Bioorganic & Medicinal Chemistry 20 (2012) 521–530

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and evaluation of novel 4-[(3H,3aH,6aH)-3-phenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid derivatives as potent acetylcholinesterase inhibitors and anti-amnestic agents

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ARTICLE INFO

Article history:
Received 17 March 2011
Revised 16 May 2011
Accepted 17 May 2011
Available online 12 July 2011

Keywords:
Acetyl cholinesterase
Pyrrolo-isoxazole benzoic acid
Morris water maze
Azomethine-*N*-oxides

ABSTRACT

The present study was designed to synthesize and evaluate pyrrolo-isoxazole benzoic acid derivatives as potential acetylcholinesterase (AChE) inhibitors for the management of Alzheimer's disease. The synthesis of pyrrolo-isoxazole benzoic acid derivatives involved ring opening cyclization of *p*-aminobenzoic acid with maleic anhydride to yield maleanilic acid, which in turn afforded *N*-arylmaleimide via ring closed cyclization. Azomethine-*N*-oxides were obtained by condensation of *N*-arylhydroxylamine with differently substituted benzaldehydes followed by refluxing of *N*-arylmaleimide with differently substituted azomethine-*N*-oxides to pyrrolo-isoxazole benzoic acid derivatives as *cis*- and *trans*-stereoisomers. The synthesized compounds were evaluated in vitro for AChE inhibitory activity in rat brain homogenate with donepezil as standard AChE inhibitor. Thereafter, the most potent test compound was evaluated for in vitro butyrylcholinesterase inhibitory activity and in vivo memory evaluation in scopolamine (0.4 mg/kg)-induced amnesia in mice by employing Morris water maze test. All pyrrolo-isoxazole benzoic acid derivatives demonstrated potent AChE inhibitory activity. Most of compounds exhibited similar activity to donepezil and four of them (**7h**, **7i**, **8i**, and **8h**, IC_{50} = 19.1 ± 1.9–17.5 ± 1.5 nM) displayed higher inhibitory activity as compared to donepezil (21.5 ± 3.2 nM) with compound **8ia** (IC_{50} = 17.5 ± 1.5 nM) being the most active one. The test compound **8ia** also ameliorated scopolamine-induced amnesia in mice in terms of restoration of time spent in target quadrant (TSTQ) and escape latency time (ELT). It may be concluded that pyrrolo-isoxazole benzoic acid derivatives may be employed as potential AChE inhibitors.

1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain and is the most common form of dementia among the elderly especially in industrialized countries. According to the cholinergic hypothesis, the decreased levels of acetylcholine in the brain areas dealing with learning, memory, behavior, and emotional responses (neocortex and hippocampus) are of critical importance in AD. The reduced levels of neurotransmitter acetylcholine are due to its rapid hydrolysis by an enzyme, acetylcholinesterase (AChE).[1] There have also been several reports showing that the enzyme AChE plays a key role in the development of the senile plaques by accelerating amyloid-beta deposition.[2] Thus, AChE inhibition has been documented as a critical target for the effective management of AD by an increase in the availability of acetylcholine in the brain regions and decrease in the deposition of amyloid beta.

The previous studies had shown that *meta*-, *ortho*- and *para*-aminobenzoic acid derived arylamides and arylimides exhibit potent acetylcholinesterase inhibitory activities.[3] It has also been documented that *p*-aminobenzoic acid derived arylamides and arylimides are more potent as compared to corresponding *m*-aminobenzoic acid and *o*-aminobenzoic acid derivatives.[3] Furthermore, the studies have shown that addition of different chemical moieties to tacrine including addition of substituted pyrazolo groups enhances the effectiveness of tacrine (one of the standard AChE inhibitor).[4] Therefore, it is proposed that substituted pyrrolo groups (bioisostere of pyrazolo) may be added to the aryalimdes of *p*-aminobenzoic acid to derive pyrrolo-isoxazole derivatives with potential acetylcholinesterase inhibitory activities. The studies have suggested that different chemical moieties with central nucleus related to pyrrolo-isoxazole posses acetylcholinesterase inhibitory properties. The different derivatives of pyrrolo-benzisoxazols, such as CP-118,954 and CP-126,998 have been reported as potent AChE inhibitor and are used for in vivo imaging of AChE.[5] The pyrazolo-pyridine and pyrazolo-naphthyridine derivatives have been shown to be very effective in inhibiting AChE.[4] Further-

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0968-0896/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2011.05.027

more, derivatives of pyrrolo-quinoline have been demonstrated to significantly ameliorate scopolamine-induced amnesia suggesting its acetylcholinergic dysfunction improving properties.[6]

Based on these reports, the present study was designed to synthesize pyrrolo-isoxazole benzoic acid derivatives as potential AChE inhibitors followed by evaluation of in vitro acetyl cholinesterase inhibitory activity and in vivo anti-amnestic activity of the most potent AChE inhibitor.

2. Results and discussion

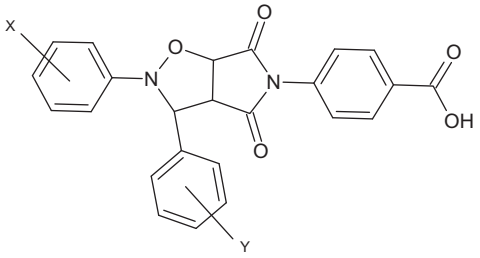
2.1. Chemistry

Ring opening cyclization of *p*-aminobenzoic acid with maleic anhydride in presence of tetrahydrofurane (THF) provided maleanilic acid (**1**). Subsequently, refluxing of maleanilic acid (**1**) in acetic anhydride with an equimolecular amount of sodium acetate via ring closed cyclization afforded *N*-arylmaleimide (**2**).[3] Reduction of substituted nitrobenzene with zinc in presence of water and ammonium chloride yielded *N*-arylhydroxylamine (**3**). Condensation of *N*-arylhydroxylamine (**3**) with substituted benzaldehyde (**4a–k**) in presence of chloroform gave respective azomethine-*N*-oxides (**5a–k** and **6a–k**). Refluxing of substituted azomethine-*N*-oxides (**5a–k** and **6a–k**) with *N*-arylmaleimide (**2**) in presence of toluene and ethanol afforded products (**7a–k** and **8a–k**) which on fractional crystallization from toluene provided two stereoisomers. These stereoisomers were characterized as *cis*- and *trans*-isomers (a and a′), respectively.[7]

2.2. Biological activity

All synthesised pyrrolo-isoxazole benzoic acid derivatives demonstrated higher inhibitory activity against AChE than *p*-aminobenzoic acid (PABA) in in vitro tests. Most of compounds exhibited similar activity to donepezil and four of them (**7h**, **7i**, **8i**, and **8h**, IC_{50} = 19.1 ± 1.9–17.5 ± 1.5 nM) displayed higher inhibitory activity as compared to donepezil (21.5 ± 3.2 nM) with test compound **8ia** (IC_{50} = 17.5 ± 1.5 nM) being the most active one. Furthermore, the *cis*-isomers displayed equipotency or slightly more potency than corresponding *trans*-isomers with respect to AChE inhibition. From IC_{50} values of tested compounds, it appears that in this series the electronic effects of the susbstituents in the aromatic rings is almost negligible, and have almost no effect on the biological activities. The compounds with methoxy substitution group (**7i** and **8i**) were highly potent than compounds with others substituted groups like hydroxy, halogen, and nitro group. Besides, the shifting of substituted group from para to *meta* and *ortho* position resulted in drop in AChE inhibitory potency (Table 1). The most potent AChE inhibitor (compound **8ia**) was evaluated for BuChE inhibitor activity and its IC_{50} value was 15.3 (±2.1) × 10^3 nM along with BChE/AChE selectivity of 874 indicating more selective inhibition of AChE than BChE. Butyrylcholinesterase is an enzyme mainly localized in the peripheral tissues including plasma and very small amount is present in the brain region. The potential advantage of selective inhibition of AChE over BChE may include lesser degree of associated side effects due to peripheral inhibition of cholinesterase enzyme. The compound **8ia** was also evaluated for memory restoration in scopolamine-induced

Table 1
Acetylcholinesterase inhibitory activity of pyrrolo-isoxazole benzoic acid derivatives



S. No.	Compound	X	Y	*Cis*-isomer	IC_{50} (nM)	*Trans*-isomer	IC_{50} (nM)
1	PABA			35.2 ± 2.1			
2	Donepezil			21.5 ± 3.2			
3	**7a**	H	H	**7aa**	23.9 ± 1.8	**7aa′**	24.4 ± 2.2
4	**7b**	H	2-OH	**7ba**	23.9 ± 1.7	**7ba′**	24.8 ± 2.2
5	**7c**	H	4-OH	**7ca**	22.5 ± 2.1	**7ca′**	22.7 ± 1.6
6	**7d**	H	2-Cl	**7da**	21.8 ± 1.2	**7da′**	22.4 ± 1.5
7	**7e**	H	3-Cl	**7ea**	20.1 ± 2.2	**7ea′**	21.8 ± 1.9
8	**7f**	H	4-Cl	**7fa**	19.7 ± 1.8	**7fa′**	20.9 ± 1.8
9	**7g**	H	2-OMe	**7ga**	19.2 ± 1.9	**7ga′**	20.6 ± 1.8
10	**7h**	H	3-OMe	**7ha**	19.1 ± 2.1	**7ha′**	20.5 ± 2.2
11	**7i**	H	4-OMe	**7ia**	18.8 ± 2.1	**7ia′**	19.1 ± 1.9
12	**7j**	H	2-NO_2	**7ja**	26.1 ± 2.4	**7ja′**	26.9 ± 2.8
13	**7k**	H	4-NO_2	**7ka**	25.8 ± 2.6	**7ka′**	26.4 ± 2.2
14	**8a**	CH_3	H	**8aa**	22.8 ± 2.9	**8aa′**	22.4 ± 2.8
15	**8b**	CH_3	2-OH	**8ba**	23.2 ± 2.8	**8ba′**	24.1± 2.1
16	**8c**	CH_3	4-OH	**8ca**	21.9 ± 2.4	**8ca′**	22.5 ± 2.5
17	**8d**	CH_3	2-Cl	**8da**	21.3 ± 2.8	**8da′**	22.1 ± 2.9
18	**8e**	CH_3	3-Cl	**8ea**	22.2± 2.8	**8ea′**	23.8 ± 2.1
19	**8f**	CH_3	4-Cl	**8fa**	19.2 ± 2.4	**8fa′**	20.4 ± 2.2
20	**8g**	CH_3	2-OMe	**8ga**	19.1± 2.4	**8ga′**	20.2 ± 2.5
21	**8h**	CH_3	3-OMe	**8ha**	18.8 ± 1.8	**8ha′**	19.9 ± 1.9
22	**8i**	CH_3	4-OMe	**8ia**	17.5 ± 1.5	**8ia′**	18.0 ± 1.8
23	**8j**	CH_3	2-NO_2	**8ja**	24.3 ± 1.9	**8ja′**	25.2 ± 2.1
24	**8k**	CH_3	4-NO_2	**8ka**	23.4 ± 1.8	**8ka′**	24.1 ± 1.9

Table 2
Effect of different interventions on escape latency time (ELT) using Morris water maze for memory evaluation

S. No	Group	Dose	Day 1 ELT	Day 4 ELT
1.	Normal	–	86.2 ± 5.5	37.2 ± 5.2[a]
2.	Scopolamine	0.5 mg/kg (ip)	89.8 ± 4.8	79.3 ± 6.3[b]
3.	Compound 8ia in scopolamine	2 mg/kg (ip)	83.7 ± 3.8	74.8 ± 5.6
4.	Compound 8ia in scopolamine	5 mg/kg (ip)	85.2 ± 6.1	69.5 ± 6.9
5.	Compound 8ia in scopolamine	10 mg/kg (ip)	81.2 ± 4.9	49.1 ± 5.7[c]
6.	Vehicle in scopolamine	5 ml/kg (ip)	89.7 ± 3.8	78.7 ± 5.7
7.	Donepezil	25 mg/kg (ip)	84.8 ± 4.1	52.6 ± 4.0[c]

Values are expressed as mean ± S.E.M. for six animals.
[a] $p < 0.05$ versus day 1 ELT in normal.
[b] $p < 0.05$ versus day 4 ELT in normal.
[c] $p < 0.05$ versus day 4 ELT in scopolamine.

amnesia in mice. Administration of scopolamine significantly decreased day 4 ELT and TSTQ on day 5 indicating an impairment of memory as assessed on Morris water maze as compared to normal mice. However, treatment with test compound **8ia** (5 and 10 mg/kg) along with donepezil (25 mg/kg) attenuated scopolamine-induced decrease in day 4 ELT and TSTQ on day 5 in a significant manner (Table 2 and Fig. 4).

2.3. Molecular docking

To disclose a possible binding mode of compound **8ia** with human AChE enzyme's binding pockets, docking simulations were performed using the available crystallographic structure of enzyme (PDB code 1B41) using Molegro Virtual Docker. The docking simulation revealed that the enzyme and compound **8ia** interacted through π–π aromatic interactions and hydrogen bonding (Fig. 5). One of the oxygen of terminal carboxyl group attached to phenyl ring may form a hydrogen bond with –NH-group of Arg 296 (3.58 Å) which is in-turn is a part of Leu 289, Pro 290, Arg 296 loop that helps in completing the binding by creating a hydrophobic environment.[8] The nitrogen of pyrrolo ring may interact with terminal hydroxyl group of Tyr 337 through hydrogen bond (3.53 Å) which is a constituent of "anionic" sub-site in the gorge and is involved in optimally positioning the ester at the acylation site along with binding to trimethylammonium choline through *p*-cation interactions. The nitrogen of pyrrolo ring may also form hydrogen bond with terminal hydroxyl group of Tyr 124 (2.71 Å), one of the five residues of peripheral anionic site which in turn is clustered around the entrance to the active site gorge. One of the oxo moiety attached to the pyrrolo ring may interact with terminal hydroxyl of Tyr 341 through hydrogen bond (3.35 Å), which is again an important residue of peripheral anionic site.[9] An oxygen of methoxy substituent attached to one of the phenyl ring may form a favorable hydrogen bond with amino terminal of Asn 87 (3.41 Å) which is a part of a disulfide-linked loop (Cys 69–Cys 96) (omega loop) covering an active site of AChE buried at the bottom of a 20 Å deep gorge.[10] This loop is associated with peripheral anionic site and forms the part of the outer wall of the gorge and it also includes Trp 86 which is a principal component of "anionic" sub-site. The docking results also revealed the potential π–π aromatic interactions between compound **8ia** and amino acid residues of human AChE. The phenyl ring of terminal benzoic acid may show π–π interactions with Phe 338, a part of "anionic sub-site" along with constituents of peripheral anionic site, that is, Tyr 341 and Tyr 72. One of the phenyl ring attached to isoxazole moiety may also show π–π interactions with ring structure of Trp 86, the principal component of "anionic sub-site".

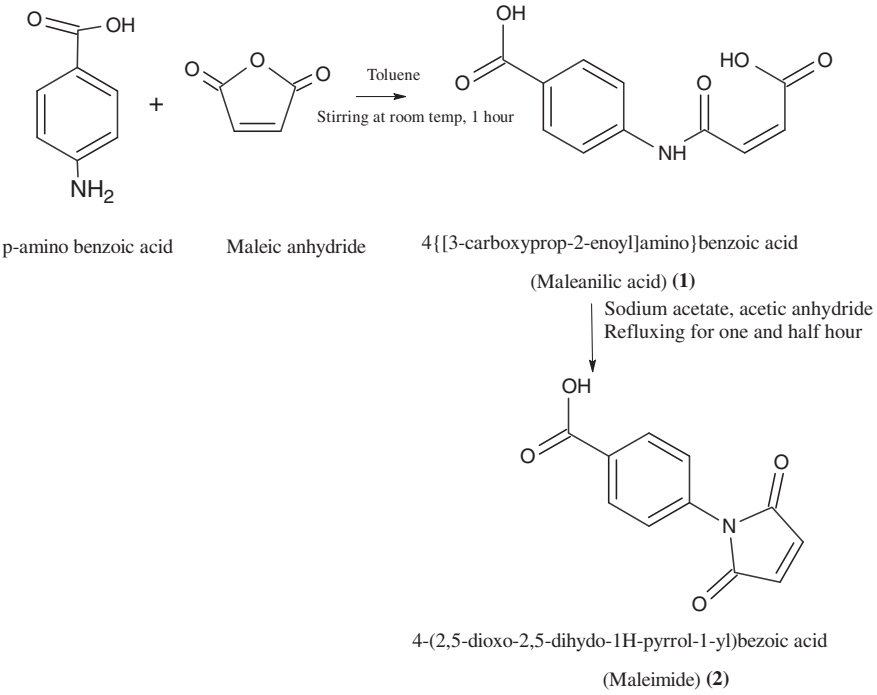


Figure 1. Schematic diagram describing the steps in the synthesis of maleimide from *p*-aminobenzoic acid.

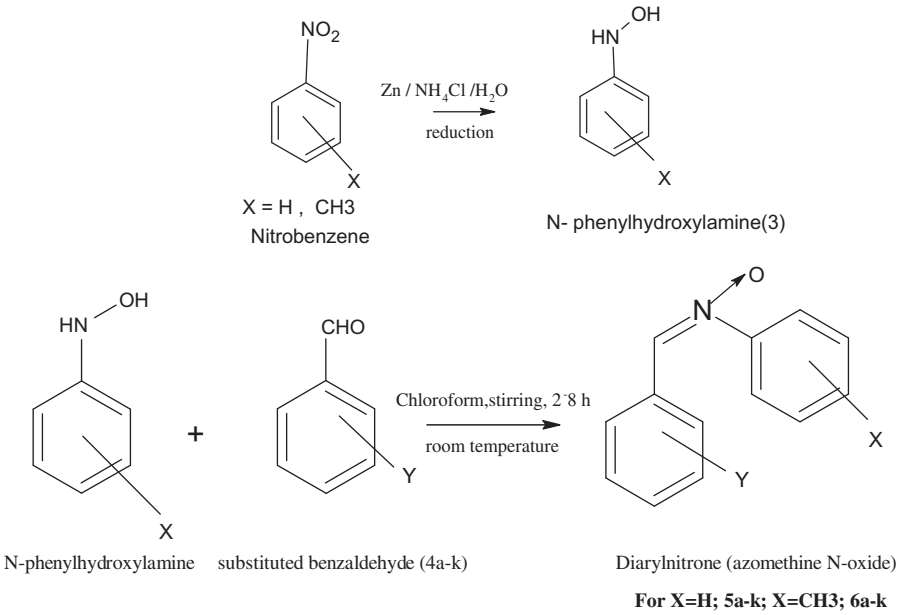


Figure 2. Schematic diagram describing the synthesis of differently substituted nitrones.

3. Conclusion

It may be concluded that pyrrolo-isoxazole benzoic acid derivatives may be serve as key ligands as AChE inhibitors for effective management of amnesic disorders including Alzheimer's disease.

4. Experimental

4.1. Chemistry

All the reagents and the solvents used were of analytical grade. Melting points were recorded on Gallen–Kamp apparatus and uncorrected. IR spectra were recorded on a Perkin Elmer spectrum RX IFT-IR system. 1H NMR spectra were recorded on Bruker Advance II 400 MHz spectrometer in DMSO with TMS as an internal standard and chemical shifts were recorded in parts per million (ppm). Coupling constants (*J* values) are in Hertz. Multiplicities are designated as singlet (s), doublet (d), triplet (t), quadruplet (q) and multiplet (m). Mass spectra (MS) signals were given in *m*/*z*. Element analysis (EA) was measured on an Elementar Analysensysteme GmbH. All the reactions were monitored using TLC.

4.1.1. General procedure for the synthesis of N-arylmaleimide

Equimolar quantities of *p*-aminobenzoic acid and maleic anhydride were stirred in tetrahydrofuran (THF) at room temperature for one and half hours to yield maleanilic acid (**1**) in the form of yellow solid with yield 80%, mp 210 °C.[3] Equimolar quantities of maleanilic acid (**1**) and sodium acetate were refluxed for one and half hours in the presence of acetic anhydride followed by cooling at room temperature. Thereafter, the contents were poured in crushed ice containing beaker and kept overnight to afford light yellow precipitate, which were filtered and washed with water to get light yellow solid *N*-arylmaleimide (**2**) (Fig. 1). Yield 70%, mp 155–157 °C, 1H NMR (400 MHz, DMSO-d_6).[3]

4.1.2. General procedure for the synthesis of N-aryl nitrones

The mixture of nitrobenzene/4-tolynitrobenzene (50 g, 0.41 M) and ammonium chloride (25 g) in 800 ml of water was stirred for one hour with slow addition of zinc (59 g, 0.83 M). The rate of addition of zinc was such that rise in temperature was not more than 65 °C. After the complete addition of zinc, the stirring was continued for further 15 min to complete the reduction and it was observed by noting the decrease in temperature. The resulting warm solution was filtered and washed with warm water (100 ml). Thereafter, the filtrate was used to collect pale yellow precipitates of *N*-phenyl hydroxylamine (**3**). Equimolar quantities of *N*-phenyl hydroxylamine (**3**) and different substituted benzaldehydes (**4a–k**) were stirred in the presence of chloroform (15–20 ml) for 2–8 h until the solid precipitates of different nitrones were obtained and recrystallized from to give corresponding different compounds (**5a–k and 6a–k**) (Fig. 2).[7]

4.1.3. General procedure for the synthesis of pyrrolo-isoxazole benzoic acid derivatives

Equimolar quantities of maleimide (**2**) and nitrones (**5a–k** and **6a–k**) were refluxed in toluene (20 ml) and ethyl alcohol (5 ml) for 8–10 h (TLC monitoring using petroleum ether and hexane 1:1) followed by cooling with addition of dry ether. The products (**7a–k** and **8a–k**) were separated out after filtration and recrystallized from toluene and petroleum ether mixture (1:1) to yield *cis*-isomers (**7aa–7ka** and **8aa–8ka**). The mother liquor on further work up provided *trans*-isomers which were recrystallized from ethanol and diethyl ether mixture (1:1) (**7aa′–7ka′** and **8aa′–8ka′**) (Fig. 3).[7] These stereoisomers were characterized by their 1H NMR, IR and mass spectra in addition to their melting points and elementary analysis. These stereoisomers have identical IR spectra and elemental analysis but differ in their melting points, 1H NMR and mass spectra.

4.1.3.1. *Cis*-4-[(3H,3aH,6aH)-3-phenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid(7-aa). White solid, yield 39%, mp 190 °C; IR (Nujol) υ 1715, 1681, 1597, 1370, 1280, 1212 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.41–5.43 (1H, d, H_3, J = 7.64 Hz), 4.89–4.94 (1H, q, H_{3a}, J = 7.61 and 7.96 Hz), 4.16–4.18 (1H, d, H_{6a}, J = 7.92 Hz), 7.0–8.0 (14H, m, aromatic H), 10.61 (1H, s, COOH); MS: *m*/*z* 415 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_5$: C, 69.56; H, 4.38; N, 6.76; O, 19.30. Found: C, 68.23; H, 4.21; N, 6.01; O, 18.19.

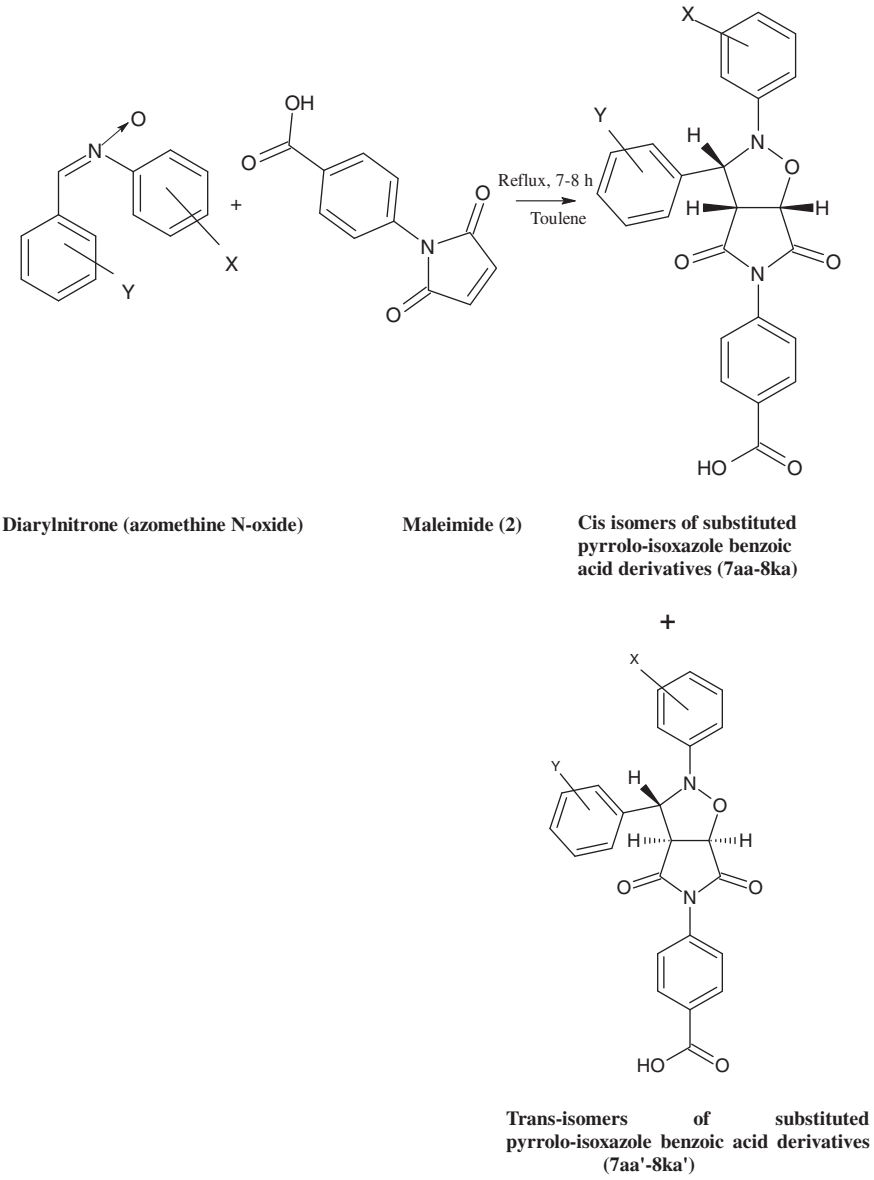


Figure 3. Schematic diagram describing the steps in the synthesis of substituted *cis*- and *trans*-isomers from differently substituted diaryl nitrones and maleimide.

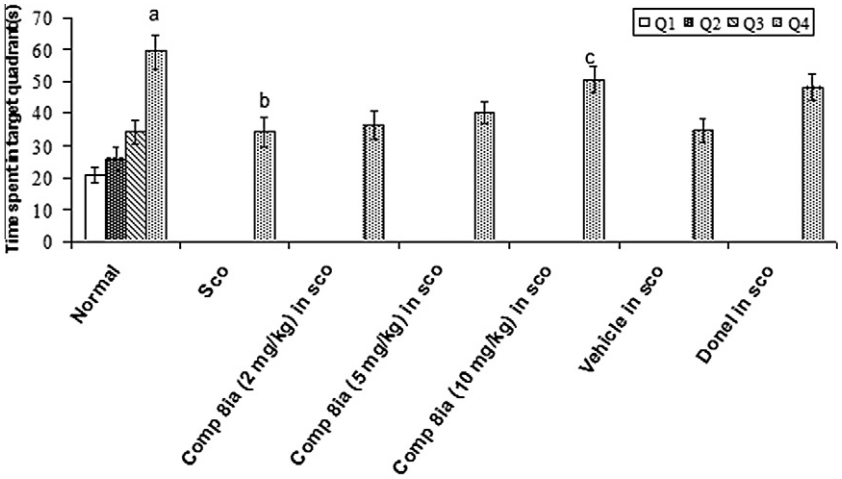


Figure 4. Effect of different interventions on time spent in target quadrant (TSTQ), that is, Q4 in Morris water maze test for memory evaluation. Values are expressed as mean ± S.E.M for six animals. Sco, scopolamine; Donel, donepezil. a = p <0.05 versus time spent in other quadrants (Q1, Q2, and Q3) in normal; b = p <0.05 versus TSTQ in normal; c = p <0.05 versus TSTQ in scopolamine treated. The data were analyzed using One way ANOVA followed by Tukey's multiple range test.

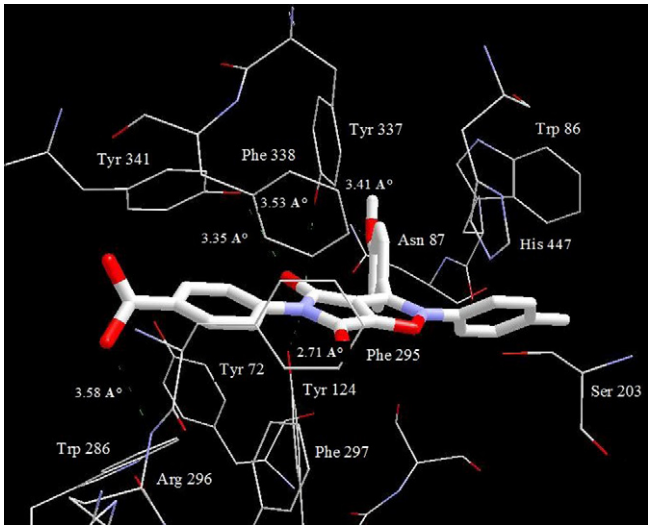


Figure 5. The docking view of compound **8ia** with AChE (PDB code 1B41) showing five hydrogen bond interactions (shown by broken lines) among the different amino acid residues and structural parts of compound. The different atoms are shown in different colors, that is, nitrogen with blue, oxygen with red, and carbon with white.

4.1.3.2. *Trans*-4-[(3H,3aH,6aH)-3-phenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7aa′). White solid, yield 22%, mp 170 °C; IR (Nujol) υ 1714, 1682, 1598, 1372, 1280, 1210 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.72 (1H, s, H_3), 5.23–5.24 (1H, d, H_{3a}, J = 7.4 Hz), 4.13–4.15 (1H, d, H_{6a}, J = 7.4 Hz), 6.7–8.0 (14H, m, aromatic H), 10.8 (1H, s, COOH); MS: m/z 415 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_5$: C, 69.56; H, 4.38; N, 6.76; O, 19.30. Found: C, 67.23; H, 4.14; N, 6.23; O, 18.92.

4.1.3.3. *Cis*-4-[(3H,3aH,6aH)-3-(2-hydroxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ba). White solid, yield 40%, mp 204 °C; IR (Nujol) υ 3310, 1717, 1690, 1599, 1380, 1285, 1210 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.33–5.35 (1H, d, H_3, J = 7.68 Hz), 5.17–5.21 (1H, q, H_{3a}, J = 6.92 and 7.14 Hz), 4.22–4.24 (1H, d, H_{6a}, J = 7.76 Hz), 6.65–8.14 (13H, m, aromatic H), 12.5 (1H, s, COOH), 8.35 (1H, s, OH); MS: m/z 431 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_6$: C, 66.97; H, 4.22; N, 6.51; O, 22.30. Found: C, 65.23; H, 4.12; N, 6.24; O, 21.13.

4.1.3.4. *Trans*-4-[(3H,3aH,6aH)-3-(2-hydroxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ba′). White solid, yield 20%, mp 190–192 °C; IR (Nujol) υ 3305, 1716, 1691, 1600, 1381, 1286, 1210 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 6.04 (1H, s, $H_{3,}$), 5.19–5.21 (1H, d, H_{3a}, J = 7.44 Hz), 4.17–4.19 (1H, d, H_{6a}, J = 7.44 Hz), 6.65–8.12 (13H, m, aromatic H), 9.64 (1H, s, COOH), 8.25 (1H, s, OH); MS: m/z 431 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_6$: C, 66.97; H, 4.22; N, 6.51; O, 22.30. Found: C, 65.36; H, 4.02; N, 6.12; O, 21.35.

4.1.3.5. *Cis*-4-[(3H,3aH,6aH)-3-(4-hydroxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid(7ca). White solid, yield 53%, mp 217–218 °C; IR (Nujol) υ 3308, 1712, 1698, 1605, 1378, 1287, 1204 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.43–5.45 (1H, d, H_3, J = 8.02 Hz), 5.25–5.28 (1H, q, H_{3a}, J = 7.12 and 7.56 Hz), 4.35–4.37 (1H, d, H_{6a}, J = 7.92 Hz), 6.75–8.40 (13H, m, aromatic H), 12.25 (1H, s, COOH), 8.75 (1H, s, OH); MS: m/z 431 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_6$: C, 66.97; H, 4.22; N, 6.51; O, 22.30. Found: C, 66.79; H, 4.21; N, 6.49; O, 22.32.

4.1.3.6. *Trans*-4-[(3H,3aH,6aH)-3-(4-hydroxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ca′). White solid, yield 22%, mp 199 °C; IR (Nujol) υ 3312, 1711, 1697, 1605, 1378, 1287, 1204 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 6.14 (1H, s, $H_{3,}$), 5.31–5.33 (1H, d, H_{3a}, J = 7.53 Hz), 4.38–4.40 (1H, d, H_{6a}, J = 7.43 Hz), 6.18–8.13 (13H, m, aromatic H), 11.34 (1H, s, COOH), 8.74 (1H, s, OH); MS: m/z 431 $(M+H)^+$; Anal. Calcd for $C_{24}H_{18}N_2O_6$: C, 66.97; H, 4.22; N, 6.51; O, 22.30. Found: C, 66.75; H, 4.23; N, 6.42; O, 22.19.

4.1.3.7. *Cis*-4-[(3H,3aH,6aH)-3-(2-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7da). White solid, yield 42%, mp 202 °C; IR (Nujol) υ 1715, 1685, 1600, 1387, 1270, 1200 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.19–5.21 (1H, d, H_3, J = 7.65 Hz), 4.31–4.36 (1H, q, H_{3a}, J = 7.56 and 8.01 Hz), 4.24–4.26 (1H, d, H_{6a}, J = 7.52 Hz), 7.01–8.45 (13H, m, aromatic H), 10.95 (1H, s, COOH); MS: m/z 449.9 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 63.21; H, 3.21; N, 6.10; O, 16.23; Cl, 7.85.

4.1.3.8. *Trans*-4-[(3H,3aH,6aH)-3-(2-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7da′). White solid, yield 25%, mp 180–181 °C; IR (Nujol) υ 1716, 1684, 1602, 1388, 1271, 1202 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 6.02 (1H, s, $H_{3,}$), 5.54–5.56 (1H, d, H_{3a}, J = 7.45 Hz), 5.41–5.43 (1H, d, H_{6a}, J = 8.21 Hz), 7.08–8.31 (13H, m, aromatic H), 10.6 (1H, s, COOH); MS: m/z 449.5 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 63.21; H, 3.12; N, 5.89; O, 17.10; Cl, 7.87.

4.1.3.9. *Cis*-4-[(3H,3aH,6aH)-3-(3-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ea). White solid, yield 39%, mp 168–170 °C; IR (Nujol) υ 1714, 1688, 1608, 1391, 1274, 1205 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.12–5.14 (1H, d, H_3, J = 7.34 Hz), 4.26–4.30 (1H, q, H_{3a}, J = 7.23 and 7.80 Hz), 4.19–4.21 (1H, d, H_{6a}, J = 8.23 Hz), 7.02–8.41 (13H, m, aromatic H), 10.7 (1H, s, COOH); MS: m/z 449.9 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 64.19; H, 3.64; N, 6.17; O, 17.79; Cl, 7.82.

4.1.3.10. *Trans*-4-[(3H,3aH,6aH)-3-(3-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ea′). White solid, yield 26%, mp 143–145 °C; IR (Nujol) υ 1715, 1686, 1607, 1392, 1273, 1204 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.83 (1H, s, $H_{3,}$), 5.46–5.48 (1H, d, H_{3a}, J = 7.66 Hz), 5.30–5.32 (1H, d, H_{6a}, J = 7.12 Hz), 7.08–8.19 (13H, m, aromatic H), 10.5 (1H, s, COOH); MS: m/z 449.9 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 64.24; H, 3.85; N, 6.12; O, 17.65; Cl, 7.88.

4.1.3.11. *Cis*-4-[(3H,3aH,6aH)-3-(4-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7fa). White solid, yield 50%, mp 205–207 °C; IR (Nujol) υ 1717, 1690, 1603, 1389, 1275, 1140 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 4.93–4.95 (1H, d, H_3, J = 8.26 Hz), 4.16–4.21 (1H, q, H_{3a}, J = 7.32 and 8.68 Hz), 4.11–4.13 (1H, d, H_{6a}, J = 7.41 Hz), 6.76–8.13 (13H, m, aromatic H), 10.1 (1H, s, COOH); MS: m/z 449.9 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 62.32; H, 3.12; N, 6.02; O, 16.92; Cl, 7.84.

4.1.3.12. *Trans*-4-[(3H,3aH,6aH)-3-(4-chlorophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7fa′). White solid, yield 26%, mp 190–192 °C; IR (Nujol) υ 1715, 1686, 1607, 1382, 1273, 1157 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.76 (1H, s, $H_{3,}$), 5.39–5.41 (1H, d, H_{3a}, J = 7.64 Hz), 5.26–5.28 (1H, d, H_{6a}, J = 7.36 Hz), 6.76–8.07 (13H, m, aromatic H), 10.0 (1H, s, COOH); MS: m/z 449.9 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}ClN_2O_5$: C, 64.22; H, 3.82; N, 6.24; O, 17.82; Cl, 7.90. Found: C, 62.10; H, 3.24; N, 5.78; O, 17.04; Cl, 7.81.

4.1.3.13. *Cis*-4-[(3H,3aH,6aH)-3-(2-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ga). White solid, yield 42%, mp 200–201 °C; IR (Nujol) υ 1711, 1695, 1599, 1391, 1283, 1187 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.12–5.14 (1H, d, H_3, J = 8.04 Hz), 4.92–4.89 (1H, q, H_{3a}, J = 7.95 and 8.21 Hz), 4.01–4.02 (1H, d, H_{6a}, J = 7.84 Hz), 6.76–7.89 (13H, m, aromatic H), 10.3 (1H, s, COOH), 3.73 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 66.03; H, 4.02; N, 6.19; O, 20.12.

4.1.3.14. *Trans*-4-[(3H,3aH,6aH)-3-(2-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ga′). White solid, yield 24%, mp 180–182 °C; IR (Nujol) υ 1711, 1694, 1598, 1392, 1283, 1187 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.23 (1H, s, $H_{3,}$), 5.12–5.14 (1H, d, H_{3a}, J = 7.37 Hz), 4.02–4.04 (1H, d, H_{6a}, J = 7.42 Hz), 6.51–8.01 (13H, m, aromatic H), 10.3 (1H, s, COOH), 3.72 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 66.89; H, 4.28; N, 5.98; O, 21.13.

4.1.3.15. *Cis*-4-[(3H,3aH,6aH)-3-(3-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ha). White solid, yield 40%, mp 205–206 °C; IR(Nujol) υ 1712, 1690, 1600, 1386, 1284, 1180 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.36–5.38 (1H, d, H_3, J = 7.41 Hz), 5.17–5.21 (1H, q, H_{3a}, J = 7.62 and 8.19 Hz), 4.02–4.04 (1H, d, H_{6a}, J = 7.12 Hz), 6.50–8.12 (13H, m, aromatic H), 10.2 (1H, s, COOH), 3.74 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 67.42; H, 4.52; N, 6.24; O, 21.45.

4.1.3.16. *Trans*-4-[(3H,3aH,6aH)-3-(3-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ha′). White solid, yield 21%, mp 170–172 °C; IR (Nujol) υ 1710, 1692, 1601, 1387, 1284, 1181 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.70 (1H, s, $H_{3,}$), 5.26–5.28 (1H, d, H_{3a}, J = 7.47 Hz), 4.13–4.15 (1H, d, H_{6a}, J = 7.31 Hz), 6.51–8.05 (13H, m, aromatic H), 10.7 (1H, s, COOH), 3.82 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 66.46; H, 4.48; N, 6.21; O, 21.42.

4.1.3.17. *Cis*-4-[(3H,3aH,6aH)-3-(4-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ia). White solid, yield 43%, mp 195–196 °C; IR (Nujol) υ 1720, 1695, 1604, 1389, 1271, 1170 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.55–5.57 (1H, d, H_3, J = 7.25 Hz), 5.36–5.40 (1H, q, H_{3a}, J = 7.28 and 7.56 Hz), 4.15–4.17 (1H, d, H_{6a}, J = 7.44 Hz), 6.8–8.12 (13H, m, aromatic H), 10.6 (1H, s, COOH), 3.80 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 65.20; H, 4.24; N, 5.68; O, 20.34.

4.1.3.18. *Trans*-4-[(3H,3aH,6aH)-3-(4-methoxyphenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ia′). White solid, yield 20%, mp 160–162 °C; IR (Nujol) υ 1719, 1695, 1604, 1390, 1272, 1170 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.85 (1H, s, $H_{3,}$), 5.42–5.44 (1H, d, H_{3a}, J = 7.35 Hz), 4.26–4.28 (1H, d, H_{6a}, J = 7.65 Hz), 6.86–8.12 (13H, m, aromatic H), 10.7 (1H, s, COOH), 3.85 (1H, s, OCH_3); MS: m/z 445.4 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 66.32; H, 4.25; N, 6.06; O, 21.02.

4.1.3.19. *Cis*-4-[(3H,3aH,6aH)-3-(2-nitrophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ja). White solid, yield 45%, mp 212–214 °C; IR (Nujol) υ 1712, 1680, 1608, 1368, 1271, 1150 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.29–5.31 (1H, d, H_3, J = 7.7 Hz), 4.39–4.41 (1H, q, H_{3a}, J = 7.38 and 7.56 Hz), 4.28–4.27 (1H, d, H_{6a}, J = 7.44 Hz), 6.6–8.03 (13H, m, aromatic H), 10.5 (1H, s, COOH); MS: m/z 460 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}N_3O_7$: C, 62.75; H, 3.73; N, 9.15; O, 24.38. Found: C, 62.71; H, 3.74; N, 9.10; O, 24.08.

4.1.3.20. *Trans*-4-[(3H,3aH,6aH)-3-(2-nitrophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ja′). White solid, yield 35%, mp 181–182 °C; IR (Nujol) υ 1711, 1681, 1608, 1369, 1272, 1151 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.46 (1H, s, $H_{3,}$), 4.74–4.72 (1H, d, H_{3a}, J = 7.38 Hz), 4.36–4.38 (1H, d, H_{6a}, J = 7.44 Hz), 6.92–8.04 (13H, m, aromatic H), 10.6 (1H, s, COOH); MS: m/z 460 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}N_3O_7$: C, 62.75; H, 3.73; N, 9.15; O, 24.38. Found: C, 61.49; H, 3.70; N, 9.04; O, 24.18.

4.1.3.21. *Cis*-4-[(3H,3aH,6aH)-3-(4-nitrophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ka). White solid, yield 50%, mp 225–227 °C; IR (Nujol) υ 1713, 1685, 1605, 1372, 1275, 1161 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.12–5.14 (1H, d, H_3, J = 7.80 Hz), 4.31–4.28 (1H, q, H_{3a}, J = 7.42 and 7.86 Hz), 4.12–4.14 (1H, d, H_{6a}, J = 8.02 Hz), 6.84–8.10 (13H, m, aromatic H), 10.6 (1H, s, COOH); MS: m/z 460 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}N_3O_7$: C, 62.75; H, 3.73; N, 9.15; O, 24.38. Found: C, 62.72; H, 3.64; N, 9.07; O, 24.20.

4.1.3.22. *Trans*-4-[(3H,3aH,6aH)-3-(4-nitrophenyl)-4,6-dioxo-2-phenyldihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (7ka′). White solid, yield 30%, mp 170–172 °C; IR (Nujol) υ 1714, 1684, 1605, 1372, 1276, 1162 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.35 (1H, s, H_3), 4.51–4.54 (1H, d, H_{3a}, J = 7.50 Hz), 4.24–4.26 (1H, d, H_{6a}, J = 7.37 Hz), 6.74–8.11 (13H, m, aromatic H), 10.5 (1H, s, COOH); MS: m/z 460 $(M+H)^+$; Anal. Calcd for $C_{24}H_{17}N_3O_7$: C, 62.75; H, 3.73; N, 9.15; O, 24.38. Found: C, 62.65; H, 3.69; N, 9.08; O, 24.35.

4.1.3.23. *Cis*-4-[(3H,3aH,6aH)-3-phenyl-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8aa). White solid, yield 45%, mp 215–217 °C; IR (Nujol) υ 1714, 1682, 1596, 1372, 1286, 1210 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.39–5.41 (1H, d, H_3, J = 7.65 Hz), 4.87–4.92 (1H, q, H_{3a}, J = 7.61 and 7.95 Hz), 4.14–4.16 (1H, d, H_{6a}, J = 7.95 Hz), 6.73–8.14 (13H, m, aromatic H), 9.99 (1H, s, COOH), 2.23 (3H, s, CH_3); MS: m/z 430 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_5$: C, 70.08;

H, 4.71; N, 6.54; O, 18.67. Found: C, 70.01; H, 4.72; N, 6.50; O, 18.60.

4.1.3.24. *Trans*-4-[(3H,3aH,6aH)-3-phenyl-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8aa′). White solid, yield 30%, mp 161–162 °C; IR (Nujol) υ 1716, 1684, 1595, 1370, 1284, 1212 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.65 (1H, s, $H_{3,}$), 5.12–5.14 (1H, d, H_{3a}, J = 7.57 Hz), 4.15–4.17 (1H, d, H_{6a}, J = 7.52 Hz), 6.29–7.96 (13H, m, aromatic H), 10.01 (1H, s, COOH), 2.25 (3H, s, CH_3); MS: m/z 430 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_5$: C, 70.08; H, 4.71; N, 6.54; O, 18.67. Found: C, 69.95; H, 4.35; N, 6.43; O, 18.42.

4.1.3.25. *Cis*-4-[(3H,3aH,6aH)-3-(2-hydroxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ba). White solid, yield 48%, mp 204 °C; IR (Nujol) υ 3310, 1715, 1693, 1600, 1383, 1286, 1206 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.23–5.25 (1H, d, H_3, J = 7.90 Hz), 5.02–5.05 (1H, q, H_{3a}, J = 7.39 and 7.89 Hz), 4.17–4.19 (1H, d, H_{6a}, J = 7.80 Hz), 6.45–8.01 (12H, m, aromatic H), 11.8 (1H, s, COOH), 8.35 (1H, s OH), 2.19 (3H, s, CH_3); 8MS: m/z 446 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 67.52; H, 4.49; N, 6.32; O, 21.47.

4.1.3.26. *Trans*-4-[(3H,3aH,6aH)-3-(2-hydroxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ba′). White solid, yield 26%, mp 185–186 °C; IR (Nujol) υ 3300, 1714, 1693, 1602, 1382, 1287, 1204 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.92 (1H, s, $H_{3,}$), 5.05–5.07 (1H, d, H_{3a}, J = 7.47 Hz), 4.07–4.09 (1H, d, H_{6a}, J = 7.42 Hz), 6.45–7.98 (12H, m, aromatic H), 9.2 (1H, s, COOH), 8.34 (1H, s, OH), 2.21 (3H, s, CH_3); MS: m/z 446 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 66.99; H, 4.36; N, 6.27; O, 21.51.

4.1.3.27. *Cis*-4-[(3H,3aH,6aH)-3-(4-hydroxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ca). White solid, yield 59%, mp 227–228 °C; IR (Nujol) υ 3310, 1713, 1688, 1607, 1380, 1287, 1160 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.23–5.25 (1H, d, H_3, J = 7.82 Hz), 5.35–5.38 (1H, q, H_{3a}, J = 7.02 and 7.51 Hz), 4.25–4.27 (1H, d, H_{6a}, J = 7.72 Hz), 6.50–8.24 (12H, m, aromatic H), 12.1 (1H, s, COOH), 8.50 (1H, s, OH), 2.25 (3H, s, CH_3); MS: m/z 446 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 67.02; H, 4.39; N, 6.09; O, 20.85.

4.1.3.28. *Trans*-4-[(3H,3aH,6aH)-3-(4-hydroxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ca′). White solid, yield 24%, mp 210–212 °C; IR (Nujol) υ 3312, 1711, 1679, 1606, 1380, 1285, 1164 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 6.04 (1H, s, $H_{3,}$), 5.37–5.39 (1H, d, H_{3a}, J = 7.23 Hz), 4.28–4.30 (1H, d, H_{6a}, J = 7.23 Hz), 6.71–8.20 (12H, m, aromatic H), 11.95 (1H, s, COOH), 8.74 (1H, s, OH),(2.27 (3H, s, CH_3); MS: m/z 446 $(M+H)^+$; Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30; O, 21.60. Found: C, 67.50; H, 4.53; N, 6.28; O, 21.55.

4.1.3.29. *Cis*-4-[(3H,3aH,6aH)-3-(2-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8da). White solid, yield 43%, mp 200 °C; IR (Nujol) υ 1715, 1686, 1601, 1386, 1272, 1201 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.25–5.27 (1H, d, H_3, J = 7.40 Hz), 4.21–4.25 (1H, q, H_{3a}, J = 7.46 and 8.11 Hz), 4.12–4.14 (1H, d, H_{6a}, J = 7.25 Hz), 6.83–8.01 (12H, m, aromatic H), 9.92 (1H, s, COOH), 2.22 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 64.85; H, 4.15; N, 6.01; O, 17.22; Cl, 7.60.

4.1.3.30. *Trans*-4-[(3H,3aH,6aH)-3-(2-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8da′). White solid, yield 30%, mp 182–183 °C; IR (Nujol) υ 1717, 1685, 1601, 1389, 1272, 1205 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.82 (1H, s, $H_{3,}$), 5.14–5.16 (1H, d, H_{3a}, J = 7.22 Hz), 5.02–5.04 (1H, d, H_{6a}, J = 7.88 Hz), 6.51–7.89 (12H, m, aromatic H), 10.01 (1H, s, COOH), 2.24 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 64.75; H, 4.12; N, 5.85; O, 17.15; Cl, 7.52.

4.1.3.31. *Cis*-4-[(3H,3aH,6aH)-3-(3-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ea). White solid, yield 53%, mp 202–203 °C; IR (Nujol) υ 1715, 1689, 1609, 1392, 1276, 1206 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.21–5.23 (1H, d, H_3, J = 7.24 Hz), 4.46–4.49 (1H, q, H_{3a}, J = 7.32 and 7.88 Hz), 4.29–4.31 (1H, d, H_{6a}, J = 8.33 Hz), 6.72–7.84 (12H, m, aromatic H), 9.7 (1H, s, COOH), 2.22 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 63.84; H, 4.02; N, 5.97; O, 17.25; Cl, 7.57.

4.1.3.32. *Trans*-4-[(3H,3aH,6aH)-3-(3-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ea′). White solid, yield 26%, mp 146–148 °C; IR (Nujol) υ 1717, 1682, 1602, 1381, 1271, 1200 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.73 (1H, s, $H_{3,}$), 5.40–5.42 (1H, d, H_{3a}, J = 7.52 Hz), 5.22–5.24 (1H, d, H_{6a}, J = 7.16 Hz), 6.88–8.02 (12H, m, aromatic H), 10.01 (1H, s, COOH), 2.19 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 64.85; H, 4.10; N, 5.90; O, 17.20; Cl, 7.46.

4.1.3.33. *Cis*-4-[(3H,3aH,6aH)-3-(4-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8fa). White solid, yield 50%, mp 220 °C; IR (Nujol) υ 1711, 1691, 1605, 1390, 1285, 1145 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 4.95–4.97 (1H, d, H_3, J = 8.20 Hz), 4.18–4.23 (1H, q, H_{3a}, J = 7.12 and 8.58 Hz), 4.09–4.11 (1H, d, H_{6a}, J = 7.31 Hz), 6.70–8.02 (12H, m, aromatic H), 10.4 (1H, s, COOH), 2.25 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 64.25; H, 4.03; N, 6.01; O, 17.16; Cl, 7.33.

4.1.3.34. *Trans*-4-[(3H,3aH,6aH)-3-(4-chlorophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8fa′). White solid, yield 31%, mp 165–167 °C; IR (Nujol) υ 1712, 1687, 1607, 1378, 1283, 1158 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.65 (1H, s, $H_{3,}$), 5.25–5.27 (1H, d, H_{3a}, J = 7.66 Hz), 5.16–5.18 (1H, d, H_{6a}, J = 7.16 Hz), 6.67–7.98 (12H, m, aromatic H), 9.99 (1H, s, COOH), 2.15 (3H, s, CH_3); MS: m/z 464.5 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_5$: C, 64.87; H, 4.14; N, 6.05; O, 17.28; Cl, 7.66. Found: C, 64.23; H, 4.07; N, 6.02; O, 17.23; Cl, 7.42.

4.1.3.35. *Cis*-4-[(3H,3aH,6aH)-3-(2-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ga). White solid, yield 35%, mp 200–202 °C; IR (Nujol) υ 1713, 1693, 1600, 1390, 1282, 1189 cm^{-1}; ^1H NMR1H NMR (DMSO, 400 MHz) δ 5.02–5.04 (1H, d, H_3, J = 7.884 Hz), 4.78–4.83 (1H, q, H_{3a}, J = 7.75 and 8.11 Hz), 424–4.26 (1H, d, H_{6a}, J = 7.87 Hz), 6.46–7.97 (12H, m, aromatic H), 10.2 (1H, s, COOH), 3.70 (1H, s, OCH_3), 2.27 (3H, s, CH_3); MS: m/z

459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 68.09; H, 4.74; N, 5.95; O, 20.85.

4.1.3.36. *Trans*-4-[(3H,3aH,6aH)-3-(2-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ga′). White solid, yield 24%, mp 161–162 °C; IR (Nujol) υ 1714, 1680, 1609, 1386, 1263, 1210 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.20 (1H, s, $H_{3,}$), 5.02–5.04 (1H, d, H_{3a}, J = 7.44 Hz), 4.34–4.36 (1H, d, H_{6a}, J = 7.46 Hz), 6.61–8.01 (12H, m, aromatic H), 10.2 (1H, s, COOH), 3.65 (1H, s, OCH_3), 2.17 (3H, s, CH_3); MS: m/z 459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 68.10; H, 4.86; N, 6.02; O, 20.78.

4.1.3.37. *Cis*-4-[(3H,3aH,6aH)-3-(3-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ha). White solid, yield 40%, mp 180 °C; IR (Nujol) υ 1717, 1692, 1603, 1385, 1265, 1188 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.26–5.28 (1H, d, H_3, J = 7.34 Hz), 5.08–5.11 (1H, q, H_{3a}, J = 7.68 and 8.20 Hz), 4.62–4.64 (1H, d, H_{6a}, J = 7.12 Hz), 6.57–8.07 (12H, m, aromatic H), 10.1 (1H, s, COOH), 3.67 (1H, s, OCH_3), 2.21 (3H, s, CH_3); MS: m/z 459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 67.94; H, 4.80; N, 6.09; O, 20.68.

4.1.3.38. *Trans*-4-[(3H,3aH,6aH)-3-(3-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ha′). White solid, yield 25%, mp 150–152 °C; IR (Nujol) υ 1715, 1687, 1607, 1377, 1274, 1161 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.46 (1H, s, $H_{3,}$), 5.22–5.24 (1H, d, H_{3a}, J = 7.57 Hz), 4.13–4.15 (1H, d, H_{6a}, J = 7.33 Hz), 6.25–8.05 (12H, m, aromatic H), 10.02 (1H, s, COOH), 3.72 (1H, s, OCH_3), 2.23 (3H, s, CH_3); MS: m/z 459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 67.77; H, 4.76; N, 5.42; O, 20.60.

4.1.3.39. *Cis*-4-[(3H,3aH,6aH)-3-(4-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ia). White solid, yield 53%, mp 201–203 °C; IR (Nujol) υ 1714, 1675, 1608, 1378, 1273, 1207 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.45–5.47 (1H, d, H_3, J = 7.15 Hz), 5.25–5.29 (1H, q, H_{3a}, J = 7.34 and 7.65 Hz), 4.18–4.20 (1H, d, H_{6a}, J = 7.41 Hz), 6.28–8.01 (12H, m, aromatic H), 10.12 (1H, s, COOH), 3.78 (1H, s, OCH_3), 2.26 (3H, s, CH_3); MS: m/z 459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 67.42; H, 4.62; N, 5.46; O, 20.49.

4.1.3.40. *Trans*-4-[(3H,3aH,6aH)-3-(4-methoxyphenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ia′). White solid, yield 26%, mp 182–183 °C; IR (Nujol) υ 1718, 1691, 1605, 1380, 1262, 1160 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.79 (1H, s, $H_{3,}$), 5.35–5.38 (1H, d, H_{3a}, J = 7.25 Hz), 4.56–4.58 (1H, d, H_{6a}, J = 7.45 Hz), 6.01–7.89 (12H, m, aromatic H), 10.2 (1H, s, COOH), 3.82 (1H, s, OCH_3), 2.23 (3H, s, CH_3); MS: m/z 459 $(M+H)^+$; Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11; O, 20.94. Found: C, 67.84; H, 4.72; N, 6.01; O, 19.84.

4.1.3.41. *Cis*-4-[(3H,3aH,6aH)-3-(2-nitrophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ja). White solid, yield 44%, mp 218–220 °C; IR (Nujol) υ 1711, 1685, 1603, 1378, 1281, 1140 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.31–5.33 (1H, d, H_3, J = 7.43 Hz), 4.57–4.60 (1H, q, H_{3a}, J = 7.28 and 7.47 Hz), 4.38–4.40 (1H, d, H_{6a}, J = 7.22 Hz), 6.24–8.12 (12H, m, aromatic H), 11.01 (1H, s, COOH), 2.24 (3H, s, CH_3); MS: m/z 475 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}N_3O_7$: C, 63.42; H, 4.05; N, 8.88; O, 23.66. Found: C, 63.41; H, 4.01; N, 8.78; O, 22.33.

4.1.3.42. *Trans*-4-[(3H,3aH,6aH)-3-(2-nitrophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ja′). White solid, yield 23%, mp 195–197 °C; IR (Nujol) υ 1713, 1682, 1609, 1368, 1271, 1154 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.44 (1H, s, $H_{3,}$), 4.56–4.58 (1H, d, H_{3a}, J = 7.38 Hz), 4.26–4.28 (1H, d, H_{6a}, J = 7.24 Hz), 6.42–7.64 (12H, m, aromatic H), 10.4 (1H, s, COOH), 2.22 (3H, s, CH_3); MS: m/z 475 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}N_3O_7$: C, 63.42; H, 4.05; N, 8.88; O, 23.66. Found: C, 63.35; H, 3.85; N, 8.35; O, 23.57.

4.1.3.43. *Cis*-4-[(3H,3aH,6aH)-3-(4-nitrophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl]benzoic acid (8ka). White solid, yield 48%, mp 229–231 °C; IR (Nujol) υ 1712, 1686, 1606, 1373, 1276, 1163 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.03–5.05 (1H, d, H_3, J = 7.67 Hz), 4.23–4.26 (1H, q, H_{3a}, J = 7.44 and 7.68 Hz), 4.05–4.07 (1H, d, H_{6a}, J = 8.12 Hz), 6.68–8.04 (12H, m, aromatic H), 10.2 (1H, s, COOH), 2.21 (3H, s, CH_3); MS: m/z 475 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}N_3O_7$: C, 63.42; H, 4.05; N, 8.88; O, 23.66. Found: C, 63.25; H, 3.97; N, 8.39; O, 23.10.

4.1.3.44. *Trans*-4-[(3H,3aH,6aH)-3-(4-nitrophenyl)-4,6-dioxo-2p-tolydihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3H,6H,6aH)-yl] benzoic acid (8ka′). White solid, yield 27%, mp 205–208 °C; IR (Nujol) υ 1715, 1685, 1607, 1373, 1275, 1185 cm^{-1}; 1H NMR (DMSO, 400 MHz) δ 5.26 (1H, s, $H_{3,}$), 4.55–4.56 (1H, d, H_{3a}, J = 7.67 Hz), 4.29–4.31 (1H, d, H_{6a}, J = 7.48 Hz), 6.54–8.02 (12H, m, aromatic H), 10.1 (1H, s, COOH), 2.19 (3H, s, CH_3); MS: m/z 475 $(M+H)^+$; Anal. Calcd for $C_{25}H_{19}N_3O_7$: C, 63.42; H, 4.05; N, 8.88; O, 23.66. Found: C, 63.45; H, 3.98; N, 8.53; O, 23.48.

4.2. Biological evaluation

4.2.1. Experimental animals

Swiss albino mice of either sex, weighing 20–25 g (procured from Punjab Agriculture University, Ludhiana) were employed for in vivo evaluation of learning and memory. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) 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. 107/1999/CPCSEA).

4.2.2. Drugs and chemicals

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

4.2.3. In vitro estimation of cholinesterase inhibitory activity

The brain AChE inhibitory activity of the test compounds was measured spectrophotometerically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm by the method of Ellmann et al.[11] The potency of test compounds were expressed IC_{50} with donepezil as standard acetyl cholinesterase inhibitor. The most potent test compound (compound **8ia**) was further evaluated for butyrylcholinesterase (BChE) inhibitory activity in rat plasma using Ellmann method[11] and its potency was also expressed in IC_{50}.

4.2.4. Assessment of learning and memory by Morris water maze

Morris water maze is one of the most commonly used animal models to test memory.[12] It consists of large circular pool and is di-

vided in to 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, (ii) time spent in target quadrant (Q4) on fifth day of trial, that is, the day of retrieval.

4.2.5. Experimental protocol

Seven groups, each group comprising six Swiss albino mice, were employed in the present study.

4.2.5.1. 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 fifth day of trial (an index of retrieval).

4.2.5.2. Group II: scopolamine treated control. Scopolamine bromide (0.4 mg/kg ip) 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.

4.2.5.3. Group III, IV, and V: test compound(compound 8ia) (2, 5, and 10 mg/kg) in scopolamine treated control. The test compound with most potent acetyl cholinesterase inhibitory activity (compound **8ia**) was administered in scopolamine treated mice (30 min prior to scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

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

4.2.5.5. Group VII: donepezil in scopolamine treated control. Donepezil (25 mg/kg) was administered in scopolamine treated mice (30 min prior to scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

Acknowledgments

The authors duly acknowledge the financial support from UGC New Delhi, India, through the Research Scheme No. 39-716/2010 (SR). The authors are also thankful to Dr. Nirmal Singh, Reader in Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, for his valuable suggestions.

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