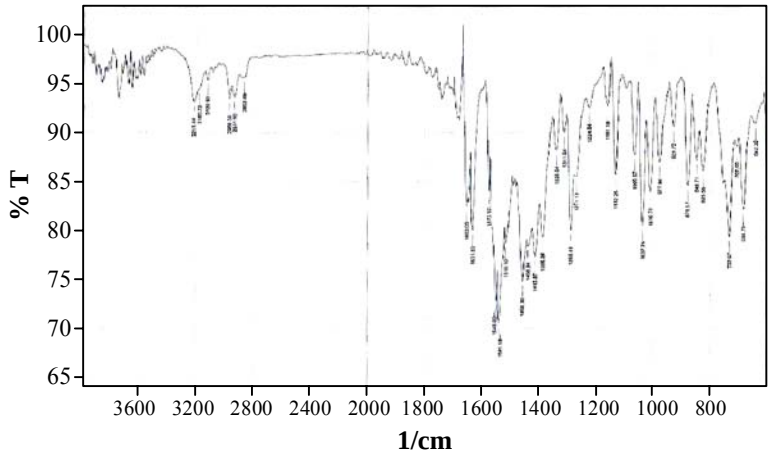



**Fig. 3: IR spectra for compound 3a**

The GC-MS spectrum of compound **3a** as illustrated in Fig. 4 showed the parent ion peak at an m/z value of ($M^+$) 187. The fragments at 158, 130 and 92 strongly enhanced the elucidation of compound. The suggested fragments for the compound **3a** depicted in Fig. 5.

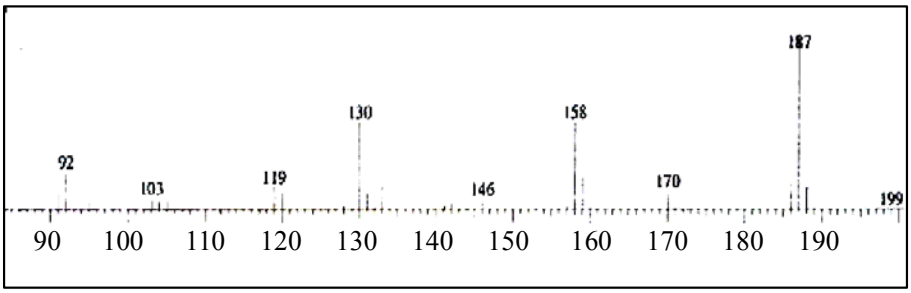



**Fig. 4: GC-MS spectrum for compound 3a**

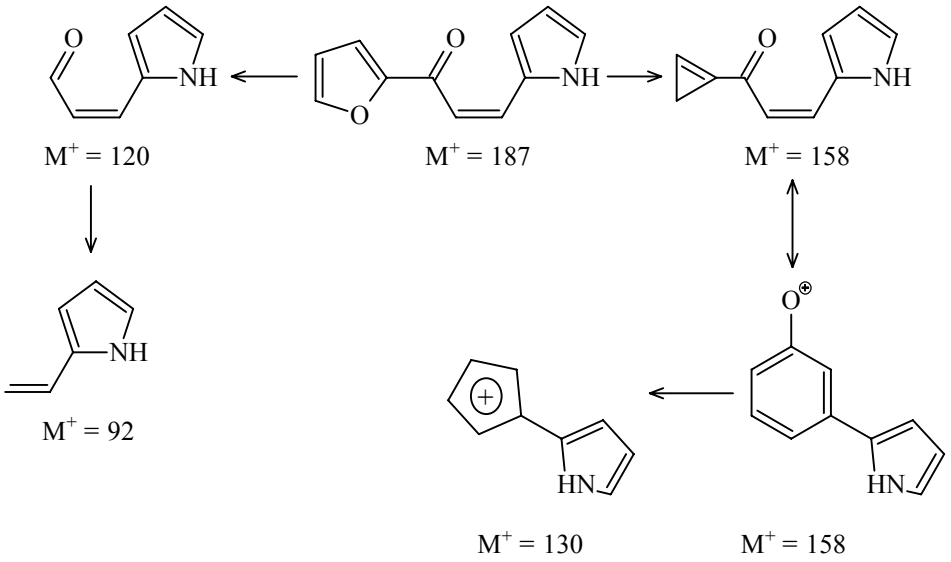



**Fig. 5: Mass fragments of compound 3a**

The physical properties, spectral data and mass analysis of all the synthesized compounds are given in the experimental section.

### *In vitro* assay

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 56.89% for compound **3d**, the most active hit, to 51.10% for compound **3a**, 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-d)** as promising AChE inhibitors.

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

| | Calculated inhibition constant (Ki) (µM) | | | | | | | | | | Actual affinity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compounds** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **% Inhibition at $4.5 \times 10^{-3}$ M** |
| Rivastigmine | 3.22 | 3.94 | 4.85 | 5.68 | 5.83 | 6.43 | 7.74 | 4.22 | 6.48 | 8.57 | - |
| **3a** | 4.02 | 12.17 | 33.95 | 27.89 | 29.35 | 29.56 | 30.46 | 51.79 | 51.9 | 66.63 | 51.10 |
| **3b** | 8.37 | 9.35 | 9.56 | 16.62 | 16.98 | 25.13 | 11.0 | 13.19 | 14.58 | 26.75 | 55.52 |
| **3c** | 6.09 | 6.72 | 7.57 | 8.94 | 9.03 | 9.60 | 9.75 | 10.33 | 11.62 | 13.78 | 51.34 |
| **3d** | 5.63 | 6.48 | 6.67 | 8.58 | 9.60 | 6.28 | 6.44 | 10.09 | 10.50 | 10.97 | 56.89 |

## CONCLUSION

Chalcone derivatives as new skeleton of AChE inhibitors were discovered using Auto Dock 4.2 software. Several chalcone derivatives from our data base were docked within the binding gorge of enzyme to explore their affinities against AChE. Rivastigmine, the known AChE inhibitors was used as standard. The high ranking derivatives comparing with the standard were synthesized and *in vitro* tested as serum AChE inhibitors. The inhibitory activity of synthesized compounds **(3a-d)** strongly enhanced the potential of using docking study to identified potent inhibitors against AChE.

## ACKNOWLEDGEMENT

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