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Design and discovery of Novel Thiazole acetamide derivatives as anticholinesterase agent for possible role in the management of Alzheimer's

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ABSTRACT

A novel series of thiazole acetamides was synthesized in excellent yields and characterized with the aid of various spectroscopic and elemental analysis. These compounds were evaluated for in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities for possible benefit in Alzheimers disease (AD). Among the synthesized compound, **6d** was identified as the most potent compound of AChE (IC_{50} = 3.14 ± 0.16 μM) with a selectivity index (SI) of 2.94 against BuChE. These compounds were further tested for inhibition of Aβ aggregation and β-secretase, where it showed potent inhibition which confirmed its multifactorial benefits in AD. The toxicity and docking study were also carried out to exemplify the pharmacological profile of compound **6d** as prospective lead molecule against AD.

Across a globe, the greater number of elder population was affected by progressive, never-ending, neurodegenerative disorder known as Alzheimers disease (AD).[1–3] It is characterized by a loss of memory and cognitive abilities affecting about 6% of the population worldwide aged over 65. Studies suggest that, the loss of cholinergic neurons in the hippocampus and the cerebral cortex of the central nervous system (CNS) are the ultimate cause of cognitive and behavioral abnormalities that characterize AD.[4–6] Therefore, early therapeutic approaches to treat AD were aimed at escalating the availability of the cholinergic neurotransmitter acetylcholine (ACh). The levels of ACh could be maintained by two types of agents, viz., drugs directly acting on cholinergic receptor or drugs preventing its degradation via inhibiting the activity of acetylcholinesterase (AChE) enzyme. The latter class of drug, that is, AChE inhibitors (AChEIs) is considered superior over directly acting ACh ligands for the symptomatic relief of AD by enhancing the cholinergic action.[7,8]

Thiazoles are an important class of heterocyclic molecules exhibiting numerous pharmacological activities, for instance, antimicrobial[9], anticancer[10] and antimalarial[11] including anticholine esterase activity.[12] More recently, acotiamide hydrochloride, a thiazole based selective AChEI for the treatment of functional dyspepsia has been identified and now being in advanced stages of clinical studies.[13]

Therefore, on the basis of above, we are encouraged to design and develop Novel Thiazole acetamides as anticholinesterase inhibitors for potential benefit in AD.

The development of target analogues **6** (**a**–**j**) were realised in an efficient synthetic manner as depicted in Scheme 1. The substituted benzaldehyde (**1**) was allowed to react with thiourea to afford phenyl thiazole (**3**) in efficient yield via cyclo-condensation reaction. This reaction was carried out in the presence of Bromine which leads to the formation of α-haloketones and reaction generally referred as Hantzsch thiazole synthesis. Moreover, in a second step, the chloro acetyl chloride was used to replace the free amine of the above synthesized thiazole to afford compound **4**. This resultant compound was then reacted with distinguished anilines **5** (**a**–**j**) to furnish target analogues **6** (**a**–**j**). Here, various electron donating and withdrawing substituent were introduced on the R^1 positions to determine its potential effect as anti-choline esterase.

The structure of various title derivatives **6** (**a**–**j**) was ascertained by spectral analysis. The FT-IR peaks of title compounds showed at 3368–3385 cm^{-1} (strong, broad) show characteristic of amide hydrogen (—CONH—). All compounds exhibits stretching vibration between 1658 to 1678 cm^{-1} which attributable to C=O group. All compounds displayed characteristic carbon, carbon double bonds (C=C) peak at 1559–1571 cm^{-1} in its expected region. Furthermore, the ^1H NMR ^{13}C NMR and MS signals of the title compounds were also observed in their expected region.

The bioactivities of the organic medicinal agents (OMAs) have been greatly dependent upon physicochemical properties.

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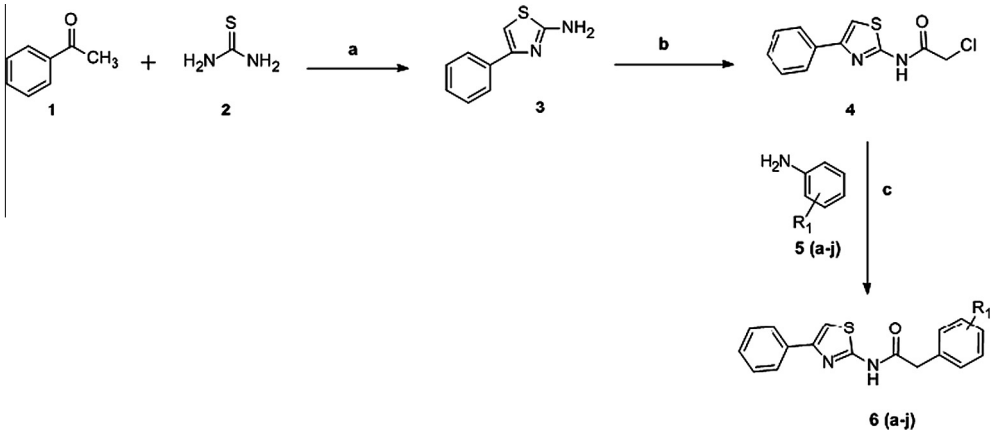

Scheme 1. Synthesis of Thiazole acetamides derivative. Reagents and conditions: (a) Br_2, Reflux; (b) DCM, Et_3N, 0 °C – rt, 4 h; (c) ACN, K_2CO_3, Reflux 4–8 h.

Therefore, the developed thiazole acetamides analogues were rigorously analyzed by Molinspiration, a web based software. It was used to predict parameter such as MiLogP, TPSA and druglikeness.[14]

The MiLogP (octanol/water partition coefficient) is able to envisage the ability of the molecule to cross the cell membrane. This methodology is based on the sum of fragment based contributions and correction factors for the compounds analyzed. Molecular Polar Surface Area or Total Polar Surface Area (TPSA) is calculated as a sum of fragment based contributions in which surface areas correspond to polar fragments centered to N- and O- and by hydrogen atoms attached to them are to be considered.[15] It has been deemed as a very efficient descriptor illustrating the drug absorption (including intestinal absorption, bioavailability, Caco-2 permeability) and blood brain barrier permeability. This method is based on contribution of group obtained by comparing the sum of fragment contributions to "real" 3D volume for a training set of about 12,000 drug-like molecules. Moreover, the semiempirical AM1 method was used for the optimization of the 3D molecular geometries for a training set.

The ability of a molecule to iterate for adopting the best possible conformation in binding pocket was determined by the Number of Rotatable Bonds. It has been directly related with an oral bioavailability of drugs and defined as any single bond except the aromatic, bonded to non-terminal non-hydrogen atom. Due to high rotational energy barrier, amide C—N bonds are not considered.[16] The results of the rigorous analysis of compounds **6** (**a**–**j**) in terms of molecular properties which depicts pharmacokinetics tolerability on the basis of above discussed parameters has been shown in Table 1. It was found that, the entire set of molecules obeyed Lipinski rule of five recommendations suggesting to have good bioavailability necessary for new chemical entities (Nviolation = 0). As shown by MilogP value, these molecules were also able to cross the cell membrane which is a key parameter for transportation across and in turn is needed for the generation of bioactivity. Moreover, it will be suitable for drug absorption owing to the acceptable TPSA value.[16]

BuChE was found in elevated concentration in AD and responsible for hydrolyzing the acetyl choline, thus, inhibition of BuChE was also considered as a fruitful target for symptomatic relief in ADs. Consequently, the inhibitory effect of target analogues was analyzed for both AChE and BuChE using Ellman method. Rivastigmine was used as standard in this comparison test. The inhibitory potency (IC_{50}) of tested compounds along with selectivity index has been outlined in Table 2. According to the results, the entire set of the synthesized compound showed considerable inhibition of both the enzymes. It has been found that, compound **6a**, bearing no substituent showed least activity against the tested enzymes. Whereas, on the introduction of the methyl as a substitutent (**6b**) render compound much more active in regard of inhibitory potency and SI. On changing the substitution pattern, from *para* to *ortho*, the activity was decreased marginally. The highest inhibitory potency was reported by the compound **6d** having *p*-methoxy against the tested enzymes along with high SI. Whereas, it's isomeric counterpart, compound **6e** exhibit less pronounced activity than the parent. The activity was significantly abolished in the presence of an electron withdrawing group. Particularly, in the case of compound **6f** and **6g**, marked reduction in activity was observed with minimal difference. On replacing the nitro with chloro, the activity was declined more than twofold with a mild reduction in SI. No significant difference was observed in the case of compound **6j** against both the tested enzymes with less selectivity for AChE. It is surprising to note that, none of the synthesized compound exhibited activity similar or better than Rivastigime. However, the structure activity relationship (SAR) suggests that, presence of electron donating group seemed to be suitable for

Table 1
Molecular properties of compounds **6** (**a**–**j**)

Compound	miLogP	TPSA	Natoms	MW	nON	nOHNH	nviolations	nrotb	Volume
6a	3.65	41.99	21	294.38	3	1	0	4	261.60
6b	4.10	41.99	22	308.41	3	1	0	4	278.17
6c	4.05	41.99	22	308.41	3	1	0	4	278.17
6d	3.70	51.22	23	324.40	4	1	0	5	287.15
6e	3.66	51.22	23	324.40	4	1	0	5	287.15
6f	3.61	87.81	24	339.38	6	1	0	5	284.94
6g	3.56	87.81	24	339.38	6	1	0	5	284.94
6h	4.33	41.99	22	328.82	3	1	0	4	275.14
6i	4.28	41.99	22	328.82	3	1	0	4	275.14
6j	3.81	41.99	22	312.37	3	1	0	4	266.54

Table 2
In vitro inhibition of AChE and BuChE of compound **6** (**a**–**j**)

Compound	R	AChE Inhibition (IC_{50}, μM)	BuChE Inhibition (IC_{50}, μM)	Selectivity index[a]
6a	H	32.45 ± 0.24	30.67 ± 0.55	0.94
6b	4-CH_3	6.18 ± 0.61	9.43 ± 0.42	1.52
6c	2-CH_3	7.71 ± 0.54	8.11 ± 0.35	1.05
6d	4-OCH_3	3.14 ± 0.16	9.24 ± 0.32	2.94
6e	2-OCH_3	5.23 ± 0.42	10.83 ± 0.37	2.07
6f	4-NO_2	8.21 ± 0.25	6.32 ± 0.39	0.76
6g	2-NO_2	8.25 ± 0.37	11.28 ± 0.25	1.36
6h	4-Cl	22.43 ± 0.34	25.27 ± 0.28	1.12
6i	2-Cl	23.12 ± 0.26	26.42 ± 0.18	1.14
6j	4-F	26.63 ± 0.14	26.33 ± 0.33	0.98
Rivastigmine		12.65 ± 0.22	1.12 ± 0.45	0.08

[a] Selectivity of inhibitor potency BChE/AChE.

Figure 1. Structure activity realationship (SAR) study of compound 6 (a-j) against AChE inhibition.

activity, while electron withdrawing group lessen the activity. The position of the group also has marked influence on the activity, such as, *para* is found much more active than meta substituted derivatives. The observation of SAR has been elucidated in Figure 1.

Current drug discovery approaches to treat AD were aimed at development of multi-target directed ligands concentrated to attack on distinct AD-relevant goals.[17,18] Consequently, according to the amyloid hypothesis, accumulation of Aβ peptide produced after cleavage of amyloid precursor protein by β-secretase in protein triggers numerous pathophysiological changes that eventually lead to cognitive dysfunction, particularly in AD.[19] Therefore, inhibition of β-secretase and Aβ aggregation by selective inhibitors offers great interest in the discovery for agents modifying the disease condition. Thus, the inhibitory activity of compound **6d** was determined against Aβ aggregation and β-secretase, using curcumin as reference control showed in Table 3. The inhibitory ability was evaluated at a single concentration (100 μM) against Aβ

Table 3
Inhibitory effects of compounds **6d** against Aβ aggregation and β-secretase

Compound	IC_{50}, μM	
	Aβ aggregation	β-secretase
6d	10.6 ± 1.2	13.2 ± 0.4
Curcumin	11.3 ± 0.3	6.1 ± 0.8

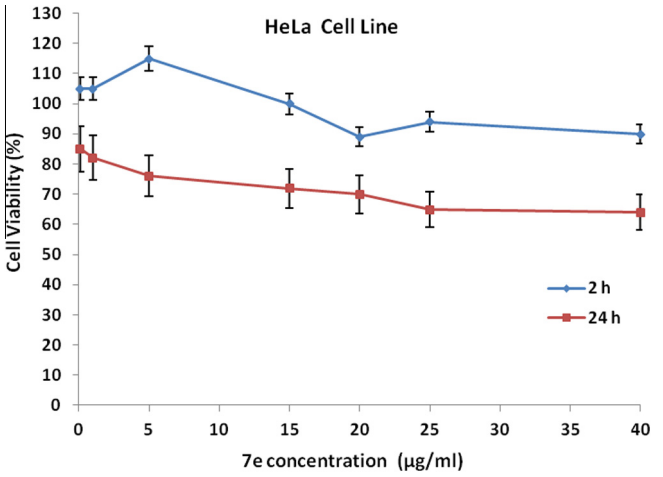

Figure 2. Percentage of viability of HeLa cells in the presence of compound 6d at various concentrations.

aggregation and β-secretase by fluorometric assay. It has been found that, compound **6d** (IC_{50} = 10.6 ± 1.2 μM) exhibit better activity than curcumin against the Aβ aggregation. Where, it has been found a nearly twofold less active than standard against β-secretase.

Toxicity is a much critical factor to be considered during the drug development process. Thus, after confirmation of the high bioactivity and mechanism of action of most active compound **6d**, it is worthwhile to perform its toxicity study. The human cervical carcinoma cell line (HeLa 229) was used for cytotoxicity assessment using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. As shown in Figure 2, it was revealed that compound **6d** did not exhibit significant toxicity within 2 h of administration, but viability has been reduced in the

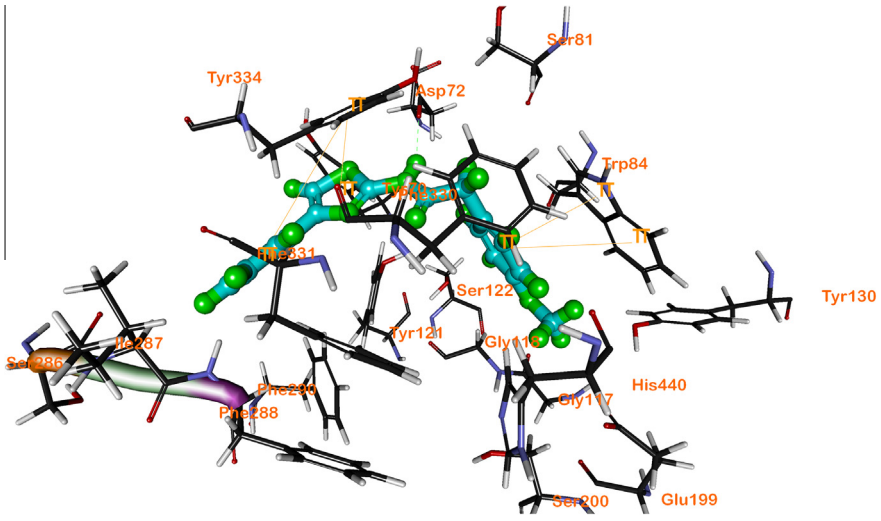

Figure 3. Docked orientation of compound **6d** in the catalytic site of acetyl choline esterase.

24 h study period. Thus, it was corroborated that it did not appreciably lower the cell viability and hence could be termed as non-toxic at the maximum tested dose of 40 μg/mL.

As expected, docking study confirmed that, the planar structure of thiazole in compound **6d** led to the important interactions showed in Figure 3. It was revealed Phe330 and Trp84, a key catalytic residue present at the anionic site (CAS) are responsible for the corresponding interactions. It was revealed the formation of non covalent, pi–pi interaction with Tyr334 and Trp84 with phenyl, thiazole and substituted phenyl moiety fragments and it seemed that the designed inhibitor was efficient enough to engage the key catalytic contact of the active site for its effect.

As a concluding remark, a novel series of thiazole acetamides was synthesized in excellent yield and characterised with the aid of various spectroscopic and elemental analysis. These compounds were evaluated for in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities for possible benefit in Alzheimers disease (AD). Among the synthesized compound, **6d** was identified as most potent compound of AChE (IC_{50} = 3.14 ± 0.16 μM) with SI of 2.94 against BuChE.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.001.

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