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LETTER ARTICLE

Cholinesterase Inhibitory Activities of Selected Halogenated Thiophene Chalcones

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Abstract: ***Background*:** Dual-acting human monoamine oxidase B (hMAO-B) and cholinesterase (ChE) inhibitors are more effective than the classic one-drug one-target therapy for Alzheimer's disease (AD).

***Methods*:** The ChE inhibitory ability of some halogenated thiophene chalcone-based molecules known to be selective hMAO-B inhibitors was evaluated.

***Results*:** Based on the IC_{50} values, the selected compounds were found to moderately inhibit ChE, with IC_{50} values in the range of 14-70 μM. Among the synthesised molecules, **T8** and **T6** showed the most potent inhibitory activity against AChE and BChE, respectively.

***Conclusion*:** Taken together, the data revealed that **T8** could be further optimized to enhance its AChE inhibitory activity.

Keywords: Acetylcholinesterase, butyrylcholinesterase, chalcone, docking, monoamine oxidase-B, thiophene.

Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disease that is characterized by loss of cholinergic neurons in the basal forebrain and which is difficult to treat [1]. The death of these nerve cells leads to impairment of memory and other cognitive dysfunctions. The pathophysiology of AD is based on the cholinergic hypothesis, a widely accepted theory [2], and the inability of the cholinesterase enzyme (ChE) to degrade acetylcholine due to an increase in the availability of the neurotransmitter. There are two types of ChE enzymes found in the central nervous system, namely acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [3]. AChE is the main cholinesterase that breaks down acetylcholine, whereas BChE has no specific substrate but can degrade acetylcholine as well. Symptomatic treatment of mild to moderate AD relies on ChE inhibitors. Galanthamine, donepezil and rivastigmine are the three ChE inhibitors approved by the FDA for clinical use. Donepezil and galanthamine are more selective for AChE while rivastigmine is a mixed inhibitor, having activity against both AChE and BChE [4].

The active site of ChE is located on the bottom of a long and narrow gorge, made up of 5 important regions. The substrate, acetylcholine, is brought down to the site of reaction by interactions with aromatic side-chain residues lining the gorge wall. The breakdown of acetylcholine happens at the catalytic triad located at the end of the active site. The overall structure of BChE is very similar to that of AChE, except that hydrophobic amino acids make up the gorge residues in BChE. These changes make it possible for bulkier butyrate substrate moieties and inhibitors to bind onto BChE but not onto AChE [5].

Monoamine oxidase B (MAO-B) inhibitors are more promising candidates for the treatment of AD than MAO-A inhibitors because MAO-B inhibitors can regulate the neurotransmitters, reduce oxidative stress in dopaminergic neurons by reducing H_2O_2 production, and have neuroresuing effects

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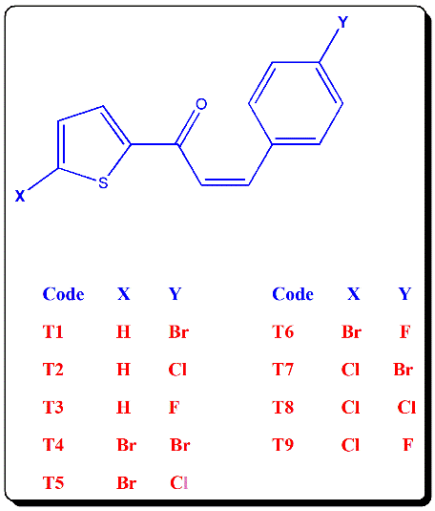
$$\text{Percentage of inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

[6]. Since the etiology of AD is multifactorial, therapeutic approaches of a broader nature are required to combat the disease. Dual ChE and MAO inhibitors developed over the past years may considered as promising candidates for therapeutic intervention in AD. Accordingly, optimum treatment is now based on a multi-target direct-ligand strategy (MTDLs] [7].

Chalcones have a potential promiscuous profile in treating various diseases and have been proven to be effective MAO-B inhibitors [8-17]. Inspired by the following outcomes, we developed a series of halogenated thiophene chalcone-based molecules, previously reported by our group as selective hMAO-B inhibitors [18-21]. Recently we investigated the bio-distribution in the human blood for the titled derivatives, thus contributing to the development of strategies which allow their safe therapeutic use [22]. Herein, we describe the evaluation of acetylcholinesterase and butyrylcholinesterase inhibitory activities of 9 synthesized halogenated thiophene chalcones (Fig. **1**).



Code	X	Y	Code	X	Y
T1	H	Br	T6	Br	F
T2	H	Cl	T7	Cl	Br
T3	H	F	T8	Cl	Cl
T4	Br	Br	T9	Cl	F
T5	Br	Cl			

Fig. (1). Structures of halogenated thiophene chalcones.

Cholinesterase inhibitory activity of the synthesized moiety was tested using an extremely sensitive 96-well microplate Ellman's assay as described by Khaw *et al*. [23]. 140 µL of 0.1M sodium phosphate buffer (pH 8) was added to each 96- well microplate followed by 20 µL each of test sample and 0.09 unit/mL of AChE. The pre-incubation was done at room temperature, followed by the addition of 10 µL each of 10Mm DTNB and 14mM acetylthiocholine iodide as the substrate. The absorbance of the coloured solution was measured at 412nm using Multiskan GO microplate reader (Thermo Fischer Scientific, USA). A similar procedure was adopted for the inhibitory assay of BChE except that the enzyme and substrate used were equine serum BChE and S-butyrylthiocholine chloride respectively. The reference standard used was of a known inhibitor, galanthamine. The initial concentration of the test solutions in DMSO was 1mg/mL and the final concentration was kept at 1% where DMSO had no inhibition on both acetylcholinesterase and butyrylcholinesterase enzymes. A set of 5 concentrations done in triplicate was used to estimate IC_{50}. Percentage inhibition was calculated using the equation:

The ChE inhibitory activity of the synthesized compounds is given in Table **1**. The compounds were initially tested at 10µg/mL. In general, all the compounds showed higher AChE inhibition than BChE. The compounds with over 50% inhibition on AChE were chosen for IC_{50} determination (except **T5** due to its solubility at higher concentration), whereas for BChE, IC_{50} of compounds **T6-T8** was determined for calculation of selectivity index for cholinesterase inhibition. Based on the IC_{50} values, the selected compounds were found to be the moderate ChE inhibitors, with IC_{50} values in the range of **14-70** µM. Among them, **T8** and **T6** showed the most potent inhibitory activity against AChE and BChE respectively. On a molar basis, **T8** was 7 times less potent in comparison to clinically used inhibitor galanthamine. Thus, **T8** could be further optimized to enhance its AChE inhibitory activity.

From the Table **1** of cholinesterase inhibition of halogenated thiophene-based chalcones some interesting structure activity relationships (SARs) principles could be derived.

- Unsubstituted thiophene chalcone (**T1**) didn't have any impact on both AChE and BChE inhibition. This clearly highlighted the importance of the presence of halogens in the thiophene chalcones against ChE inhibition.
- Among the series, the introduction of chlorine substituent at the 5th position of thiophene ring showed favorable inhibitory activity profile against both AChE and BChE with IC_{50} values ranging from 14.76-16.15 and 59.03-69.29 µM respectively.
- Presence of chlorine substituents on the *para* position of phenyl system (**T2** and **T5**) didn't have much impact on both inhibitions. However, the combination of one or more chlorine substituents at the thiophene system (**T8**) showed favorable inhibition against AChE with an IC_{50} value of 14.76 µM.
- Combination of two bromine substituents (**T4**) also had moderate inhibition against AChE with an IC_{50} value 24.75 µM.
- Combination of bromine and chlorine on thiophene and phenyl ring showed (**T5**) had no significant activity on both AChE and BChE inhibition. Interestingly the exchange of both atoms in (**T7**) motif showed moderate inhibition against both AChE and BChE with IC_{50} values ranging from 16.15 and 59.03 µM respectively.

Previous studies document that halogenated thiophene-based chalcones under the current study were highly selective MAO-B inhibitors [18-21]. High-blood barrier penetration and good bioavailability profiles encouraged to resolve

Table 1. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of T1-T9.

Sample	% of Inhibition at 10µg/mL		AChE Inhibition, IC_{50}		BChE Inhibition, IC_{50}		Selectivity for	
	AChE	BChE	µg/mL	µM	µg/mL	µM	AChE[a]	BChE[b]
T1	48.85±10.78	18.64±1.44	-	-	-	-	-	-
T2	68.24±4.36	25.30±1.53	12.65±1.90	50.86	-	-	-	-
T3	32.66±7.85	32.55±1.86	-	-	35.42±1.73	152.49	-	-
T4	52.75±5.90	14.59±0.76	9.21±1.83	24.75	-	-	-	-
T5	54.89±14.88	19.04±4.92	-	-	-		-	-
T6	64.23±16.09	28.46±0.53	5.65±0.68	18.16	16.27±4.52	52.29	2.88	0.35
T7	67.82±8.82	30.28±4.29	5.29±0.86	16.15	19.34±1.11	59.03	3.65	0.27
T8	71.02±6.00	32.04±1.12	4.18±0.54	14.76	19.62±1.30	69.29	4.69	0.21
T9	45.64±2.65	12.37±4.65	-	-	-	-	-	-
Galanthamine			-	2.09	-	19.34	9.25	0.11

Data presented as Mean ± SD (n=3-6)
[a] Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE)
[b] Selectivity for BChE is defined as IC_{50}(AChE)/IC_{50}(BChE)
-: not determined

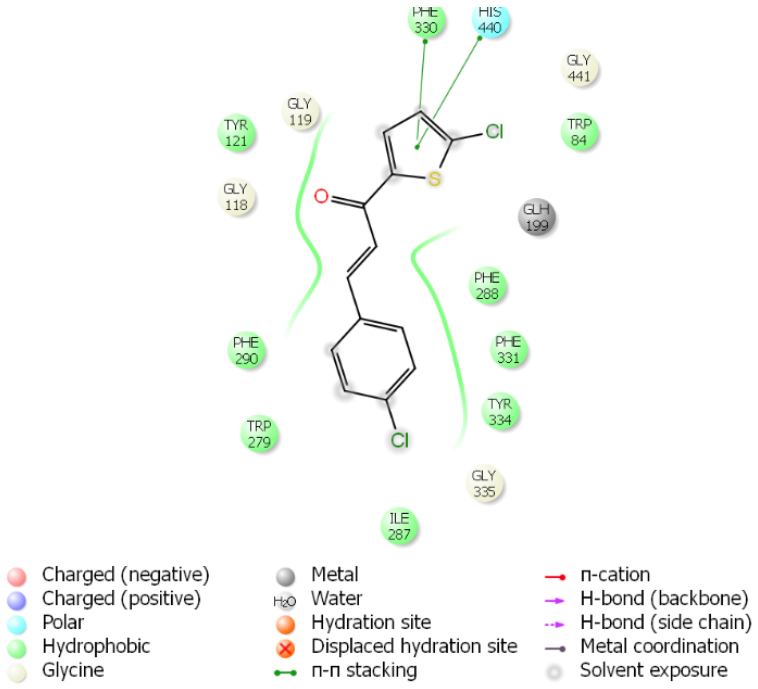


Fig. (2). Hypothetical binding mode of **T8** in AChE binding site.

the discussion about their potential activities in AD. Positive results of cholinesterase inhibition activity of lead compounds give merit to further in-depth evaluation of known dual ChE/MAO-B inhibitors that might be able to potentially cure AD.

To gain insight into the molecular determinants that modulate the AChE inhibitory activities of the lead compound, molecular docking studies of **T8** with AChE (PDB code: 1GQR) were performed using the Glide software. The Built module of Maestro was used to create the molecules and a conformational search for all molecules performed exhaustively by the OPLS-2005 force field. During the conformational search, the OPLS-2005 force field was exhaustively utilized on all the molecules with a limit of total conformational energy versus lowest energy. Default figures used during the conjugate gradient and steepest descent minimization were 0.05Å and 1.00Å for the initial and maximum step sizes respectively. The default option of 10^{-7} for the en-

ergy change and 0.001 kcal/mol for the gradient change were used during minimization as convergence criteria [24]. Docking pose of **T8** in the active site of AChE is shown in Fig. (**2**). Using X-ray crystallography, it was demonstrated that the AChE has two distinct ligand binding sites. The first site is a catalytic active site (CAS) that is situated at the opening while the second site is situated at the base of the active site gorge and is the peripheral cationic site (PAS). The concomitant binding of inhibitors onto the CAS and PAS sites is an important functional consideration during the design of targeted AChE inhibitors [25]. Evaluation of the docking studies demonstrated that chlorinated thiophene nuclei have dual interactions with Phe330 and His440 and that T8 orients itself spatially through hydrophobic interactions with Phe331 and Trp279 when binding to PAS.

In summary, we have designed, synthesized and evaluated the ChE inhibitory effect of a series of halogenated thiophene chalcone-based molecules which were previously reported by our group as selective hMAO-B inhibitors. Structure-activity relationship (SAR) studies demonstrated that AChE is favourably inhibited by **T8** due with an IC_{50} value 14.76 μM. This inhibition was attributed to the presence of one or more chlorine substituent at the thiophene system of **T8**. Based on these results, compound **T8**, is a promising candidate for one- compound multi target direct ligand for Alzheimer's disease treatment and needs further optimization.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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