Synthesis, molecular docking and biochemical analysis of aminoalkylated naphthale-based chalcones as acetylcholinesterase inhibitors

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ABSTRACT

Twelve novel chalcones were synthesized using 2-alkyloxy-naphthaldehydes and Mannich bases of 4-hydroxyacetophenone. The chalcones were characterized using FTIR, 1D and 2D NMR and HRMS spectroscopy. Comparative docking analysis was carried out to screen their affinity towards the AChE enzyme (PDB 1EVE). All chalcones showed lower binding energy (−13.06 to −10.43 kcal/mol) against AChE better than donepezil (−10.52 kcal/mol). All chalcones were potent inhibitors towards AChE, with IC50 values ranging between 0.11 and 5.34 nM better than donepezil (IC50 33.4 nM) and selectivity indexes (0.66–23.83), despite the fact that chalcones 10 and 13 were inactive. The structure activity relationship indicated that introducing diethyl amine in ring A of the chalcone skeleton and the propargyl moiety at ring B was affirmed to be a prospective drug against AChE. The multifunctional properties of chalcone 15 were all advantages that demonstrate an excellent candidate for the development of an effective drug against AChE.

1. Introduction

Cholinesterases (ChE), including Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE), belong to serine hydrolase family; both enzymes are esterase catalyzing the hydrolytic breakdown of neurotransmitter acetylcholine (ACh) [1]. According to the classical cholinergic hypothesis, AChE terminates the neurotransmission at the cholinergic synapse by hydrolyzing the neurotransmitter ACh causing the cognitive impairment in Alzheimer’s disease (AD) patients [2, 3]. AChE and BuChE differ in kinetics and substrate selectivity since the BuChE lacks six aromatic amino acids out of the fourteen that line the catalytic gorge of AChE [4, 5]. Strong evidence of the correlation between high selectivity for AChE versus BuChE and therapeutic index of the inhibitor was investigated in vivo by Liston et al. [6]. It was suggested that high selectivity for AChE might contribute to the clinically favourable tolerability profile of drugs in Alzheimer’s disease patients. AChE inhibitors are still the best available pharmacotherapy for AD patients [7].

The Food and Drug Administration approved (FDA) treatments for AD belong to a category of acetylcholinesterase inhibitors (AChEIs) namely rivastigmine, donepezil, and galantamine [8–10]. Although Tacrine was the first drug approved for the AD treatment in 1993, it was withdrawn from the market because of a high incidence of hepatotoxicity [11]. Unfortunately, most of the commercial medications were observed to be associated with adverse side effects [12]. Hence, the search for novel AChE is still of great interest.

Recently, it became a trend to use natural products such as chalcones in discovering cholinesterase inhibitors due to their slight side-effects [13]. Chalcone based derivatives have gained attention due to their simple structures with diverse pharmacological actions [14]. The presence of a reactive α, β-unsaturated keto function in chalcones, is found to be responsible for their bioactivities. In the past years, a variety of chalcones have been reviewed to highlight the recent evidence of chalcone as a privileged scaffold in medicinal chemistry [15–17]. Fascinatingly, it has been reported that some chalcone derivatives can be considered as a multifunctional agent for AD treatment [18]. By screening AChEIs inhibitors in clinical application, some researchers claimed that amino substituent was possibly important pharmacophore of them [19]. Thereby, a series chalcone derivatives containing amino substituents were designed and synthesized in their investigations [20, 21]. Their results suggested that dimethylamine, diethylamine, dipropylamine, pyrrolidine-containing chalcones had more
potent effects in inhibiting AChE compared with other nitrogen-containing chalcones.

Liu et al. (2014) were inspired to synthesize novel cholinesterase inhibitors by introducing Mannich bases to the well-known chalcone flavokawin B due to its diverse bioactivities (Figure 1). As a matter of fact, the AChE inhibitory activity was poor for the parent compound \( \text{IC}_{50} > 500 \, \mu \text{M} \). However, most of its Mannich base derivatives were potential AChE inhibitors especially piperidine derivative, which was more active than rivastigmine by two-fold. Meanwhile, it can bind with both the catalytic site and the peripheral site of AChE binding pocket according to the molecular docking result[22].

Furthermore, many researchers have argued that molecules bearing phenolic Mannich base moieties may exhibit good antioxidant, AChE inhibitory activity and chelating metal properties [23–25]. It has conclusively been shown that targets with these properties have been majorly included in the multi-target directed ligands regimen (MTDLs) of AChE inhibitors [2]. More recent studies in AD have confirmed that incorporate Mannich bases moiety and chalcone scaffold in one pharmacophore exerted moderate inhibitory potency for EeAChE with good multifunctional properties [26,27].

This project emphasizes on structure-based drug design of AChE inhibitors following the MTDLs approach to design chalcone analogues. Assuming that the novel synthesized chalcones will be an efficient AChE inhibitor with antioxidant property.

Furthermore, recent evidence suggests that the presence of a different ring or fused ring system, make the drug structure more rigid [28]. This rigidity improves the probability of binding to the active site in the correct conformation. Hence, we aimed to introduce naphthalene in a chalcone skeleton to satisfy the primary structural necessity for the deep hydrophobic active site, as presented in Figure 2. Moreover, we proposed side chain R₁ to form three different alkoxy derivatives, namely propyloxy, propargyloxy and benzyloxy. This modification was done to increase the probability of cation-\( \pi \) interaction between the proposed ligands and the CAS residues. Likewise, the discrepancy of the side alkyl chain on the naphthalene motif was to facilitate the interaction with the high aromatic content of gorge walls.

**2. Results and discussion**

**2.1. Synthesis of chalcone derivatives**

The known precursors \( 2(\text{a-c}) \) were obtained via SN2 nucleophilic substitution reaction of commercially available 2-hydroxynaphthaldehyde (1) with alkyl halide under basic conditions using the sonication procedure in good to excellent yields as reported in the literature [30–32]. Simultaneously, Mannich bases \( 4(\text{a-d}) \) were prepared in a one-step condensation reaction of 4-hydroxyacetophenone (3), formaldehyde and different secondary amines using microwave irradiations according to our published method [33].

The target chalcones were obtained via Claisen–Schmidt condensation reaction of 2-alkoxy naphthaldehyde derivatives \( 2(\text{a-c}) \) with appropriate Mannich bases of 4-hydroxyacetophenone \( 4(\text{a-d}) \) using a catalytic amount of \( \text{SOCl}_2 \) in ethanol to furnish the novel chalcones \( 5–16 \) in an excellent yield (83–98%) as illustrated in Scheme 1. The structures of the newly chalcones \( 5–16 \) were characterized based on their spectroscopic analysis. Chalcone 6 was chosen as a model to verify the pattern of such compounds (Figure 3). The IR spectrum
of compound 6 showed a broad absorption band at 3433 cm\(^{-1}\) for hydroxyl group and stretching vibration at 2938 cm\(^{-1}\) for aliphatic CH (\(sp^2\)). Also, it displayed absorption frequency at 1645 cm\(^{-1}\) indicating the presence of conjugated carbonyl group besides the characteristic band C=O, olefinic peak at 1602 cm\(^{-1}\) and absorption band of C-N at 1297 cm\(^{-1}\).

Moreover, \(^1H\) NMR spectrum confirmed the formation of chalcone 6 due to the presence of an AB spin system at \(\delta_H 7.97\) and 8.27 (\(J = 16.0\) Hz each) attributed to H-\(\alpha\) and H-\(\beta\). These two doublets are characteristic of trans-olefinic protons of chalcone. The spectrum also disclosed an ABX spin system at \(\delta_H 8.29\) (1H, d, \(J = 2.0\) Hz), 8.07 (1H, d, \(J = 8.5\) and 2.0 Hz), and 7.17 (1H, d, \(J = 8.5\) Hz) attributed to three aromatic protons in the A ring. While the prominent triplet signal at \(\delta_H 5.15\) and doublet peak at the downfield region \(\delta_H 4.26\) were attributed to propargyl protons’ H-3\(^{\prime}\) and H-1\(^{\prime}\), respectively. Interestingly, this multiplicity pattern is due to long-range \(^1H–^1H\) couplings between H-3\(^{\prime}\) and H-1\(^{\prime}\) as depicted in the COSY spectrum (Supplementary Figure S5). Additionally, DEPT and \(^13\)C NMR spectra of chalcone 6 displayed three signals at downfield region \(\delta_C 57.27\) (C-1\(^{\prime}\)), 79.77 (C-2\(^{\prime}\), C), 79.18 (C-3\(^{\prime}\), C) which confirmed the existence of propargyl moiety. The HMBC spectrum of chalcone 6 supported the assignment of the quaternary carbons and the connectivity within the carbon framework. For example, a long range \(^1H–^1H\) correlations were observed between H-\(\alpha\) (\(\delta_H 7.97\)) and H-\(\beta\) (\(\delta_H 8.27\)) with C-1(\(\delta_C 132.59\)) and carbonyl carbon (\(\delta_C 187.98\)), H-\(\beta\) (\(\delta_H 8.27\)) with C-\(\alpha\) (\(\delta_C 129.22\)) and C-2 (\(\delta_C 155.29\)). The protons and carbons assignment of chalcone 6 was also supported and reconfirmed by HMQC and HMBC spectra. The pseudo molecular ion peak detected at \(m/z\) 426.2064 ([M + H]\(^+)\) (calcd. 425.1991) recorded in the HRESIMS spectrum was in good agreement with the molecular formula C\(_{28}\)H\(_{27}\)NO\(_3\). The complete elucidation of chalcone 6 is listed in Table 1.

### 2.2. In silico forecasts drug properties prediction of library compound/chalcone derivatives

Although considerable efforts were devoted to achieve selectivity for AChE as a target, and indeed, these days, many ligands endowed with outstanding in vitro selectivity are available [34]. However, it should be noted that a highly selective ligand for a given target does not always result in a clinically efficient drug. Experimental in vivo investigations of such drugs are not only significantly intricate but also expensive. Computational methods such as docking are commonly used to simulate the ligand interactions with the target to highlight its affinity. Apart from the docking functions, computational biology approaches have led the researchers to have an idea of structure–activity relationship (SAR) and pharmacokinetic properties (absorption, distribution, metabolism, excretion, and toxicity or ADMET) of the potential ligands [35]. The application of various computational tools, thus, helps save time that was spent in traditional combinatorial chemistry screening experiments [36].

#### 2.2.1. In silico prediction of physiochemical properties, drug likeness and bioactivity of the synthesized chalcones

The analyses of the physiochemical properties have been widely used to filter out compounds with undesirable properties, especially poor ADMET profile [37]. Furthermore, drug-likeness is another characteristic, which provides the base for the compound to be an efficient drug candidate. The most famous drug-likeness filter the “Rule of Five” has been proposed by Lipinski et al.[38], which provides five rules to determine whether a molecule is well orally absorbed or

![Figure 3. Chalcone 6.](image)
Bioactivity of the synthesized chalcones. The bioactivity of the drug, as their drug-likeness scores range from 1.08-1.88. The novel chalcones are most likely to be active drugs towards GPCR ligands, nuclear receptor ligands; and other enzyme targets. Besides, chalcone derivatives are moderately active towards kinase inhibitors, ion channel modulators and protease inhibitors. Though, for more specific target prediction, the Swiss Target Prediction server was used [41]. Strikingly, the target prediction result confirms our suggestions that the modified chalcones could be AChE inhibitors, as exemplified in Figure 4.

2.2.2. In silico pharmacokinetic and toxicity predictions of chalcones 5–16
Pharmacokinetic and toxicity screening are tabulated in Tables 4 and 5. All tested derivatives 5–16 have shown high gastrointestinal (GI) absorption, which is a good indicator of oral bioavailability. Also, most of the chalcones have shown blood–brain permeability except compounds 7, 10, 14, 16. The ability to cross the BBB is an essential feature for the potency of AChE inhibitors. All chalcones are P-gp inhibitors, implies that active efflux across biological membranes is not possible. All chalcones were inhibitors for CYP2C19 and CYP2D6 implicating potential increased in other drug concentration as these compounds might not be metabolized by the liver enzymes hence accumulate inside the body except chalcones except 7 and 13. Toxicity screening showed that all chalcones are noncarcinogenic and non-mutagenic, excluding compound 16, which is predicted to be mutagenic. The computed LD50 in the rat from the acute toxicity prediction appears to be adequately benign in the range between 2.56 and 2.82 mol/kg.

Table 2. Physicochemical properties and drug-likeness of synthesized chalcones 5-16.

| Compound No. | Mol. Wt. | RB | HBA | HBD | TPSA | ilogP | Lipinski | Bioavailability Score | Drug Likeness Score |
|--------------|----------|----|-----|-----|------|-------|----------|----------------------|-------------------|
| 5            | 429.55   | 8  | 4   | 1   | 49.77| 4.41  | Yes      | 0.55                | 1.88              |
| 6            | 425.52   | 7  | 4   | 1   | 49.77| 3.93  | Yes      | 0.55                | 1.61              |
| 7            | 477.59   | 8  | 4   | 1   | 49.77| 4.06  | Yes      | 0.55                | 1.45              |
| 8            | 415.52   | 8  | 4   | 1   | 49.77| 4.37  | Yes      | 0.55                | 1.78              |
| 9            | 411.49   | 7  | 4   | 1   | 49.77| 3.63  | Yes      | 0.55                | 1.51              |
| 10           | 463.57   | 8  | 4   | 1   | 49.77| 3.85  | Yes      | 0.55                | 1.36              |
| 11           | 431.52   | 8  | 5   | 1   | 59.00| 3.96  | Yes      | 0.55                | 1.68              |
| 12           | 427.49   | 7  | 5   | 1   | 59.00| 4.09  | Yes      | 0.55                | 1.41              |
| 13           | 479.57   | 8  | 5   | 1   | 59.00| 4.17  | Yes      | 0.55                | 1.28              |
| 14           | 417.54   | 10 | 4   | 1   | 49.77| 4.29  | Yes      | 0.55                | 1.41              |
| 15           | 413.51   | 9  | 4   | 1   | 49.77| 3.87  | Yes      | 0.55                | 1.36              |
| 16           | 465.58   | 10 | 4   | 1   | 49.77| 4.24  | Yes      | 0.55                | 1.08              |

RB: number of rotatable bonds; HBD: number of hydrogen bond donor and acceptor; TPSA: total polar surface area.

Table 3. Molinspiration bioactivity score.

| Compd. No. | GI absorption | BBB permeability | P-gp CYP2C19 inhibitor | CYP2D6 inhibitor |
|------------|---------------|------------------|------------------------|------------------|
| 5          | High          | Yes              | Yes                    | Yes              |
| 6          | High          | Yes              | Yes                    | Yes              |
| 7          | High          | No               | Yes                    | No               |
| 8          | High          | Yes              | Yes                    | Yes              |
| 9          | High          | Yes              | Yes                    | Yes              |
| 10         | High          | No               | Yes                    | Yes              |
| 11         | High          | Yes              | Yes                    | Yes              |
| 12         | High          | Yes              | Yes                    | Yes              |
| 13         | High          | Yes              | Yes                    | Yes              |
| 14         | High          | No               | Yes                    | Yes              |
| 15         | High          | Yes              | Yes                    | Yes              |
| 16         | High          | No               | Yes                    | Yes              |

GI, gastrointestinal; P-gp, P-glycoprotein; BBB, blood-brain barrier; CYP2C19 and CYP2D6, cytochrome P450.
2.2.3. Molecular docking
Comparative docking analysis was carried out on twelve different chalcone derivatives to screen their binding affinity on the Torpedo californica acetylcholinesterase (TcAChE) (PDB 1EVE). Firstly, to validate the docking parameter, the co-crystallized ligand donepezil (E20) was re-docked into the active site of the AChE enzyme. The parameters are considered successful with Root Mean Square Deviation (RMSD) value of the docking structure is less than 1.5 Å and the ligand orientation display similar interactions as reported in the crystal structure [42]. The validation experiment is illustrated in Figure 5, which shows the superimposition of both the re-docked donepezil (red) and its respective conformation in the crystal structure (blue) within the active site of AChE, indicating that the selected docking parameters are acceptable. The RMSD value for docking conformation is 1 Å. Docking analysis of the synthesized chalcone derivatives 5–16 demonstrated lower binding energy than donepezil (−10.52 kcal/mol) indicating increased in affinity towards AChE enzyme as illustrated in Figure 6.

The interaction mode of the docked chalcones demonstrated that the piperidine moiety in chalcones 5 and 7, was stacked against the amino acids PHE331 and TRP84 at the anionic site, respectively, whereas, in chalcone 6, it was stacked against TYR334 in PAS (see Appendix, Tables S41-S44). Moreover, it illustrated that diethyl amine moiety in chalcone 14 possessed a flexible structure that enabled it to be stacked against
Table 5. Toxicity predictions of the synthesized chalcones using admetSAR.

| Compound Number | Mutagenicity Ames Test | Carcinogenicity | Acute Oral Toxicity mol/kg |
|-----------------|------------------------|-----------------|----------------------------|
| 5               |                        |                 | 2.60                       |
| 6               |                        |                 | 2.82                       |
| 7               |                        |                 | 2.81                       |
| 8               |                        |                 | 2.61                       |
| 9               |                        |                 | 2.78                       |
| 10              |                        |                 | 2.77                       |
| 11              |                        |                 | 2.69                       |
| 12              |                        |                 | 2.82                       |
| 13              |                        |                 | 2.82                       |
| 14              |                        |                 | 2.56                       |
| 15              |                        |                 | 2.80                       |
| 16              |                        |                 | 2.77                       |

Figure 5. Superimposition of docking conformation of TcAChE-donepezil complex (red stick) and its crystal structure (blue stick) (PDB: 1EVE).

Table 6. DPPH radical scavenging activity of the chalcones 5-16.

| Compound | DPPH Radical IC₅₀ μg/ml |
|----------|-------------------------|
| 5        | 39.68 ± 0.9             |
| 6        | 42.47 ± 1.8             |
| 7        | 55.52 ± 1.1             |
| 8        | 41.66 ± 1.5             |
| 9        | 12.57 ± 0.8             |
| 10       | 19.34 ± 0.7             |
| 11       | 42.10 ± 1.3             |
| 12       | 35.36 ± 1.5             |
| 13       | 42.70 ± 1.1             |
| 14       | 44.50 ± 0.7             |
| 15       | 40.58 ± 1.7             |
| 16       | 37.52 ± 1.8             |
| Ascorbic acid | 17.96 ± 0.4              |

Figure 6. Lowest binding energy of the top-ranked conformations of the resulted complexes of docking experiments.

TRP279 while in chalcone 15 and 16, the same moiety stacked against TRP84. Meanwhile, the pyrrolidine moiety in chalcone 8 stacked against PHE331 and TRP279 in chalcones 9 and 10. Whereas the morpholine moiety in chalcones 11, 12 was stacked against the anionic site (PHE331), but in chalcone 13, it showed interaction with the acyl pocket amino acid (PHE288).

To consider the overall efficacy for chalcones as AChEIs in terms of substitution at ring B, it was necessary to screen the binding profiles of chalcone derivatives in depth. As a comparison, it was found that chalcone 5 showed competitive inhibition via direct catalytic active sites (CAS) only among propyl series. In contrast, the remaining derivatives of the propyl series demonstrated several potent interactions with the CAS and weak interaction with PAS without any interaction with the critical amino acid TRP279, despite chalcone 14. For the propargyl series, the binding network of 9 revealed non-competitive inhibition due to the absence of the interaction with the CAS residues, especially Trp84, as presented in Figure 7. At the same time, chalcone 15 exhibited CAS and PAS’s dual binding site inhibitor. The overlay of the AChE-15 complex with the complex of
2.3. Bioactivities

2.3.1. DPPH radical scavenging activity

The radical scavenging potential of chalcone derivatives 5–16 with ascorbic acid (AA) as positive control is shown in Table 6. All chalcones displayed potent DPPH radical scavenging activity (IC$_{50}$ 12.57-55.52 µg/ml). It is interesting to note that chalcones 9 and 10 were the most potent antioxidant with IC$_{50}$ 12.57 and 19.34 µg/ml, respectively. All chalcones were found to scavenge DPPH in a dose-dependent manner, as portrayed in Figure 9. Chalcones 5–16 owning hydroxyl group at para position in ring A that readily reacted with the radicals and converted to phenoxy radical due to the electron delocalization of the relative coplanar structure of the chalcone, which also responsible for the excellent scavenging activity [43].

2.3.2. Cholinesterases enzyme inhibitory activity

Ellman’s spectrophotometric method was followed as described by Koay et al. to evaluate the cholinesterase inhibitory activities of chalcones 5–16 [44]. The AChE from an electric eel, BuChE from an equine serum and donepezil as the reference standard was utilized for this evaluation, as shown in Table 7, Figures 10 and 11. It is apparent from this table that chalcones 5–16 clearly showed higher potency against AChE (IC$_{50}$ ranging from 0.11–5.34 nM) than donepezil (IC$_{50}$ 33.4 nM) despite chalcones 10 and 13. The present findings seem to be generally consistent with the docking results, which suggested that chalcones bearing the Mannich base might be better inhibitors than donepezil. Besides, all synthesized chalcones were found to be more effective inhibitors towards AChE than BuChE, with high selectivity indexes (0.66–23.83).
Scheme 1. Synthesis of 2-alkoxynaphthyl chalcone bearing Mannich bases 5–16.

2.4. Structure–activity relationships

The \textit{in vitro} studies declared that chalcone with a piperidine substituent is potent AChEIs excluding propargyl derivative. However, piperidine derivatives’ potency was accompanied by lower selectivity indexes towards AChE ranging between 0.66–3.34. On the other hand, diethylamine derivatives disclosed higher efficacy and selectivity as AChEIs. While pyrrolidine derivatives 8–10
Table 7. Cholinesterase inhibitory activity of chalcones 5–16.

| Compound | AChE (IC₅₀) [nM] ± SD | BuChE (IC₅₀) [nM] ± SD | SI |
|----------|-----------------------|------------------------|----|
| 5        | 0.33 ± 0.002          | 0.80 ± 0.003           | 2.44 |
| 6        | 0.57 ± 0.005          | 1.90 ± 0.002           | 3.34 |
| 7        | 1.01 ± 0.003          | 0.67 ± 0.003           | 0.66 |
| 8        | 0.86 ± 0.004          | 2.68 ± 0.003           | 3.10 |
| 9        | 0.48 ± 0.003          | 11.35 ± 0.005          | 23.79 |
| 10       | ND                    | ND                     | ND |
| 11       | 2.80 ± 0.001          | 18.58 ± 0.001          | 6.64 |
| 12       | 5.01 ± 0.015          | 2.69 ± 0.005           | 5.33 |
| 13       | > 500                 | 2.69 ± 0.003           | -   |
| 14       | 0.66 ± 0.002          | 3.88 ± 0.005           | 5.89 |
| 15       | 0.11 ± 0.002          | 2.59 ± 0.003           | 23.83 |
| 16       | 5.34 ± 0.002          | 4.84 ± 0.002           | 0.91 |
| Donepezil| 33.4 ± 0.002          | 2246.0 ± 0.003         | 67.25 |

ND = not determined; IC₅₀ ± SD: Inhibitor concentration (mean ± SD of three experiments) needed for 50% inhibition of the enzyme; SI: selectivity index = IC₅₀(BuChE)/IC₅₀(AChE).

were less potent than piperidine and diethylamine analogues with selectivity indexes (3.10 – 23.79). It was evident that chalcones with a morpholine substituent at ring A demonstrated the lowest inhibitory activity towards AChE among the four series. These findings corroborate the ideas of Zhang et al. (2019). They suggested that the electron-withdrawing effects of oxygen atoms at the morpholine unit might reduce the electronic density of amines and further impact its protonation, affecting the interaction between the terminal nitrogen and AChE.

A correlation between the structure and inhibitory activity attributes of the novel chalcones towards AChE was established. In brief, modifications at ring A using different amines showed that introducing diethyl amine is favourable due to its flexibility that enabled the chalcone to be extended into the PAS and CAS region of the active site and increase AChE inhibition. This structural flexibility is absent when introduced cyclic amines such as piperidine, pyrrolidine or morpholine due to their structural rigidity. Moreover, introducing the hydrophilic cyclic amine morpholine demonstrated practically the lowest inhibitory activity towards AChE among the four series.

On the other hand, the general tendency for AChE inhibition from the perspective of substitution at ring B based on the in vitro analysis was propargyl > propyl > benzyl, as presented in the histogram illustration in Figure 6. However, this finding contrasts with pre-evaluations of docking simulations based on the binding affinity. The efficacy of the propargyl derivatives might be attributable to the high electron density and structural rigidity of the propargyl fragment when compared to propyl. Furthermore, it was predicted for propyl analogues that chalcone 11 (NR₂R₃: morpholine) had almost similar binding energy for chalcone 5 (NR₂R₃: piperidine) (Figure 4). Interestingly, the in vitro potency of 11 was eight folds lower than 5. This distinctive difference in efficacy can be explained based on the binding profiles of both chalcones, as presented in Appendix, Table S41, chalcone 5 showed three hydrogen bonds with critical amino acids of the active site SER200, HIS400 and PHE330, while chalcone 11 showed only one hydrogen bond with PHE330 at the anionic site with distance 2.13Å.

It is notable that propargylated chalcones 6, 9 and 12 (R₂: piperidine, pyrrolidine and morpholine) demonstrated nearly the same affinity against AChE, however, chalcone 9 was more selective to inhibit AChE with nearly four folds with SI = 23.79. This variation in the selectivity towards AChE was due to the missing interaction with TRP279 in chalcones 6 and 12. It has been reported that TRP279 and PHE330 amino acid residues are conserved in AChE but absent in BuChE, which leads to the selectivity that may be important for clinical consideration, as inhibition of BuChE may cause potentiating side effects (Kryger et al., 1999). Likewise, chalcone 15 (NR₂R₃: diethylamine) shows five folds higher potency than chalcone 6 (NR₂R₃: piperidine) with the highest selectivity index 23.83. Moreover, potency of chalcone 15 as AChEI increased by four folds from 9, although both chalcones showed similar selectivity index towards AChE (SI = 23.79).

It is worth noting that increasing the aromaticity by introducing the benzyl moiety ended with a bulky structure of chalcone that selectively inhibits BuChE more than AChE, as presented in Figure 11. Moreover, the presence of the benzylic group causes a steric hindrance during the interaction between the chalcone and the amino acids residues of the receptor.

To sum it up, the modification at ring B using the propargyl moiety led to a competitive inhibition via direct catalytic active site (CAS), which is unfavourable. Introducing the benzyl moiety at naphthalene ring ended with a bulky structure of chalcone that selectively inhibits BuChE more than AChE. Thus, it decreased the potential AChEI of the associated chalcones. The modification using propargyl disclosed characteristic dual interactions with amino acids of both the PAS and CAS of AChE binding site in a similar manner to the reference drug, donepezil.

3. Conclusion

Four series of aminoalkylated naphthalene-based chalcones 5–16 were synthesized successively through a Claisen–Schmidt condensation reaction. The condensation reaction was done using thionyl chloride between 2-alkoxynaphthaldehyde derivatives 2(a-d) and Mannich bases 4(a-d). In silico predictions revealed that the novel chalcones are most likely to be a drug, as their drug-likeness scores range from 1.08-1.88. Pharmacokinetic screening disclosed that most of the chalcones were able to permeate through the BBB except chalcones 7,10,14 and 16, while toxicity screening showed that all chalcones are noncarcinogenic and
non-mutagenic, excluding chalcone 16, which is predicted to be mutagenic. The Swiss Target Prediction server was used to predict the suitable target for the synthesized chalcones. Consistently, the predictions confirmed our hypothesis regarding the selected target AChE before embarking the docking and the in vitro assessments.

Docking analysis was carried out on the acetylcholinesterase (TcAChE) to compare the binding affinity of chalcone derivatives. The results predicted that all chalcones 5–16 have a higher affinity compared with donepezil. Based on the bioactivities study, all of the chalcones were found to scavenge DPPH radicals with IC$_{50}$ values that ranged between 12.57 and 55.52 µg/ml. The cholinesterase inhibitory activities of donepezil (IC$_{50}$ 33.4 nM), despite that chalcones 5–16 were investigated in vitro using two enzymes AChE and BuChE, while the positive control was donepezil. Steadily with docking pre-evaluations, the novel chalcones 5–16 showed potent inhibitory activity against AChE (IC$_{50}$ 0.11–5.34 nM) more than donepezil (IC$_{50}$ 33.4 nM), despite that chalcones 10 and 13 were inactive against AChE. From the structural activity relationship (SAR), it is concluded that the potent dual site AChEI bears diethylamine at ring A and the propargyl moiety at ring B. Thus, among the promising candidates against AChE, chalcone 15 demonstrated enormous advantages, including an excellent AChE inhibitory activity, good antioxidant activity (IC$_{50}$ 40.58 µg/ml), low logP 3.87 and was able to permeate through the BBB. These multifunctional properties promoted 15 as an excellent candidate for the development of an effective drug against AChE.

4. Experimental

4.1. Chemistry

All solvents and reagents were purchased from Sigma-Aldrich and used without further purification. The monitoring of reaction was done by the utilization of pre-coated silica gel plates (60 F254), thin-layer chromatography (TLC). The normal phase silica gel (Merck, 70–230 mesh) was used to perform column chromatography (CC) purification, while the Merck silica gel (230–400 mesh) was utilized to perform the vacuum liquid chromatography (VLC). Melting points were measured using a Sanyo MPD350 apparatus with a digital display. A Perkin Elmer ATR spectrophotometer was used to record the infrared (IR) spectra without KBr. A Bruker Avance 400 MHz spectrometer was used to record $^1$H NMR and $^{13}$C NMR spectra. NMR samples were measured in DMSO, CDCl$_3$ and MeOD at room temperature. Mass spectral data were obtained from Mass Spectrometry Laboratory, King Abdulaziz University (Saudi Arabia). The absorbance data for bioactivity assays were recorded on BIOTEK Microplate reader (USA) spectrophotometer.

4.1.1. General synthesis of alkoxy naphthaldehydes (2 a-c)

2-Hydroxy-1-naphthaldehyde (1) (5.17 g, 30 mmol) was mixed with 36 mmol of potassium carbonate anhydrous in (60 mL) of N, N-dimethylformamide (DMF) as an aprotic solvent. This mixture was stirred at room temperature. Different alkyl halide, namely 1-Iodopropane, propargyl bromide, benzyl chloride (42 mmol), was added to the activated mixture and heated to 40°C using ultrasound sonication for 30 min until the reaction complete. The mixture was cooled to room temperature and poured into crushed ice until precipitation. The resulting precipitate was filtered, washed with cold water, air-dried and recrystallized using ethanol. Three alkoxy-naphthaldehydes namely 2-propanoylnaphthalene-1-carbaldehyde (2a), 2-((prop-2-yn-1-yl)oxy)naphthalene-1-carbaldehyde (2b) and 2-(benzoyloxy)naphthalene-1-carbaldehyde (2c) were accomplished.

2-propanoylnaphthalene-1-carbaldehyde (2a)

It was obtained as colourless crystals (4.5 g, 70%) with R$_f$ = 0.9 (n-hexane: EtOAc; 3:1), mp 63–65°C (Lit. mp 63–64 °C $^{30}$). IR$_{\text{max}}$ cm$^{-1}$ (ATR): 1663 (C = O), 137.50 (CH), 131.59 (C), 129.79 (CH), 128.41 (C), 128.22, 124.92, 124.67 (CH), 117.99 (C), 116.72 (C), 113.58 (CH), 71.08 (OCH$_2$), 22.70 (CH$_2$), 10.58 (CH$_3$).$^{13}$C NMR (100 MHz, CDCl$_3$): δ 192.00 (C = O), 161.90 (C-H sp$^3$), 131.44 (C), 129.91 (CH), 128.41 (C), 128.22, 124.92, 124.67 (CH), 117.99 (C), 113.58 (CH), 71.08 (OCH$_2$), 22.70 (CH$_3$), 10.58 (CH$_3$).

2-((prop-2-yn-1-yl)oxy)naphthalene-1-carbaldehyde (2b)

It was obtained as colourless crystals (4.9 g, 77%) with R$_f$ = 0.8 (n-hexane: EtOAc; 3:1), mp 113–115°C (Lit. mp 113–115 °C $^{31}$). IR$_{\text{max}}$ cm$^{-1}$ (ATR): 1659 (C = O), 137.31 (CH), 131.44 (C), 129.91 (CH), 128.11 (CO), 128.22, 124.92, 124.67 (CH), 117.99 (C), 77.68 (C = CH), 76.82 (C = CH), 57.38 (OCH$_2$).$^{13}$C NMR (100 MHz, CDCl$_3$): δ 192.00 (C = O), 161.90 (C-2), 137.31 (CH), 131.44 (C), 129.91 (CH), 128.41 (C), 128.22, 124.92, 124.67 (CH), 117.99 (C), 113.58 (CH), 71.08 (OCH$_2$), 22.70 (CH$_3$), 10.58 (CH$_3$).

2-(benzoyloxy)naphthalene-1-carbaldehyde (2c)

It was obtained as colourless crystals (6.7 g, 85%) with R$_f$ = 0.9 (n-hexane: EtOAc; 3:1), mp 113–115°C (Lit. mp 113–115 °C $^{31}$). IR$_{\text{max}}$ cm$^{-1}$ (ATR): 1663 (C = O), 137.50 (CH), 131.59 (C), 129.79 (CH), 128.41 (C), 128.22, 124.92, 124.67 (CH), 117.99 (C), 113.58 (CH), 71.08 (OCH$_2$), 22.70 (CH$_3$), 10.58 (CH$_3$).
(100 MHz, CDCl₃); δ 192.12 (CHO), 163.21 (C-2), 137.54 (CH), 135.96, 131.57 (C), 129.93, 128.83 (CH), 128.70 (C), 128.44, 128.27 (CH), 127.40, 125.01, 124.95 (CH), 117.20 (C), 113.95 (CH), 71.47 (OCH₂).

4.1.2. General synthesis of mannich base precursors (4a-d)

To a solution of 4-Hydroxy-acetophenone (3) (16 mmol) and formaldehyde (CH₂O) (1.5 equivalent) in 1,4-dioxane (15 mL), was added to the corresponding secondary amine (piperidine (a), pyrrolidine (b), morpholine (c) or diethyl amine (d)) using the same equivalent of (3). This mixture was placed in the MW vessel with stirring and capped with a rubber cap. The reaction mixture was irradiated for 15-30 min, at 120°C (power 300 W)³³. TLC was used to monitor the progress of the reaction. After the complete consumption of the starting materials, the vessel was removed and cool down to room temperature. The reaction mixture was concentrated under reduced pressure and purified using Column chromatography.

1-[4-hydroxy-3-[(piperidin-1-yl)methyl]phenyl]ethan-1-one (4a)

The reaction of compound (3), CH₂O and piperidine using the respective ratio 1:1.5:1.5 gave the crude product of (4a). The obtained product was purified using hexanes/ EtOAc (6:4) as an eluent over SiO₂ to yield (4a) as colourless crystals (2.15 gm, 58%) with Rf = 0.55, mp 82-83°C. IR: vmax cm⁻¹ (ATR): 2946 (C-H sp³), 1661 (C = O), 1594 (C = C), 1284 (C-N), 1259 (C-O). ¹H NMR (400 MHz, CDCl₃): δ 7.81 (1H, d, J = 8.4 and 2.2 Hz, H-6), 7.67 (1H, d, J = 2.2 Hz, H-2), 6.84 (1H, d, J = 8.4 Hz, H-5), 3.75 (2H, s, Benzylic-CH₂), 2.55 (7H, br s, 2 × N-CH₂CH₂, CH₃), 1.67 (6H, br s, 3 × CH₃), ¹³C NMR (100 MHz, CDCl₃): δ 196.86 (C-O), 163.32 (C-4), 130.24 (C-6), 129.35 (C-2), 128.73 (C-1), 121.06 (C-3), 115.93 (C-5), 61.51 (Benzylic-CH₂), 53.73 (2 × N-CH₂CH₂), 26.22 (CH₃), 25.60 (2 × N-CH₂CH₂), 23.71 (N-CH₂CH₂), ElMS, m/z (% rel. intensity): 233 (10) [M⁺, C₁₄H₁₉NO₂], 149 (4), 133 (2), 106 (1), 98 (5), 84 (100), 77 (3), 56 (2).

1-[4-hydroxy-3-[(pyrrolidin-1-yl)methyl]phenyl]ethan-1-one (4b)

A ratio of 1:1.5:1.5 of compound (3), CH₂O and pyrrolidine respectively were used to synthesize (4b). Eluting system of hexanes/ EtOAc (6:4), was used to purify the crude product to yield pale-yellow crystals, (1.72 g; 49%) with Rf = 0.45, mp 92-95°C. IR: vmax cm⁻¹ (ATR): 2945 (C-H sp³), 1661 (C = O), 1595 (C = C), 1287 (C-N), 1247 (C-O). ¹H NMR (400 MHz, MeOD): δ 7.83 (1H, d, J = 8.6 and 2.2 Hz, H-6), 7.79 (1H, d, J = 2.2 Hz, H-2), 6.72 (1H, d, J = 8.6 Hz, H-5), 4.03 (2H, s, Benzylic-CH₂), 2.92 (4H, t, J = 7.0 Hz, 2 × NCH₂CH₂), 2.51 (3H, s, CH₃), 1.95-1.99 (4H, m, 2 × NCH₂CH₂). ¹³C NMR (100 MHz, MeOD): δ 197.75 (C-O), 167.15 (C-4), 130.73 (C-6), 128.52 (C-2), 121.21 (C-1), 116.53 (C-5), 57.13 (Benzylic-CH₂), 52.88 (2 × NCH₂CH₂), 24.67 (CH₃), 23.02 (2 × NCH₂CH₂), ElMS, m/z (% rel. intensity): 219(13) [M⁺, C₁₃H₁₇NO₂], 149 (5), 133 (3), 106, 91 (2), 84 (9), 77 (4), 70 (100), 51 (2).

1-[4-hydroxy-3-[(morpholin-4-yl)methyl]phenyl]ethan-1-one (4c)

The purification of the crude product which has been synthesized in the respective ratio of 1:1.5:1.5 of compounds (3), CH₂O and morpholine was done using column chromatography with hexanes/ EtOAc (8:2) as eluent to yield (4c) as colourless crystals (3.02 g; 80%) with RF = 0.65, mp 69-70°C. IR: vmax cm⁻¹ (ATR): 2958 (C-H sp³), 1669 (C = O), 1597 (C = C), 1304 (C-N), 1113 (C-O). ¹H NMR (400 MHz, MeOD): δ 7.92 (1H, s, OH), 7.83 (2H, s, H-2 and H-6), 3.73 (12H, t, J = 4.6 Hz, 2 × Benzylic-CH₂, 4 × OCH₂), 2.58 (8H, t, J = 4.0 Hz, 4 × N-CH₂CH₂), 2.55 (3H, s, CH₃), ¹³C NMR (100 MHz, MeOD): δ 197.94 (C = O), 161.44 (C-4), 130.24 (C-2, C-6), 128.25 (C-1), 121.97 (C-3, C-5), 66.32 (4 × OCH₂), 58.18 (2 × Benzylic-CH₂), 52.78 (4 × N-CH₂CH₂), 24.90 (CH₃). ElMS, m/z (% rel. intensity): 334(14) [M⁺, C₁₃H₂₁NO₄], 276 (6), 247 (100), 217 (12), 189 (14), 162 (8), 133 (34), 119 (11), 86 (24), 56 (14).

1-3-[(diethylamino)methyl]-4-hydroxyphenyl]ethan-1-one (4d)

The crude product of (4d) was obtained from the reaction of the respective ratio 1:2:1:2:1 of compound (3), CH₂O and diethyl amine. The product was purified by column chromatography using a mixture hexanes/ EtOAc (6:4) to yield (4d) as a yellow oily liquid (3.56 g, 99%) with RF = 0.56. IR spectrum (vmax cm⁻¹): 2972 (C-H sp³), 1667 (C = O), 1589 (C = C), 1279 (C-N), 1255 (C-O). ¹H NMR (400 MHz, CDCl₃): δ 11.47 (1H, br s, OH), 7.73 (1H, d, J = 8.4 Hz, H-6), 7.60 (1H, s, H-2), 6.74 (1H, d, J = 8.4 Hz, H-5), 3.77 (2H, s, Benzylic-CH₂), 2.59 (4H, q, J = 7.0 Hz, 2 × NCH₂), 2.46 (3H, s, CH₃), 1.07 (6H, t, J = 7.0 Hz, 2 × NCH₂CH₂). ¹³C NMR (100 MHz, CDCl₃): δ 196.74 (C-O), 163.77 (C-4), 130.06 (C-6), 128.95 (C-2), 128.51 (C-1), 121.68 (C-3), 115.87 (C-5), 56.61 (Benzylic-CH₂), 46.22 (2 × NCH₂CH₂), 26.08 (CH₃), 10.99 (2 × NCH₂CH₂). ElMS, m/z (% rel. intensity): 221 (5) [M⁺, C₁₃H₁₉NO₂], 206 (6), 149 (8), 132 (2), 106 (1), 77 (2), 58 (100).

4.1.3. General synthesis of naphthoxy chalcones bearing Mannich bases

The corresponding precursors 4(a-d) and 2(a-c) were synthesized from 4-hydroxyacetophenone and 2-hydroxy-1-naphthaldehyde, as described in the reported literatures [34,36,45]. A mixture of Mannich bases 4 (a-d) (1 mmol) and 2-alkoxy-1-naphthaldehyde 2 (a-c) (1 mmol) in 10 mL of ethanol was stirred at room temperature. A catalytic amount of thionyl chloride was added dropwise, and the reaction mixture was kept overnight at room temperature. The reaction was monitored by TLC. After the reaction completion, the crude was allowed to stand under the cold condition for 2 h. The mixture was filtered or evaporated under reduced pressure to give the precipitate.
The resulting solid was subjected to column chromatography on silica gel using a mixture of CHCl3 and EtOH (9.9:0.1) as an eluent to yield the pure target chalcone.

E-1-[c-(piperidin-1-yl)methyl-4-hydroxyphenyl]-3-(2-propoxyanaphthal-en-1-yl)-2-propan-1-one (5)

Compounds 4a (0.23 g, 1 mmol) and 2a (0.21 g, 1 mmol) was treated as described above. The desired chalcone 5 was obtained as yellow powder (0.42 g, 98%) with $R_f = 0.35$ (EtOH: CHCl3; 1:9), mp 200-202°C. IR: $v_{max}$ cm$^{-1}$ (KBr): 3430 (OH), 3063 (C-H sp$^3$), 2937 (C-H sp$^3$), 1647 (C = O), 1604 (C = C) and 1269 (C-N).$^1$H NMR (400 MHz, DMSO): $\delta$ 8.36 (1H, $d$, $J = 15.8$ Hz, H-β), 8.20 (1H, $d$, $J = 8.4$ Hz, H-5), 8.16 (1H, $d$, $J = 2.0$ Hz, H-2), 8.06 (1H, $d$, $J = 9.2$ Hz, H-3), 7.97 (1H, $d$, $J = 15.8$ Hz, H-α), 7.94 (1H, $d$, $J = 8.8$ Hz, H-8), 7.67 (1H, $d$, $J = 9.0$ Hz, H-4), 7.57-7.63 (4H, m, H-6, H-3, H-7), 7.38-7.47 (4H, m, H-7, H-4'), 3.276 (H, H-6'), 7.02 (1H, $d$, $J = 8.8$ Hz, H-5'), 5.43 (2H, s, H-11'), 4.21 (2H, s, H-7'), 2.97 (4H, br s, H-8, H-12), 1.75 (4H, br s, H-9'/-H-11), 1.50 (2H, br s, H-10).$^1^3$C NMR (100 MHz, DMSO): $\delta$ 187.86 (C = O), 161.75 (C-4), 156.83 (C-2), 136.06 (C-7), 134.91 (C-2'), 132.93 (C-1), 132.54 (C-3), 132.16 (C-6), 129.74 (C-1'), 125.90 (C-8), 129.10 (C-3'), 128.74 (C-7), 128.76 (C-6), 128.67 (C-5'), 128.36 (C-6), 127.6 (C-4'), 122.76 (H-C/11), 21.62 (C-2'), 11.03 (C-3'). HR-APCI-MS: m/z 430.2362 [M + H]$^+$ (calcld for. C$_{28}$H$_{32}$NO$_{5}$ 447.2304).

E-1-[c-(piperidin-1-yl)methyl-4-hydroxyphenyl]-3-(2-propoxyanaphthalen-1-yl)-2-propan-1-one (6)

Compound 4a (0.22 g, 1 mmol) and 2a (0.21 g, 1 mmol) was treated as described above. Chalcone 6 was obtained as a yellow crystal (0.38 g, 91%) with $R_f = 0.33$ (EtOH: CHCl3; 1:9), mp 213-215°C. IR: $v_{max}$ cm$^{-1}$ (KBr): 3342 (OH), 2945 (C-H sp$^3$), 1646 (C = O), 1602 (C = C) and 1279 (C-N).$^1$H NMR (400 MHz, MeOD): $\delta$ 8.98 (1H, $d$, $J = 15.6$ Hz, H-β), 8.26 (1H, $d$, $J = 8.4$ Hz, H-5), 8.19 (1H, $d$, $J = 2.0$ Hz, H-2), 8.13 (1H, $d$, $J = 8.5$ and 2.0 Hz, H-6), 8.10 (1H, $d$, $J = 15.6$ Hz, H-α), 7.98 (1H, $d$, $J = 9.2$ Hz, H-3), 7.88 (1H, $d$, $J = 8.0$ Hz, H-8), 7.59 (1H, $d$, $J = 4.6$ Hz, H-7), 3.41 (4H, t, $J = 4.6$ Hz, H-7), 1.64 (2H, t, $J = 4.6$ Hz, H-7). HR-APCI-MS: m/z 478.2377 [M + H]$^+$ (calcld for. C$_{29}$H$_{33}$NO$_{5}$ 497.2304).
sp²), 2923 (C-H sp³), 2119 (C ≡ C), 1656 (C ≡ O), 1605 (C ≡ C) and 1266 (C-N), 1132 (C-O).¹ HNMR (400 MHz, DMSO): δ 8.18 (1H, d, J = 15.6 Hz, H-β), 8.17 (1H, d, J = 2.1 Hz, H-2), 8.11 (1H, d, J = 8.4 Hz, H-5), 7.98 (1H, dd, J = 8.7 and 2.1 Hz, H-6), 7.98 (1H, d, J = 9.0 Hz, H-3), 7.87 (1H, d, J = 15.6 Hz, H-α), 7.87 (1H, d, J = 8.0 Hz, H-8), 7.52 (1H, ddd, J = 8.4, 5.2, 1.2 Hz, H-6), 7.51 (1H, d, J = 9.0 Hz, H-4), 7.38 (1H, dd, J = 8.0, 0.8 Hz, H-7), 7.07 (1H, d, J = 8.7 Hz, H-5), 5.15 (2H, d, 2H, J = 2.1 Hz, H-1″), 4.26 (2H, s, H-7), 3.68 (1H, t, J = 2.4 Hz, H-3″), 3.16 (4H, br s, H-8 / H-10), 1.84 (4H, br s, H-9 / H-11).¹³C NMR (100 MHz, DMSO): δ 187.90 (C = O), 161.45 (C-14), 155.25 (C-1), 133.60 (C-β″), 134.34 (C-2), 132.54 (C-1), 132.37 (C-3), 131.14 (C-6), 129.72 (C-1′″), 129.46 (C-9), 129.24 (C-α″), 128.37 (C-6′), 127.55 (C-8), 124.79 (C-7′), 123.58 (C-5), 118.54 (C-10), 117.85 (C-3), 116.26 (C-5′), 115.18 (C-4), 79.76 (C-2″′), 79.26 (C-3″), 57.20 (C-1″′), 53.49 (C-8′), 51.14 (C-9′), 22.93 (C-9′/10′). HR-APCI-MS: m/z 412.1907 [M + H]^+ (calcd for C_{27}H_{32}NO_{3} 411.1834).

E-1-[3-(pyrrolidin-1-yl)methyl-4-hydroxy phenyl]-3-(2-(benzoyloxy)naphthalen-1-yl)-2-propen-1-one (10)

Compound 4b (0.22 g, 1 mmol) and 2c (0.26 g, 1 mmol) was treated as described above. The product 10 was obtained as a light yellow amorphous powder (0.45 g, 97%) with Rf = 0.35 (EtOH: CHCl₃: 1:9), mp 225-228°C. IR: ν_{max} cm⁻¹ (KBr): 3435 (OH), 2929 (C-H sp³), 1635 (C = O), 1593 (C = C) and 1289 (C-N), 1117 (C-O).¹ HNMR (400 MHz, DMSO): δ 8.8 (1H, d, J = 15.6 Hz, H-β), 8.22 (1H, d, J = 8.8 Hz, H-5), 8.18 (1H, d, J = 2.0 Hz, H-2), 8.07 (1H, d, J = 9.2 Hz, H-3), 7.99 (1H, d, J = 15.6 Hz, H-α), 7.94 (1H, d, J = 9.6 Hz, H-8), 7.68 (1H, d, J = 9.2 Hz, H-4), 7.58-7.65 (4H, m, H-6, H-3′, H-7′, H-5′-6′), 7.07 (1H, d, J = 8.4 Hz, H-5′), 5.43 (2H, s, H-1′), 4.32 (2H, s, H-7′), 3.12 (4H, br s, H-8′, H-11′), 1.99 (2H, br s, H-10′), 1.88 (2H, br s, H-9′),¹¹C NMR (100 MHz, DMSO): δ 187.91 (C = O), 161.31 (C-4), 158.63 (C-2), 137.14 (C-2′), 136.09 (C-β), 134.13 (C-1), 127.32 (C-3), 123.55 (C-3′), 123.07 (C-6), 129.78 (C-1′), 129.29 (C-8), 129.17 (C-9), 129.09 (C-3″), 128.73 (C-4′′), 128.68 (C-5′′), 128.6 (C-6′′), 126.75 (C-α″), 124.53 (C-7′′), 123.18 (C-5′′), 118.54 (C-5′), 116.78 (C-10), 116.06 (C-5′), 115.15 (C-4), 71.20 (C-1′), 53.46 (C-8′/11′), 51.92 (C-7′), 22.95 (C-9′/10′). HR-APCI-MS: m/z 464.2017 [M + H]^+ (calcd for C_{31}H_{32}NO_{3} 463.2147).

E-1-[3-(morpholinomethyl)-4-hydroxyphenyl]-3-(2-(benzoyloxy)naphthalen-1-yl)-2-propen-1-one (13)

Compound 4c (0.24 g, 1 mmol) and 2b (0.21 g, 1 mmol) was treated as described above. The product 13 was obtained as a light yellow needle (0.40 g, 84%) with Rf = 0.54 (EtOH: CHCl₃: 1:9), mp 205-208°C. IR: ν_{max} cm⁻¹ (KBr): 3431 (OH), 2972 (C-H sp³), 2926 (C-H sp²), 1664 (C = O), 1622 (C = C), 1274 (C-N) and 1128 (C-O).¹ HNMR (400 MHz, DMSO): δ 8.36 (1H, d, J = 15.8 Hz, H-β), 8.20 (1H, d, J = 8.4 Hz, H-5), 8.17 (1H, d, J = 2.4 Hz, H-2′), 8.05 (1H, d, J = 9.2 Hz, H-3), 7.97 (1H, d, J = 15.8 Hz, H-α), 7.94 (1H, d, J = 9.2 Hz, H-8), 7.66 (1H, d, J = 9.2 Hz, H-4), 7.56-7.63 (4H, m, H-6, H-3″, H-7″), 7.39-7.46
(4H, m, H-7, H-4', H-5', H-6''), 7.05 (1H, d, J = 8.8 Hz, H-5'), 5.07 (2H, s, H-1'), 4.13 (2H, s, H-7), 3.65 (4H, s, H-8 / 10), 3.05 (4H, brs, s, H-8 / 11). 13C NMR (100 MHz, DMSO): δ 187.94 (C = O), 161.33 (C-4), 158.86 (C-2), 137.17 (C-2'), 131.16 (C-β), 131.14 (C-2), 132.95 (C-1), 132.58 (C-3), 132.09 (C-6), 129.81 (C-1'), 129.32 (C-8), 129.20 (C-9), 129.11 (C-3', C-7''), 128.75 (C-4', C-6''), 128.71 (C-5''), 128.39 (C-6), 126.77 (C-α), 124.56 (C-7), 123.21 (C-5), 118.54 (C-3), 116.81 (C-10), 116.09 (C-5), 115.17 (C-4), 71.24 (C-1'), 63.62 (C-9 / 10), 54.24 (C-7'), 51.92 (C-8' / 11). HR-APCI-MS: m/z 480.2169 [M + H]+ (calcd. for. C32H32NO5 479.2097).

E-1-[3-(diethylenamino)methyl-4-hydroxy phenyl]-3-(2-propoxy-naph-thalen-1-yl)-2-propen-1-one (14)

Compound 4d (0.22 g, 1 mmol) and 2a (0.21 g, 1 mmol) was treated as described above. The product of 14 was obtained as a dark yellow amorphous powder (0.35 g, 84%), with Rf = 0.45 (EtOH: CHCl3: 1:9), m.p 188-191°C. IR: νmax cm⁻¹ (KBr): 3433 (OH), 2941 (CH sp³), 1642 (C = O), 1605 (C = C), 1278 (C = C) and 1093 (C-O). 1H NMR (400 MHz, DMSO): δ 8.34 (1H, d, J = 16.0 Hz, H-β), 8.25 (1H, d, J = 1.6 Hz, H-2'), 8.19 (1H, d, J = 8.4 Hz, H-5), 8.02-8.05 (1H, m, H-6'), 8.03 (1H, d, J = 9.2 Hz, H-3), 8.01 (1H, d, J = 16.0 Hz, H-α), 7.93 (1H, d, J = 8.0 Hz, H-8), 7.59 (1H, t, J = 7.6 Hz, H-6), 7.53 (1H, d, J = 9.2 Hz, H-4), 7.43 (1H, t, J = 7.6 Hz, H-7), 7.19 (1H, d, J = 8.8 Hz, H-5'), 4.29 (2H, s, H-7), 4.23 (2H, t, J = 6.4 Hz, H-1'), 3.10 (4H, q, J = 7.2 Hz, 2×CH3), 1.82-1.91 (2H, m, H-2''), 1.26 (6H, t, J = 7.2 Hz, 2×CH3), 1.03 (3H, t, J = 7.4 Hz, H-3'). 13C NMR (100 MHz, DMSO) δ 187.98 (C = O), 161.87 (C-4), 156.96 (C-2), 136.44 (C-β), 134.75 (C-2), 132.80 (C-1'), 132.56 (C-3), 132.17 (C-6), 129.78 (C-1'), 129.25 (C-8), 128.99 (C-6'), 128.31 (2 C-α), 126.75 (C-7), 124.34 (C-9), 123.34 (C-5), 117.48 (C-3'), 116.67 (C-10), 116.32 (C-5'), 114.80 (C-4), 70.88 (C-11), 49.99 (C-7'), 46.70 (2×CH3), 22.76 (C-2''), 11.06 (C-3''), 8.95 (2×CH3). HR-APCI-MS: m/z 418.2359 [M + H]+ (calcd. for. C32H31NO5 417.2304).

E-1-[3-(diethylenamino)methyl-4-hydroxy phenyl]-3-(2-prop-2-yn-1-yloxy) naphthalen-1-yl)-2-propen-1-one (15)

Compound 4d (0.22 g, 1 mmol) and 2b (0.21 g, 1 mmol) was treated as described above. The product of 15 was obtained as an orange amorphous powder (0.43 g, 92%), with Rf = 0.38 (EtOH: CHCl3: 1:9), m.p 146-148°C. IR: νmax cm⁻¹ (KBr): 3386 (OH), 3060 (C-H sp²), 2933 (C-H sp³), 1651 (C = O), 1607 (C = C) and 1267 (C-N), 1139 (C-O). 1HNMR (400 MHz, DMSO): δ 11.58 (1H, s, OH), 8.38 (1H, d, J = 15.4 Hz, H-β), 8.21 (1H, d, J = 8.8 Hz, H-5), 8.20 (1H, d, J = 2.0 Hz, H-2'), 8.06 (1H, d, J = 9.0 Hz, H-3), 8.04 (1H, d, J = 15.4 Hz, H-α), 7.95 (1H, d, J = 8.0 Hz, H-8), 7.67 (1H, d, J = 9.0 Hz, H-4), 7.58 -7.63 (4H, m, H-6, H-6', H-3', H-7'), 7.42 -7.47 (4H, m, H-7, H-4', H-5', H-6'), 7.11 (1H, d, J = 8.4 Hz, H-5), 5.44 (2H, s, H-1''), 4.25 (2H, s, H-7), 3.06 (4H, q, J = 7.2 Hz, 2×CH3), 1.25 (6H, t, J = 7.0 Hz, 2×CH3). 13C NMR (100 MHz, DMSO): δ 187.81 (C = O), 161.71 (C-4'), 158.85 (C-2'), 137.16 (C-2''), 136.05 (C-β'), 134.54 (C-2'), 132.92 (C-1), 132.58 (C-3), 132.10 (C-6'), 129.70 (C-1'), 129.31 (C-8), 129.15 (C-9), 121.10 (C-3', C-7''), 128.76 (C-4', C-6''), 128.70 (C-5''), 128.38 (C-6), 126.67 (C-α), 124.52 (C-7), 123.20 (C-5), 117.65 (C-3'), 116.68 (C-10), 116.08 (C-5'), 115.10 (C-4), 71.13 (C-1''), 50.02 (C-7'), 46.72 (2×CH2), 8.93 (2×CH3). HR-APCI-MS: m/z 466.2353 [M + H]+ (calcd. for. C32H31NO5 465.2304).

4.2. Bioactivities analysis

4.2.1. DPPH radical scavenging activity

The antioxidant evaluation was performed against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical based on the method described by Hamad et. al. [46]. Briefly, a stock solution of chalcones 5–16 in methanol were diluted to final concentrations from 1280 to 10 µg/mL. An aliquot of 40 µL of each test sample (8 serial dilutions) was mixed with 160 µL of freshly prepared methanolic solution of (DPPH) radical 100 µM and kept at 37°C for 30 min. The absorbance at 517 nm was determined. The absorbance of the DPPH radical without antioxidant (blank) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition of the DPPH radical was calculated according to the formula:

Percentage Inhibition (%) = \( \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 \)
Where \(A_{\text{blank}}\) = absorbance of the blank solution (containing DPPH solution without sample) and \(A_{\text{sample}}\) = absorbance of a sample solution. The concentration affording 50% inhibition (IC\(_{50}\)) values were calculated by plotting scavenging percentages against concentrations of the sample.

4.2.2. Acetylcholinesterase inhibitory assay

The acetylcholinesterase inhibitory activity of chalcones 5–16 were determined by Ellman’s microplate assay described by Koay et al. [44]. 140 µl of 0.1 M sodium phosphate buffer (pH 8) was first added followed by 20 µl of each test sample (in 10% methanol) and 20 µl of 0.09 unit/ml AChE. After pre-incubation at room temperature, 10 µl of 10 mM 5,5′-dithiobis (2- nitrobenzoic acid) DTNB was added into each well followed by 10 µl of 14 mM acetylthiocholine iodide as substrate. The absorbance of the coloured product was measured using a microplate reader at 412 nm following 30 min incubation. Donepezil was used as a positive control. Percentage inhibition was calculated using the following formula for different eight concentrations:

\[
\text{Percentage inhibition} = \left(\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100.
\]

Three replicates of each sample were used for statistical analysis with values reported as mean ± S.D. Standard curves were generated and calculations of the 50% inhibitory concentration (IC\(_{50}\)) values were done using GraphPad Prism for Windows (version 8.3.0) software.

4.3. In silico predictions of drug-likeness; pharmacokinetic; toxicity and target predictions

The in silico studies of the synthesized chalcones were predicted using online web tools: http://www.swissadme.ch [32]; https://www.molinspiration.com/ and http://lmmd.ecust.edu.cn/admetsar2/ [47].

4.4. Molecular docking

Docking study was carried out using AUTODOCK 4.2 as a programme to screen the binding affinity of all chalcones on the Torpedo california acetylcholinesterase (TcAChE) [48]. The X-ray crystal structure of the acetylcholinesterase complexed with donepezil E20 (PDB code: 1EVE) was obtained from the Protein Data Bank (https://www.rcsb.org/structure/1eve). All ligands and water molecules were removed from the retrieved protein using Discovery Studio Visualizer v17.2.0.16349 [49]. Docking calculations were carried out using the Lamarckian genetic algorithm (LGA), and all parameters were the same for each docking. The grid box size was set at 40,40, 40 Å³, while the centre of the grid box was set at 2.023(x), 63.295(y) and 67.062(z). The spacing between the grid points was 0.375Å. The 2D structures of the novel chalcones were sketched in ChemBio Draw Ultra 12.0, which were then converted to three-dimensional structures in ChemBio3D Ultra 12.0. and then the structures geometry optimization was performed using the PM3 process for the MOPAC Ultra 2009 programme to build the 3D pdbqt format.[50] The chalcones-protein interactions for the most stable binding modes of each chalcone in the active site of TcAChE were analyzed and visualized in Two-dimensional (2D) diagrams using Discovery Studio Visualizer.

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