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In-vitro evaluation and *in-silico* studies applied on newly synthesized amide derivatives of *N*-phthaloylglycine as Butyrylcholinesterase (BChE) inhibitors

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ABSTRACT

Amide derivatives of *N*-phthaloylglycine were synthesized under Schotten Baumann reaction condition. The structures of synthesized compounds (4a–d) were characterized by using FTIR, ^{1}HNMR and EI-MS. The compounds were evaluated for their *in-vitro* Butyrylcholinesterase inhibition and all of them exhibited good activity against this enzyme. Compound 4a ($IC_{50} = 6.5 \pm 0.1$) was found to be most potent compared with the reference compound Galantamine ($IC_{50} = 6.6 \pm 0.00038$) and the other compounds (4b,4c,4d) were also possess that activity and hence can be employed for the discovery of lead compounds against Alzheimer's disease.

The depth analysis of the binding mechanism of these newly synthesized compounds inside the binding gorge of BChE, an *in silico* technique, molecular docking was performed. All the compounds were found to be well accommodated within the binding pocket of BChE. Compounds 4a, 4b and 4c showed hydrogen bonding interaction with binding site residue TYR332. Moreover, hydrophobic and π–π interaction assisted the compounds to attain their enzyme inhibitory activity. These theoretical studies showed significant correlation with experimental results.

1. Introduction

Studies on enzymatic inhibition has been attaining a great interest in pharmaceuticals to discover the useful drugs for the variety of the disease (David and Paul, 1991; Nese, 2003).

Animal cholinesterase is widespread enzyme present in cholinergic and non-cholinergic tissues as well as in their plasma and other body fluid (Greigh et al., 2002; Vladimir et al., 2011; Gilberto et al., 1999). They are divided into two classes depending upon their substrate specificity and susceptibility to the inhibitor, acetylcholinesterase (true cholinesterase; AChE) and butyrylcholinesterase (pseudocholinesterase; BChE). These serine hydrolase belongs structurally to the class of protein known as esterase/lipase family (Azizurrehman et al., 2014). Both cholinesterase inhibitors are used in treatment of various neuromuscular disorders and have provided the first generation of drugs for the treatment of Alzheimer's disease (AD) (Harry et al., 2003). The inhibition of cholinesterase enzyme is directly connected with the treatment of AD.

N-Phthaloyl derivative of amino acids possess the vast significance in synthetic organic chemistry because of having the ability to form various derivatives that are biologically active. They are applicable as analgesic (Robert et al., 2003), antimicrobial (Julija and Zdenka, 2005), antiepileptic (Omar et al., 1994), anticonvulsant (Cyril et al., 2001), hypolipidemic (Cutinho Neto et al., 1993) and also having DNA cleaving abilities (Brana and Ramose, 2001). Among the *N*-phthaloylamino acids, *N*-phthaloylglycine has been most widely studied for the metal complexation with supramolecular structure (Nilotpal et al., 2006a), cleavage with various amines (Khan and Ismail, 2002), Adduct formation with various aromatic amines (Nilotpal et al., 2006b) and other heterocyclic derivatives of *N*-phthaloylglycine such as Oxadiazole (Antunes et al., 1998), benzoxazinone (Mehdi and Sohrab, 2004), and 1,2,4-triazole compounds are also reported (Uzma et al., 2008).

Molecular docking is an extremely powerful tool in computer aided drug designing CADD which is used to study interaction

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between ligands and the target protein at atomic level, and to prioritize these ligands according to their binding affinity towards the receptor. This aim is achieved in two steps, the first step consists of generation of different poses of ligand into the active site of target and the second step involves ranking of these poses by assessment of binding affinity through a scoring function (Yuriev and Ramsland, 2013; Taylor et al., 2002). Various types of sampling algorithms and scoring functions have been developed to get most accurate results of molecular docking studies (Kroemer, 2007; Douglas et al., 2004). There are two major types of molecular docking, rigid docking and flexible docking. In rigid docking both ligand and target protein are treated as rigid bodies. This type of docking takes into account lock and key theory to elucidate binding mechanism of ligand into the target. Whereas, flexible docking considers the fact that the active site of target protein is reshaped during its interaction with ligand and therefore both ligand and the target protein are treated as flexible entities. This type of docking is based on induced-fit theory (Meng et al., 2011). Numerous software programs for molecular docking simulation have been developed, according to the requirement of protein-ligand treatment in the theoretical experiment, such as AutoDock Vina (Trott and Olson, 2010), FlexX (Bernd et al., 1999), GOLD (Joy et al., 2006), FRED (McGann, 2012), Surflex-DOCK (Jain, 2007). In the present study, Molecular docking simulation was performed on all the four synthesized compounds using Surflex software program, in order to predict mode of inhibition of these compounds against human BChE enzyme. Surflex is a fully automated algorithm used for flexible docking of ligands into the target protein. It integrates scoring functions from the hammerhead docking systems with a surface-based molecular similarity sampling algorithm in order to produce appropriate poses for molecular fragments (Jain, 2003).

Often the scoring functions that are incorporated in docking programs do not always exhibit the best affinity predictions (Warren et al., 2006). To tackle this problem, rescoring is performed where the poses generated by a docking program are taken and one or more alternative scoring functions are applied to those poses (Kroemer, 2007). A large number of scoring functions are available for this purpose, these scoring functions are categorized as force-field based, empirical and knowledge based (Sheng-You et al., 2010). Force field based scoring functions evaluate binding energies of protein-ligand complexes by calculating the sum of electrostatic and van der Waals interactions. One typical force field scoring function in molecular docking is the scoring function of DOCK (Elaine et al., 1992). Empirical scoring functions assess binding energy of protein-ligand complexes by taking into account hydrogen bond, hydrophobic effect and binding entropy *etc*., and multiplying them with a coefficient obtained from the regression analysis of protein-ligand complex. Examples of these scoring functions include LUDI, PLP and Chem-Score (Wang et al., 2002). Knowledge-based scoring functions use statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing the favored interaction frequencies of all interatomic pairs in the protein-ligand complex. Examples of Knowledge based scoring functions include Bleep, Drug-Score and PMF. Consensus scoring combines different scoring functions to assess binding affinity of protein-ligand complex so, a ligand having better affinity toward target protein according to a number of different scoring functions will have a high consensus score. For example, C-Score is a consensus scoring function that combines Chem-Score, DOCK, PMF, GOLD and FlexX scoring functions (Meng et al., 2011; Sheng-You et al., 2010). In this study, Different kinds of scoring functions were also employed to evaluate docking results.

In continuation of our previous work for the synthesis of *N*-phthaloylglycine amide (Samreen et al., 2014) the present research work was a successful attempt to extend the biological activity results of the synthesized derivatives of *N*-phthaloylglycine amide against butyrylcholinsterase inhibition.

2. Experimental

All the reagents and solvents were purchased from sigma or Merck companies and used without further purification. Melting points are uncorrected and were determined with digital electro thermal equipment. ^{1}H NMR spectra were recorded on Avance Bruker 300 and 400 MHz in $CDCl_3$. The EI-MS were measured on Finnigan MAT-312, Germany, and JEOL MS Route JMS. 600 Hz, Japan Instruments. TLC analysis was performed on pre-coated silica gel aluminum plates (Kieselgel 60F_{254}, E. Merck, Germany).

2.1. N-*Phthaloylglycine(1)*

A well pulverized mixture of 6.0 g (0.02 mol) of phthalic anhydride and 3.0 g glycine (0.02 mol) was heated until the solid melted, stirred gently with a glass rod and then heated the molten mass at 150–190 °C for 15 min, the mixture was allowed to cool and recrystallized with water (Samreen et al., 2014; Charles, 1984).

Yield 72%, mp 196–198 °C. R_f 0.78. IR spectrum, ν, cm^{-1}: 3450–3000 (OH-br, carboxylic acid), 1770,1721 (C=O-Imides), 1669 (C=O-acid), 1560 (Ar, C=C), 1398 (C—O), 711 (*ortho* substituted Ar ring). ^{1}H NMR spectrum (DMSO), δ, ppm: 4.30 (s, 2H, N—CH_2—C=O), 7.85–7.93 (m, 4H, Ar-H), 13.22 (s, 1H, OH). Mass spectrum (EI), *m/z* (I_{rel}. %): 205 $[M]^+$ (10), 160 (100), 149 (4), 133.0 (70), 104 (70), 77 (65), 50 (30).

2.2. N-*Phthaloylglycyl chloride(2)*

Pure *N*-phthaloylglycine (2.4 g) was refluxed with thionyl chloride ($SOCl_2$) (9.0 ml) for 1 h. The excess of thionyl chloride was evaporated from the reaction mixture and the synthesized *N*-phthaloylglycyl chloride was used for the synthesis of amide derivatives without purification (Samreen et al., 2014; Charles, 1984).

Yield 96%, mp 82–84 °C. R_f 0.75. IR spectrum, ν, cm^{-1}: 1755, 1765 (C=O-imide), 1750 (C=O-Acid chloride), 1654 (Ar C=C), 711 (*ortho* subst. Ar ring), 605 (C—Cl). ^{1}H NMR spectrum (DMSO), δ, ppm: 4.30 (s, 2H, N—CH_2—C=O), 7.85–7.93 (m, 4H, Ar-H)

2.3. *General procedrure for the synthesis of* N-*phthaloylglycine amides (4a–d)*

To a solution of amines (3a–d) in $CHCl_3$ (2–5 ml), 2 M sodium acetate (10 ml) was added and stirred vigorously. To a reaction mixture, *N*-phthaloylglycyl chloride (1.5 eq) solution in $CHCl_3$ (3.5 ml), was added and stirred for 2 h, the organic layer was separated and washed with aqueous Na_2CO_3 solution three times. The resulting organic layer ($CHCl_3$) was dried with Na_2SO_4 and concentrated. The solid residue was washed with methanol to get the pure product and the purity was checked by using TLC analysis (Samreen et al., 2014; Haishan and Ganesan, 2000).

2.4. N,N-*Dibutyl-2-(1,3-dioxo-1,3-dihydro-2*H-*isoindol-2-yl) acetamide (4a) (Blicke and Zienty, 1941)*

Yield 10%, mp 80–82 °C. R_f 0.72. IR spectrum, ν, cm^{-1}: 1770, 1720 (C=O-imide), 1646 (C=O-amide), 1629 (aromatic), 1418 (C—N), 713 (*ortho* subst, Ar. Ring). ^{1}H NMR spectrum (MeOD), δ, ppm: 0.94–0.99 (t, *J* 5.4 Hz, 3H, CH_3), 1.00–1.04 (t, *J* 6.4 Hz, 3H, CH_3), 1.26–1.36 (m, 4H, $2CH_2$), 1.37–1.55 (m, 4H, $2CH_2$), 1.65–1.73 (m, 4H, $2CH_2$), 3.29–3.42 (m, 4H, $2CH_2$), 4.82 (s, 2H, CH_2), 7.82–7.89 (m, 4H, Ar-H). Mass spectrum (EI), *m/z* (I_{rel}. %): 316 $[M]^+$ (5), 273 (8), 188 (18), 160 (80), 156 (68), 104 (20), 86 (60).