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Synthesis and characterization of novel 1,2-oxazine-based small molecules that targets acetylcholinesterase

Alexey Yu. Sukhorukov [a], Anilkumar C. Nirvanappa [b], Jagadish Swamy [c], Sema L. Ioffe [a], Shivananju Nanjunda Swamy [d], Basappa [b,*], Kanchugarakoppal S. Rangappa [c,*]

[a] N.D. Zelinsky Institute of Organic Chemistry, Leninsky Prospect, 47, Moscow 119991, Russia
[b] Laboratory of Chemical Biology, Department of Chemistry, Bangalore University, Palace Road, Bangalore 560001, India
[c] Department of Chemistry, University of Mysore, Manasagangotri, Mysore 560001, India
[d] Department of Biotechnology, Sri Jayachamarajendra College of Engineering, JSS, Technical Institutions Campus, Mysore 570006, India

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ABSTRACT

Thirteen 2-oxazine-based small molecules were synthesized targeting 5-lipoxygenase (LOX), and acetylcholinesterase (AChE). The test revealed that the newly synthesized compounds had potent inhibition towards both 5-LOX and AChE in lower micro molar concentration. Among the tested compounds, the most active compound, 2-[(2-acetyl-6,6-dimethyl-4-phenyl-5,6-dihydro-2*H*-1,2-oxazin-3-yl)methyl]-1*H*-isoindole-1,3(2*H*)-dione (**2a**) showed inhibitory activity towards 5-LOX and AChE with an IC_{50} values of 1.88, and 2.5 μM, respectively. Further, the in silico molecular docking studies revealed that the compound **2a** bound to the catalytic domain of AChE strongly with a highest CDOCKER score of −1.18 kcal/mol when compared to other compounds of the same series. Additionally, **2a** showed a good lipophilicity ($\log P = 2.66$), suggesting a potential ability to penetrate the blood–brain-barrier. These initial pharmacological data revealed that the compound **2a** could serve as a drug-seed in developing anti-Alzheimer's agents.

Alzheimer's disease (AD) is a progressive age related neurodegenerative disorder and it is clinically characterized by impairment in memory, visuospatial skills, complex cognition, language, emotion and personality. Although the exact cause of AD remains elusive, mounting evidence continues to support the involvement of inflammation in the development of AD.[1] An inflammatory marker, interleukin-1 known to play a major role in enhancing the neuronal acetylcholinesterase (AChE) activity.[2–4] These physiological mechanism or systemic inflammation process is termed 'cholinergic anti-inflammatory pathway' because is mediated by the neurotransmitter acetylcholine (ACh).[5] Based on the compelling evidence that inflammatory processes are involved in the pathogenesis of AD, research has looked into the use of anti-inflammatory drugs as a treatment option for patients with AD. Epidemiological evidence continues to build up indicating that non-steroidal anti-inflammatory drugs (NSAIDs) may lower the risk of developing AD.[6] A possible mode of action for the effectiveness of NSAIDs is by the blockage of cyclooxygenase (COX)-2 in the brain.[7–10] Evidently, it has been shown that COX-2 mRNA and protein are considerably up-regulated in affected areas of AD brain,[11–13] suggesting the involvement of COX-2 in AD. So, we herein attempted to design and synthesize, 1,2-oxazine-based small molecules that could show anti-inflammatory activity and also play a major role in inhibiting the AChE activity that involved in AD. Since the discovery of 2-amino-1,3-oxazine scaffold was identified as the selective and better inhibitors of b-site amyloid precursor protein cleaving enzyme 1, and also projected to be the suitable starting point for further development of brain penetrating compounds for potential Alzheimer's disease treatment.[14] In addition, the neuroprotective effect of 2-ethoxy-4,5-diphenyl-1,3-oxazine-6-one against H_2O_2-induced cell death in rat pheochromocytoma cells was reported.[15] Evidently, the design, synthesis and results on 1,4-oxazines revealed that the oxazine-based small molecules significantly inhibited the transthyretin (TTR) amyloid fibril formation.[16] In continuation of our effort to synthesize novel anti-inflammatory[17] and anti-cholinergic agents,[18] we herein report the synthesis, characterization, anti-inflammatory and anti-cholinergic activity of novel 1,2-oxazine-based small molecules for the first time.

A library of racemic tetrasubstituted functionalized 1,2-oxazines (5,6-dihydro-4*H*-1,2-oxazines **1** and *N*-acetyl-5,6-dihydro-2*H*-1,2-oxazines **2**) required for the biological assays was generated according to the synthetic strategy previously developed by us[19,20] (Scheme 1, Table 1). Stereoselective assembly of 1,2-oxazine

* Corresponding authors. Tel.: +91 8022961346 (B.), tel./fax: +91 8212419363 (KSR)
E-mail addresses: salundibasappa@gmail.com (Basappa), rangappaks@gmail.com (K.S. Rangappa).

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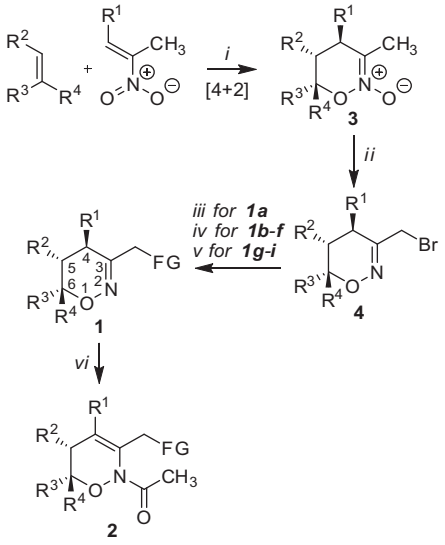


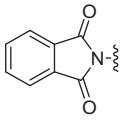
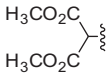
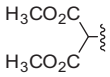
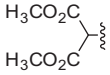
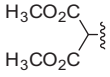
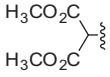
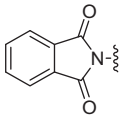
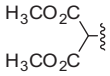
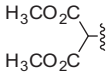
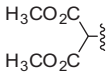
Scheme 1. Synthesis of 3,4,5,6-tetrasubstituted-1,2-oxazine-based small molecules. Reagents and conditions: (i) $SnCl_4$, CH_2Cl_2, −94 °C to −30 °C; (ii) $(CH_3)_3SiBr$, Et_3N, CH_2Cl_2, −30 °C, 24 h; (iii) potassium phthalamide, DMF, 50–60 °C, 2 h; (iv) $CH_2(CO_2CH_3)_2$, KO^tBu, DMF, 60 °C, 2 h; (v) 15 bar CO/$Pd(PPh_3)_2Cl_2$, CH_3OH, 100 °C, 3 h; (vi) AcBr, Ac_2O, CH_2Cl_2, rt, 2 h.

core was achieved by inverse electron demand Diels–Alder (IEDDA) reaction of nitroalkenes derived from nitroethane to olefines. The resulting diastereomerically pure 1,2-oxazine-*N*-oxides **3** were subjected to silylation with an excess of trimethylsilyl bromide in the presence of Et_3N to give 3-bromomethyl-substituted 5,6-dihydro-4*H*-1,2-oxazines **4**, which serve as key precursors for the synthesis of C-3 functionalized 1,2-oxazines **1** and **2**. Thus, nucleophilic substitution of bromide for phthalimide or dimethylmalonate anions furnished 1,2-oxazines **1a–f**. 1,2-Oxazines **1g–i** with FG = CO_2CH_3 were obtained by catalytic carbonylation of corresponding bromides **3** in methanol being followed our previously reported protocol.[21] Transformation of 5,6-dihydro-4*H*-1,2-oxazines **1** into and *N*-acetyl-5,6-dihydro-2*H*-1,2-oxazines **2** was achieved by acetylation of the former with AcBr/Ac_2O mixture in high yields (Scheme 1).

All compounds were obtained in analytically pure form by column chromatography on silica gel and crystallization. The structure and stereochemistry of previously unknown products was confirmed by 1D and 2D NMR spectroscopy and elemental analysis.

Effect of 2-oxazines on LOX-5 and AChE. Oxazin-2-thione-based small molecule exhibited LOX and COX-1 inhibitory action.[22] In particular, 5-LOX catalyses the biosynthesis of leukotrienes play a pivotal role in inflammatory and allergic disorders as well as in cardiovascular diseases and cancer.[23] Additionally, tetrahydro-1,4-

Table 1
Synthesis of 3,4,5,6-tetrasubstituted-1,2-oxazine-based small molecules

Entry	R^1	R^2	R^3	R^4	FG	Yield[a] (%)	Melting point
1a	C_6H_5	H	CH_3	CH_3		99	149–155 °C
1b	4-CH_3O–C_6H_4–	H	CH_3	CH_3		84	76–79 °C
1c	4-CH_3O–C_6H_4–	–$(CH_2)_4$–		H		86	100–102 °C
1d	C_6H_5	–$(CH_2)_3$–		H		90	123–131 °C
1e	C_6H_5			H		61	85–90 °C
1f	CH_3	H	CH_3	CH_3		70	71–79 °C
1g	4-CH_3O–C_6H_4–	H	CH_3	CH_3	CO_2CH_3	89	63–65 °C
1h	C_6H_5	–$(CH_2)_4$–		H	CO_2CH_3	71	81–84 °C
1i	C_6H_5			H	CO_2CH_3	92	75–78 °C
2a	C_6H_5	H	CH_3	CH_3		84	137–139 °C (dec)
2b	4-CH_3O–C_6H_4–	H	CH_3	CH_3		97	90–92 °C
2c	C_6H_5	H	CH_3	CH_3		82	64–69 °C
2d	4-Cl–C_6H_4–	H	CH_3	CH_3		73	91–93 °C

[a] Yield for the last step (average of two experiments).

Table 2
5-LOX, and AChE inhibitory potential of 3,4,5,6-tetrasubstituted-1,2-oxazine-based small molecules

Compounds	IC_{50} 5-LOX (μM)	IC_{50} AChE (μM)
1a	30.04	65.32
1b	66.87	NS[a]
1c	33.75	NS
1d	81.68	75.35
1e	21.09	NS
1f	NS	NS
1g	NS	NS
1h	16.09	35.12
1i	16.68	6.3
2a	1.88	2.5
2b	1.23	14.35
2c	6.32	12.0
2d	NS	46.76
NEOSTIGMINE		2.4

[a] NS: not significant.

Table 3
CDOCKER Energies of 3,4,5,6-tetrasubstituted-1,2-oxazine small molecules

Compounds	-CDOCKER energy (kcal/mol)	-CDOCKER interaction energy (kcal/mol)
1a	17.6109	43.3683
1b	46.4932	57.429
1c	37.026	52.236
1d	31.4987	48.0747
1e	39.8809	47.9319
1f	30.7859	33.7126
1g	35.6327	42.8039
1h	28.9761	41.6439
1i	−11.8795	44.2924
2a	1.14435	45.1691
2b	25.3233	53.8689
2c	20.7983	51.7264
2d	30.7952	57.8437

oxazines are known to be biologically active against inflammatory drug metabolizing enzymes.[24] The potential of all the synthesized compounds to inhibit 5-LOX and AChE was determined on pure enzymes. Since inflammation occurs in the pathological regions of Alzheimer's disease (AD) brain, and a few animal models and clinical studies clearly suggest that AD inflammation significantly contributes to AD pathogenesis.[1] Some of the tested 1,2-oxazines exhibited 5-LOX inhibitory activity, with the exception of **1f**, **1g**, and **2d**. Amongst the new compounds **2a**, **2b**, and **2c** exhibited most potent 5-LOX inhibitory activities, whereas compounds **2a** and **1i** were found to inhibit the activity of AChE (Table 3) effectively. Interestingly, the most active compound **2a** showed inhibitory activity towards both the enzymes 5-LOX and AChE with an IC_{50} values of 1.88, and 2.5 μM, respectively. These results indicated that the 1,2-oxazine pharmacophore plays an important role in deciding the preferences for these enzyme sites. The present work thus confirms that the 1,2-oxazine-based ligands are effective LOX/AChE inhibitors, which can serve as promising therapeutic agents against Alzheimer disease.

Structure based in silico molecular docking analysis on 1,2-oxazine-based small molecules that target AChE. Many cholinesterase inhibitors are used for symptomatic treatment of AD.[25] The drug, Aricept (E2020) was known to enhance performance in animal models of cholinergic hypofunction and has a high affinity for AChE.[26] Prior to the elucidation of three-dimensional structure of Torpedo californica AChE (TcAChE), E2020 was designed on the basis of QSAR studies, which inhibited both electric eel and mouse AChE in the nanomolar range. Later, the co-crystal structure of the E2020–TcAChE complex at 2.5 Å resolution (PDB ID: 1EVE) was reported. This structure was taken for our molecular docking studies.[26] The molecular docking studies on 1,2-oxazine-based small molecules into the crystal structure of tcAChE were performed using CDOCKER of Accelrys as reported earlier.[27] Out of 10 docked complexes, the high score of CDOCKER energy was selected and described (Table 3). The analysis of the molecular docking revealed that the compound **1i**, and **2a** bound to the active site of the tcAChE with a greater extent of CDOCKER energies of 11.8, and −1.14 (kcal/mol), when compared to other structurally compounds (Table 2). E2020 that bound to the active site of AChE is compared with the 1,2-oxazines. The binding of compound **2a** towards the binding of AChE is depicted in Figure 1. The compound **2a** makes primary interactions at the active-site gorge of the enzyme through its three major functional groups such as phthalamide moiety, *N*-acetyl-2-oxazine ring, and the phenyl rings. These functional groups responsible for three inter-hydrogen bonding formation between Tyr334, Asp72, and Asn85 amino acids, Gly118, and Ser122 amino acids, and also with Gln69, Gly123, and Tyr121 amino acids. These results indicate that the strong affinity of compound **2a** on AChE could lead to the potent inhibition of the catalytic activity of the enzyme.

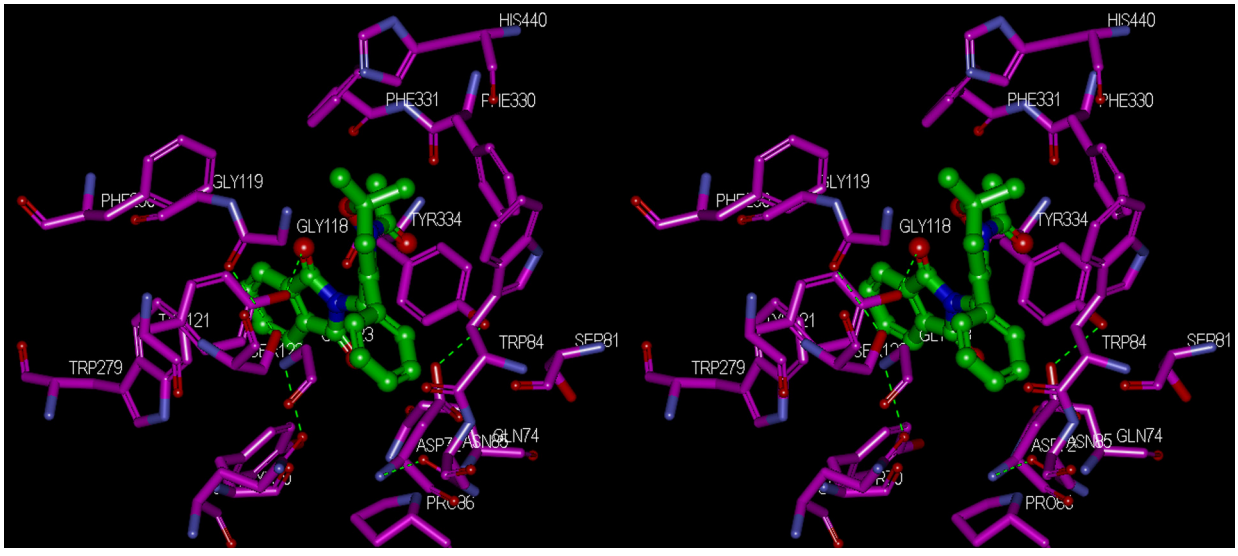


Figure 1. Stereoview of the interaction map of compound **2a** that bound to the active site of AChE (PDB ID: 1EVE). The stick model (carbon-pink and other atoms are in parent color) of the key amino acids that are interacting with compound **2a** (ball and stick model, carbon-green and other atoms are in parent color) was shown.

We herein report the synthesis of new 2-oxazine-based small molecules targeting 5-LOX and AChE. Among the tested compounds, the most active compound **2a** showed inhibitory activity towards 5-LOX and AChE with an IC_{50} value of 1.88, and 2.5 μM, respectively. Further, the in silico molecular docking studies revealed that the compound **2a** and **1e** bound to the catalytic domain of AChE strongly with a highest CDOCKER score about −1.18, and 11.8 (kcal/mol), when compared to other compounds of the same series. These initial pharmacological data revealed that the compound **2a** could serve as a drug-seed in developing anti-Alzheimer's agents.

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Supplementary data

Supplementary data (detailed experimental procedures for the synthesis, pharmacological investigations, and spectral datum) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.05.040.

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