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A New Indenone from *Echiochilon fruticosum*, a Potential Beta-secretase 1 (BACE1) and Acetylcholinesterase (AChE) Inhibitor

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ABSTRACT

A new indenone (3-(methyl-acetoxy), 6-methoxy indenone) was isolated from Echiochilon fruticosum. Its chemical structure was established by different spectroscopic analyses. Molecular docking studies were performed to investigate its interaction with the pocket sites of Beta-secretase-1 (BACE1) and acetyl cholinesterase. This compound may serve as potential dual anti-alzheimer scaffolds.

Keywords: *Echiochilon fruticosum*, Indenone, Alzheimer, Acetylcholinesterase inhibitor

INTRODUCTION

Echiochilon fruticosum (family: Boraginaceae) is distributed in the sandy and stony grounds of North Africa, Sinai, Syria and Arabia. A variety of chemical entities has been reported from *E. fruticosum*, including volatile components, eugenol glycoside, vomifoliol, *trans*-syringin, several flavone derivatives, anhydroalkanin, echiochiloquinone and caffeic acid. *E. fruticosum* extracts as well as echiochiloquinone have demonstrated good anti-inflammatory activity when assessed using cotton pellet-induced and carrageenan-induced rat paw edema [1]. Several members of family Boraginaceae exhibited acetyl cholinesterase inhibitory activity as *Cordia* species [2] and *Arnebia* species and this activity was not attributed to the main constituents "shikonin derivatives" [3].

Alzheimer's Disease (AD) is a chronic, progressive, neurodegenerative disorder of the brain characterized clinically by deterioration in the key symptoms of activities of daily living, behavior, and cognition. The cholinergic hypothesis states that the cognitive decline in AD is secondary to deficits in central cholinergic neurotransmission resulting from a loss of Acetylcholine (ACh). AD pathophysiology is characterized by the presence of microscopic neuropathological hallmarks which are extracellular amyloid plaques. These are composed of the Beta-amyloid peptide (Abeta), which are mainly generated by the action of Beta-secretase-1 (BACE1) a trans-membrane aspartic protease that cleaves the beta-amyloid precursor protein. Much evidence suggests that Abeta deposition is known to cause neuronal death by a number of possible mechanisms including oxidative stress, inflammation and apoptosis. Thus, therapeutic strategies to lower cerebral Abeta levels through BACE inhibition should prove beneficial for AD treatment.

Recently, a few number of molecules acting as BACE1 inhibitors have advanced to clinical development. However, up to 2016 no BACE1 inhibitor has passed phase II/III clinical trials. So, the further development of compounds targeting BACE1 is required [4]. AChE inhibitors are widely used for the symptomatic treatment of Alzheimer's disease and other dementias.

Recent evidence indicates that the one molecule, multiple targets paradigm is effective for the treatment of complex diseases due to the drug's ability to interact with multiple targets responsible for the pathogenesis of the disease. Given the complex nature of alzeheimer's and the fact that a single drug acting on a specific target (AChE) may have undesirable clinical effects, the "one molecule, multiple targets" paradigm strategy has find its way in anti-AD drug design and was recently proven to be a successful strategy for its treatment.

Several indanone derivatives have been designed, synthesized and proved to possess significant inhibitory activity against Abeta aggregation [5], Aricept the drug approved by FDA for the treatment of alzeheimer's (Figure 1) contains three major functional groups, of which, the dimethoxyindenone moiety is essential for its selective binding with acetyl cholinesterase as it interacts with TRP 279 at the entrance of the gorge of the enzyme [6], several derivatives were synthesized from Aricept as inhibitors for both acetyl-and butyrylcholinesterase [5,7].

On the other hand, molecular docking simulation is a powerful and increasingly substantial tool for drug discovery. It can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes.

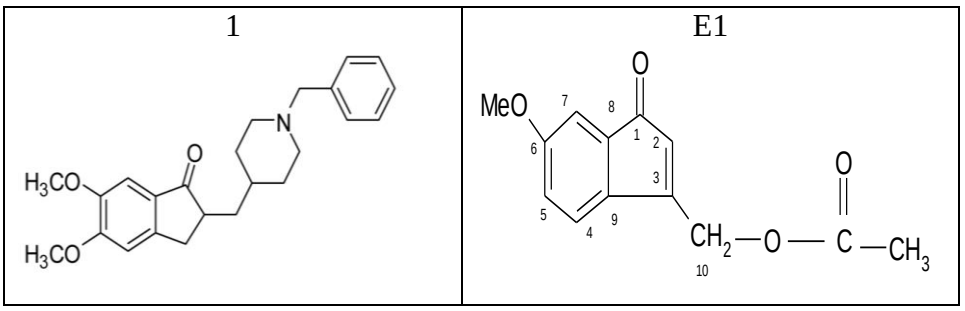

Figure 1: Structure of 1-Aricept, E1: 3-(methyl-acetoxy), 6-methoxy indenone

MATERIALS AND METHODS

Plant material

Echiochilon fruticosum Desf. was collected from Omayed district, 90 km west Alexandria, Egypt. The plant material was identified by Prof. Dr. Lotfy Boulos, National Research Center, Dokki, Cairo, Egypt. A voucher was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt.

Isolation of compound E1

The powdered air-dried roots (400 g) were defatted with light petroleum. The marc was dried and then macerated in 70% ethanol. The alcoholic extract was filtered and the solvent evaporated under reduced pressure to give dark red semisolid residue (25 g), this residue was dissolved in dichloromethane/MeOH and adsorbed on celite. The adsorbed material was extracted successively with light petroleum, $CHCl_3$ and MeOH. The light petroleum soluble fraction afforded a major spot which was purified on a silica gel column (50 g, 30 × 2 cm) and compound E1 (10 mg) was eluted with dichloromethane.

Docking studies

Docking studies were performed to examine qualified binding poses against BACE1. The three dimensional structure of indenone was derived by Avogadro software and then the energy minimized compound was captured. The X-ray crystallographic structure of human BACE complexed with an inhibitor (3TPR Crystal structure of BACE1) and that of acetyl cholinesterase (1EVE, complexed with the anti-alzheimer drug, e2020 (Aricept®), with a resolution of 2.50 Å was obtained from the RCSB Protein Data Bank. The accurate docking was performed using iGEMDOCK v2.0® software based on the binding energy in kcal/mol. The numbers of runs taken are 70 and max interactions were 2000 with population size 200 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lowest energy is chosen. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was identified at a distance 8Å.

General experimental procedures

Silica gel (230-400 mesh) E. Merck was used for column chromatography. Precoated TLC plates 0.25 mm, silica gel 60 (GF-254) E. Merck, were used for TLC analysis. 1D and 2D-NMR analyses were obtained using Jeol 500 MHz spectrometer for ^{1}H and 125 MHz for ^{13}C-NMR. Deuterated solvents were used to reference the spectra. EIMS was obtained on a Delsi-Nermag R30-10. UV-Visible spectra were carried on a Helios α thermo spectronic, England, supported with software Vision 32®.

RESULTS AND DISCUSSION

Chemistry

The mass spectrum showed a prominent peak at m/z 232 corresponding to the molecular formula $C_{13}H_{12}O_4$ indicating 8 degrees of unsaturation. The spectrum also showed additional peaks at m/z 233 (M^++1), 205, 191 and 173. The UV spectrum (MeOH) showed two absorption maxima λ_{max}=220, 245 nm. The ^{1}H-NMR spectrum (Table 1) showed an ABX system at δ=7.1 (1H, d, *J*=8.4 Hz), δ=8.09 (1H, dd, J=2 and 8.4 Hz) and 8.67 (1H, d, J=2 Hz) suggesting a 1,2,4-trisubstituted aromatic ring. The ^{13}C-NMR spectrum showed an acetyl group as depicted by the two signals at δ=23.0 and δ=152.9, this was further supported by the peaks at m/z 173 and 189 in the mass spectrum corresponding to (232-59) and (232-43) respectively, along with signals in the ^{1}H-NMR spectrum at δ=2.07 (3H, s). The signals at δ=187.6, 131.6 and 162.7 suggest the presence of α, β-unsaturated carbonyl group.

Table 1: H^1 and ^{13}C-NMR data of compound E1

S. No.	δ H ($CDCl_3$)	δ C ($CDCl_3$)
1	-	187.6
2	6.35 (1H, s)	131.6
3	-	162.7
4	7.1 (1H, d, *J*=8.4 Hz)	121.6
5	8.09 (1H, dd, *J*=2 and 8.4 Hz)	135.5
6	-	166.1
7	8.67 (1H, d, *J*=2 Hz)	133.8
8	-	125.9
9	-	128.5
10	4.67 (2H,s)	73.0
Acetyl	-	152.9
Acetyl	2.07 (3H,s)	23.0
OCH_3	3.90 (3H, s)	52.3

As shown from Heteronuclear Multiple Bond Correlation (HMBC) and Correlation Spectroscopy (COSY) correlations (Figures 2 and 3) the methylene proton at 4.67 interacts with the carbon at δ=162.7 and that of the acetyl group at δ=152.9 indicating that the acetyl group is attached to the olefinic carbon at δ=162.7 through the methylene at δ=73.03. HMBC spectrum also showed that the singlet proton at δ=6.35 interacts with the methylene at δ=73.0 and with the methyl at 23.0 indicating its attachment to the olefinic carbon at δ=162.7 Thus we can confidently lay down the following moiety, O=C-C=C-CH_2-$OCOCH_3$.

The presence of olefinic carbon (with two degrees of unsaturation) and the carbonyl group at δ 187.6 with the aromatic adds up to seven degrees of unsaturation suggesting that compound E1 possess a bicyclic structure. The proposed indenone moiety is rarely found in natural products; however, euplectine (indenone derivative) was isolated from the lichen *Flavoparmelia euplecta* [8] as well as ochracenoid B from marine sources [9].

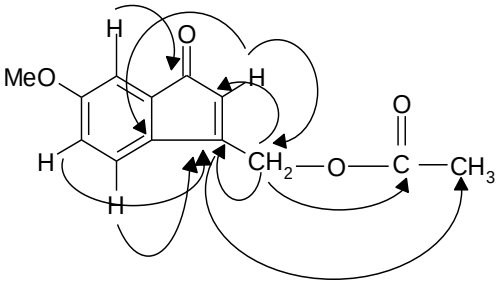

Figure 2: HMBC correlations of compound E3

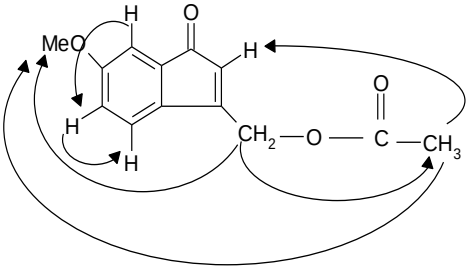

Figure 3: COSY correlations of compound E3

Docking

The structure of BACE1 site is generally composed of 11 pockets (also referred to as the "subsite"), i.e., S7, S6, S4, S3, S2, S1, S1′, S2′, S3′ and S4′, located between the N-terminal and C-terminal domains The S1 pocket, hydrophobic in nature, is located at one of the entries of the cavity formed by the target protein interface, the S1′ and S2′ pockets are located at the other entry of the BACE1 active cavity. The S1′ pocket is amphiphilic in nature, surrounded by two hydrophobic residues Val326 and Ile226, as well as a few hydrophilic residues, such as Thr329, Lys224, Tyr198, Arg235, Asp228, Thr72 and Thr231 the S2′ pocket is amphiphilic in nature, formed by both hydrophilic residues such as Ser35 and Arg128 and hydrophobic residues such as Ile126 and Pro70 [4]. An important feature is the loop located in the S3 pocket of β-secretase, right between two β strands. It allows for greater binding between the substrate and the S3 pocket. It also contains within it a glycine residue GLY11 with which the substrate can form a hydrogen bond, allowing for further stabilization of β-secretase-substrate interaction.

The energy of binding of indenone towards BACE1 was -75.7, GLY11, GLY12, GLY13, SER10, TYR14, VAL 170, ARG307 and GLU339 were identified as interacting residues. It forms hydrogen bonds with GLY11, GLY12 (at the S5 pocket), GLY13 and Vanderwal forces with SER10, GLY11, SER10, TYR14, VAL 170, TYR14, ARG307 and GLU339 (Figure 3).

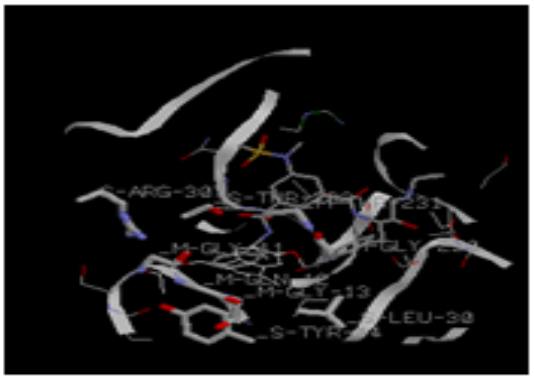

Figure 3: Predicted binding model indenone to BACE1. The BACE1 was shown as a ribbon drawing. The side chains of the active site residues and indenone is represented as stick model

The 3D crystallographic structures of AChE revealed a deep gorge lined by aromatic amino acids with two possible binding sites: the peripheral site located at the entrance of the gorge and the active site which is located at the bottom contains two sub-sites: the catalytic triad and the 'anionic site' composed of aromatic residues, that play an important role guiding the substrate towards the active site [10].

The energy of binding of indenone towards ACHE was -91, ASP 72, PHE 288, ARG 289, TYR 70, TYR 121, PHR 331 and TYR 334 were identified as interacting residues. It forms hydrogen bonds with ASP 72, PHE 288 and ARG 289 and Vanderwal ones with TYR 70 (located at the gorge), TYR 121 and TYR 334 (at the peripheral anionic site that lies at the entrance of the gorge), PHE 331 (at the bottom of the gorge) (Figure 4) [11].

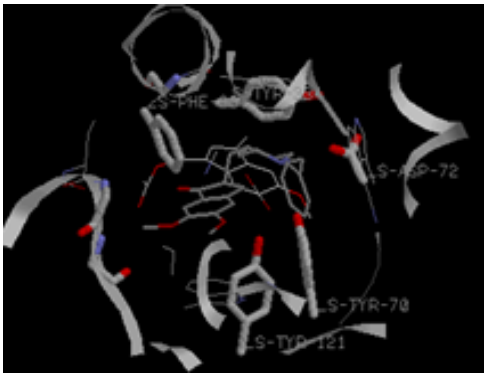

Figure 4: Predicted binding model indenone to AChE. The AChE was shown as a ribbon drawing. The side chains of the active site residues and indenone is represented as stick model

CONCLUSION

A new indenone was isolated from *E. fruiticosum*. Computational studies has proven its potential use as dual Acetyl cholinesterase and butyrylcholinesterase inhibiting activity it is anticipated that this indenone may help the development of novel multi-target BACE1 and Ache inhibitors anti-AD agents.

CONFLICT OF INTEREST

The authors report no declaration of conflict of interest.

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