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# Synthesis, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities, and molecular docking studies of a novel compound based on combination of flurbiprofen and isoniazide†

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Synthesis of a compound with balanced bioactivities against a specific target is always a challenging task. In this study, a novel compound (**1**) has been synthesized by combination of flurbiprofen and isoniazide and shows ~2.5 times enhanced acetylcholinesterase (AChE) inhibition activity and ~1.7 times improved butyrylcholinesterase (BuChE) inhibition activity compared to flurbiprofen and a standard drug (*i.e.* physostigmine). A comparative AutoDock study has been performed, based on the optimized structure, by the DFT/B3LYP method, which confirmed that compound (**1**) is more active against AChE and BuChE, with calculated binding energies of  $-12.9 \text{ kcal mol}^{-1}$  and  $-9.8 \text{ kcal mol}^{-1}$  respectively as compared to flurbiprofen and an eserine (physostigmine) standard for which the binding energy was calculated to be  $-10.1 \text{ kcal mol}^{-1}$  and  $-8.9 \text{ kcal mol}^{-1}$ , respectively. A mixed mode of inhibition of AChE and BuChE with compound **1** was confirmed by Lineweaver–Burk plots. AChE and BuChE inhibition activity alongside docking results suggests that compound (**1**) could be used for treatment of Alzheimer's disease. Moreover, compound (**1**) also exhibit better  $\alpha$ -chymotrypsin activity compared to flurbiprofen. Furthermore, *in vitro* and *in vivo* analysis confirmed that compound (**1**) exhibit more activity and less toxicity than the parent compounds.

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## Introduction

The history of non-steroidal anti-inflammatory drugs (NSAIDs) dates back thousands of years but the mechanism of NSAID therapies was first fully understood by John Vane in 1971.<sup>1–3</sup> Nowadays, NSAIDs (naproxen, indomethacin, aspirin,

ibuprofen, flurbiprofen and diclofenac) have been recommended for diseases such as pain, short term fever and inflammation.<sup>4–7</sup> Scientists have worked hard to understand the kinetics and structure–activity relationships of these drugs.<sup>8,9</sup> In this regard, computer-aided drug design (CADD) is one of the most powerful tools.<sup>10–13</sup> It enables us to search and understand the interactions of ligands with potential protein targets.<sup>14</sup> Moreover, structure–activity relationships can be established in a better way by comparing the experimental results with theoretical studies. Therefore, CADD has an intrinsic benefit of understanding the phenomenon at the molecular level and providing correct assignments.<sup>15</sup>

A patient suffering from Alzheimer's disease (AD) experience a progressive and irreversible disorder of brain which slowly destroy thinking and memory skills at large.<sup>16</sup> AD is currently ranked as the sixth leading cause of death in United States and there are almost 50 million people around the world suffering from this disease.<sup>16–18</sup> It has been observed that change in acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in cerebral cortex and hippocampus contribute to the disease progression.<sup>17–19</sup> Increased or unchanged BuChE activity and decreased AChE activity are commonly observed in certain

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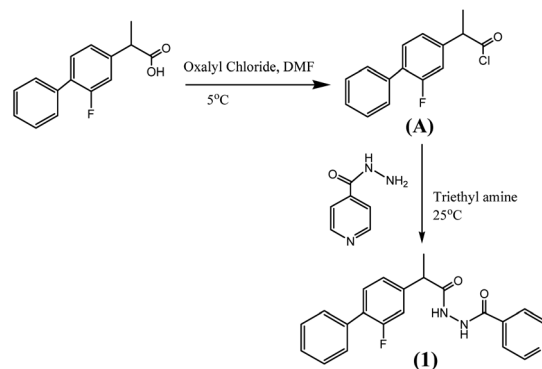
brain regions of patients suffering from AD.<sup>20–22</sup> Human AChE active site, a long gorge with overall length of approximately 20 Å, mainly consisting of catalytic active site (CAS) at bottom of gorge while peripheral anionic site (PAS) is situated near the entrance of gorge. These two are linked by a narrow groove. CAS forms the catalytic triad, Ser200, Glu327 and His440, and is responsible for hydrolysis of AChE inside the triad.<sup>23,24</sup> PAS consists of several aromatic residues, including Tyr70, Tyr121, and Trp279. Compounds that can interact with both CAS and PAS are believed to exert multiple therapeutic effects.<sup>25</sup> The shape and arrangement of the active site of BuChE is similar to that of AChE; however, the volume of the catalytic site in BuChE is much larger than that of AChE.<sup>26</sup> Researchers from all over the world are trying to explore new and novel strategies to develop effective methods for treatment of AD and other diseases as well, but it is a challenging task to obtain a specific compound with balanced activities against the specific targets most importantly retaining the drug-like properties.<sup>27</sup> Synthesis of novel drug by using existing NSAIDs is an area which is largely unexplored and it has been observed that NSAIDs can be tuned for better lipophilicity, reduced toxicity and better bio-availability.<sup>28</sup> Therefore a clever design and synthesis of compound having aforementioned properties is need of the day in order to grasp and improve the pharmacological benefits of NSAIDs.<sup>29</sup>

Keeping in view of the facts about AD and limitations of existing drugs, a novel compound based on combination of flurbiprofen and isoniazide, *N'*-(2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoyl) isonicotinohydrazide (**1**), has been synthesized. The synthesized compound (**1**) was subjected to *in vitro* analysis which shows ~2.5 times and ~1.7 times enhanced AChE and BuChE inhibition activity respectively compared to flurbiprofen and standard physostigmine. These experimental results has been supported by comparative AutoDock study of compound (**1**) with AChE and BuChE which also confirmed that compound (**1**) is more active against AChE and BuChE with binding energy of  $-12.9 \text{ kcal mol}^{-1}$  and  $-9.8 \text{ kcal mol}^{-1}$  respectively as compared to flurbiprofen for which binding energy was calculated to be  $-8.2 \text{ kcal mol}^{-1}$ . Compound (**1**) showed improved  $\alpha$ -chymotrypsin activity as well. Lineweaver–burk plot suggests mixed mode of inhibition of AChE and BuChE with compound **1**. Experiments suggests that the compound **1** is slightly more lipophilic compared to flurbiprofen. AChE and BuChE inhibition activities aided with docking results suggested that compound (**1**) could be used for Alzheimer's disease. To the best of our knowledge, no report has been published yet which describes synthesis, experimental procedures, molecular docking and *in vitro* studies of the compound (**1**). Furthermore, the present data as theoretically and experimentally can be helpful for further studies of compounds/derivatives of flurbiprofen.

## Results and discussion, experimental

### Synthesis

Synthesis of the compound (**1**) was done by preparing acid chloride of flurbiprofen using oxalyl chloride which was then allowed to react with isoniazide. Scheme 1 (detail in ESI†).



Scheme 1 Synthesis of novel compound (**1**) based on combination of flurbiprofen and isoniazide.

### Biological activities

**Anti-inflammatory activities.** Percent inhibitions of edema by the flurbiprofen, isoniazide and the compound (**1**) at dose rate of  $50 \text{ mg kg}^{-1}$  were found to be 95.81, 28.51 and 75.21 respectively (Table 1). Although anti-inflammatory activity of the compound is slightly less than flurbiprofen but  $\text{IC}_{50}$  value is far greater than the parent drug which can be advantageous in treatment. A control experiment had also been performed using flurbiprofen and isoniazide at half dose. Again the compound **1** showed more activity compared to flurbiprofen and isoniazide at half dose. The existence of polar groups *i.e.* fluoro and amide alongwith hydrogen bond acceptor (pyridyl) side of compound **1** might be the main cause of better anti-inflammatory activity.

### Enzyme inhibition activities

***In vitro* acetylcholinesterase (AChE) inhibition assays.** Acetylcholine (ACh) has been proved to employ an anti-inflammatory property with action that involves down-modulating of pro-inflammatory cytokines.<sup>30</sup> Therefore, AChE is responsible for hydrolysis of acetylcholine and is being modulated in inflammation. Results showed that AChE inhibitory activity of the compound (**1**) was considerably greater (~2.5 times) than that of flurbiprofen (Table 2). Physostigmine (eserine) has been used as a standard compound in order to compare the activity with that of flurbiprofen and compound **1**. These results suggest that compound (**1**) offer selective and far improved inhibition of AChE as compared with flurbiprofen and standard (physostigmine). Based on experimental results and further confirmed by molecular studies it has been suggested that the synthesized compound (**1**) can be used for treatment of AD.

***In vitro* butyrylcholinesterase (BuChE) inhibition assays.** Compound **1** was also checked for its inhibitory activity against

Table 1 Anti-inflammatory assay of compound (**1**) and parent drugs

Anti-inflammatory drug	Inhibition (%)	$\text{IC}_{50}$ ( $\mu\text{mol}$ )
Isoniazide	$28.51 \pm 0.12$	—
Flurbiprofen	$95.81 \pm 0.16$	$50.51 \pm 0.14$
Flurbiprofen : isoniazide (50 : 50)	$62.31 \pm 0.11$	—
Compound <b>1</b>	$75.21 \pm 0.16$	$352.2 \pm 0.27$



Table 2 AChE assay of compound (1) compared with parent drugs

Drugs used as standard	Inhibition (%)	IC <sub>50</sub> (μmol)
Isoniazide	15.19 ± 0.04	1.11 ± 0.17
Flurbiprofen	31.43 ± 0.05	50.51 ± 0.14
Flurbiprofen : isoniazide (50 : 50)	22.31 ± 0.07	—
Physostigmine (eserine)	45.35 ± 0.06	—
Compound 1	78.92 ± 0.24	112.11 ± 0.14

Table 3 BuChE assay of compound (1) compared with parent drugs

Drugs used as standard	Inhibition (%)	IC <sub>50</sub> (μmol)
Isoniazide	15.51 ± 0.15	20.25 ± 0.18
Flurbiprofen	36.17 ± 0.24	<600
Flurbiprofen : isoniazide (50 : 50)	23.41 ± 0.05	—
Physostigmine (eserine)	41.42 ± 0.04	—
Compound 1	61.25 ± 0.85	238.51 ± 0.11

BuChE as well. Here again physostigmine (eserine) has also been used as a standard. The results showed that the BuChE inhibitory activity of compound 1 was almost two times higher as compared to flurbiprofen (Table 3) and standard drug. These results suggest that compound (1) also offer selective and improved inhibition of BuChE compared with flurbiprofen.

Structure–activity relationship can be inferred by comparison of the experimental data with molecular docking studies. According to the docking simulation it is clear that compound (1) is able to fit well in active site of acetylcholinesterase and butyrylcholinesterase and interact with important amino acid residues. The enhanced inhibition activity for compound (1) might be due to the presence of fluoro group at ortho position of the extended phenyl ring and pyridyl group on other side of molecule. Compound (1) is able to form potential  $\pi$ - $\pi$  interaction with residue Tyr332 and hydrophobic interactions with other residues within the esteratic pocket of the active site (Fig. 2a). Fluoro and pyridyl groups on sides aided by amide group in the middle tend to stabilize the ground state

Table 4 The  $V_{\max}$  and  $K_m$  values of compound 1 in kinetic studies with AChE and BuChE

Concentration (μM)	$V_{\max}$ (μM min <sup>-1</sup> )	$K_m$ (μM)	$R^2$
<b>Against AChE</b>			
0	3.86 ± 0.61	354.37 ± 10.34	0.99
0.2	3.69 ± 0.73	429.06 ± 13.26	0.99
0.4	2.52 ± 0.50	317.93 ± 11.95	0.99
0.6	1.70 ± 0.21	254.53 ± 7.32	0.99
1	1.27 ± 0.14	260.56 ± 8.22	0.99
<b>Against BuChE</b>			
0	0.46 ± 0.05	299.69 ± 9.21	0.99
0.2	0.39 ± 0.03	364.54 ± 14.54	0.99
0.4	0.32 ± 0.02	425.17 ± 16.87	0.99
0.6	0.25 ± 0.02	474.13 ± 18.15	0.99
1	0.17 ± 0.02	393.30 ± 17.92	0.99

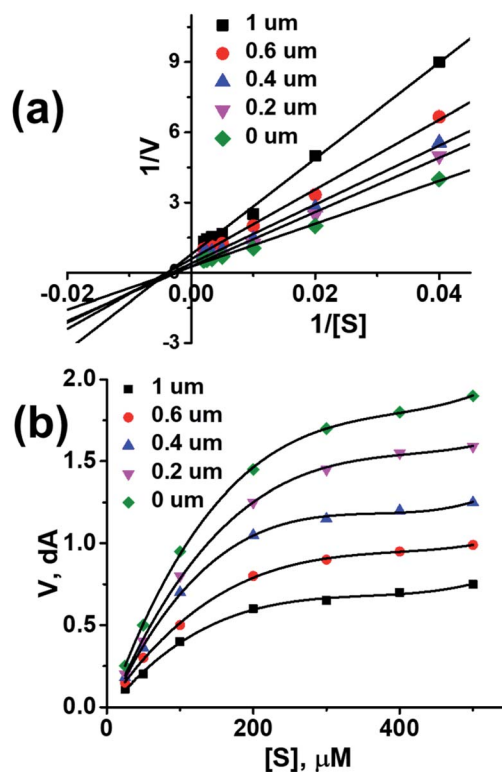


Fig. 1 (a) Lineweaver–burk plot of compound 1 with AChE. (b) Enzyme inhibition activity of compound 1 with AChE.

orientation by engaging in hydrophobic and hydrophilic interactions of amino acids (Fig. 4).

To further investigate the binding manner compound 1 was subjected to kinetic studies with AChE and BuChE, respectively. Lineweaver–Burk reciprocal plots were applied as described previously to elucidate the kinetic properties and inhibitory modes of the compounds.<sup>26</sup> Generally, Lineweaver–Burk plots can be described by reciprocal rates *versus* reciprocal substrate concentrations for different inhibitor concentrations resulting from the substrate–velocity curves for ChEs. The detailed values of  $K_m$  and  $V_{\max}$  of compound against AChE and BuChE at different concentrations are listed in Table 4. From the Fig. 1a its evident that both slopes (decreased  $V_m$ ), and the intercepts (higher  $K$ ) varied with increasing concentration (0.2, 0.4, 0.6, and 1.0 mM). This behaviour suggests mixed inhibition of AChE by compound 1. The substrate–velocity curve (Fig. 1b) showed that compound 1 reduced the enzymatic velocity of the AChE–substrate catalytic reaction in a dose-dependent manner. From Fig. 2 it is proved that compound 1 also exhibited mixed inhibition of BuChE and a dose-dependent decrease of the enzymatic velocity of the BuChE–substrate catalytic reaction. These results indicated that the compound 1 may simultaneously bind to CAS and PAS when interacting with the targets.

*In vitro*  $\alpha$ -chymotrypsin activity of compound (1) and parent drugs. Chymotrypsin is a digestive enzyme component of pancreatic juice acting and performs proteolysis.<sup>31</sup> Chymotrypsin preferentially cleaves peptide amide bonds by hydrolysis reaction and helps in digestion. Generally this enzyme is



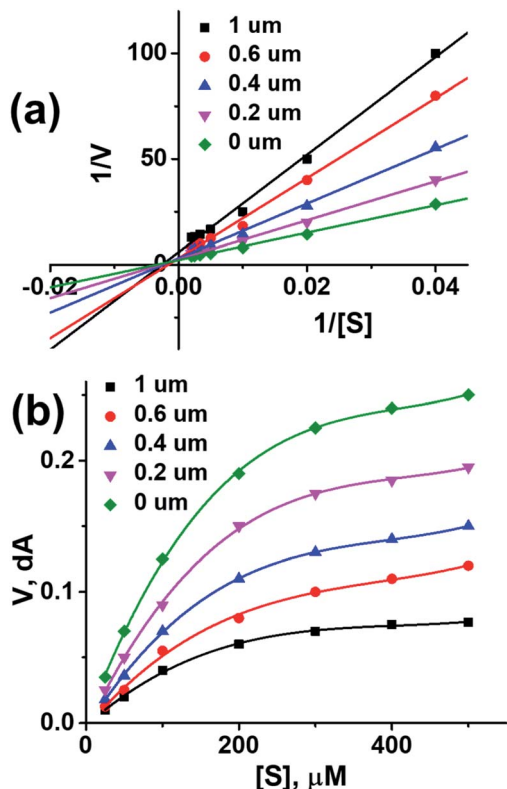


Fig. 2 (a) Lineweaver–burk plot of compound 1 with BuChE. (b) Enzyme inhibition activity of compound 1 with BuChE.

activated in the presence of trypsin. Compound (1) showed enhanced  $\alpha$ -chymotrypsin activity compared with parent drugs (Table 5).

### Toxicity study

The  $LD_{50}$  (oral, rats) values were found to be isoniazide:  $645 \text{ mg kg}^{-1}$ , flurbiprofen:  $115 \text{ mg kg}^{-1}$  and compound (1):  $3431 \text{ mg kg}^{-1}$ . Toxicity results proved that the compound (1) is much safer to use than the parent analogues.

### Lipophilicity studies

In context of drug discovery one of the most important parameter, despite the fact that the drug should show a significant activity against the potential target, is identification of drugs which are more likely to be well absorbed and distributed

Table 5  $\alpha$ -Chymotrypsin activity of compound (1) compared with parent drugs

Parent drug	Concentration of solutions (mM)	Inhibition (%)
Isoniazide	0.5	$5.13 \pm 0.12$
Flurbiprofen	0.5	$27.05 \pm 0.16$
Flurbiprofen : isoniazide (50 : 50)	0.5	$16.38 \pm 0.12$
Compound 1	0.5	$33.01 \pm 0.15$

in human body. It suggests that a drug must be lipophilic enough to penetrate the lipid cores of membranes, but not so lipophilic that they get stuck there.<sup>32</sup> Lipophilicity of the compound (1) was calculated by following the recently published method.<sup>33</sup>  $\log P$  of compound (1) was calculated to be 3.94 which is slightly higher than flurbiprofen ( $\log P$  3.82). Flurbiprofen possess carboxylic moiety, therefore it is less lipophilic than compound (1) that contains amide linkage.

### Molecular docking studies

The study was designed for compound (1) against acetylcholinesterase and butyrylcholinesterase enzymes with the following communications; Intel(R) core i7 @ 3.50 GHz system having 8 GB RAM with windows 7 operating platform. Protein–ligand docking was carried out using Autodock Vina software.<sup>34</sup> X-ray crystal structures of human BChE (Pdb: 1P0P with 2.30 Å resolution) from the RCSB protein data bank<sup>35</sup> was selected as the target protein based on suitable resolution and co-crystallized ligands, *i.e.* AChE and BuChE. The energy of ligands was minimized using MMFF94x force field. 3D and 2D interactions diagrams were generated through BIOVIA Discovery Studio visualizer V17.2.<sup>36</sup> Before proceeding for docking studies structure of compound (1) was optimized with the help of density functional theory (DFT)/B3LYP<sup>37–42</sup> method with 6-311G(d, p) as basis sets (Fig. 3). This optimized structure of compound 1 was then used for docking studies with AChE and BuChE enzymes.

### Acetylcholinesterase activity

By performing *in vitro* studies aided with molecular docking simulation it has been observed that compound 1 (%)

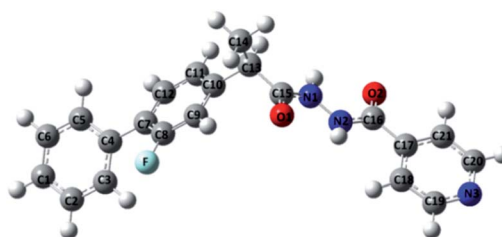


Fig. 3 Optimized structure of compound (1).

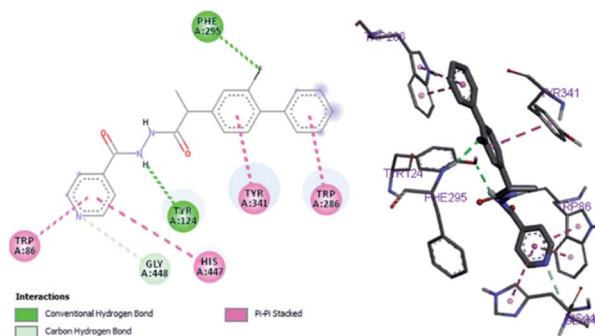


Fig. 4 Binding position for compound (1) in the active site of AChE.





Table 6 Important interactions of compound 1, flurbiprofen and serine with AChE

Compound code	Binding energies kcal mol <sup>-1</sup>	H-bonds interactions	Hydrophobic interaction ( $\pi$ - $\pi$ )
Compound 1	-12.9	Tyr124, Phe295	Trp286, Trp86, Tyr341, His447
Eserine	-8.9	—	Tyr341,
Flurbiprofen	-8.3	Phe298	Phe338, Tyr341, Tyr337

inhibition =  $78.92 \pm 0.24$ ) is the most active analogue with binding energy  $-12.9$  kcal mol<sup>-1</sup> for AChE inhibition activity. In active site, two most important residues Trp286, Trp86, Trp341 and His447 of AChE are frequently involved through hydrogen bonding or  $\pi$ - $\pi$  interaction and play important inhibitory roles. The compound 1 also involves in hydrogen bonding interaction with Trp124 and Phe295 of AChE. On the basis of docking results it is clear that the compound 1 is able to fit well in active site of acetylcholinesterase and interact with important amino acid residues. The enhanced inhibition activity for compound 1 might be due to the presence of fluoro group at ortho position of the extended phenyl ring. The top-ranked docking conformation of compound 1 showed that it is able to form potential  $\pi$ - $\pi$  interaction with residue Tyr286 and hydrophobic interactions with other residues within the esteratic pocket of the active site (Fig. 4). On the other hand, several amino acid residues appear to stabilize the ground state binding orientation of the phenyl rings by engaging in hydrophobic

interaction with the side chains of residues Try124, Gly448 and Phe295 (Table 6).

Flurbiprofen and eserine were observed to be least active compound having binding score of  $-10.1$  kcal mol<sup>-1</sup> and  $-8.9$  kcal mol<sup>-1</sup>. Binding interactions are shown in Fig. 5 and 6.

### Butyrylcholinesterase activity

*In vitro* studies aided with molecular docking simulation revealed that compound 1 (% inhibition =  $61.25 \pm 0.85$ ) is the active compound with binding energy  $-9.8$  kcal mol<sup>-1</sup> compared to eserine ( $-8.5$  kcal mol<sup>-1</sup>) which is used as standard drug for BuChE inhibition activity and flurbiprofen ( $-8.2$  kcal mol<sup>-1</sup>). In active site there are two most important residues Trp82 and Tyr332 of BuChE involving through hydrogen bonding or  $\pi$ - $\pi$  interaction which play an important inhibitory role.

The docking results suggest that compound 1 is able to fit well in active site of butyrylcholinesterase and interact with important amino acid residues. The inhibition activity for compound 1 might be due to the presence of fluoro group at ortho position of the extended phenyl ring. Compound 1 showed hydrogen bond interaction with the Thr120 amino acid residue. The top-ranked docking conformation of compound 1 showed that it is able to form potential  $\pi$ - $\pi$  interaction with residue Tyr332 and hydrophobic interactions with other residues within the esteratic pocket of the active site (Fig. 7). On the other hand, several amino acid residues appear to stabilize the ground state binding orientation of the phenyl rings by engaging in hydrophobic interaction with the side chains of residues Tyr332, Ala328 and Gly116 (Table 7).

Flurbiprofen was observed to be least active compound having binding score  $-8.2$  kcal mol<sup>-1</sup>. Binding interactions are

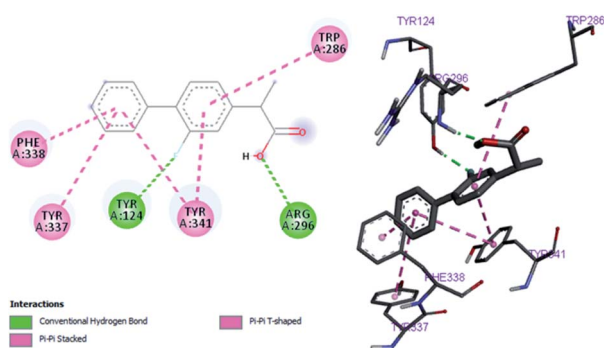


Fig. 5 Binding position for flurbiprofen in the active site of AChE.

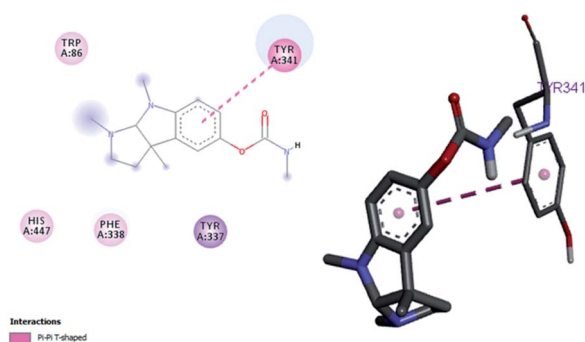


Fig. 6 Binding position for eserine in the active site of AChE.

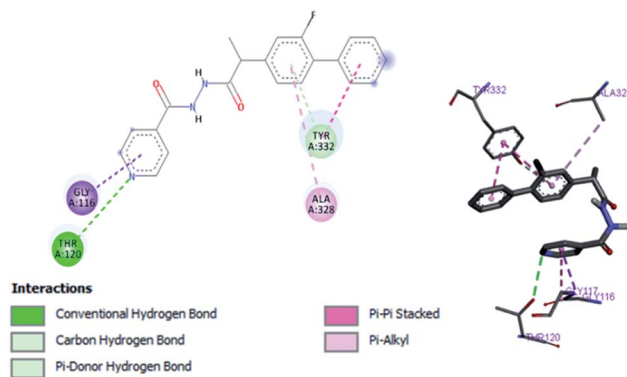


Fig. 7 Binding position for compound (1) in the active site of BuChE.



Table 7 Important interactions of compound 1, flurbiprofen and eserine with BuChE

Compound code	Binding energies kcal mol <sup>-1</sup>	H-bonds interactions	Hydrophobic interaction ( $\pi$ - $\pi$ )
Compound 1	-9.8	Trp82, Trp332, Thr120	Trp82, Trp332, Ala332, Gly116, Ala328
Eserine	-8.5	Tyr128, His438, Phe329	Trp82, Phe329
Flurbiprofen	-8.2	Tyr128, His438	Trp82, Ala328, Phe329

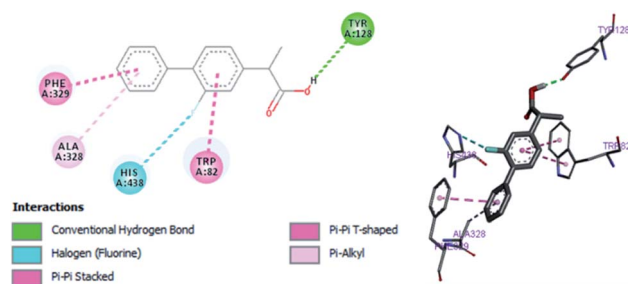


Fig. 8 Binding position for flurbiprofen in the active site of BuChE.

shown in Fig. 8. Docking and BuChE inhibition activity results revealed that compound 1 could be used for Alzheimer's disease.<sup>43</sup>

## Conclusions

A novel compound (1) based on combination of flurbiprofen and isoniazide has been designed and synthesized. AChE and BuChE inhibition activities of compound (1) were found to be  $\sim 2.5$  and  $\sim 1.7$  folds higher respectively as compare to standard physostigmine and parent drug *i.e.* flurbiprofen. Molecular docking studies prove that compound (1) is more active against AChE and BuChE having calculated binding energies of  $-12.9$  kcal mol<sup>-1</sup> and  $-9.8$  kcal mol<sup>-1</sup> respectively, as compared to flurbiprofen and eserine (physostigmine) for which binding energy was calculated to be  $-10.1$  kcal mol<sup>-1</sup> and  $-8.9$  kcal mol<sup>-1</sup> respectively. Lineweaver-Burk plot suggests mixed mode of inhibition of AChE and BuChE with compound 1. AChE and BuChE inhibition activities aided with docking results suggested that compound (1) could be used for Alzheimer's disease. Moreover, compound (1) also exhibit improved  $\alpha$ -chymotrypsin activity as well. Compound (1) was found to be more active and less toxic than the parent analogues in various *in vitro* and *in vivo* tests.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- 1 J. R. Vane, *J. Physiol. Pharmacol.*, 2000, **51**, 573-586.
- 2 J. R. Vane, *Nat. New Biol.*, 1971, **43**, 232-235.
- 3 W. L. Smith, *Metal ions in Biological systems*, ed. H. Siegel and A. Siegel, Marcel Dekker, New York, 1994.
- 4 J. C. Otto and W. L. Smith, *J. Lipid Mediat. Cell Signal*, 1995, **12**, 139-156.
- 5 A. Inotai, B. Hanko and A. Meszaro, *Pharmacoepidemiol. Drug Saf.*, 2010, **19**, 183-190.
- 6 J. J. Talley, *Prog. Med. Chem.*, 1999, **36**, 201-234.
- 7 W. C. Black, *Annu. Rep. Med. Chem.*, 2004, **39**, 125-138.
- 8 T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang and P. C. Isakson, *J. Med. Chem.*, 1997, **40**, 1347-1365.
- 9 P. Prasit, Z. Wang, C. Brideau, C. C. Chan, S. Charleson, W. Cromlish, D. Ethier, J. F. Evans, A. W. Ford-Hutchinson, J. Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Léger, J. Mancini, G. P. O'Neill, M. Ouellet, M. D. Percival, H. Perrier, D. Riendeau, I. Rodger and R. Zamboni, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1773-1778.
- 10 B. G. Johnson, P. M. W. Gill and J. A. Pople, *J. Chem. Phys.*, 1993, **98**, 5612-5626.
- 11 N. Oliphant and R. T. Bartlett, *J. Chem. Phys.*, 1994, **100**, 6550.
- 12 Y. Zhang, Z. J. Guo and X. Z. You, *J. Am. Chem. Soc.*, 2001, **123**, 9378.
- 13 M. J. Frisch, G. W. Trucks, H. B. Schlegel, *et. al.*, *Gaussian 03, Revision E.01*, Gaussian Inc., Wallingford, CT, 2004.
- 14 G. Fitzgerald and J. Andzelm, *J. Phys. Chem.*, 1991, **95**, 10531-10534.
- 15 A. A. Seliman, M. Altaf, A. T. Onawole, S. Ahmad, M. Y. Ahmed, A. A. Al-Saadi, S. Altuwaijri, G. Bhatia, J. Singh and A. A. Isab, *J. Organomet. Chem.*, 2017, **848**, 175-183.
- 16 M. Prince, A. Comas-Herrera, M. Knapp, M. Guerchet and M. Karagiannidou, *Alzheimer's Disease International. World Alzheimer Report 2016*. September 2016. <http://www.alz.co.uk/research/WorldAlzheimerReport2016.pdf>.
- 17 E. K. Perry, R. H. Perry, G. Blessed and B. E. Tomlinson, *Neuropathol. Appl. Neurobiol.*, 1978, **4**, 273-277.
- 18 P. Davies and A. J. Maloney, *Lancet*, 1976, **8000**, 1403.



- 19 P. J. Whitehouse, D. L. Price, R. G. Struble, A. W. Clark, J. T. Coyle and M. R. Delon, *Science*, 1982, **215**, 1237–1239.
- 20 A. Ciro, J. Park, G. Burkhard, Y. Nicole and G. Changiz, *Curr. Alzheimer Res.*, 2012, **9**, 138–143.
- 21 G. Mushtaq, N. H. Greig, J. A. Khan and M. A. Kamal, *CNS Neurol. Disord.: Drug Targets*, 2014, **13**, 1432–1439.
- 22 A. Morsy and P. C. Trippier, *J. Alzheimer's Dis.*, 2019, **72**, S145–S176.
- 23 Y. Bourne, P. Taylor, Z. Radic and P. Marchot, *EMBO J.*, 2003, **22**, 1–12.
- 24 J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker and I. Silman, *Science*, 1991, **253**, 872–879.
- 25 M. Bajda, N. Guzior, M. Ignasik and B. Malawska, *Curr. Med. Chem.*, 2011, **18**, 4949–4975.
- 26 Y. Chen, W. Li, H. Lin, L. Wu, H. Yang, Y. Pei, R. Tan and H. Sun, *RSC Adv.*, 2017, **7**, 3429.
- 27 D. Panek, A. Więckowska, J. Jończyk, J. Godyń, M. Bajda, T. Wichur, A. Pasięka, D. Knez, A. Pišlar, J. Korabecny, O. Soukup, V. Sepsova, R. Sabaté, J. Kos, S. Gobec and B. Malawska, *ACS Chem. Neurosci.*, 2018, **9**, 1074–1094.
- 28 A. Asghar, M. Yousuf, H. Mubeen, R. Nazir, K. Haruna, A. T. Onawole and L. Rasheed, *Bioorg. Med. Chem.*, 2019, **27**, 2397–2404.
- 29 A. Jaramillo, P. Bhattacharjee, G. Sonnenfold and G. Paterson, *Curr. Eye Res.*, 1992, **11**, 571–579.
- 30 P. Bacalhau, A. A. S. Juan, C. S. Marques, D. Peixoto, A. Goth, C. Guarda, M. Silva, S. Arantes, A. T. Calderia, R. Martins and A. J. Burke, *Bioorg. Chem.*, 2016, **67**, 1–8.
- 31 E. K. Ulleberg, I. Comi, H. Holm, E. B. Herud, M. Jacobsen and G. E. Vegarud, *Food Dig.*, 2011, **2**, 52–61.
- 32 A. Andrés, M. Rosés, C. Ràfols, E. Bosch, S. Espinosa, V. Segarra and J. M. Huerta, *Eur. J. Pharm. Sci.*, 2015, **76**, 181–191.
- 33 A. Czyrski, *J. Chem.*, 2019, **3407091**, 1–6.
- 34 O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455–461.
- 35 <https://www.rcsb.org/structure/1p0p>.
- 36 K. Stierand and M. Rarey, *ACS Med. Chem. Lett.*, 2010, **9**, 540–545.
- 37 R. Dennington, T. Keith and J. Millam, *Gauss View, Version 4.1.2*, Semichem Inc., Shawnee Mission, KS, 2007.
- 38 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
- 39 R. Ditchfield, W. J. Hehre and J. A. Pople, *J. Chem. Phys.*, 1971, **54**, 724–728.
- 40 I. Dennington, R. T. Keith and J. Millam, *GaussView*, Semichem, Inc., Shawnee Mission, KS, 2003.
- 41 M. Haroon, T. Akhtar, M. Yousuf, M. W. Baig, M. N. Tahir and L. Rasheed, *J. Mol. Struct.*, 2018, **1167**, 154–160.
- 42 M. Yousuf, I. S. Youn, J. Yun, L. Rasheed, R. Valero, G. Shi and K. S. Kim, *Chem. Sci.*, 2016, **7**(6), 3581–3588.
- 43 P. Bacalhau, A. A. S. Juan, C. S. Marques, D. Peixoto, A. Goth, C. Guarda, M. Silva, S. Arantes, A. T. Calderia, R. Martins and A. J. Burke, *Bioorg. Chem.*, 2016, **67**, 1–8.

