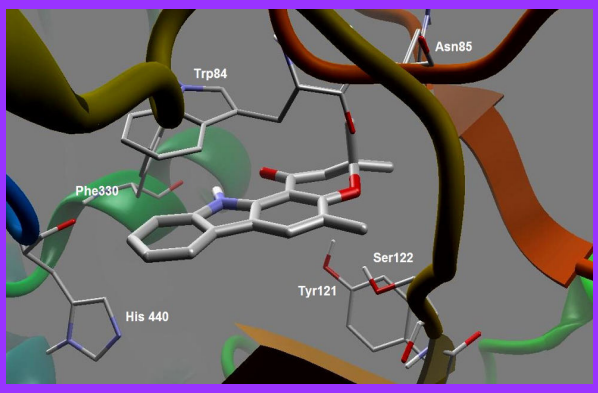

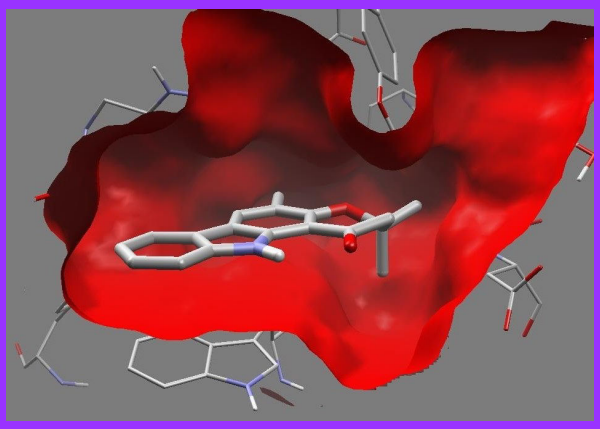

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Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2013; 8: 361-364
ISSN: 1991-0088

Molecular interactions of an alkaloid euchrestifoline as a new acetylcholinesterase inhibitor

Fazal-Ur-Rehman[1], Muhammad Faird Khan[1], Inamullah Khan[2] and Roohullah[3]

[1]Faculty of Pharmacy Gomal University, Dera Ismail Khan, KPK, Pakistan; [2]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan; [3]Department of Pharmacy, University of Abasyn, Peshawar, Pakistan.

Article Info

Received: 15 September 2013
Accepted: 3 October 2013
Available Online: 20 October 2013

DOI: 10.3329/bjp.v8i4.16417

Cite this article:
Fazal-Ur-Rehman, Khan MF, Khan I, Roohullah. Molecular interactions of an alkaloid euchrestifoline as a new acetylcholinesterase inhibitor. Bangladesh J Pharmacol. 2013; 8: 361-64.

Abstract

Acetylcholinesterase (AChE) inhibitors are well established therapeutic agents for clinical management of Alzheimer's diseases and other disorders associated with deficient cholinergic neurotransmission. In the current investigation, a new inhibitor has been reported for its significant AChE inhibitory. Euchrestifoline was isolated from the extract of *Murraya paniculata.* Isolated compound showed significant enzyme inhibitory activity (IC_{50} value: 93.1 ± 0.0 µM). Molecular docking revealed structural insights behind its significant inhibitory activity. Various molecular interaction were found between euchrestifoline and AChE especially Ser122, Trp84 and Tyr121. This study indicated promising potential of euchrestifoline to be further developed and explored as potential lead compound.

Introduction

Murraya paniculata (Orange jasmine) is an evergreen shrub, belongs to family Rutaceae. It is native to southwest Asia like Pakistan, India and China, but also found in Indonesian, Brazil, and Philippines. In traditional system of treatment, different parts of the plant are frequently used for multiple purposes. Roots and leaves are used for their analgesic, cough, and effectiveness against hysteria. The antibacterial activity of the plant is also documented (Sastri, 1962). Additionally, it is also used for the treatment of various skin disorders, stomachache, and dysentery (Kinoshita et al., 1985). Two previously known compounds, murranganone and paniculatin from the leaves of *Murraya paniculata*, have also been found to be moderate inhibitors of cholinesterase.

Phytochemical studies showed the isolation of alkaloids prenylatedcoumarins, polymethoxyflavones and flavornoids (Kinoshita et al., 1997; Proença et al., 2005; Zhang et al., 2011).

Cholinesterase inhibitors isolated from medicinal plants belongs to diverse classes like alkaloids, terpenoids, coumarines, flavonoids, lignans, lactones etc. Physostigmine and galanthamine are the well-established AChE inhibitors that are approved as drugs. Cholinesterase inhibition screening is considered to be a promising approach for the treatment of Alzheimer's disease and for possible therapeutic applications in the treatment of Parkinson's disease, aging and myasthenia gravis.

In the present work, here we reported the isolation of a compound euchrestifoline. Furthermore, the isolated compound was tested in established experimental paradigms to evaluate its role as a cholinesterase inhibitor.

Materials and Methods

Plant material

Arial parts of the Plant were collected from District Peshawar of the Khyber Pakhtunkhwa Province and authenticated by Dr. Sarfaraz Khan. A sample with voucher number GH-040 (2011) was deposited in the Gomal Herbarium of Faculty of Pharmacy Gomal University. After shade drying of arial parts of the plant

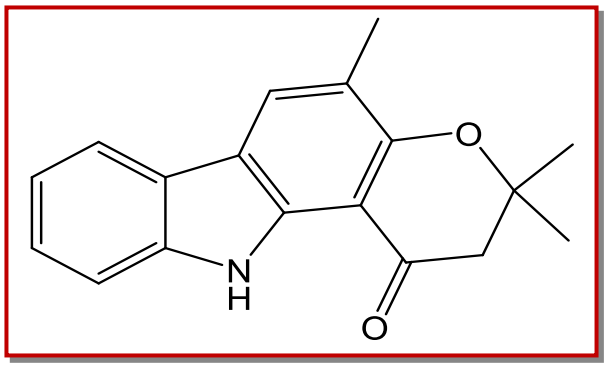

Figure 1: Chemical structure of euchrestifoline (Ecf)

for two weeks, it was crushed and stored under refrigeration for further experimental use.

Extraction and isolation

The dried powder of the leaves (13.5 kg) were macerated in methanol (11.5 L) for 48 hours and filtered. This process was repeated three times and the combined filtrate was concentrated in vacuo at 40°C to give crude methanol extract (1.15 kg, 8.5% (w/w). It was redissolved in distilled water and sequentially extracted with hexane, chloroform; ethyl acetate and finally water give the respective fractions. Chloroform fraction (27 g) was subjected to column chromatography and was eluted using chloroform : *n*-hexane (1:1 to 100:0) gradient on Si gel with gradual increase of polarity to chloroform (100%). This afforded 9 fractions (fr.1-9). Further elution with 1 to 10% methanol/chloroform gradient has afforded 7 fractions (fr.10-16) fractions. Subfraction 11 (1.29 g) was rechromatographed over Si gel and eluted with 1%methanol-chloroform gradient resulting in 2 subfractions. Further purification by using 1.5%acetone-chloroform gradient provided euchrestifoline (211 mg). Structure (Figure 1) of euchrestifoline (Wu et al., 1996) was recognized via similarity of spectral data with the spectral data cited in literature.

Acetylcholinesterase inhibitory activity

To investigate the ACE-inhibitory activity we followed the method previously described (Khan et al., 2010; 2011), with slight modified spectrophotometric procedure. Electric-eel AChE (type VI-S, Sigma) were utilized as source of cholinesterases. Acetylthiocholine iodide (Sigma) was used as substrate for AChE, to perform the reaction. 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB, Sigma) was utilized for the determination of cholinesterase assay. Investigated sample and the positive control (galanthamine) were solubilized in ethanol. Reaction mixture contained 150 µL of (100 mM) sodium phosphate buffer (pH 8.0), 10 µL of DTNB, 10 µL of test-compound solution and 20 µL of acetylcholinesterase solution were mixed and incubated for 15 min (25°C). 10 mL of acetylthiocholine was added to initiate the reaction. Hydrolysis of acetylthiocholine 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 412 nm (15 min). All the reactions were performed in triplicate in 96-well micro-plate. The percentage inhibition was measured using the formula (E−S)/E×100. Where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound. The method of (Khan et al., 2011) was followed to calculate the IC_{50} values.

Molecular docking molecular docking

FRED 2.1 (Khan et al., 2011; Martino et al., 2006) was used in this study to dock the OMEGA pre-generated multi-conformer library. FRED 2.1 strategy is to exhaustively dock/score all possible positions of each ligand in the binding site. The exhaustive search is based on rigid rotations and translations of each conformer within the binding site defined by a box. FRED filtered the poses ensemble by rejecting the ones that clash with the protein (TcAChE) or that does not have enough contacts with the protein. The final poses can then be scored or re-scored using one or more scoring functions. In this study, the smooth shape-based Gaussian scoring function (shapegauss) was selected to evaluate the shape complementarily between each ligand and the binding pocket. Default FRED protocol was used except for the size of the box defining the binding sites. In an attempt to optimize the docking–scoring performance, we performed exhaustive docking with shapegauss applying the "Optimization" mode. The "Optimization" mode involves a systematic solid body optimization of the top ranked poses from the exhaustive docking. Three different boxes were explored for AChE (PDB ID: 1ACL) and 3 different simulations were carried out with an added value of 8 Å around the reference ligand.

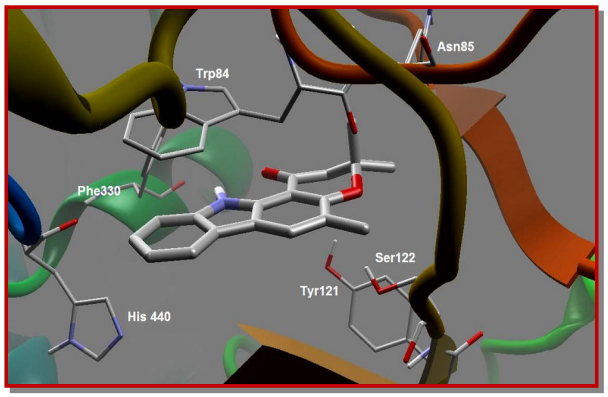

Figure 2: Molecular binding mode of euchrestifoline (Ecf) inside active site of AChE. Hydrogen atoms except polar ones are omitted for clarity

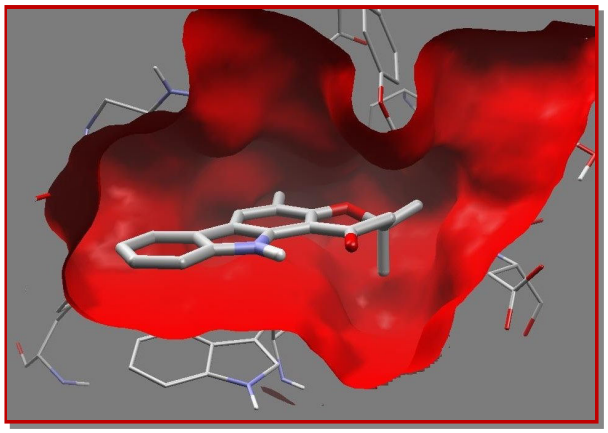

Figure 3: A closer view of molecular interactions between residues inside active site of AChE with euchrestifoline (Ecf). Hydrogen atoms except polar ones are omitted for clarity

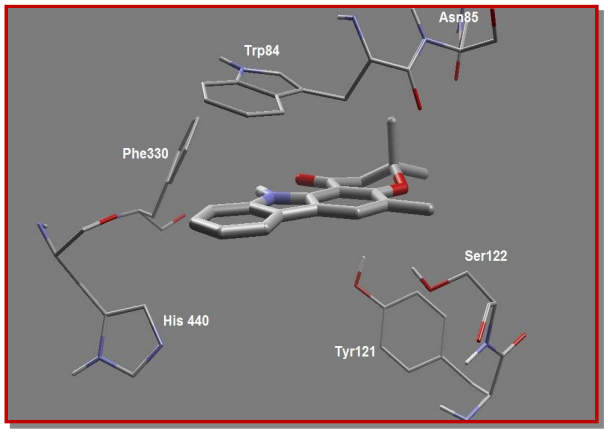

Figure 4: Favorable steric indications of euchrestifoline (Ecf) with binding pocket inside active site of AChE. Hydrogen atoms except polar ones are omitted for clarity

Results and Discussion

AChE (EC 3.1.1.7), a complex protein composed of α/β hydrolase fold type with an overall ellipsoid structure having a deep and narrow groove of 20 Å deep referred to as 'aromatic gorge' or 'active site gorge' as shown in (Figure 2). The actual hydrolysis of acetylcholine occurs at the very *bottom* of the gorge, where four main catalytic subsites; the "Esteratic (ES)", the "oxyanion hole (OX)", the "Anionic (AS), also termed as choline binding subsite or hydrophobic subsite)" and the "Acyl Pocket (AP)" subsites exists. The ES possesses the catalytic machinery of the enzyme which is dependent on a catalytic triad of Ser200–His440–Glu327 (Harel et al., 1996) while the OX comprise of Gly118, Gly119 and Ala201 (Khan et al., 2010; Szegletes et al., 1998; Zhang et al., 2002). The "Anionic Subsite" (AS) is majorly comprised of aromatic residues and contains Trp84, Phe330 and Glu199; which are believed to bind with the quaternary ammonium ligands through π-cation interactions. The AP is composed of Phe288 and Phe290, and is believed to play a role in limiting the dimension of substrates which are able to enter the active site (Nisar et al., 2011). There is also a peripheral site (also called peripheral active site, PAS) which consists of Tyr70, Asp72, Tyr121, Tyr334, and Trp279 residues (Khan et al., 2010). The PAS is involved in the initial binding of Ach and ligand binding at this site in general may result in the changes in the allosteric conformation of the active catalytic center (Harel et al., 1996; Nisar et al., 2011; Szegletes et al., 1998; Zhang et al., 2002). Furthermore, PAS has been shown to interact with Aβ peptide and implicated in Aβ aggregation that appears to be the hallmark feature of AD. Hence, compounds interacting with both the ES and the PAS of AChE could not only alleviate cognitive deficits associated with cholinergic neuron depletion but also inhibit Aβ peptide aggregation. These so-called bivalent ligands are now being developed as a new avenue of AD therapeutic approach (Gaggeri et al., 2010; 2011; Khan et al., 2011; Langjae et al., 2007; Martino et al., 2006; Martino et al., 2008; Nisar et al., 2011). The PAS varies among AChEs and that from *Torpedo californica* (TcAChE), for example, consists of aromatic and carboxylic acid residues, Asp72, Tyr70, Tyr121, Trp279, and Phe290 (Szegletes et al., 1998).

In view of the above discussed background, the present study focuses on computational studies on euchrestifoline (Ecf) that exhibited considerable inhibitory activity (IC_{50}: 93.1 ± 0.0 µM) against AChE. Molecular docking simulations uncovered the molecular mechanism for the observed potent inhibitory effect and mode of inhibition (Figure 2 and 3). Euchrestifoline possesses a slightly rigid skeleton that appears to undergo molecular interactions with the ES, PAS, AS, OX and AP subsites inside the catalytic pocket of AChE (Figure 2 and 3). Favorable molecular interactions are involved between the heterocyclic oxygen atoms of Ecf with Ser122 residue of the AChE, through hydrogen bonding at a distance of 3.49Å. Interestingly, the carbonyl oxygen of Ecf was found to be involved in holdingthe molecular contact with Tyr121 of PAS of AChE via dipole-dipole electrostatic interactions. Involvement of PAS subsite of AChE in macromolecular complex of inhibitor and enzyme seems to be an additional factor behind considerable inhibitory activity of the compound. Apart from involvement of PAS subsite, AS subsite also seems to be involved in favorable molecular contacts of the Ecf and AChE. Trp84 residue is lying flat and in parallel conformation to the aromatic frame of Ecf, which ultimately lead to favorable π-π stacking effects with the inhibitor showing favorable hydrophobic interactions. The aromatic rings of Ech were found to be favorably surrounded by His440, Phe330, Gly441 and Ile444, providing support to the macromolecular complex. No steric clash was observed between Ecf and AChE (Figure 4).

Conclusion

Our detailed computational insights study showed that Ecf has a strong potential to penetrate in and bind with the active subsites of AChE binding pocket. Instantaneous and good bonding interactions of Ecf with PAS subsite provided a major clue behind its significant AChE inhibitory activity. On the basis of recommendations of the present study, new and better derivatives of Ecf could be designed as a potential AD drugs. Nevertheless, such new derivatives should be subjected to both structure-based design techniques and 3D-QSAR prior to being considered as candidate for clinical drug development.

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Author Info
Fazal-Ur-Rehman (Principal contact)
e-mail: fazleepharmacist@yahoo.com