SHORT REPORT

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Cholinesterase Inhibition and Molecular Docking Studies of Sesquiterpene Coumarin Ethers from *Heptaptera cilicica*

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Abstract: Five sesquiterpene coumarin ethers: umbelliprenin, umbelliprenin-10',11'-monoepoxide, conferone, mogoltacin and feselol were isolated from the fruits of *Heptaptera cilicica*. Their structures were identified by means of spectroscopic methods. AChE and BuChE inhibitory activities of the compounds were determined by molecular docking method which were confirmed by *in vitro* experiments. According to molecular docking results, total score of feselol and umbelliprenin were 5.69 and 3.23 kcal/mol against acetylcholinesterase, respectively. Total score for butyrylcholinesterase inhibitory effect of them were 2.76 and 4.99 kcal/mol, respectively. Feselol and umbelliprenin exhibited significantly high inhibitory potency against acetylcholinesterase (IC_{50} = 1.26 ± 0.01 and 5.86 ± 0.03 µM, respectively) and butyrylcholinesterase (IC_{50} = 9.98 ± 0.24 and 1.10 ± 0.19 µM, respectively). This is the first report of isolation of natural bioactives obtained from the chloroform extract of *Heptaptera cilicica* fruits with anticholinesterase activity.

Keywords: *Heptaptera cilicica*; sesquiterpene coumarin ether; acetylcholinesterase; butyrylcholinesterase.

1. Plant Source

The genus *Heptaptera* (Apiaceae) has four species growing in Turkey [1]. The plant material *H. cilicica* (Apiaceae) was collected from Mersin, between Tarsus-Çamlıyayla, Beylice Village, roadside of Kayabaşı (Turkey) on 10.06.2013 (540 m) and identified by Dr. Gülderen Yılmaz from Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University. A voucher specimen (AEF 26679) has been deposited in the herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey.

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2. Previous Studies

Several coumarin derivatives have been isolated from *Heptaptera* species [2-4]. The antioxidant capacity and AChE inhibitory activity of the extracts of these species were reported previously [5].

3. Present Study

Dried fruits of *H. cilicica* (412 g) were extracted with chloroform at 40°C (2 L × 4) and it was concentrated in vacuo to give a residue (74.9 g). The chloroform extract was subjected to column chromatography (CC) on silica gel using *n*-hexane with increasing amounts of ethyl acetate (from 100:0 to 0:100, v/v) to give four main fractions (Fr. A-D). Fr. A (5.6 g) was submitted to a silica gel column with a solvent gradient of *n*-hexane-ethyl acetate (from 100:0 to 82:18, v/v). Then, the subfraction was crystallized with the same solvent system to yield umbelliprenin (**1**) (32 mg). Fr. B (4.6 g) was separated by silica gel CC using *n*-hexane-ethyl acetate (from 100:0 to 88:12, v/v) to four subfractions (Fr. B1-4) which were crystallized with the same solvent system to yield umbelliprenin-10′,11′-monoepoxide (**2**) (20.3 mg), conferone (**3**) (188.8 mg), mogoltacin (**4**) (98 mg) and feselol (**5**) (40.5 mg). The structures of the isolated compounds are shown in Figure 1.

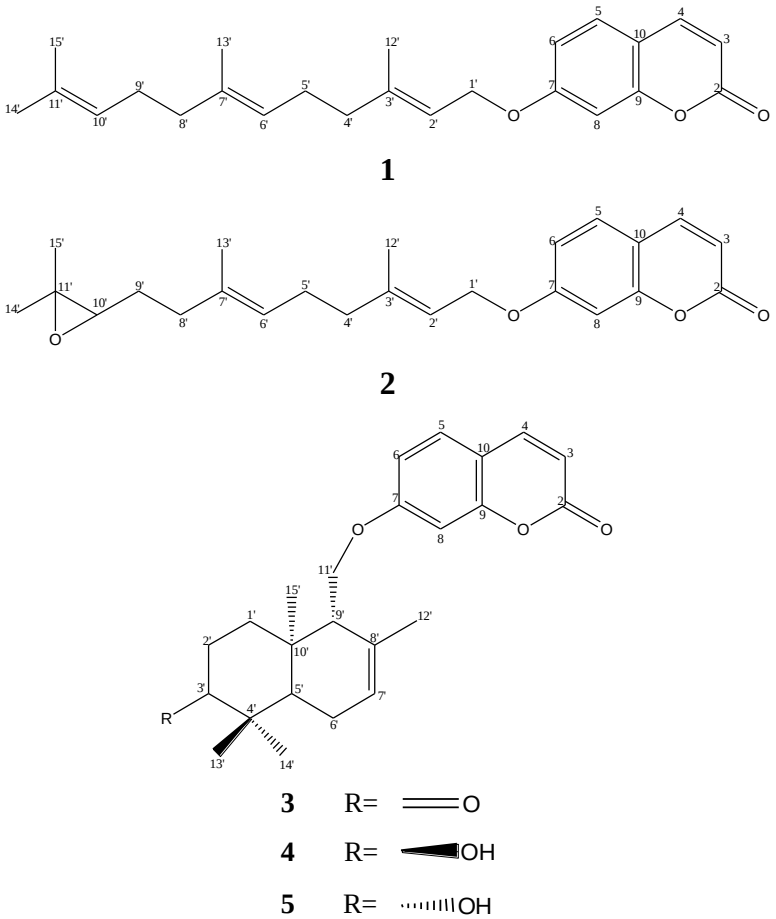

Figure 1. Structures of compounds **1-5** isolated from *H. cilicica*.

The microplate assay for anticholinesterase activity: Inhibitory activities of AChE and BuChE of the chloroform extract and the test compounds were evaluated against AChE and BuChE spectrophotometrically by Ellman's method [6] with some modifications using commercially available

neostigmine bromide as the reference compound [7]. Stock solutions were dissolved in dimethylsulfoxide and then diluted in a 50 mM Tris buffer (pH 8.0) to provide a final concentration range. In a 96-well polystyrene photometric microplates, the assay medium in each well consisted of 50 µL of a Tris buffer, 125 µL of 3 mM 5,5-dithiobis-(2-nitrobenzoic acid) DTNB, 25 µL of 0.2 U/mL enzyme (AChE or BuChE) and a 15 mM substrate ATCI or BTCI. The assay mixture containing the enzyme, buffer, DTNB and 25 µLof the inhibitor compound was preincubated for 15 min at 37^{o}C before the substrate was added to begin the reaction. All test compounds were prepared at 11 different concentrations: 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL. The absorbance of the reaction mixture was then measured three times at 412 nm every 45 s using a microplate reader. The measurements and the calculations were determined with GraphPad Prism 6.

Docking procedure: The docking study was performed using Surflex-Dock in Sybyl-X 2.0 by Tripos Associates. 3D structures of the compounds were constructed using the Sybyl sketcher module. The structures were minimized using the conjugated gradient method until the gradient was 0.001 kcal/mol, max iterations: 1,000 with the Tripos force field with the Gasteiger Huckel charge. The simulation system was built on the crystal structures of 1ACJ and 1P0I, which were obtained from the Protein Data Bank. At the commencement of docking, all the water and ligands were removed and the random hydrogen atoms were added. Docking calculations using Surflex-Dock for 1ACJ and 1P0I were performed through protomol generation by ligand. The parameters used were threshold 0.5 and bloat 0.

The chloroform extract of *H. cilicica* fruits showed 45 and 79% inhibition towards AChE and BuChE at 200 µg/mL. These results led us to further studies and five sesquiterpene coumarin ethers were isolated from the chloroform extract of *H. cilicica* fruits. The structures of the isolated compounds were identified by comparing their spectroscopic data with that reported in the literature umbelliprenin **(1)** [8], umbelliprenin-10′,11′-monoepoxide **(2)** [9], conferone **(3)**, mogoltacin **(4)** and feselol **(5)** [10]. Among them umbelliprenin has been reported previously as AChE inhibitor [11]. In this study umbelliprenin-10′,11′-monoepoxide was isolated from Apiaceae family and mogoltacin from *Heptaptera* genus for the first time.

In the last few years, we have performed an intensive screening program, using both conventional and *in silico* approaches, with the aim of discovering novel potent and selective AChE and BuChE inhibitors [12].

Molecular modelling studies were performed to investigate possible interactions between the most active compounds, feselol with AChE, and umbelliprenin with BuChE. The possible interactions were simulated by the docking program SYBYL X 2.0. The structures of the enzymes were obtained from the Protein Data Bank.

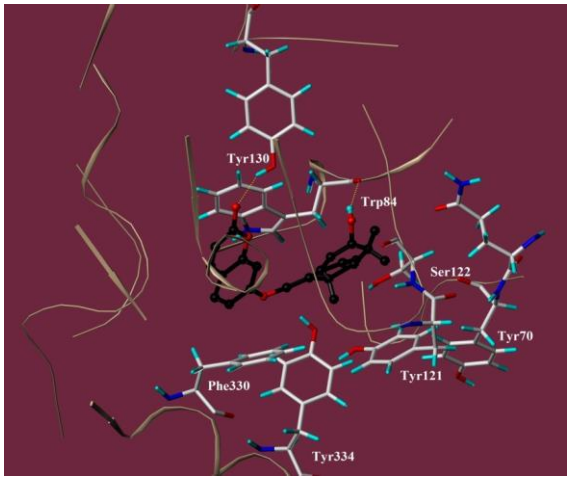

Figure 2. Docking model of feselol and AChE complex

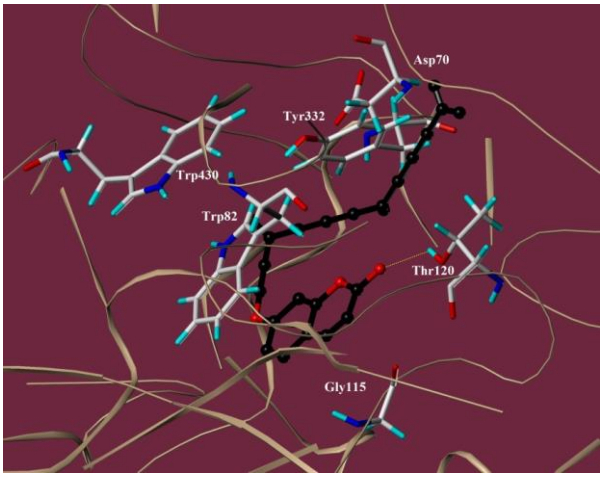

Figure 3. Docking model of umbelliprenin and BuChE complex.

The docking results showed that feselol displayed two hydrogen bonding interactions with Torpedo californica acetylcholinesterase (TcAChE-1ACJ), as shown in Figure 2. In the 1ACJ-feselol complex, the hydroxyl group of feselol created a hydrogen bond with carbonyl group of Trp84 (2.11 Å) at the catalytic active site (CAS) of the enzyme. And the carbonyl group of the chromene ring formed an interaction: H-bond with OH group of Tyr130 (2.14 Å). The most potent BuChE inhibitor, umbelliprenin, showed interactions with Thr120 and Trp82 residues of Human butyrylcholinesterase enzyme (HuBuChE-1P0I) (Figure 3). Hydrogen bond interaction between the carbonyl group and OH group of Thr120 (2.69 Å) at the CAS of HuBuChE was occured. And the other interaction was seen as a π-π hydrophobic interaction between chromene ring of umbelliprenin and phenyl ring of Trp82 (3.19-3.38 Å) at the peripheral anionic site.

For a comparative binding affinity analysis of all compounds, some docking parameters (e.g., D_ [13], PMF_ [14], G_ [15] and Chem_ scores [16]) were estimated using in silico analysis module of the Sybyl-X 2.0. *Surflex*-Dock (***T_***) and polarity scores, which provide multiple approaches to better evaluate ligand–receptor interactions, were also used to verify the high binding affinity of the compounds against the related enzymes. It is hoped that by using different scoring functions, the limitations of one function may be overcome.

The docking scores revealed that the most potent ChE inhibitors, the compound **5** (*T_score* [5.69] and polarity_score [2.12]) against AChE and compound **1** (*T_score* [4.99] and polarity_score [1.27]) against BuChE, have better scores than all other molecules in the series. These high scores indicate increased interactions with the related enzymes than the other compounds. As can be seen, high values of *T_scores* and polarity_scores parallels the experimental IC_{50} results accurately.

The other scoring functions (PMF, Chem, G-, and D- scores) were evaluated during the computational analysis. Charge and van der Waals interactions between the protein and the ligand (D_score) suggests that the most potent compound **1** is the superior ligand than neostigmine and other compounds to bind with BuChE. In addition, compounds **1** and **5** showed better hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies (G_score) than other compounds in the series against BuChE and AChE, respectively. All docking score values of the compounds are shown in Table 1.

Table 1. Surflex-Dock scores (kcal/mol) of the compounds **1-5**.

AChE						
Compounds	**T_score**	**Polarity_ score**	**D_score**	**PMF_score**	**G_score**	**Chem_score**
1	3.23	0.00	-425.092	-69.662	-263.242	-41.260
2	2.01	0.72	-207.887	-69.088	-253.430	-44.783
3	3.21	1.60	-204.278	-110.543	-210.975	-38.771
4	5.20	1.97	-298.770	-85.861	-265.916	-40.722
5	5.69	2.12	-233.079	-89.841	-269.802	-41.044
Neostigmine bromide	5.81	2.62	-194.127	-80.741	-270.668	-41.698
BuChE						
Compounds	**T_score**	**Polarity_ score**	**D_score**	**PMF_score**	**G_score**	**Chem_score**
1	4.99	1.27	-444.021	1.372	-227.541	-32.698
2	3.33	0.00	-433.813	-10.673	-120.280	-32.534
3	2.17	0.00	-304.333	-43.105	-151.288	-26.378
4	3.32	1.07	-288.657	-98.099	-187.698	-39.234
5	2.76	1.08	-316.341	-68.258	-179.160	-39.218
Neostigmine bromide	3.57	1.23	-396.474	-69.366	-207.000	-17.247

After the docking results, the sesquiterpene coumarin ethers isolated from chloroform extract of *H. cilicica* were tested for their AChE and BuChE inhibitory activities by *in vitro* Ellman method [8]. According to the IC_{50} values of the isolated compounds, umbelliprenin was found the most potent compound which showed maximum inhibitory activity against BuChE. In a previous study, umbelliprenin showed weak acetylcholinesterase inhibitory activity [17] which is similar to our present study. On the contrary, it showed about four times stronger inhibitory effect (IC_{50}=1.10 µM) than neostigmine (IC_{50} = 4.36 µM) against BuChE. And the other potent compound, feselol (IC_{50} = 1.26 µM), exhibited similar activity to the reference compound neostigmine (IC_{50} = 1.08 µM) on AChE inhibition. The IC_{50} values of the compounds are given in Table 2. These results confirmed the molecular docking studies.

Table 2. Inhibitory activities of the compounds **1-5** against AChE and BuChE.

Compounds	AChE[a] IC_{50} (µM)	BuChE[b] IC_{50} (µM)	Selectivity index[c]
1	5.86 ± 0.030	1.10 ± 0.190	0.18
2	> 100	12.59 ± 0.021	-
3	3.31 ± 0.014	9.31 ± 0.280	2.81
4	1.95 ± 0.050	9.74 ± 0.003	0.68
5	1.26 ± 0.010	9.98 ± 0.240	7.92
Neostigmine bromide	1.08 ± 0.012	4.36 ± 0.015	4.03

[a] 50% inhibitory concentration (means ± SD of three experiments) of AChE.
[b] 50% inhibitory concentration (means ± SD of three experiments) of BuChE.
[c] Selectivity for AChE = IC_{50} (BuChE) / IC_{50} (AChE).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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