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Synthesis, molecular modelling and biological evaluation of tetrasubstituted thiazoles towards cholinesterase enzymes and cytotoxicity studies

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ABSTRACT

Alzheimer is a neurodegenerative disease and requires the development of new scaffold to treat it. In this regard, thiazoles derivative are playing their significant role. In the current research paper we have focused our attention for the development of tetrasubstituted thiazole **(3a-h)** derivatives using domino synthesis by mixing the thiourea as a precursor, with acetophenone in the presence of iodine and tosic acid in DMSO and refluxed for 12–22 h. The structures of the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and EIMS analysis. Thiazole derivatives were analyzed for their biological significances against acetyl and butylcholinesterase enzymes and compound **3b** and **3d** were found more active against these enzyme, respectively. The mode of inhibition was determined for the potent compounds against both the enzymes. Moreover, the molecular docking studies were carried out to explore the interactive behavior of the compounds within the active pocket of enzymes. Furthermore, the derivatives **(3a-h)** were evaluated for their anticancer potential against HeLa cancer cell lines. Most potent inhibition was observed by compound **3b**.

1. Introduction

Heterocycles are the major class of organic compounds and have got their significance in the field of pharmacy and industry. The concept of drug like specie is very common in the field of medicinal chemistry. The use of molecular descriptors founded on already known drugs to help in the planing of new molecules is a broadly known policy to exploit the chance of clinical success. One of the Nobel Laureate James Black stated "the most fruitful basis for the discovery of a new drug is to start with an old drug" [1]. Organic chemists are playing their significant role for the development of new synthetic strategies towards the synthesis of industrially and pharmaceutically active heterocyclic motifs. Problem associated with the organic synthesis is the multisteps synthesis leads towards small yields. Domino synthesis is the tool to overcome this issue by reducing the steps and end up with high yields [2,3]. Alzheimer's disease (AD), with time becoming a neurodegenerative disorder which is associated with cognitive insufficiency and memory loss. Its major side effects comprises of the deficiency of cholinergic neurons and with their cortical extension from the basalis nucleus and areas related with the basal forebrain. For the treratment of Alzheimer's disease cholinesterase is targeted. Cholinesterase is consisted of two chief enzymes coming from the group of serine hydrolases, includes the butyrylcholinesterase (BChE, EC 3.1.1.8; also known as pseudocholinesterase) and acetylcholinesterase (AChE, EC 3.1.1.7). Structurally, these serine hydrolases fit into the esterase/lipase family, a class of proteins. The AChE plays its role to catalyse the hydrolysis of acetylcholine (ACh) in cholinergic synapses, while the function of BChE is not as much of clearly explained because it has the ability to hydrolyse ACh as well as other esters. By inhibiting these enzymes increase in the concentration of ACh in cholinergic synapses can be done and a number of pathogenic pro-

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cesses can be controlled. The inhibitors of ChE (ChEIs) are used in the cure of several neuromuscular disorders and become the first generation of drugs for the treatment of Alzheimer's disease, myasthenia gravis and glaucoma. An increase in the concentration of ACh can leads towards the increase in the symptoms of these diseases. Thus, research on new ChEIs may be appreciated for further progress in the treatment of these diseases [4,5]. Thiazole and its derivatives, are medicinally and industrially [6–10] very significant class of heterocycles playing their significant role against these enzymes. Coumaryl thiazole derivatives containing aryl urea/thioures showed excellent inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes [11]. Fluorobenzo[*d*]thiazol-2-yl)ethanamine showed excellent activity againt the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes [12]. In the course of development of cholinesterases inhibitor, here we will describe our efforts to report the domino synthesis of multisubstituted thiazoles starting from 1,3-disubstituted thioureas and their *in vitro* and *in silico* cholinesterases inhibition assay.

2. Results and discussion

2.1. Chemistry

Domino synthesis of *N*-(5-(2-oxo-2-phenylacetyl)-3,4-diphenyl thiazol-2(3*H*)-ylidene)benzamides (**3a-h**) was carried out by mixing thiourea (1a-h) with acetophenone dissolved in DMSO in the presence of iodine and p-toluene sulfonic acid for 12–22 h reflux under nitrogen atmosphere [13] (Scheme 1). We were expecting 3′ as the major product but after coloum chromatography product 3 was isolated with high yield. Physical data of *N*-(5-(2-oxo-2-phenylacetyl)-3,4-diphenylthiazol-2(3H)-ylidene)benzamides (**3a-h**) are presented in Table 1.

FT-IR data of the N-(5-(2-oxo-2-phenylacetyl)-3,4-diphenylthiazol-2(3*H*)-ylidene)benzamide (**3a-h**) confirmed the presence of the functional groups such as CH_3 (2950–3070), presence of carbonyl groups (C=O) was confirmed by the appearance of stretching bands on (1672–1709), (1672–1718) and (1633–1683), thiazolidine ring (1417–1488) and aromatic rings (1530–1632), (1532–1581), (1533–1578) cm^{-1} [14]. ^1H NMR data for the compounds **3a-h** confirmed the phenyl diketone substitution at the 5 position by the appearance of the phenyl group signals at 7.58 (m, 4HAr) and 7.47 (t, 6HAr). ^{13}C NMR further confirmed the product **3a-h** by the presence of three carbonyl signals at 183–185, 165–166 and 155–158 ppm. Further EIMS spectra confirmed the structure of compound **3a-h** by the appearance of molecular ion peak and two more peaks confirming the presence of substituted benzoyl group coming from benamide and base peak of benzoyl group peak coming from phenyl diketone group of the thiazoylidene benzamide [15].

2.2. Biological activity

2.2.1. Cholinesterases (chess) inhibition

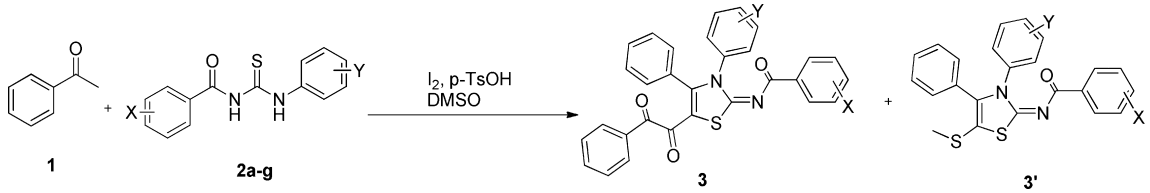
All the newly synthesized compounds (**3a-h**) were investigated for their potential to inhibit commercially available electric eel acetylcholinesterase (AChE) and horse serum butyrylcholinesterase (BChE). The inhibition studies were carried out and experimental results were reported in Table 2. All compounds were tested at 100 μM concentration initially and the inhibition (%) were calculated. Derivatives showing more than 50% inhibition potential were further tested at eight different concentrations to calculate their IC_{50} values. Galanthamine and donepezil were as used as standard for both the enzymes with an IC_{50} values of 0.62 ± 0.01 and 0.032 ± 0.003 μM for AChE and 0.87 ± 0.03 and 6.41 ± 0.34 μM for BChE, respectively.

2.2.2. Structure- activity relationships (SAR)

All the compounds showed promising and potent AChE inhibitory activity, except **3a**. While for BChE, except three derivatives (**3a, 3c** & **3h**), all other compounds showed promising and potent inhibitory activity. Most of the compounds are selective inhibitors of either AChE or BChE, but **3c** and **3h** are the most selective inhibitor of AChE. Experimental results revealed that compounds showed relatively more inhibitory activity to BChE as compared to AChE. This may due to the larger active site gorge of BChE that can accommodate the bulky groups unlike the narrow active site gorge of AChE. Although all the structural features were actively participating in inhibitory activity but alteration of different groups on main scaffold was actually responsible for a change in inhibitory potential. Since, all compounds have common, (*Z*)-*N*-(5-oxo-2-phenylacetyl)-3,4-diphenylthiazol-2(3H)-ylidene) benzamide, scaffold in their structures, the observed SAR was mainly due to modification of other functionalities on this main scaffold. The most potent compound against acetylcholinesterase was **3b**, showing an IC_{50} value of 1.03 ± 0.06 μM, and when compared to standard inhibitor, donepezil (0.032 ± 0.003 μM), it exhibited ~32-fold less inhibitory potential. This compound had two methyl groups on the main scaffold, but when one or both of these methyl groups were exchanged with chloro, flouro or even with methoxy groups, inhibitory activity of AChE was reduced sufficiently. In case of butyrylcholinesterase, the most potent compound was **3d** with an IC_{50} value of 0.49 ± 0.02 μM, ~13-fold more inhibitory potential when compared with donepezil (6.41 ± 0.34 μM). This compound contained three chloro groups attached with main scaffold. When these groups were exchanged by other substituents, inhibitory potential of BChE was decreased.

2.2.3. Investigation of mechanism of enzyme inhibition

For the investigation of mechanism of enzyme inhibition of the most potent inhibitor **3b** against AChE and **3d** against BChE, double reciprocal plots of initial velocity and substrate concentration (0, 0.5, 1.0, 1.5 and 2.0 mM) were plotted (Figs. 1 and 2). At the concentration of 0, 0.5, 1.0 and 1.5 μM for both potent inhibitors of acetylcholinesterase and butyrylcholinesterase, the enzyme kinetic studies were performed. K*m* values were determined and was found to increase with the increasing concentration of inhibitors. For AChE, km values were found 1.61, 1.90, 2.45 and 6.37 at 0, 0.5, 1.0 and 1.5 μM, respectively, of **3b** and for BChE, K*m* values were found 0.15, 0.61, 1.16 and 1.27 at 0, 0.5, 1.0 and 1.5 μM, respectively, of **3d**. The results showed that both compounds **3b**



Scheme 1. Domino synthesis of tertasubstituted thiazole (**3a-h**).