Design, synthesis and evaluation of benzimidazole-NsCOXis hybrids for the treatment of Alzheimer’s disease

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Abstract

Usage of an acetylcholinesterase (AChE) inhibitor and a non-selective COX inhibitor (NsCOXi) have been documented to exhibit significantly protective and recuperative effects in AD patients. Therefore, it is hypothesised that a compound capable of exhibiting both AChE inhibition and anti-inflammatory activities can be potential pluripotent drug candidate for de-accelerating the progression of AD, as well as for providing relief from the associated inflammatory pathological indications. The present study involves the coupling of ibuprofen (IB) or naproxen (NP) to varied disubstituted amines (AChE inhibitor pharmacophore) through benzimidazole to develop two series of compounds i.e. IB01-IB05 and NP01-NP05 as pluripotent anti-AD compounds. All target compounds are evaluated for in vitro AChE inhibitory and COX inhibitory activities. Compounds IB01-IB05 are found more potent as compared to NP01-NP05. Compound IB04 being the most active in in vitro evaluation is selected for in vivo evaluation of memory restoration using scopolamine-induced amnesia model in mice. It significantly reverses the scopolamine-induced changes (i.e., escape latency time, mean time spent in target quadrant, brain AChE activity and oxidative stress) in a dose-dependent manner. IB04 at higher dose i.e. 8 mg/kg is significantly effective in lowering AD manifestation in comparison to donepezil. The findings indicate that Benzimidazole-NsCOXi derivatives having pyrrolidine moiety may prove a useful template for the development of new chemical moieties against AD with multiple potencies.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by progressive cognitive decline and memory loss. US-FDA has approved three cholinesterase inhibitors (donepezil, rivastigmine and galantamine) for the treatment of AD. Memantine is another drug approved by FDA to treat the cognitive symptoms (memory loss, confusion, and problems with thinking and reasoning) of AD, which acts through regulation of glutamate activity [1, 2]. These drugs, though modestly improve memory and cognitive function in AD patients, are unable to prevent the progression of the disease. This may be attributed to the pathological complexity of AD. Over the time, various strategies such as complex drug therapy or drug cocktails (independent dosage of the drugs) and combined drugs (fixed combination of two or more drugs in one dosage form) have evolved for the treatment of AD [3]. Compelling evidences from multiple epidemiology studies have revealed that long-term dosing with non-steroidal anti-inflammatory drugs (NSAIDs) dramatically reduces AD risk in the elderly, including delayed disease onset, reduced symptomatic severity and slowed cognitive decline [4–6]. NSAIDs are predicted to dampen the neuro-inflammatory response and impact AD progression via several mechanisms, when administered along with drugs that inhibit beta-amyloid oligomerization [7]. The simultaneous administration of an acetylcholinesterase (AChE) inhibitor and a NSAID has been documented to exhibit significantly protective and recuperative effects in AD patients [8]. Clinical studies have proven that NSAIDs belonging to the category of non-selective COX inhibitors such as ibuprofen and naproxen protect against cognitive decline and reduce the levels of β-amyloid better than other NSAIDs (flurbiprofen, fenoprofen, indomethacin, meclofenamic acid, sulindac and fenbufen). A recent molecular docking study has
suggested that (i) carboxylic group in a NSAID is not an essential requirement for inhibition of Aβ fibrils, and (ii) -O-; -NH-; -CO- and –CONH- linker groups in NSAIDs afford maximum binding affinity [9]. Based on these reports, it is hypothesized that combining the AChE inhibitory and anti-inflammatory potentials of a non-selective COX inhibitors (NsCOXi) in a single molecule may be an attractive strategy for both de-accelerating the progression of AD and providing relief from the associated inflammatory pathological indications. Therefore, the present study is designed to hybridise NsCOXi with appropriate pharmacophore for AChE inhibition to develop pluripotent molecules for the treatment of AD. A di-substituted amine in the form of an aliphatic chain or piperidine ring is common pharmacophore identified from most of the studies reported for AChE inhibition. Naproxen and ibuprofen are the most commonly used NsCOXi. Benzimidazole is a versatile heteronucleus, which is exploited as an anchor to develop numerous biologically active molecules [10]. Therefore, the compounds have been designed by coupling naproxen or ibuprofen with some di-substituted amines through benzimidazole nucleus (Fig. 1), and are evaluated for in vitro AChE inhibition, COX inhibition and in vivo behavioural study.

Results And Discussion

Chemistry

The target compounds have been synthesized by following a synthetic scheme comprising 4 steps as depicted in Scheme 1. In the first step, carboxylic acid group of 3,4-diaminobenzoic acid (1) is esterified for its protection by refluxing 1 with ethanol in the presence of dry HCl gas to produce ethyl 3,4-diamino benzoate (2). Esterification of 1 to 2 is confirmed through ¹H-NMR spectral analysis which reveals that the broad singlet due to -COOH in 1 disappears, and a 2-proton quintet at d 4.27 and a 3-proton triplet at d 1.32 appear due to ethyl group. In the second step, the esteric intermediate (2) is refluxed with ibuprofen (3a) or naproxen (3b) in the presence of orthophosphoric acid (OPA) to yield benzimidazole-NsCOXi conjugate intermediates (4a/4b). Formation of these conjugates is confirmed by IR and ¹H-NMR spectral analyses. Mainly, a double band at 3442 and 3300 cm⁻¹ due to -NH₂ in IR spectrum of 2 is replaced by a single broad band due to -NH- in IR spectrum of the conjugates; and a band appears at 1672.1 cm⁻¹ due to C=N stretching, which indicate that conversion of –NH₂ group in 2 to –NH- group involves the formation of benzimidazole nucleus. ¹H-NMR spectra of 4a and 4b show the signals due to three protons of benzimidazole nucleus along with the proton signals due to respective NsCOXi i.e., ibuprofen and naproxen. For instance, a 2-proton quartet and a 3-proton doublet due to esteric ethyl group are noted at d 4.62-3.34 and d 1.98-1.26, respectively whereas three benzimidazole protons are noted in the range of d 7.78-6.98 in the spectra of both 4a and 4b. In spectrum of 4a, ibuprofen-specific signals include two doublets at d 7.15 and d 7.00 due to four protons of the para-disubstituted phenyl ring, and a set of signals in the range d 3.04-1.06 due to isobutyl group, whereas the signals characteristic to naproxen include a 3 proton singlet at d 3.87 due to methoxyl group and aromatic protons at d 7.75-7.58. De-esterification of 4a/4b by 5 M NaOH in THF results in free carboxylic acid intermediates 5a/5b, which is confirmed by the disappearance of protons of ethyl group in their ¹H-NMR spectra.
Finally, the intermediate 5a/5b are coupled to varied disubstituted amines by stirring in the presence of dicyclohexycarbodiimde (DCC) and dichloromethane (DCM) to obtain the target compounds IB 01-05/NP 01-05. IR spectrum of each target compound is found to lack the C=O stretching band corresponding to the free –COOH group, which shows that –COOH group in intermediates 5a/5b is couple to the disubstituted amine. Further, Amide I and Amide II bands at about 1680 and 1575 cm\(^{-1}\), respectively, suggest an amide linkage in the compound due to coupling of –NH with –COOH. The \(^1\)H-NMR spectra of all target compounds have shown signals similarly as in the respective intermediates (5a/5b). Additionally, protons of the substituted amine moieties are detected at the expected chemical shift positions in the region of d 0-5. The \(^1\)C-NMR spectra of all target compound show a maximally downfield signal at d 169.8-166.9 due to carbonyl carbon, along with signals due to other carbons as expected. Finally, the high resolution mass spectral data (+ESI) of all target compounds show the parent ion peak as [M+H]\(^+\) at \(m/z\) values corresponding to their theoretical masses confirming the formation of the compounds.

**In vitro AChE inhibitory activity**

AChE inhibitory activity of target compounds (IB01-IB05 and NP01-NP05) was determined by using Ellman method [11] with slight modifications using donepezil as stranded drug. IC\(_{50}\) values of all target compounds for AChE inhibition are summarized in Table 1. The data reveal that almost all compounds have significant AChE inhibitory activity.

**Table 1:** Acetyl cholinesterase (AChE) and cyclooxygenase (COX) inhibitory activity of target compound (IB01-IB05 and NP01-NP05)
| Compound | IC$_{50}$ (µM) | AChE | COX-1 | COX-2 | Selectivity Index(COX-1/COX-2) |
|----------|----------------|------|--------|-------|-------------------------------|
| IB01     | 0.84±0.01      | 16.08±0.56 | 96.87±8.97 | 0.16 |
| IB02     | 1.01±0.02      | 14.06±0.89  | 80.69±7.28  | 0.17 |
| IB03     | 0.55±0.01      | 20.69±0.72  | 62.68±6.26  | 0.33 |
| IB04     | 0.34±0.02      | 10.96±2.81  | 39.67±2.63  | 0.27 |
| IB05     | 0.47±0.02      | 19.67±1.68  | 52.68±1.69  | 0.37 |
| NP01     | 0.67±0.02      | 27.52±1.80  | 74.12±6.98  | 0.03 |
| NP02     | 1.55±0.02      | 24.56±2.91  | 68.21±5.62  | 0.36 |
| NP03     | 1.40±0.09      | 23.61±2.24  | 48.37±2.43  | 0.48 |
| NP04     | 0.92±0.03      | 21.03±0.98  | 42.36±1.49  | 0.49 |
| NP05     | 1.33±0.05      | 18.19±0.62  | 47.28±6.69  | 0.38 |
| Donepezil| 0.13±0.02      | -             | -             | -    |
| Ibuprofen| -              | 1.30±0.58    | 15.01±0.26   | 0.08 |
| Naproxen | -              | 2.26±0.52    | 1.30±0.56    | 1.73 |

In the IB series, a heterocyclic moiety (morpholine, pyrrolidine and piperizine) appended to benzimidazole rings is found to incur significantly higher activity (IC$_{50}$ 0.34 - 0.55 µM) than a dialkylamino moiety (IC$_{50}$ 0.84 and 1.01 µM). Contrastingly in NP series, the dimethylamino substituted analog (NP01) is found to be maximally potent (IC$_{50}$ 0.65 µM).

Statistical analysis of the data by one way ANOVA followed by multiple comparison (Tukey’s) test revealed that ibuprofen-derived compounds (IB01-IB05) are more potent than the naproxen-derived ones (NP01-NP05). Compound IB04 is the most potent among all the synthesized compounds from both the series, which is however less active than donepezil.

**In vitro COX inhibitory activity**

The target compounds were tested for their inhibitory activity against both COX-1 and COX-2 enzymes as well as their selectivity index (SI = IC$_{50}$ COX-1/IC$_{50}$ COX-2) using ibuprofen and naproxen as reference drugs. Amongst the ibuprofen-derived compounds, IB04 is found to be the most potent for both COX-1 and COX-2 with a COX-1/COX-2 selectivity index of 0.27. The compound IB02 is statistically equipotent to IB-04 towards COX-1 and has maximum selectivity for COX-1 (Selectivity index 0.17) among ibuprofen derived molecules. Amongst the naproxen-derived molecules, NP05 and NP04 are most potent for COX-1 and COX-2, respectively. The methyl and ethyl substituted compounds (NP01-NP02) are less active as
compared to closed ring amine derivatives (NP03-NP05) for both COX-1 and COX-2. Interestingly, all target compounds are found to have selectivity towards COX- significantly increased as compared ibuprofen and naproxen. Further, the ibuprofen-derived compounds (IB01-IB05) are more selective towards COX-1 (SI 0.16 - 0.37) as compared to the naproxen-derived compounds (NP01-NP05) (SI 0.36-0.49).

**In vivo biological evaluation**

**IB-04** is found to be the most potent inhibitor of AChE (IC$_{50}$ 0.34 μM/ml) as well as COX-1 (IC$_{50}$ 10.96 μM/ml) with good selectivity towards COX-1 (SI 0.27). Therefore, it was selected for evaluation of learning and memory restoration through scopolamine-induced amnesia model in mice at three different doses (i.e., 2, 4 and 8 mg/kg) using Morris Water Maze. The learning and memory were assessed in terms of Escape Latency Time (Fig. 2) and Mean Time Spent in Target Quadrant (Fig. 3).

**Escape Latency Time (ELT)**

All the animals were subjected to trials in morris water maze for 5 days, and assessed for ELT for first for 4 days. Normal control group animals showed downward trend in ELT exposure during acquisition trial of day 4 that reflects normal learning abilities. However, scopolamine-treated mice showed a significant rise in ELT on day 4 in comparison to the control group mice indicating an impairment of acquisition (learning). Administration of IB04 (at 2, 4 and 8mg/kg; p.o) and donepezil (2 mg/kg, i.p) in scopolamine-treated mice resulted in fall in ELT on day indicating reversal in scopolamine-induced impaired learning and memory. Treatment with IB04 showed improvement in ELT in a dose-dependent manner with response at 8 mg/kg being comparable to donepezil.

**Mean Time Spent in Target Quadrant (TSTQ)**

Normal control mice when subjected to retrieval test on day 5 spent significantly (p<0.05) more time in the target quadrant (Q4) in search for the missing platform as compared to the time spent in other quadrants (Q1, Q2 and Q3), reflecting normal memory capacity. On the other hand, scopolamine-treated mice showed significant decrease in time spent in Q4 quadrant, which depicts memory impairment. However, administration of donepezil and IB04 in scopolamine-treated mice showed increase in day 5 TSTQ in a dose-dependent manner, which indicates normal cognitive function (Fig. 3). These findings of in vivo evaluations disclosed that IB04 exhibits significant actions against AD manifestations in a dose-dependent fashion.

**Table 2:** Effect of IB04 on AChE activity and oxidative stress (TBARS and GSH) in mice brain.
| Compound                  | AChE activity (µM/min/mg/protein) | TBARS (nM/mg/protein) | GSH (µM/mg/protein) |
|---------------------------|----------------------------------|-----------------------|---------------------|
| Control                   | 1.08±0.13                        | 14.74±0.24            | 38.25±4.63          |
| Sco                       | 3.85±0.12<sup>a</sup>            | 26.01±0.13<sup>a</sup> | 21.91±0.08<sup>a</sup> |
| Vehicle + scop            | 3.46±0.78<sup>a</sup>            | 28.33±1.48<sup>a</sup> | 22.08±0.37<sup>a</sup> |
| Donepezil + scop          | 1.41±0.15<sup>b</sup>            | 18.17±0.35<sup>b</sup> | 37.36±0.14<sup>b</sup> |
| IB04 (2mg/kg)+ scop       | 3.33±0.58<sup>b,c</sup>          | 24.44±0.22<sup>b,c</sup>| 25.70±0.38<sup>b,c</sup> |
| IB04 (4mg/kg)+ scop       | 2.14±0.58<sup>b,c</sup>          | 21.78±0.56<sup>b,c</sup> | 28.89±0.89<sup>b,c</sup> |
| IB04 (8mg/kg)+ scop       | 1.84±0.23<sup>b</sup>            | 20.99±1.01<sup>b</sup> | 33.70±1.04<sup>b</sup> |

Data are presented as mean ± standard deviation (n=5); analyzed by one way ANOVA followed by Tukey's multiple comparison test; <sup>a</sup>Significant difference (p < 0.05) in comparison to control; <sup>b</sup>Significant difference (p < 0.05) in comparison to scopolamine; <sup>c</sup>Significant difference (p < 0.05) in comparison to donepezil + scopolamine.

**Effect of IB04 on scopolamine-induced changes in brain AChE activity**

Brain AChE activity was found to increase significantly in scopolamine-treated mice as compared to that in normal control mice. Administration of donepezil and IB04 prevented this scopolamine-induced rise in brain AChE activity in a dose-dependent manner (Fig. 4).

**Effect of IB04 on scopolamine-induced changes in brain oxidative stress levels**

TBARS levels were enhanced and reduced glutathione (GSH) levels were decreased significantly (p<0.05) in scopolamine-treated mice in comparison to normal control mice, indicating increased oxidative stress in scopolamine-treated mice. Treatment of scopolamine-treated mice with donepezil and IB04 showed a significantly decrease in TBARS levels and increase in brain GSH level, which depicts a decrease in oxidative stress in a dose-dependent manner (Fig. 5). These observations suggested that IB04 is effective in reducing oxidative stress in dose-dependent fashion.

**Conclusion**

Though numerous multifunctional strategies for the treatment of AD are reported in literature, yet inhibition of both inflammatory pathways and AChE activity or level needs to be explored. Hence, the present study involves the coupling of pharmacophore from anti-inflammatory drugs (ibuprofen and naproxen) and from varied AChE inhibitors to design and synthesize benzimidazole-based two series of
compounds i.e., **IB01-05 and NP01-05**. All compounds were primarily evaluated for *in vitro* AChE and COX inhibitory activities. Compounds **IB01-IB05** are found to be more potent *in vitro* as compared as compounds **NP01-NP05**. Among all, compound **IB04** is found to be the most potent, and hence, it is evaluated for its memory restoration activity using scopolamine-induced amnesia model in mice. It significantly reversed the scopolamine-induced changes (i.e., escape latency time, mean time spent in target quadrant, brain AChE activity and oxidative stress) in a dose-dependent manner. **IB04** at 8 mg/kg is found to be significantly effective in lowering AD manifestation in comparison to donepezil. The findings of this study indicate that the benzimidazole-NsCOXi derivatives having pyrrolidine moiety may provide a useful template for the development of new chemical moieties effective against AD.

**Experimental Chemistry**

All reactions were monitored by TLC using pre-coated aluminum plates. The purity of the compounds was ascertained confirmed by the developed TLC plate visualised in UV chamber at short as well as long wavelengths. The compounds were characterized by IR, NMR and Mass spectral techniques. In $^1$H-NMR and $^{13}$C-NMR spectra, chemical shifts were reported in  $\delta$ values using tetramethylsilane as internal standard with number of protons, in the deuterated solvent CDCl$_3$ and DMSO. IR spectra were recorded as KBr pellets. Structures of all target compounds were confirmed by high resolution mass spectral (HRMS) data recorded using positive electrospray ionization mode (+ ESI), wherein the compounds were detected as [M + H]$^+$ ions at $m/z$ values corresponding to their theoretical masses.

**Ethyl-3,4-diaminobenzoate (2):** Initially 10 ml of ethanol was saturated with dry HCl gas. 3,4-Diaminobenzoic acid (0.088 mM, 6 g) was dissolved in HCl saturated ethanol and refluxed for 6 h. Completion of the reaction was checked by the disappearance of spot of reactant in TLC. After the completion of reaction, the reaction mixture was poured, while it was still hot into excess of water. The solution was then neutralized by sodium carbonate. The precipitated ester was filtered and dried under vacuum. The crude product was recrystallised from boiling water. Yield and melting point were noted.

yield: 63.33%; mp 108–109 ºC; IR (KBr), $\tilde{\nu}_{	ext{max}}$ 3442 – 3300, 3290.9, 2952.0, 1735; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 7.72 (1H, d, $J = 1.3$ Hz H-2), 7.59 (1H, dd, $J = 1.5$, 8 Hz, H-5), 7.14 (1H, d, $J = 8.2$ Hz, H-6), 4.27 (2H, q, $J = 7.1$ Hz, H-1’a), 1.32 (3H, t, $J = 7.0$ Hz, H-1'b).

**Synthesis of BZ-Ibuprofen/Naproxen conjugate**

Ethyl-3,4-diaminobenzoate (2.5 mM, 0.45 g) was refluxed with 3.75 mM of ibuprofen or naproxen in the presence of OPA in a 50 ml round bottom flask. Completion of the reaction was ascertained through the disappearance of spot of reactant in TLC. After completion of the reaction, the reaction mixture was poured into excess of cold water and basified with aqueous ammonia. The resultant product was filtered, washed with cold water and recrystallized from hot ethanol-water mixture to obtain fine crystals, which were dried in vacuum desiccator.
1-(2-(1-(4-Isobutylphenyl)ethyl-1H-benzimidazol-5-yl)propan-1-one (BZ-IB, 4a)

This compound was prepared by taking 0.45 g ethyl-3,4-diaminobenzoate, 0.76 g ibuprofen, and 12 ml OPA. It was obtained as a light brown solid, yield 68.0%; mp 185–186 ºC; IR (KBr), $\nu_{\text{max}}$ 3374.4, 3284.6, 2950.9, 1740, 1672.1; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 7.72 (1H, d, $J$ = 1.3 Hz, H-4), 7.56 (1H, dd, $J$ = 8.2, 1.36 Hz, H-7), 7.30–7.24 (2H, m, H-3', H-7'), 7.18 (1H, d, $J$ = 8.2 Hz, H-6), 7.12–7.08 (2H, m, $J$ = 7.9 Hz, H-4', H-6'), 4.10 (1H, q, $J$ = 7.0 Hz, H-1'), 3.61 (2H, q, $J$ = 7.1 Hz, H-5b), 2.39 (2H, d, $J$ = 7.08 Hz, H-5'a), 1.80 (1H, m, H-5'b), 1.39 (3H, d, $J$ = 7.0 Hz, H-1'a), 1.32 (3H, t, $J$ = 7.0 Hz, H-5c), 1.20–0.87 (6H, m, H-5'c, H-5'd)

Ethyl-2-(1-(6-Methoxynapthalen-2-yl)ethyl)-1H-benzimidazol-5-carboxylate (BZ-NP, 4b)

This compound was prepared by taking 0.45g ethyl-3,4-diaminobenzoate, 0.86 g naproxen and 8 ml OPA. It was obtained as a brown solid, yield 77 %; mp 212–213 ºC; IR (KBr), $\nu_{\text{max}}$ 3324.9, 3060, 2924.9, 1730, 1298; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 7.89–7.86 (2H, m, H-4, H-7), 7.76–7.74 (3H, m, H-5, H-8', H-4'), 7.70–7.67 (2H, m, H-9', H-3'), 7.63–7.59 (2H, m, H-7', H-5'), 4.65 (1H, q, $J$ = 7.2 Hz, H-1'), 3.87 (3H, s, H-6'a), 1.78 (3H, d, $J$ = 7.2 Hz, H-1'a), 1.30 (3H, t, $J$ = 7.0 Hz, H-5'c).

Synthesis of intermediate (5a and 5b)

BZ-IB (4a; 1.0 mmol, 0.34 g) or BZ-NP (4b; 1.0 mmol, 0.74 g) conjugate was dissolved in 7.30 ml of tetrahydrofuran. The resultant solution was mixed with 4.5 ml of 5M sodium hydroxide solution, and refluxed for 7 h. After completion of reaction, the reaction mixture was allowed to cool to room temperature, and acidified with dil HCl. The resultant precipitates were collected, washed with water, and recrystallized from in hot ethanol-water.

2-(1-(4-Isobutylphenyl)ethyl-1H-benzimidazol-5-carboxylic acid (5a)

It was obtained as a brown solid, yield 80.0 %; mp 192–193 ºC; IR (KBr), $\nu_{\text{max}}$ 3400 – 2490, 3284.6, 2950.9,1718; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 7.72 (1H, d, $J$ = 1.32 Hz, H-4), 7.58–7.56 (1H, m, H-7), 7.29–7.24 (2H, m, H-3', H-7'), 7.18 (1H, d, $J$ = 8.2 Hz, H-6), 7.12–7.08 (2H, m, H-4', H-6'), 3.61 (1H, q, $J$ = 7.0 Hz, H-1'), 2.39 (2H, d, $J$ = 7.08 Hz, H-5'a), 1.80–1.76 (1H, m, H-5'b), 1.39 (3H, d, $J$ = 7.0 Hz, H-1'a), 1.50–0.86 (6H, m, H-5'c, H-5'd)

2-(1-(6-Methoxynaphthalen-2-yl)ethyl)-1H-benzoimidazol-5-carboxylic acid (5b)

It was obtained as a dark brown solid, yield 75.0 %; mp 216–217 ºC; IR (KBr), $\nu_{\text{max}}$ 3400 – 2500, 3060, 2924.9, 1683, 1298; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 11.92 (1H, s, OH), 7.83–7.80 (2H, m, H-4, H-7), 7.75–7.70 (3H, m, H-6, H-8', H-4'), 7.64–7.60 (2H, m, H-9', H-3'), 7.58–7.62 (2H, m, H-7', H-5'), 4.68 (1H, q, $J$ = 7.2, H-1'), 3.90 (3H, s, H-6'a), 1.78 (3H, d, $J$ = 7.0 Hz, H-1'a).

General procedure Synthesis of target compound (IB01-05 and NP01-NP05 series)
Dicyclohexyl carbodiimide (DCC; 0.1 mM, 0.4 g) was added to a solution of the BZ-NsCOXi conjugates (0.1 mM) in dichloromethane (10 mL). The content were stirred for 30 min followed by addition of disubstituted amine (0.3 mM) and a solution of DMAP in dichloromethane (10 mL). The reaction mixture was stirred at 0°C for first 2 h, followed by stirring at room temperature overnight. The precipitated dicyclohexyurea (DHU) was filtered and the solvent was removed in vacuum. Ethyl acetate (10 mL) was added to the residue in order to separate the product from residual DHU. The ethyl acetate solution was filtered, washed with 10% aqueous solution of sodium bicarbonate followed by distilled water and then dried with magnesium sulphate (anhydrous). The solvent was recovered in vacuum to obtain the crude product.

2-[α-Methyl-4-(2-methylpropyl)benzyl]-5-(N,N-dimethylaminocarbonyl)benzimindazole (IB01)

This compound was prepared by taking 0.64 g ibuprofen and 0.2 ml dimethylamine. It was obtained as an offwhite solid, yield 59%; mp 173–174 °C; IR (KBr), ν<sub>max</sub> 3375.4, 3289.6, 2955.9, 1645, 1585.1; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 7.72 (1H, d, J = 1.2 Hz, H-4), 7.59 (1H, dd, J = 8.2, 1.36 Hz, H-7), 7.21 (2H, d, J = 7.6 Hz, H-3', H-7'), 7.16 (1H, d, J = 7.8 Hz, H-6), 6.98 (2H, d, J = 7.9 Hz, H-4', H-6'), 3.39 (1H, q, J = 8.2 Hz, H-1'), 3.04–2.98 (8H, m, H-5'a, H-5b, H-5c), 1.76–1.73 (1H, m, H-5'b), 1.29 (3H, d, J = 7.08 Hz, H-1'a), 0.81 (6H, d, J = 6.6 Hz, H-5'c, H-5'd); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz) δ 166.9 (C-5a), 142.7 (C, C-2, C-5'), 145.7 (C, C-7a, C-3a), 134.8 (C, C-2'), 128.76 (C, C-5, C-6', C-4'), 124.1 (C, C-7', C-3'), 120.6 (C, C-6), 110 (C, C-4), 109.1 (C, C-7), 48 (C, C-5'a, C-5b, C-5d), 42.6 (C, C-1'), 30.1 (C, C-5'b), 22.1 (C, C-5', C-5'd), 19.47 (C, C-1'a), 13.3 (C, C-5c, C-5e); HRMS (ESI+) m/z calcd for C<sub>22</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub> 350.2188, found 350.2185 (M + H<sup>+</sup>.

2-[α-Methyl-4-(2-methylpropyl)benzyl]-5-(N,N-diethylaminocarbonyl)benzimindazole (IB02)

This compound was prepared by taking 0.64 g ibuprofen and 0.4 ml diethylamine. It was obtained as an offwhite solid, yield 56%; mp 166–167 °C; IR (KBr), ν<sub>max</sub> 3377.7, 3290.9, 2952.0, 1676.8, 1645.5, 1534.2; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 7.71 (1H, d, J = 1.34 Hz, H-4), 7.53 (1H, dd, J = 8.2, 1.36 Hz, H-7), 7.19 (2H, d, J = 7.9 Hz, H-3', H-7'), 7.12 (1H, d, J = 8.2 Hz, H-6), 7.03 (2H, d, J = 7.92 Hz, H-4', H-6'), 3.34 (1H, q, J = 7.08 Hz, H-1'), 2.80 (4H, q, J = 7.2 Hz, H-5b, H-5d), 2.39 (2H, d, J = 7.0 Hz, H-5'a), 1.83–1.76 (1H, m, H-5'b), 1.39 (3H, d, J = 7.08 Hz, H-1'a), 1.11 (6H, t, J = 7.2 Hz, H-5c', H-5e), 0.85 (6H, d, J = 6.6 Hz, H-5's, H-5'd); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz) δ 167.9 (C, C-5a), 142.7 (C, C-2, C-5'), 141.7 (C, C-7a, C-3a), 136.8 (C, C-2'), 128.76 (C, C-5, C-6', C-4'), 127.1 (C, C-7', C-3'), 121.6 (C, C-6), 112 (C, C-4), 111.1 (C, C-7), 48 (C, C-5'a, C-5b, C-5d), 42.6 (C, C-1'), 30.1 (C, C-5'b), 22.1(C, C-5', C-5'd), 19.47 (C, C-1'a), 13.3 (C, C-5c, C-5e); HRMS (ESI+) m/z calcd for C<sub>24</sub>H<sub>31</sub>N<sub>3</sub>O<sub>3</sub> 378.2501, found 378.2498 (M + H<sup>+</sup>.

2-[α-Methyl-4-(2-methylpropyl)benzyl]-5-(morpholin-4-ylcarbonyl)benzimindazol (IB03)

This compound was prepared by taking 0.64 g ibuprofen and 0.3 ml morpholine. It was obtained as a yellowish solid, yield 45%; mp 176–178 °C; IR (KBr), ν<sub>max</sub> 3380.4, 3292.6, 2957.9, 1673.1, 1648, 1587.7; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 7.64 (1H, d, J = 1.32 Hz, H-4), 7.52 (1H, dd, J = 8.2, 1.3 Hz, H-7), 7.09 (2H, d, J = 7.6 Hz, H-3', H-7'), 7.03(1H, d, J = 8.4 Hz, H-6), 7.00 (2H, d, J = 7.8 Hz, H-4', H-6'), 4.27 (1H, q, J = 7.08
2-[a-Methyl-4-(2-methylpropyl)benzyl]-5-(pyrrolidin-1-ylcarbonyl)benzimidazole (IB04)

This compound was prepared by taking 0.64 g ibuprofen and 0.32 ml pyrrolidine. It was obtained as off-white solid, yield 59 %, mp 195–196°C; IR (KBr), \( \delta_{\text{max}} \) 3374.4, 3284.6, 2950.9, 1672.1, 1642, 1583.1; \(^{1}\)H NMR (DMSO-d6, 400 MHz) \( \delta \) 7.68 (1H, d, \( J = 1.2 \) Hz, H-4), 7.57 (1H, dd, \( J = 8.2, 1.36 \) Hz, H-7), 7.16 (2H, d, \( J = 8.4 \) Hz, H-3'), 7.11 (1H, d, \( J = 7.4 \) Hz, 7.00 (2H, d, \( J = 7.4 \) Hz, H-4', H-6'), 3.35 (1H, q, \( J = 7.6 \) Hz, H-1'), 2.76 (4H, t, \( J = 6.5 \) Hz, H-5b', H-5d), 2.38 (2H, d, \( J = 7.0 \) Hz, H-5'a), 1.82–1.75 (1H, m, H-5'b), 1.29 (3H, d, \( J = 7.0 \) Hz, H-1'a), 0.85 (6H, d, \( J = 6.6 \) Hz, H-5'c, H-5'd); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \) 167.6 (C, C-5a), 142.9 (C, C-2), 141.9 (C, C-7a), 138.7 (C, C-3a), 137.0 (C, C-2'), 128.9 (C, C-5a), 128.4 (C, C-4', C-6'), 126.9 (C, C-3', C-7'), 125.6 (C, C-6), 115.9 (C, C-4), 115.0 (C, C-7), 47.9 (C, C-5b, C-5d), 44.5 (C, C-5'a), 39.4 (C, C-1'), 29.0 (C, C-5'b), 25.4 (C, C-5c, C-5e), 22.8 (C, C-5'c, C-5'd), 20.4 (C, C-1'a); HRMS (ESI+) \( m/z \) calcd for C\(_{24}\)H\(_{29}\)N\(_{3}\)O 376.2344, found 376.2342 (M + H\(^{+}\)).

2-[a-Methyl-4-(2-methylpropyl)benzyl]-5-(piperazin-1-ylcarbonyl)benzimidazole (IB05)

This compound was prepared by taking 0.64 g ibuprofen and 0.34 g piperazine. It was obtained as a yellowish solid, yield 52 %; mp 188–189°C; IR (KBr), \( \delta_{\text{max}} \) 3377.4, 3293.6, 2956.9, 1678.1, 1648, 1588.1; \(^{1}\)H NMR (DMSO-d6, 400 MHz) \( \delta \) 7.66 (1H, d, \( J = 1.2 \) Hz, H-4), 7.55 (1H, dd, \( J = 8.2, 1.36 \) Hz, H-7), 7.19 (2H, d, \( J = 8 \) Hz, H-3'), 7.10 (1H, d, \( J = 8.2 \) Hz, H-6), 7.01 (2H, d, \( J = 7.96 \) Hz, H-4', H-6'), 3.34 (1H, q, \( J = 8 \) Hz, H-1'), 2.71–2.60 (8H, m, H-5b, H-5c, H-5c, H-5d), 2.38 (2H, d, \( J = 7.1 \) Hz, H-5'a), 1.82–1.75 (1H, m, H-5'b), 1.28 (3H, d, \( J = 7.12 \) Hz, H-1'a), 0.85 (6H, d, \( J = 8 \) Hz, H-5'c, H-5'd); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \) 168.9 (C, C-5a), 143.1 (C, C-2), 141.39 (C, C-7a), 136.6 (C, C-3a, C-5'), 137.0 (C, C-2'), 128.4 (C, C-5, C-4', C-6'), 126.9 (C, C-3', C-7'), 122.3 (C, C-6), 112.3 (C, C-4), 111.2 (C, C-7), 51.4 (C, C-5b, C-5d), 48.3 (C, C-5c, C-5e), 46.0 (C, C-5'a), 40.6 (C, C-1'), 29.9 (C, C-5'b), 22.0 (C, C-5'd, C-5'c), 19.37 (C, C-1'a); HRMS (ESI+) \( m/z \) calcd for C\(_{24}\)H\(_{30}\)N\(_{4}\)O 391.2453, found 391.2450 (M + H\(^{+}\)).

2-[1-6-Methoxy-2-naphthyl)ethyl]-5-(N,N-dimethylaminocarbonyl)-benzimidazole (NP01)

This compound was prepared by taking 0.74 g naproxen and 0.2 ml dimethylamine. It was obtained as a light pink solid, yield 67 %; mp 233–234°C; IR (KBr), \( \delta_{\text{max}} \) 3324.9, 3060, 2924.9, 1618, 1559.4, 1298; \(^{1}\)H NMR (DMSO-d6, 400 MHz) \( \delta \) 7.82–7.80 (2H, m, H-4, H-7), 7.75–7.71 (3H, m, H-6, H-8', H-4'), 7.67–7.65 (2H, m, H-9', H-3'), 7.60–7.53 (2H, m, H-7', H-5'), 4.68 (1H, q, \( J = 7 \) Hz, H-1), 3.9 (3H, s, H-6'a), 3.33 (6H, s, H-5b, H-5c) 1.78 (3H, d, \( J = 7 \) Hz, H-1'a); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \) 168.7 (C, C-5a), 156.1 (C, C-6'), 141.5 (C, C-2), 139.9 (C, C-7a), 138.7 (C, C-3a), 132.8 (C, C-2', C-7'a), 129.9 (C, C-3'a, C-5), 128.5 (C, C-4', C-
9'), 126.7 (C, C-3'), 126.1 (C, C-6), 118.6 (C, C-5'), 115.9 (C, C-7), 115.0 (C, C-4), 105.4 (C, C-7'), 55.8 (C, C-6'a), 39.8 (C, C-1'), 38.7 (C, C-5b, C-5c), 20.4 (C, C-1'a); HRMS (ESI+) m/z calcd for C_{23}H_{23}N_{3}O_{2} 374.1824, found 374.1821 (M + H^+).

2-[1-(6-Methoxy-2-naphthyl)ethyl]-5-(N,N-diethylaminocarbonyl)benzimidazole (NP02)

This compound was prepared by taking 0.74 g naproxen and 0.4 ml dimethylamine. It was obtained as a brown solid, yield 65%; mp 202–203°C; IR (KBr), \( \tilde{\nu}_{\text{max}} \) 3326.4, 3061, 2920.9, 1621, 1564.4, 1299; \(^1\)H NMR (DMSO-d6, 400 MHz) \( \delta \)
7.84–7.82 (2H, m, H-4, H-7), 7.73–7.70 (3H, m, H-6, H-8, H-4'), 7.64–7.60 (2H, m, H-9', H-3'), 7.58–7.62 (2H, m, H-7', H-5'), 4.68 (1H, q, \( J = 7.0 \text{ Hz} \), H-1'), 3.05 (4H, q, \( J = 7.2 \text{ Hz} \), H-5b, H-5d), 3.9 (3H, s, H-6'a), 1.78 (3H, d, \( J = 7.0 \text{ Hz} \), H-1'a), 1.21 (6H, t, \( J = 7.2 \text{ Hz} \), H-5c, H-5e); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \)
168.9 (C, C-5a), 156.1 (C, C-6'), 141.5 (C, C-2), 139.9 (C, C-7a), 138.7 (C, C-3a), 133.0 (C, C-2', C-7'a), 129.9 (C, C-5), 129.4 (C, C-3'a), 128.5 (C, C-9'), 126.7 (C, C-3'), 126.1 (C, C-8'), 122.5 (C, C-6), 118.6 (C, C-5'), 115.9 (C, C-7), 115.0 (C, C-4), 105.4 (C, C-5b, C-5d), 39.8 (C, C-1'), 20.4 (C, C-1'a), 12.8 (C, C-5c, C-5e); HRMS (ESI+) m/z calcd for C_{25}H_{27}N_{3}O_{2} 402.2137, found 402.2135 (M + H^+).

2-[1-(6-Methoxy-2-naphthyl)ethyl]-5-(morpholin-4-yl-carbonyl)benzimidazole (NP03)

This compound was prepared by taking 0.74 g naproxen and 0.3 ml morpholine. It was obtained as a light brown solid, yield 60%; mp 255–256°C; IR (KBr), \( \tilde{\nu}_{\text{max}} \) 3327.4, 3068, 2925.9, 1623, 1562.4, 1297; \(^1\)H NMR (DMSO-d6, 400 MHz) \( \delta \)
7.69–7.63 (2H, m, H-4, H-7), 7.70–7.65 (3H, m, H-6, H-8', H-4'), 7.55–7.52 (2H, m, H-9', H-3'), 7.51–7.49 (2H, m, H-7', H-5'), 4.65 (1H, q, \( J = 7 \text{ Hz} \), H-1'), 3.9 (3H, s, H-6'a), 3.58–3.55 (4H, m, H-5b, H-5d), 2.79–2.77 (4H, m, H-5c, H-5e), 1.78 (3H, d, \( J = 7 \text{ Hz} \), H-1'a); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \)
168.8 (C, C-5a), 156.1 (C, C-6'), 141.5 (C, C-2), 139.9 (C, C-7a), 138.7 (C, C-3a), 133.0 (C, C-2', C-7'a), 129.9 (C, C-5), 129.4 (C, C-3'a), 128.5 (C, C-9'), 126.7 (C, C-3'), 126.1 (C, C-8'), 122.5 (C, C-6), 118.6 (C, C-5'), 115.9 (C, C-7), 115.0 (C, C-4), 105.4 (C, C-7'), 66.2 (C, C-5c, C-5e), 55.8 (C, C-6'a), 46.5 (C, C-5b, C-5d), 39.8 (C, C-1'), 20.8 (C, C-1'a); HRMS (ESI+) m/z calcd for C_{25}H_{25}N_{3}O_{3} 416.1929, found 416.1925 (M + H^+).

2-[1-(6-Methoxy-2-naphthyl)ethyl]-5-(pyrrolidin-1-yl-carbonyl)benzimidazole (NP04)

This compound was prepared by taking 0.74 g naproxen and 0.32 ml pyrrolidine. It was obtained as a brown solid, yield 62%; mp 249–250°C; IR (KBr), \( \tilde{\nu}_{\text{max}} \) 3322.4, 3057, 2919.9, 1620, 1563.4, 1297; \(^1\)H NMR (DMSO-d6, 400 MHz) \( \delta \)
7.68–7.62 (2H, m, H-4, H-7), 7.71–7.68 (3H, m, H-6, H-8', H-4'), 7.58–7.61 (2H, m, H-9', H-3'), 7.53–7.50 (2H, m, H-7', H-5'), 4.62 (1H, q, \( J = 7 \text{ Hz} \), H-1'), 3.9 (3H, s, H-6'a), 2.74 (4H, m, H-5c, H-5e), 1.78 (3H, d, \( J = 7 \text{ Hz} \), H-1'a), 1.63–1.58 (4H, m, H-5d, H-5b); \(^{13}\)C NMR (DMSO-d6, 100 MHz) \( \delta \)
169.5 (C, C-5a), 156.1 (C, C-6'), 141.5 (C, C-2), 139.9 (C, C-7a), 138.7 (C, C-3a), 132.8 (C, C-2', C-7'a), 129.9 (C, C-5), 128.5 (C, C-9'), 126.7 (C, C-3'), 126.1 (C, C-8'), 122.5 (C, C-6), 118.6 (C, C-5'), 115.9 (C, C-7), 115.0 (C, C-4), 105.4 (C, C-7'), 55.8 (C, C-6'a), 47.9 (C, C-5b, C-5d), 25.4 (C, C-5c, C-5e), 39.8 (C, C-1'), 20.4 (C, C-1'a); HRMS (ESI+) m/z calcd for C_{25}H_{25}N_{3}O_{2} 400.1980, found 400.1978 (M + H^+).

2-[1-(6-Methoxy-2-naphthyl)ethyl]-5-(piperazin-1-yl-carbonyl)benzimidazole (NP05)
This compound was prepared by taking 0.74 g naproxen and 0.34 g piperazine. It was obtained as a pink solid, yield 64 %; mp 246–247°C; IR (KBr), \( \nu_{\text{max}} \) 3324.4, 3060, 2922.9, 1622, 1565.4, 1298; \(^1\)H NMR (DMSO-d6, 400 MHz) \( \delta \) 7.70–7.68 (2H, m, H-4, H-7), 7.64–7.61 (3H, m, H-6, H-8', H-4'), 7.52–7.48 (2H, m, H-9', H-3'), 7.54–7.49 (2H, m, H-7', H-5'), 4.62 (1H, q, \( J = 7.0 \) Hz, H-1'), 3.9 (3H, s, H-6'a), 2.71–2.50 (8H, m, H-5b, H-5c, H-5d, H-5e), 1.78 (3H, d, \( J = 7.0 \) Hz, H-1'a); \(^1\)C NMR (DMSO-d6, 100 MHz) \( \delta \) 168.9 (C, C-5a), 156.1 (C, C-6'), 141.5 (C, C-2), 139.9 (C, C-7a), 138.7 (C, C3a), 132.8 (C, C-2', C-7'a), 129.9 (C, C-5, C-3'a, C-4'), 128.5 (C, C-9'), 126.7 (C, C-3'),126.1 (C, C-8'), 122.5 (C, C-6), 118.6 (C, C-5'), 115.9 (C, C-7), 115.0 (C, C-4), 105.4 (C, C-7'), 52.6 (C, C-5b, C-5d), 55.8 (C, C-6'a), 47.2 (C, C-5c, C-5e), 39.8 (C, C-1'), 20.4 (C, C-1'a); HRMS (ESI+) \( m/z \) calcd for C\(_{25}\)H\(_{26}\)N\(_4\)O\(_2\) 415.2089, found 415.2086 (M + H\(^+\)).

**In vitro biological evaluation**

**In-vitro AChE activity**

AChE inhibitory activity of target compounds was evaluated by the spectrophotometric method developed by Ellman *et al.* [11] with some modifications.

**In-vitro COX inhibition assay**

All the target compounds were screened for their ability to inhibit COX-1 and COX-2 enzymes in mouse macrophages [12]. This was carried out using Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111) supplied by Cayman chemicals, Ann Arbor, MI, USA, according to reported method [13].

**In vivo biological activity**

**Experimental animals**: Swiss albino mice of either sex, weighing 20–25 g (procured from NIPER, SAS Nagar, Mohali Punjab, India) were used for in vivo evaluation of learning and memory. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). The animals were housed in the Animal house facility, Punjabi University Patiala, India and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No: 107/GO/ReBi/S/99/CPCSEA/2018-13).

**Drugs and chemicals**

Scopolamine bromide was dissolved in normal saline and test compounds were dissolved in 10% dimethylsulfoxide (DMSO). All other reagents used in the present study were of analytical grade and freshly prepared.

**Assessment of learning and memory by Morris water maze**: Morris water maze is one of the most commonly used animal models to test memory [14]. It consists of large circular pool and is divided into four quadrants (Q1, Q2, Q3, and Q4). Each animal was subjected to trial of 120 s in the water maze for
five consecutive days and the memory was assessed in terms of: (i) escape latency time (ELT), that is, the time taken by the animal to locate the hidden platform in the target quadrant (Q4) for the first 4 days of training, and (ii) time spent in target quadrant (Q4) on fifth day of trial, that is, the day of retrieval.

**Experimental Protocol**

Seven groups, each group comprising five mice, were employed in this study.

**Group I (normal control)**

Normal mice, without any treatment, were subjected to trials on the water maze for 5 days to note escape latency time (ELT) for first 4 days (an index of learning) and time spent in target quadrant (TSTQ) on 5th day of trial (an index of retrieval).

**Group II (scopolamine control)**

Scopolamine (0.4 mg/kg i.p.) was administered to each mouse, 30 min prior to each trial, for first 4 days of trials. In scopolamine-treated mice, the ELT and TSTQ were noted as described in group I.

**Group III (vehicle control)**

10 % DMSO (1 ml/kg) was administered in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I

**Group IV (Donepezil in scopolamine control)**

Donepezil (5 mg/kg) was administered in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

**Group V-VII (Compound IB-04 (2, 4 and 8 mg/kg) in scopolamine control)**

The test compound with the most potent AChE, i.e. compound IB04 was administered by oral route through canula in different doses (2, 4 and 8 mg/kg) in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

**Biochemical estimations**

At the end of the protocol, animals were euthanized by cervical dislocation and brains were removed carefully. Different parts of brain i.e. cortex and hippocampus were separated. Isolated parts were homogenized in ice cold phosphate buffer of pH 7.4. The homogenate was centrifuged at 14,500 rpm for 15 min at 4 C. The clear supernatant was used for estimation of thiobarbituricacid reactive substance
(TBARS), reduced glutathione (GSH), and brain AChE activity by the methods reported by Ohkawa et al [15] Beutler, et al [16], and Ellman method [11], respectively.

Statistical analysis

Data was expressed as mean ± SD of the obtained data. The statistical analysis of the data was performed using analysis of variance (ANOVA) followed by multiple comparison test. The Morris water maze data (Day 1 ELT, Day 4 ELT, and Day 5 TSTQ data) was analysed using two-way ANOVA followed by Bonferroni post hoc test whereas biochemical estimations were statistically analyzed by one-way ANOVA followed by Tukey’s test. A value of P < 0.05 was considered statistically significant.

Declarations

CONFLICT OF INTEREST

There is no conflict of interest among authors.

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**Figures**

- **Figure 1**

  ![Diagram](image_url)
Designing of novel Benzimidazole-NsCOXi targets

Figure 2

Effect of IB04 on escape latency time at day 1 and day 4 using water maze test for memory evaluation. Data is presented as Mean ± S.D. and analyzed by two way ANOVA followed by Tukey’s multiple range test; a p< 0.05 vs day 1 ELT in control; bp< 0.05 vs day 4 ELT in normal; cp< 0.05 vs day 4 ELT in scopolamine, dp< 0.05 vs day 4 ELT in donepezil + scopolamine.
Figure 3

Effect of IB04 on time spent in target quadrant (TSTQ), i.e. Q4 in water maze test for memory evaluation. Values are expressed as Mean±S.D. and analyzed by two way ANOVA followed by Tukey's multiple range test; a p< 0.05 vs time spent in other quadrant (Q1, Q2, and Q3) in control; bp< 0.05 vs TSTQ in normal; cp< 0.05 vs TSTQ in scopolamine treated, dp< 0.05 vs TSTQ in donepezil + scopolamine.
Figure 4

Effect of IB04 on brain AChE activity. Values are expressed as Mean±S.D. and analyzed by one way ANOVA followed by Tukey's multiple range test; a(p < 0.05) in comparison to normal; b(p < 0.05) in comparison to scopolamine; c(p < 0.05) in comparison to donepezil + scopolamine.
**Figure 5**

Effect of IB04 on TBARs level. Data are presented as mean ± standard deviation; analyzed by one way ANOVA. a(p < 0.05) in comparison to Normal; b(p < 0.05) in comparison to scopolamine; c(p < 0.05) in comparison to donepezil + scopolamine.
Effect of IB04 on GSH levels. Data are presented as mean ± standard deviation; analyzed by one way ANOVA. a(p < 0.05) in comparison to Normal; b(p < 0.05) in comparison to scopolamine; c(p < 0.05) in comparison to donepezil + scopolamine.

Supplementary Files
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- Scheme1.png