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

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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

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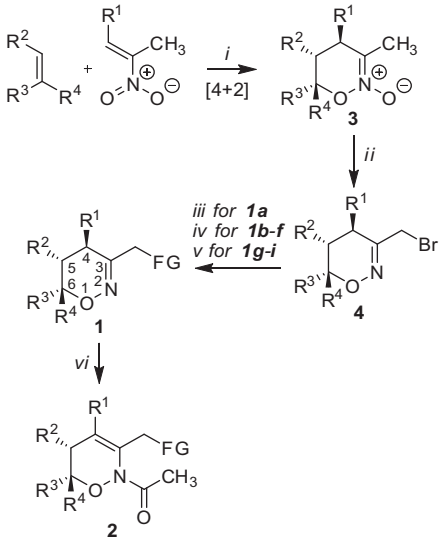


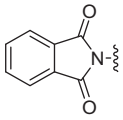
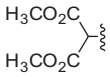
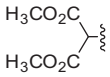
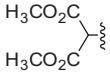
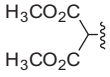
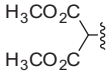
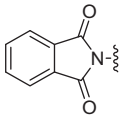
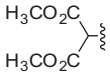
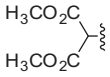
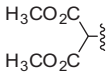
Scheme 1. Synthesis of 3,4,5,6-tetrasubstituted-1,2-oxazine-based small molecules. Reagents and conditions: (i) $SnCl_4$, CH_2Cl_2, −94 °C to −30 °C; (ii) $(CH_3)_3SiBr$, Et_3N, CH_2Cl_2, −30 °C, 24 h; (iii) potassium phthalamide, DMF, 50–60 °C, 2 h; (iv) $CH_2(CO_2CH_3)_2$, KO^tBu, DMF, 60 °C, 2 h; (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).