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Research paper

Synthesis of novel 5-(aroylhydrazinocarbonyl)escitalopram as cholinesterase inhibitors

Mehr-un Nisa [a, b], Munawar A. Munawar [a, *], Amber Iqbal [c], Asrar Ahmed [b], Muhammad Ashraf [c], Qurra-tul-Ann A. Gardener [d], Misbahul A. Khan [a, c]

[a] Institute of Chemistry, University of the Punjab, Lahore 54590, Pakistan
[b] Department of Chemistry, Division of Science & Technology, University of Education, Township, Lahore 54770, Pakistan
[c] Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[d] School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan

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ABSTRACT

A novel series of 5-(aroylhydrazinocarbonyl)escitalopram **(58–84)** have been designed, synthesized and tested for their inhibitory potential against cholinesterases. 3-Chlorobenzoyl- **(71)** was found to be the most potent compound of this series having IC_{50} 1.80 ± 0.11 μM for acetylcholinesterase (AChE) inhibition. For the butyrylcholinesterase (BChE) inhibition, 2-bromobenzoyl- (**76**) was the most active compound of the series with IC_{50} 2.11 ± 0.31 μM. Structure-activity relationship illustrated that mild electron donating groups enhanced enzyme inhibition while electron withdrawing groups reduced the inhibition except *o*-NO_2. However, size and position of the substituents affected enzyme inhibitions. . In docking study of AChE, the ligands **71, 72** and **76** showed the scores of 5874, 5756 and 5666 and ACE of −64.92,-203.25 and −140.29 kcal/mol, respectively. In case of BChE, ligands **71, 76** and **81** depicted high scores 6016, 6150 and 5994 with ACE values −170.91, −256.84 and −235.97 kcal/mol, respectively.

1. Introduction

Augustintion and Nachmansohn introduced the term acetylcholinesterase (AChE) in 1949 for the cholinesterase which can hydrolyze acetylcholine quicker than esterases. It hydrolyses the neurotransmitter acetylcholine into choline and acetate, hence playing an important role in cholinergic neurotransmission [1]. AChE is directly associated with various neuromuscular disorders *e.g.,* myasthenia gravis, glaucoma [2], and cholinergic deficiency related to Alzheimer's disease (AD) [3].

Butyrylcholinesterase (BChE) is also a serine hydrolase that is interrelated to AChE [4]. For BChE, butyrylcholine is the best substrate, neither exist naturally, nor physiologically necessary for humans [5]. BChE function has not been fully been elucidated, however, it is proposed that it scavenges anti-cholinesterase agents and defends synaptic AChE from inhibition. BChE has been explored as a possible stoichiometric bioscavenger in organophosphorus nerve agent poisoning [6]. It is extensively dispersed in the mammal's nervous system, indicating its likely participation in the neural functions and in neurodegenerative diseases [7]. Certain selective BChE inhibitors have been described to increase acetylcholine concentrations and to decrease the creation of abnormal amyloid originate in AD [8,9].

Previous studies have revealed that citalopram is an important inhibitor of cholinestrases. The selective serotonin re-uptake inhibitor antidepressant citalopram affects cholinesterase inhibition with potency comparable to that of therapeutically important donepezil and galantamine, the reversible cholinesterase inhibitors [10]. The clinical and imaging studies have indicated that the treatment of cognitive symptoms of AD with galantamine may have an extra benefit when treated with citalopram [11]. These studies have demonstrated two possibilities about better effects of galantamine after treatment with citalopram. The citalopram increases cholinesterase inhibition by inhibiting galantamine, the unactivated enzyme-substrate complex that is not affected by galantamine. Moreover, these drug combinations have been claimed to generate an inhibitory effect on BChE higher than either of the drug alone [12]. BChE synergistic inhibition results in the higher

Abbreviations: ACE, atomic contact energy; AChE, acetylcholinesterase; AD, Alzheimer's disease; BChE, butyrylcholinesterase; DTNB, 5,5′-Dithiobis-(2-nitrobenzoic acid); RCSB, the research collaboratory for structural bioinformatics.

* Corresponding author.
E-mail address: mamunawar.chem@pu.edu.pk (M.A. Munawar).

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brain acetylcholine levels. BChE remains at normal [13,14] or higher levels [15], in contrast to AChE which is reduced in AD. Conversely, BChE enzyme may truly be the core factor in the reduction of acetylcholine levels in AD [12].

In this ongoing study, we have designed and synthesized a novel series of 5-(aroylhydrazinocarbonyl)escitalopram (**58**–**84**) and have screened for their potential role in the inhibition of AChE and BChE.

2. Results and discussion

2.1. Design

Escitalopram (**1**) is very effective to inhibit cholinestrases with potency comparable to AD (*e.g.,* donepezil and galantamine) other than depression, migraine, schizophrenia *etc.* [10]. Escitalopram triazoles (**1a**) and tetrazole (**1b**) have also been reported as cholinesterase inhibitors (Figs. 1–2) [16]. Recently, we have synthesized novel 5-(aroylhydrazinocarbonyl)escitalopram (**58**–**84**) derivatives and screened for their cholinesterase inhibition.

R = H, 2-NH_2, 3-NH_2, 4-NH_2, 2-OH, 3-OH, 4-OH, 2-NO_2, 3-NO_2, 4-NO_2, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-F, 3-F, 4-F, 2-Br, 3-Br, 4-Br, 2-I, 3-I, 4-I, 2-OCH_3, 3-OCH_3, 4-OCH_3, 4-OH-3-OCH_3.

2.2. Chemistry

Escitalopram (**1**) was functionalized by partial hydrolysis of nitrile moiety into amide (**2**) by following classical method [17]. Later, amide (**2**) was converted into 5-carboxyescitalopram (**3**) using reported method (Scheme 1) [18]. Benzoic acid and its derivatives (**4**–**30**) were converted into benzohydrazides (**31**–**57**) by one pot conventional method [16]. 5-(Aroylhydrazinocarbonyl) escitalopram **(58**–**84)** were synthesized by one pot coupling (Scheme 2) [19]. A mixture of 5-carboxyescitalopram (**3**) and a benzohydrazide (**31**–**57**) was dissolved in DMF and stirred for few minutes, HBTU was incorporated. Then diisopropylethylamine (DIEA) was added and stirred. The 5-(aroylhydrazinocarbonyl) escitalopram **(58**–**84)** were purified with column chromatography.

2.3. Biological evaluation

The screening of novel 5-(aroylhydrazinocarbonyl)escitalopram (**58**–**84**) for cholinesterase inhibition was performed by Ellman method using eserine as positive control. The inhibitor interacts with AChE and BChE, and inhibits the hydrolysis of acetylthiocholine iodide, butyrylthiocholine chloride. The inhibitor lessens the enzyme activity in a dose-dependent manner. The unbound enzyme hydrolyzes the substrates and ultimately percent inhibition is calculated by quantification of the product of its reaction with DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) spectrophotometrically.

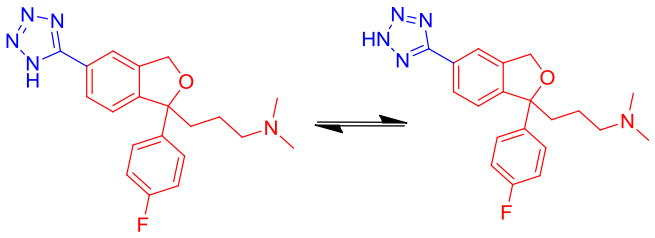


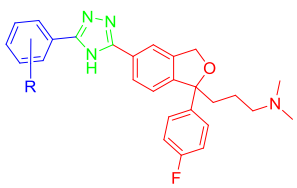
Fig. 2. Structure of escitalopram tetrazole, **1b** [16].

2.3.1. Acetylcholinesterase inhibition

In the present study, a series of compounds **(58**–**84)** were screened for AChE inhibition activities (Table 1). The AChE inhibition of this series follows the following pattern **71** > **72**>**76** > **82** > **73** > **84** > **75** > **67** > **77** > **70** > **63** > **68** > **59** > **78** > **83** > **66** > **80** > **60** > **65** > **74** > **79** > **62** > **81** > **64** > **58** > **69** > **61** which corresponds the following substituents on the aroyl group: 3-Cl>4-Cl>2-Br>2-OCH_3>2-F>4-OH-3-OCH_3>4-F>2-CH_3>3-Br>2-Cl>4-OH>3-CH_3>2-NH_2>4-Br>4-OCH_3>4-NO_2>3-I>4-NH_2>3-NO_2>3-F>2-I>3-OH>4-I>2-NO_2>H> 4-CH_3>2-OH. The results of this study indicate that 3-chlorobenzoyl- **(71)** is the most potent compound of the series having IC_{50} 1.80 ± 0.11 μM which is 45-fold less active than the standard eserine (IC_{50} 0.04 ± 0.0001 μM). Two other members of the series have also shown good inhibition <10 μM *e.g.,* 4-chlorobenzoyl- (**72**, IC_{50} 6.71 ± 0.32 μM) and 2-bromobenzoyl- (**76**, IC_{50} 7.81 ± 0.33 μM). While other compounds of series **82, 73, 84, 75, 67, 77, 70, 63, 68, 59, 78, 83, 66, 80, 60, 65, 74, 79, 62, 81, 64, 58, 69, 61** have shown good to moderate inhibition with IC_{50} values 45.81–295.53 μM.

The SAR of the series reveals that the mild electron donating groups increase AChE inhibition. It's worth noting that the substituents size and position are also important with the increased inhibition. Among *ortho, meta,* and *para* positions, the *meta* substitutions remained the most critical. In *meta* substituted ligands, the observed decreasing order of their inhibition has been found as follow 3-Cl>3-Br>3-CH_3>3-I>3-NO_2>3-F>3-OH. Among the *ortho* substituted ligands, 2-Br (**76**) has been found better inhibitor than other ligands 2-OCH_3>2-F>2-CH_3>2-Cl>2-NH_2>2-I>2-NO_2>2-OH. Similarly, among the *para* substituted ligands, 4-Cl (**72**) has shown more AChE inhibition than 4-OH-3-OCH_3>4-F>4-OH>4-Br>4-OCH_3>4-NO_2 >4-NH_2>4-I>4-CH_3.

Most of the compounds of the series have shown good AChE activities that are comparable to that of standard eserine. However, it is very hard to establish SAR of the studied 5-(aroylhydrazinocarbonyl)escitalopram **(58**–**84)** possibly because of multiple factors, *i.e.* size, shape, polarizability, and electronegativity of a ligand playing crucial role in enzyme inhibition [16].



R = H, 2-NH_2, 3-NH_2, 4-NH_2, 2-OH, 3-OH, 4-OH, 2-NO_2, 3-NO_2, 4-NO_2, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-F, 3-F, 4-F, 2-Br, 3-Br, 4-Br, 2-I, 3-I, 4-I, 2-OCH_3, 3-OCH_3, 4-OCH_3, 4-OH-3-OCH_3.

Fig. 1. Structure of escitalopram triazoles, **1a** [16].