SECTION 6

SUMMARY & CONCLUSION
The present study was designed to synthesize pyrrolo-isoxazole benzoic acid derivatives and novel carbamate derivatives of coumarin and of flavanones as potential AChE inhibitors followed by their screening for AChE inhibitory activity using *in vitro* testing along with *in-vivo* evaluation of the most potent AChE inhibitor for the memory restorative effect in a mice model of amnesia. The findings of the present study may be summarised as follows:

1. The synthesis of pyrrolo-isoxazole benzoic acid derivatives involved ring opening cyclization of p-aminobenzoic acid with maleic anhydride to yield maleanilic acid (P1), which in turn afforded N-arylmaleimide (P2) via ring closed cyclization. Azomethine-N-oxides (P5a-k, P6a-k) were obtained by condensation of N-arylhydroxylamine (P3) with differently substituted benzaldehydes (P4a-k) followed by refluxing of N-arylmaleimide with differently substituted azomethine-N-oxides to pyrrolo-isoxazole benzoic acid derivatives (P7a-k, P8a-k) as cis- and trans-stereoisomers.

2. The synthesis of carbamate substituted coumarin derivatives involved benzylation of 7-hydroxy-4-methyl coumarin (C1) with substituted benzoyl chloride (C2a-C2h) in the presence of cold 5% NaOH solution provided substituted 4-methyl-2-oxo-2H-chromen-7-ylbenzoate (C3a-C3h) that in turn was treated with H2SO4 and NaN3 to afford substituted 4-methyl-2-oxo-2H-chromen-7-ylphenylcarbamates (C4a-C4h).

3. The synthesis of carbamate substituted flavanone derivatives involved base-catalysed Claisen-Schmidt condensation reaction of 2-hydroxy acetophenone (F1) / 2-hydroxy-4,6-dimethoxyacetophenone (F1’) with differently substituted benzaldehydes (F2a-F2g) to yield differently substituted chalcones (F3a-F3g) and (F3a’-F3g’) that underwent intra-molecular oxidative cyclization on refluxing with glacial acetic acid to yield flavanone compounds (F4a-F4g) and (F4a’-F4g’). Thereafter, refluxing of
flavanone compounds (F4a-F4g) and (F4a’-F4g’) with phenyl isocyanate in the presence of petroleum-ether and triethylamine provided phenyl carbamate substituted flavanone derivatives (F5a-F5g) and (F5a’-F5g’).

4. All pyrrolo-isooxazole benzoic acid derivatives demonstrated potent AChE inhibitory activity. The most of compounds exhibited similar activity to donepezil and four of them (P7h, P7i, P8i, P8h, 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 P8ia (cis isomer) (IC$_{50}$ = 17.5 ± 1.5 nM) being the most active one. The test compound P8ia also ameliorated scopolamine-induced amnesia in mice in terms of restoration of time spent in target quadrant (TSTQ) and escape latency time (ELT).

5. All coumarin derivatives with substituted phenylcarbamate moiety (C4a-C4h) demonstrated potent AChE inhibitory as compared to parent 7-hydroxy-4-methylcoumarin. The phenylcarbamate substituted coumarin derivative C4d displayed most potent AChE inhibitory activity with IC50 = 13.5 ± 1.7 nM along with amelioration of amnesia in mice in terms of restoration of TSTQ and ELT in Morris Water maze test.

6. All the carbamate substituted 5,7-dimethoxyflavanone derivatives (F5a’-F5g’) compounds exhibited AChE inhibitory with IC$_{50}$ value ranging from 21.5 ± 1.8 to 9.9 ± 1.6 nM with compound (F5f’) being the most potent compound. Compound F5f’ also ameliorated scopolamine-induced amnesia in mice in terms of restoration of TSTQ and ELT.

7. The compounds P8ia, C4d and F5f’ also displayed number of significant interactions (hydrogen bonding and π- π) among the structural components of the synthesised compounds and amino acids residues of AChE enzyme.
8. It may be concluded that pyrrolo-isoxazole benzoic acid derivatives and carbamate substituted coumarin and 5,7-dimethoxyflavanones may serve as promising structural template for the development of novel AChE inhibitors in managing amnestic disorders including AD.
Abstract

The present study was designed to synthesize pyrrolo-isoxazole benzoic acid derivatives and novel carbamate derivatives of coumarin and of flavanones as potential AChE inhibitors followed and for memory restorative effects. The synthesis of pyrrolo-isoxazole benzoic acid derivatives involved ring opening cyclization of p-aminobenzoic acid with maleic anhydride to yield maleanilic acid (P1), which in turn afforded N-arylmaleimide (P2) via ring closed cyclization. Azomethine-N-oxides (P5a-k, P6a-k) were obtained by condensation of N- arylhydroxylamine (P3) with differently substituted benzaldehydes (P4a-k) followed by refluxing of N-arylmaleimide with differently substituted azomethine-N-oxides to pyrrolo-isoxazole benzoic acid derivatives (P7a-k, P8a-k) as cis- and trans- stereoisomers. The synthesis of carbamate substituted coumarin derivatives involved benzoylation of 7-hydroxy-4-methyl coumarin (C1) with substituted benzoyl chloride (C2a-C2h) in the presence of cold 5% NaOH solution provided substituted 4-methyl-2-oxo-2H-chromen-7-ylbenzoate (C3a-C3h) that in turn was treated with H2SO4 and NaN3 to afford substituted 4-methyl-2-oxo-2H-chromen-7-ylphenylcarbamates (C4a-C4h). The synthesis of carbamate substituted flavanone derivatives involved base-catalysed Claisen-Schmidt condensation reaction of 2-hydroxy acetophenone (F1)/2-hydroxy-4,6-dimethoxyacetophenone (F1’) with differently substituted benzaldehydes (F2a-F2g) to yield differently substituted chalcones (F3a-F3g) and (F3a’-F3g’) that underwent intra-molecular oxidative cyclization on refluxing with glacial acetic acid to yield flavanone compounds (F4a-F4g) and (F4a’-F4g’). Thereafter, refluxing of flavanone compounds (F4a-F4g) and (F4a’-F4g’) with phenyl isocyanate in the presence of petroleum-ether and triethylamine provided phenyl carbamate substituted flavanone derivatives (F5a-F5g) and (F5a’-F5g’). All the synthesised derivatives demonstrated potent AChE inhibitory activity. The compounds P8ia C4d and F5f exhibited potent
AchE inhibitory activity and also ameliorated scopolamine-induced amnesia in mice. It may be concluded that pyrrolo-isoxazole benzoic acid derivatives and carbamate substituted coumarin and 5,7-dimethoxyflavanones may serve as promising structural template for the development of novel AChE inhibitors in managing amnestic disorders including AD.