Unveiling Piperazine-Quinoline Hybrids as Potential Multi-Target Directed Anti- Alzheimer's Agents: Design, Synthesis and Biological Evaluation

Afzal Nagani
Parul University
Moksh Shah
Parul University
Salman Patel
Parul University
Harnisha Patel
Parul University
Vruti Parikh
Parul University
Ashish Patel
Ramanbhai Patel College of Pharmacy, Charusat University
Sagar Patel
University of Toronto
Kirti Patel
The M.S University of Baroda
Hardik Parmar
The M.S University of Baroda
Bhargav Bhimani
Piramal Pharma Solution
M R Yadav
mryadav11@yahoo.co.in

Parul University

Research Article

Keywords: Piperazine, Quinoline, Acetylcholinesterase, Butyrylcholinesterase, Metal chelation, Antioxidant

Posted Date: March 20th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4098574/v1

Additional Declarations: No competing interests reported.

Abstract

Multi-target directed ligands (MTDLs) have recently been popularized due to their outstanding efficacy in combating the complicated features of Alzheimer's disease. This study details the synthesis of piperazine-quinoline-based MTDLs through a multicomponent Petasis reaction, targeting multiple factors such as AChE, BuChE, metal chelation to restore metal dyshomeostasis, and antioxidant activity. Some of the synthesized compounds exhibited notable inhibitory activity against AChE and BuChE enzymes at specific concentrations. Among the synthesized compounds compound (**95**) containing a 4-chloroaniline moiety and a 4-methoxybenzyl group displayed the most promising inhibitory activities against AChE (IC_{50} 3.013 μM) and BuChE (IC_{50} = 3.144 μM). Compound (**83**) featuring 2-methoxyaniline and 4-fluorobenzyl substituents, exhibited the highest BuChE inhibition (IC_{50} 1.888 μM). Notably, compound (**79**) demonstrated 93-times higher selectivity for BuChE over AChE. Out of these compounds nine compounds were assessed for antioxidant activity, displaying significant potential at a concentration of 100 μM. Moreover, all the compounds demonstrated metal chelating activity with Cu^{+2}, Zn^{+2}, Fe^{+2}, Fe^{+3} and Al^{+3}. This study provides insights into the design of novel MTDLs, highlighting compound (**95**) as a potential candidate for Inhibiting Alzheimer's disease and emphasizing its role in the development of anti-AD medication.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory loss and dementia which poses a significant global health challenge accounting for more than 55 million cases worldwide, with nearly 10 million new cases emerging annually. The disease is named after a German psychiatrist Alois Alzheimer who first described it in the year 1906 [1–3]. The exact etiology of the disease still remains unknown. Certain hypotheses, such as deposition of β-amyloid (Aβ) plaques in the neurons, increase in the levels of acetylcholinesterase and butyrylcholinesterase neurotransmitters, neurofibrillary tangles (NFT), tau protein hyperphosphorylation, dyshomeostasis of biometals, and oxidative stress have been proposed as the causative factors for the genesis of the disease. Currently, a limited number of drugs are available to treat Alzheimer’s disease which include donepezil, rivastigmine, galantamine (all acetylcholinesterase inhibitors) and memantine, an *N*-methyl-*D*-aspartate (NMDA) receptor antagonist [4], that can either temporarily delay clinical deterioration or improve the symptoms associated with AD (Fig. 1). Due to the involvement of multiple factors in AD, the conventional approach of “one molecule one target” pattern proves inadequate for the management of the disease. Hence, an appropriate strategy for developing multi-targeted directed therapy could be adopted to counter the causative factors involved in the pathogenesis of AD [5].

Acetylcholine (ACh) a neurotransmitter, vital for cognitive functions including memory and physiological regulation, is found in the synapses of the neurons. ACh is broken down into acetic acid and choline, primarily by the enzyme acetylcholinesterase (AChE) and, to a lesser extent by butyrylcholinesterase (BuChE) secreted by glial cells. AChE's interaction with nonamyloidogenic amyloid-β (Aβ) motivated the

researchers to target the AChE in cognitive disorder studies [6]. In a healthy brain, Ach is hydrolyzed by AChE, but with progression of the Alzheimer's, the level of AChE drops, and the level of BuChE enhances by 40 to 90% in the brain's hippocampus and temporal cortex areas. BuChE is also correlated with the abnormal β-amyloid (Aβ) deposition [7]. Therefore, BuChE can be a promising target for the development of novel drugs for the treatment of AD [8, 9].

As the brain ages, body's antioxidant defense mechanism weakens and an imbalance in reactive oxygen species (ROS) production occurs, increasing the risk of AD. Oxidative stress aggravates AD's progression leading to the formation of amyloid plaques and neurofibrillary tangles in the brain. To tackle AD, researchers are focusing on reducing the levels of free radicals in the brain. Recent research has revealed the therapeutic potential of compounds that can simultaneously inhibit AChE, disaggregate amyloid beta, and reduce inflammation. This multifaceted approach targets multiple aspects of AD's origin and progression, offering new avenues for developing anti-Alzheimer's therapeutics [10–12].

AD is marked by higher levels of metal ions in the brain which include Cu^{+2}, Zn^{+2}, Fe^{+2}, Fe^{+3} and Al^{+3}, with particular emphasis on Cu^{+2} and Zn^{+2}. These metals readily bind to Aβ, causing toxic Aβ oligomer aggregation in the brain [13, 14]. Iron is instrumental in impacting neurotransmitters, oxygen transport, cellular respiration, and DNA synthesis in the brain [15]. Elevated levels of iron are found in brain-damaged areas of AD patients, correlating significantly with Aβ plaques and Tau pathology [16, 17]. Zinc, the second most abundant trace element in the human body after iron, also plays a role in AD. A meta-analysis from 1984 to 2014 showed decreased serum zinc levels in AD individuals [18]. Conversely, increased Zn levels in the cerebral cortex are associated with Aβ pathology and severity of dementia [19]. Research in recent years, has explored the link between AD and abnormal copper (Cu) metabolism. Genetic evidence suggests that genes regulating copper pathways contribute to AD susceptibility, which is supported by various studies [20–22]. Variations in copper levels in serum, plasma, cerebrospinal fluid (CSF) and the brain are linked to cognitive deficits and AD development [23].

2. Designing Strategy

Structure-based drug design approach was used to design new multi-target directed ligands as promising anti-Alzheimer's agents. Piperazine scaffold has displayed versatile applications and played a vital role in drug discovery. It is associated with molecules exhibiting various activities such as anti-cancer, anti-diabetic, anti-histaminic, anti-Alzheimer's, and also it has shown improved ADME properties along with better BBB penetration when incorporated into a molecular system. Piperazine, a bioisostere of piperidine, has been used to mimic the piperidine ring present in donepezil, and many piperazine-based AChE inhibitors have been developed, such as a piperazine derivative FK960, which has shown beneficial effects in memory deficits in Alzheimer's rats and monkeys [24–29]. Therefore, in the current study we have designed some novel molecules by incorporating piperazine into a molecular frame work utilizing multi-component Petasis reaction in the synthetic scheme.

Quinoline is a privileged scaffold present in a wide variety of natural and synthetic compounds demonstrating an array of pharmacological properties. Quinoline derivatives have been found to possess a range of biological activities, such as anti-cancer, anti-malarial, analgesic, anti-tubercular, anti-bacterial, anti-protozoal, anti-glycemic, anti-inflammatory, anti-fungal, anti-hypertensive, anti-HIV, and anti-helminthic [30, 31]. Recent research indicates that certain quinoline derivatives possess significant anti-acetylcholinesterase (AChE) and anti-butyrylcholinesterase (BuChE) effects. Molecular docking studies suggest that the quinoline fragment can bind to the peripheral anionic site (PAS) of AChE through π-π stacking interaction [32]. Also, the reported metal chelation property of quinoline in desferrioxamine [33], clioquinol (CQ) [34], and 8-hydroxyquinoline derivative (PBT2) [35] makes quinoline a potential molecular framework for anti-Alzheimer's drug discovery crusade [36]. Hence, in the present study, we report the design and development of some piperazine-quinoline analogs as multi-target directed ligands (MTDLs) using multicomponent Petasis reaction, which may open new horizons for a fundamentally novel treatment for Alzheimer's disease (AD). These MTDLs were evaluated for their efficacy for AChE inhibition, BuChE inhibition, metal chelation, and antioxidants. The designing strategy is being displayed in Fig. 2.

3. Results and discussion

3. 1 Molecular Docking

To validate the rationale behind the design of the hybrid molecules the designed compounds and some reference molecules were subjected to molecular modeling studies (**Figure 3**)**.** Molecular docking studies were performed to check drug-receptor interactions, which are responsible for binding the ligands to the target proteins leading to enzyme inhibitory activity by the designed molecules. Hence, different interactions between the ligands and the target proteins were analyzed. The molecular superposition approach was validated by comparison with the original crystallographic structure of the AChE-Donepezil complex (PDB ID 7E3H) (*Human)* [37], and BuChE-Tacrine complex (PDB ID 4BDS) (*Human*) [38]. The most accurately positioned slots obtained were assessed. The outcomes are depicted in **Figure 3**, demonstrating the alignment of the proposed binding modes for the inhibitors within the active sites of AChE and BuChE. This alignment yielded a superposition RMSD of 1.07 Å for donepezil (PDB ID 7E3H) [37] and 0.70 Å for Tacrine (PDB ID 4BDS) [2, 38]. These values fall very much within the widely accepted tolerance threshold of 2.0 Å.

The study employed molecular docking with AutoDock Tools 1.5.7 and AutoDock Vina to calculate the binding energies of the synthesized ligands with the target proteins, acetylcholinesterase (*h*AChE, PDB ID: 7E3H) [38] and butyrylcholinesterase (*h*BuChE, PDB ID: 4BDS) [37]. The results are summarized in **Table 1**, revealing the docking scores of the designed compounds ranging from **-12.6 kcal/mol** to **-9.4 kcal/mol** for *h*AChE and -**12.4 kcal/mol** to **-10.3 kcal/mol** for BuChE respectively.

Table 1: Docking score of the designed compounds for *h*AChE & *h*BuChE

Comp.	Affinity (kcal/mol)		Comp.	Affinity (kcal/mol)	
	*h*AChE (7E3H)	*h*BuChE (4BDS)		*h*AChE (7E3H)	*h*BuChE (4BDS)
71	-10.2	-11.7	**86**	-12.5	-11.1
72	-9.6	-12.2	**87**	-12.3	-11.2
73	-11.3	-11.2	**88**	-12.1	-11.5
74	-12.1	-11.7	**89**	-11.8	-11.7
75	-10.8	-11.4	**90**	-11.2	-12.1
76	-12.4	-11.4	**91**	-12.2	-11.3
77	-9.4	-10.9	**92**	-9.3	-11.7
78	-12.5	-10.9	**93**	-11.0	-10.3
79	-10.9	-11.2	**94**	-11.2	-11.5
80	-12.3	-11.8	**95**	-11.1	-11.1
81	-11.3	-12.4	**96**	-12.6	-11.0
82	-12.1	-11.4	**97**	-11.0	-11.3
83	-12.1	-11.8	Tacrine	-	-8.4
84	-12.1	-10.7	Donep-ezil	-11.4	-
85	-9.6	-11.7			

The findings indicated that all of the designed compounds assumed a consistent configuration when binding to AChE and BuChE enzymes, engaging with various amino acid fragments present in the enzymes' catalytic active sites (CAS) and peripheral anionic sites (PAS) (**Tables 2** and **3**). The findings demonstrated that all the compounds exhibited favorable fitting into the catalytic active site (CAS) and displayed effective interactions with the peripheral anionic site (PAS) of AChE and showed good binding affinity, akin to the reference inhibitor donepezil (-11.4 kcal/mol), as depicted in **Figure 4**. Upon close examination of the compounds, it was observed that in the PAS, the amino acid residues TYR341 and TRP286 were engaged in π-π stacking interactions with the quinoline ring of the designed molecules. Additionally, SER289 and ARG289 formed hydrogen bonds, while PHE331 participated in π-π stacking. TRP84 and GLN69 were also involved in hydrogen bonding. Furthermore, amino acid residues SER125 and GLY121 in the CAS, interacted via hydrogen bonding with the oxygen atom of the amide linker. In the mid gorge region, TRP86 was involved in π-π stacking and TYR337 in π-sigma bonding. Other amino acid residues, including GLU202, PHE288, ASP74, and GLY448 also contributed to favorable interactions with the molecules. **Figure 5 and Table 2** depicting amino acid interactions of *in vitro* most active compound **95** and compound having lowest binding energy **96** for AChE.

Table 2: Docking scores and amino acid interactions of the standard drug donepezil and compounds (**95** & **96**) in the specific regions of *h*AChE (7E3H).

Compound	Score	Interaction with the amino acid fragments		
		Peripheral anionic site (PAS)	Catalytic active site (CAS)	Mid-gorge
Donepezil	-11.4	TRP286, TYR341,	PHE295, VAL294	TRP86, TYR337, TYR72, PHE228
95	-11.1	TRP286, TYR341	GLY448, GLY121	PHE338
96	-12.6	TRP286, PHE330	SER125	TRP86, TYR337

In case of BuChE, docking scores of the designed compounds ranged from -12.4 kcal/mol to -10.3 kcal/mol. Further analysis of the interactions between the designed compounds and the protein was conducted. The results showed that all designed compounds effectively occupied the catalytic active site (CAS) and interacted favorably with the peripheral anionic site (PAS) of BuChE, mirroring the behavior of the reference inhibitor tacrine (-8.4 kcal/mol). Upon closer examination of all of the designed compounds, it was observed that the amino acid residue TRP231 was engaged in a π-alkyl interaction with the designed molecules in the PAS. Additionally, LEU286 and PHE329 fragments exhibited π-alkyl interactions in the mid-gorge region of the enzyme, and GLY116 and GLY119 also showed interactions in the same region. In the CAS, amino acid residues TRP82 and HIS438 displayed π-π stacking interactions with the quinoline ring, ALA328 displayed π-alkyl interaction, and many compounds displayed interactions with MET437 and TYR440 residues of the CAS region. **Figure 6 and Table 3** depicting amino acid interactions of *in vitro* most active compounds **83 and 92 for BuChE.**

Table-3: Docking score and amino acid interactions of the standard drug tacrine and the compounds (**83** & **92**) in the specific regions of BuChE (4BDS).

Compound	Score	Interaction with the amino acid fragments		
		Peripheral anionic site (PAS)	Catalytic active site (CAS)	Mid-gorge
Tacrine	-8.4	TRP231	HIS438, SER198	GLY116, GLY117, PHE329
83	-11.2	TRP231	TRP82, HIS438, TYR440, SER198	LEU286, PHE329, GLY117, GLY119
92	-11.8	TRP231	TRP82, HIS438, SER198	GLY116, GLY117, LEU286, PHE329

3.2 Chemistry

The designed compounds were synthesized using a sequence of reactions as shown in **Scheme-1**. *N-Boc*-piperazine (**1**), glyoxalic acid (**3**), and boronic acids (**2a – 2c**) were reacted in the first step utilizing Petasis-Mannich multicomponent reaction in the presence of ACN solvent to obtain the intermediates (**4 - 6**). In step 2, these intermediates were coupled with substituted aromatic/cyclic amines (**7 – 15**) through acid-amine coupling reaction in the presence of EDC.HCl, HOBt and triethylamine to obtain the amides (**16 – 42**), followed by deprotection of Boc using dioxane HCl. The designed compounds were obtained by reacting the resulting intermediates (**43 – 69**) with 5-chloromethyl-8-hydroxyquinoline (**70**) in the presence of triethylamine at 100 ^{o}C in the presence of DMSO as a solvent. The chloromethyl derivative (**70**) was obtained by chloromethylation of 8-hydroxyquinoline using formaldehyde and hydrogen chloride gas.

3.3 Anti-Alzheimer's Activity

All of the synthesized piperazine derivatives (**71 – 79, 80 – 88,** and **89 – 97**) were evaluated for anticholinesterase activity against human AChE and equine BuChE enzymes, and for their metal chelation and antioxidant properties to determine their potential application against Alzheimer's disease. AChE and BuChE inhibitory assays were performed by Ellman's enzyme assay where we determined the IC_{50} values of all the designed compounds and compared them with standard drugs, donepezil for AChE inhibition and tacrine for BuChE inhibition.

Anti-oxidant activity of nine compounds were evaluated using the DPPH method with ascorbic acid as the reference compound. The metal chelation potential of the synthesized compounds was assessed for the biologically significant metal ions such as Fe^{+2}, Fe^{+3}, Cu^{+2}, Zn^{+2}, and Al^{+3}. The results indicated that most of the compounds showed moderate AChE inhibitory activity but excellent BuChE inhibitory activity. These compounds also exhibited significant antioxidant and metal chelating properties.

3.4 Cholinesterase inhibitory activity

IC_{50} values of all the synthesized compounds were determined using Ellman's essay. Human AChE enzyme was used for determining acetylcholinesterase inhibition and Equine BuChE enzyme was utilized for butyrylcholinesterase inhibitory activities. The synthesized compounds showed low to moderate IC_{50} values for AChE inhibition, wherein twenty-seven compounds offered **IC_{50}** values under 100 µM with compound (**95)** showing the **highest activity** with an IC_{50} value of **3.013 µM**. Compounds (**81, 82** and **78**) offered 50 % inhibition at concentrations of 8.06, 21.85, and 30.92 µM. It is important to note that substitution with electron releasing group (OCH_3) (**89 - 97**) or a small sized atom (F) (**80 - 88**) on the 4^{th} position of the benzyl ring shows improved activity compared to the un-substituted derivatives (**71 - 79**). Furthermore, substitution on the aniline ring also has a significant effect in improving or reducing the inhibitory activity whereby substitution with electron-withdrawing groups (Cl or F) on the 4^{th} position in the series containing 4-methoxybenzyl ring (**89 – 97**) showed improved activity with IC_{50} values of 3.013 µM and 45.27 µM for compounds (**95** and **89)**. Additionally, attachment of electron releasing groups (CH_3,

and OCH_3) on *ortho* or *para* position in the series with electron-withdrawing group (F) on the benzyl ring showed excellent activity wherein *ortho* substitution of methyl **(81)** and methoxyl **(83)** groups showed IC_{50} values of 8.056 and 14.09 μM respectively, and *para* substitution of methyl **(82)** group gave 50 % inhibition at 21.85 μM. Moreover, it is important to note that unsubstituted aniline or cyclohexamine showed the poorest activity against AChE enzyme. Hence, we can say that substitution of electron releasing or electronegative groups on both the rings, benzyl as well as aniline is important for activity**.** When both the functional groups are present in the compounds it offered significant AChE inhibition.

For the butyrylcholinesterase inhibition, all the compounds exhibited excellent inhibitory activity with IC_{50} values below 12.42 μM, with compounds containing *o*-methoxy substituent on the aniline ring showing the best IC_{50} value of 1.88 μM for the 4-fluoro substituted benzyl derivative **(83),** 2.217 μM for 4-methoxy benzyl derivative **(92),** and 3.732 μM for unsubstituted benzyl derivative **(74).** 4-Chloro substituted aniline derivatives also showed excellent BuChE inhibition with IC_{50} values of 5.182, 2.02, and 3.133 μM for compounds (**77, 86** and **95)** respectively. Notably, cyclohexylamino and unsubstituted anilino derivatives, which proved poor AChE inhibitors offered high selectivity for BuChE with low IC_{50} values. The anilino derivative **(71)** showed an IC_{50} value of 5.740 μM which was 25 times lower than the value obtained for AChE inhibition, whereas the cyclohexylamino derivative **(79)** offered 93 times higher selectivity for BuChE with an IC_{50} of 2.288 μM**.** Anilino derivatives **(80** and **89)** accounted for IC_{50} values of 8.17 and 5.94 μM respectively, and cyclohexylamino derivatives **(88** and **97)** yielded IC_{50} values of 6.509 and 8.368 μM for compounds **(88** and **97)** yielding more than 18 times higher selectivity for butyrylcholinesterase.

3.5 Anti-oxidant and Metal chelation properties

The antioxidant property was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of some selected compounds. Top nine cholinesterase inhibitors **(78, 79, 81, 82, 83, 84, 86, 92 and 95)** were evaluated for their antioxidant properties and the activities were compared with ascorbic acid as a standard. The essay was performed by taking 20-100 μg/ml concentrations of the test and the standard compounds and evaluated for inhibition of 0.1 mM DPPH free radicals. Results indicated that all the nine compounds showed inhibition wherein compounds (**78, 83** and **86**) have shown the highest activity amongst the screened compounds. These three compounds (**78, 83** and **86**) showed **42.13 %, 39.33 %,** and **37.18 %** inhibition respectively, at **20 μg/ml** concentration against **52.54 %** shown by the ascorbic acid. At **100 μg/ml** concentration, **78** exhibited an inhibition of **55.17 %,** and its IC_{50} value was found to be **73.12 μg/ml**, and **83** exhibited an inhibition of **52.91 %,** and its IC_{50} value was found to be **81.65 μg/ml**. Similarly, **86** exhibited an inhibition of **51.4 %** in the DPPH radical scavenging activity, and its IC_{50} value was found to be **90.73 μg/ml**. Ascorbic acid was used as a reference compound which exhibited a percent inhibition of **87.56 %** and offered an IC_{50} value of **13.98 μg/ml** (**Table 5)**. All the compounds displayed metal chelating ability with (Fe^{+2}, Fe^{+3}, Zn^{+2}, Cu^{+2}, and Al^{+3}) due to the presence of 8-hydroxyquinoline moiety present in these molecules (**Table 4**)

Table 5: Antioxidant potential of compounds (**78, 79, 81, 82, 83, 84, 86, 92 and 95**)

Compounds	**Concentration (µg/ml)**					IC_{50}
	20	40	60	80	100	**(µg/ml)**
	% Inhibition					
Ascorbic Acid	52.538	61.450	70.362	79.792	87.564	13.98
78	42.133	44.935	46.443	50.862	55.172	**73.125**
79	44.145	45.284	47.150	48.290	49.326	108.787
81	34.590	38.362	41.163	44.935	49.137	107.134
82	35.668	39.331	41.702	44.181	48.599	113.202
83	39.331	42.887	47.090	49.568	52.909	**81.65**
84	43.316	44.041	44.870	45.595	46.424	197.105
86	37.176	40.301	46.012	47.737	51.400	**90.726**
92	41.761	42.072	43.730	44.455	46.943	159.365
95	31.896	35.991	40.409	43.965	48.060	109.601

3.6 ADME Prediction

In order to exhibit anti-Alzheimer activity, crossing of blood brain barrier by a test compound, is one of the key attributes, which was determined using SwissADME along with other key pharmacokinetic properties of the synthesized compounds. The results were promising, and all the compounds except compounds (**74, 75, 78, 87, 92, 93** and **96)** were found to cross BBB. Moreover, all the compounds indicated good bioavailability of **0.55** and high GI absorption. Thus, it could be said that twenty compounds out of the twenty-seven synthesized compounds, including those exhibiting promising *in vitro* cholinesterase inhibition, possess excellent pharmacokinetic properties and they have high probability to reach the active site and show anti-Alzheimer activity.

4. Experimental

4.1 Docking protocol

The ADT software and Autodock vina program were employed for molecular docking to assess the interaction between the designed analogs and the targeted enzymes (AChE & BuChE). This was aimed to corroborate the findings from both *in vitro* and *in silico* analyses. Utilizing PDB codes 7E3H for AChE and 4BDS for BuChE from the RCSB protein databank (http://www.rcsb.org), crystal structures of the targets were retrieved. Autodock vina necessitates the ligand as well as the receptor in pdbqt format. The ADT software was utilized to prepare the two enzymes and the ligands. In the process of protein preparation, all water molecules were removed, followed by the addition of polar hydrogens and Kollman charges.

Subsequently, active sites were determined by creating grid boxes sized 40 × 40 × 40 Å for AChE and for BuChE around the binding domains of each co-crystallized ligands with the respective enzyme coordinates: center_x = -43.36, center_y = 37.72, center_z = -30.31 for AChE, and center_x = 132.8, center_y = 115.68, center_z = 41.43 for BuChE. To validate the docking protocol, the docked ligands were removed from the co-crystallized structures, and re-docking both of the ligands, i.e. donepezil for AChE and tacrine for BuChE, followed by calculating the Root-Mean-Square Deviation (RSMD) between the co-crystalized ligands and the re-docked poses. For analysis of the docking results and visualization of ligand-receptor interactions, Discovery Studio 2021 client was employed.

4.2 Chemistry

For the synthesis of compounds, all the chemicals were procured from Spectrochem PrivateLimited, Sigma Aldrich, and Avra Synthesis Private Limited.All the reagents and solvents used for the synthesis of the proposed compounds were purified using standard laboratory techniques prior to use. Progress of the reactions was monitored using pre-coated silica gel GF_{254} TLC plates, and spots were visualized under UV light at 254 or 365 nm. Different solvent systems, like hexane-ethyl acetate (7:3 and 6:4) and dichloromethane-methanol (9:1 v/v), were used as eluents. A Rota evaporator (BUCHI R-300) was used for removing the solvents during the workups. Chromatographic purification was performed by column chromatography using Silica gel #100-200. Melting points of the compounds were measured using a digital melting point apparatus (Veego VMP-D) and were uncorrected. Bruker FT-IR, model ALPHA-T (Germany) spectrophotometer was used for recording the IR spectra of individual compounds (wave numbers in cm^{-1}) using ATR. Molecular weights of the synthesized compounds were determined using a Mass spectrophotometer, (Waters Acquity QDA). ^{1}NMR data was collected using an NMR instrument (Bruker 400 MHz) in $CDCl_3$ or DMSO-d_6 solvents (TMS used as internal standard). Purity and composition of the compounds were confirmed by elemental analysis using Thermo Fisher FLASH 2000 organic elemental analyser. The analysed compounds offered results within ± 0.4 % of the theoretical values of carbon, hydrogen and nitrogen.

4.2.1 *General Method for the Synthesis of Compounds (4 – 6):* (Method-A)

To a solution of 1-Boc-piperazine (2.0 g, 10.74 mM) and glyoxylic acid monohydrate (0.98 g, 10.74 mM) in acetonitrile (20 mL), the corresponding boronic acid (10.74 mM) was added. The reaction mixture was stirred at 85 °C for 16 h, and progress of the reaction was monitored by TLC using (10 % methanol in dichloromethane). After the consumption of the starting materials, the solvent was removed under reduced pressure, and the residue was washed with hexane, and purified by column chromatography using silica gel as a stationary phase to afford the desired products (**4 - 6**).

4.2.1.1 ***2-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-2-phenylacetic acid***(**4**): Prepared by **Method A** using phenylboronic acid (1.3 g, 10.74 mM) (**2a**) to offer compound (**4**) as a white solid (3.22 g, 93.6 %), m.p. 180-183 ^oC; TLC (R_f): 0.50 (10 % Methanol in dichloromethane); IR: 3445, 2977, 2930, 1697, 1621, 1423, 1365, 1345, 1136, 1166, 1080, 965 cm^{-1}; ^{1}H-NMR: δ 7.43-7.41 (d, 2H, Ar*H*), 7.31-7.28 (m, 3H, Ar*H*), 6.98 (s,

1H, Ar*H*), 4.16 (s, 1H, C*H*), 3.52-3.37 (m, 4H, CH_2), 2.73-2.66 (d, 4H, CH_2), 1.47-1.45 (d, 9H, CH_3); Mass (m/z): 321.2 (M+1).

4.2.1.2 ***2-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-2-(4- fluorophenyl)acetic acid*** (**5**): Prepared by **Method A** using 4-fluorophenylboronic acid (1.5 g, 10.74 mM) (**2b**) to offer compound (**5**) as a white solid (3.45 g, 95 %), m.p. 176-178 ^oC; TLC(R_f): 0.60 (10 % methanol in dichloromethane); IR: 3405, 2978, 2932, 1700, 1635, 1510, 1457, 1245, 1004, 757 cm^{-1}; ^1H NMR δ 7.45-7.42 (m, 2H, Ar*H*), 7.21-7.17 (m, 2H, Ar*H*), 4.02(s, 1H, C*H*), 3.31-3.30 (d, 4H, CH_2), 2.39-2.29 (m, 4H, CH_2), 1.37 (s, 9H, CH_3); Mass (m/z): 339.3 (M+1).

4.2.1.3 ***2-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid*****(6)**: Prepared by **Method A** using 4-methoxyphenylboronic acid (1.63 g, 10.74 mM) (**2c**) to offer compound (**6**) as a white solid (3.42 g, 90.95 %), m.p. 135-138 ^oC, TLC (R_f): 0.55 (10 % Methanol in dichloromethane); IR: 3422, 2931, 1700, 1617, 1517, 1461, 1412, 1259, 1134, 1038, 966, 869 cm^{-1}; ^1H NMR δ 7.35-7.33 (d, 2H, Ar*H*), 6.89-8.87 (d, 2H, Ar*H*), 4.42 (s, 1H, C*H*), 3.81 (s, 3H, OCH_3), 3.61 (s, 4H, CH_2), 2.83 (s, 4H, CH_2), 1.44 (s, 9H, CH_3); Mass (m/z): 351.2 (M+1).

4.3.1 *General method for acid-amine coupling for preparing compounds (16 - 42)*: (Method B)

To a solution of the corresponding products (**4 - 6**) (1.0 g) in THF (10 mL), EDC.HCl (1 equiv), and HOBt (1 equiv) were added, and the reaction mixture was stirred at a temperature between 5-10 °C for a time period of 20 min. The corresponding aniline/substituted aniline (1 equiv) was added to the above solution followed by *N,N*-diisopropylethylamine (3 equiv). Stirring was continued at RT for 16 h and THF was removed under reduced pressure. The resulting residue was extracted in DCM and washed with water; the organic layer was removed under reduced pressure to obtain the desired products (**16 - 42**).

4.3.1.1 ***tert-Butyl-4-(2-oxo-1-phenyl-2-(phenylamino)ethyl)piperazine-1-carboxylate*** **(16):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and aniline (0.29 g, 3.12 mM) to obtain compound (**16**) as white solid (0.92 g, 74.79 %) m.p. 88-90 ^oC, TLC (R_f): 0.40 (20 % Ethyl acetate in hexane), IR: 3501, 3259, 2862, 1676, 1601, 1559, 1447, 1249, 1171, 735 cm^{-1}.

4.3.1.2 ***tert-Butyl-4-(2-oxo-1-phenyl-2-(o-tolylamino)ethyl)piperazine-1-carboxylate*** **(17):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 2-methylaniline (0.33 g, 3.12 mM)) to obtain compound (**17**) as brown solid (0.89 g, 69.53 %), m.p. 84-87 ^oC, TLC (R_f): 0.42 (20 % Ethyl acetate in hexane), IR: 3362, 2926, 1691, 1587, 1521, 1454, 1365, 1286, 1169, 1003 cm^{-1}.

4.3.1.3 ***tert-Butyl-4-(2-oxo-1-phenyl-2-(p-tolylamino)ethyl)piperazine-1-carboxylate*** **(18):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 4-methylaniline (0.33 g, 3.12 mM) to obtain compound (**18**) as brown solid (0.9 g, 70.31%), m.p. 80-82 ^oC, TLC (R_f): 0.44 (20 % Ethyl acetate in hexane), IR: 3326, 2974, 2857, 1706, 1668, 1597, 1452, 1364, 1287, 1170, 1018 cm^{-1}.

4.3.1.4 *tert-Butyl-4-(2-((2-methoxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (19): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 2-methoxyaniline (0.38 g, 3.12 mM) to obtain compound (**19**) as white solid (0.92 g, 69.17 %), m.p. 92-95 ^{o}C, TLC (R_f): 0.38 (20 % Ethyl acetate in hexane), IR: 3324, 2970, 2836, 1683, 1598, 1512, 1480, 1423, 1304, 1170, 1018 cm^{-1}.

4.3.1.5 *tert-Butyl-4-(2-((4-methoxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (20): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 4-methoxyaniline (0.38 g, 3.12 mM) to obtain compound (**20**) as white solid (0.94 g, 70.67 %), m.p. 98-100 ^{o}C, TLC (R_f): 0.38 (20 % Ethyl acetate in hexane), IR: 3307, 2974, 1692, 1601, 1514, 1456, 1165, 1170, 1129, 1033 cm^{-1}.

4.3.1.6 *tert-Butyl-4-(2-((4-fluorophenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (21): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 4-fluoroaniline (0.34 g, 3.12 mM) to obtain the desired product (**21**) as brown solid (0.92 g, 71.32 %), m.p. 68-70 ^{o}C, TLC (R_f): 0.46 (20 % Ethyl acetate in hexane), IR: 3504, 2978, 1677, 1623, 1576, 1426, 1409, 1289, 1172, 1005 cm^{-1}.

4.3.1.7 *tert-Butyl-4-(2-((4-chlorophenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (22): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic (**4**) (1.0 g, 3.12 mM), and 4-chloroaniline (0.39 g, 3.12 mM) to obtain compound (**22**) as brown solid (0.95 g, 70.89 %), m.p. 74-76 ^{o}C, TLC (R_f): 0.48 (20 % Ethyl acetate in hexane), IR: 3319, 2976, 2857, 1704, 1677, 1592, 1400, 1635, 1244, 1170, 1001 cm^{-1}.

4.3.1.8 *tert-Butyl-4-(2-((4-hydroxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (23): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and 4-hydroxyaniline (0.34 g, 3.12 mM) was added to obtain compound (**23**) as brown solid (0.95 g, 74.21 %), m.p.104-107 ^{o}C, TLC (R_f): 0.32 (20 % Ethyl acetate in hexane), IR: 3295, 2975, 1690, 1607, 1514, 1247, 1169, 1132, 1005 cm^{-1}.

4.3.1.9 *tert-Butyl-4-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate* (24): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic **(4)** (1.0 g, 3.12 mM), and cyclohexanamine (0.30 g, 3.12 mM) was added to obtain compound (**24**) as white solid (0.98 g, 78.4 %), m.p. 88-90 ^{o}C, TLC (R_f): 0.40 (20 % Ethyl acetate in hexane), IR: 3304, 2931, 2856, 1696, 1658, 1527, 1452, 1405, 1288, 1120, 1006 cm^{-1}.

4.3.1.10 *tert-Butyl-4-(1-(4-fluorophenyl)-2-oxo-2-(phenylamino)ethyl)piperazine-1-carboxylate* (25): Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and aniline (0.27 g, 2.95 mM) was added to obtain compound (**25**) as white solid (0.91 g,

74.59 %) m.p. 82-84 ^{o}C, TLC (R_f): 0.52 (20 % Ethyl acetate in hexane), IR: 3308, 2976, 1690, 1600, 1507, 1440, 1366, 1247, 1169, 1027 cm^{-1}.

4.3.1.11 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-oxo-2-(o-tolylamino)ethyl)piperazine-1-carboxylate*** **(26):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 2-methylaniline (0.31 g, 2.95 mM) was added to obtain compound (**26**) as brown solid (0.76 g, 60.31 %), m.p. 94-97 ^{o}C, TLC (R_f): 0.48 (20 % Ethyl acetate in hexane), IR: 3309, 2924, 1693, 1601, 1510, 1421, 1285, 1366, 1168, 1001 cm^{-1}.

4.3.1.12 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-oxo-2-(p-tolylamino)ethyl)piperazine-1-carboxylate*** **(27):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 4-methylaniline (0.31 g, 2.95 mM) was added to obtain compound (**27**) as brown solid (0.82 g, 65 %), m.p. 97-99 ^{o}C, TLC (R_f): 0.50 (20 % Ethyl acetate in hexane), IR: 3316, 2976, 1692, 1600, 1512, 1457, 1421, 1285, 1127, 1001 cm^{-1}.

4.3.1.13 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-((2-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate*** *(28):* Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 2-methoxyaniline (0.36 g, 2.95 mM) was added to obtain compound (**28**) as white solid (0.98 g, 69.5 %), m.p. 78-81 ^{o}C, TLC (R_f): 0.45 (20 % Ethyl acetate in hexane), IR: 3305, 2976, 2853, 1682, 1603, 1511, 1417, 1247, 1107, 1035, 1003 cm^{-1}.

4.3.1.14 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-((4-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate*** **(29):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 4-methoxyaniline (0.36 g, 2.95 mM) was added to obtain compound (**29**) as brown solid (0.96 g, 68 %), m.p. 77-80 ^{o}C, TLC (R_f): 0.46 (20 % Ethyl acetate in hexane), IR: 3298, 2975, 1689, 1511, 1419, 1246, 1170, 1004 cm^{-1}.

4.3.1.15 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-((4-fluorophenyl)amino)-2-oxoethyl)piperazine-1-carboxylate*** **(30):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 4-fluoroaniline (0.32 g, 2.95 mM) was added to obtain compound (**30**) as brown solid (0.89 g, 70 %), m.p. 63-66 ^{o}C, TLC (R_f): 0.54 (20 % Ethyl acetate in hexane), IR: 3296, 2976, 2930, 1692, 1509, 1423, 1403, 1286, 1170, 1004 cm^{-1}.

4.3.1.16 ***tert-Butyl-4-(2-((4-chlorophenyl)amino)-1-(4-fluorophenyl)-2-oxoethyl)piperazine-1-carboxylate*** **(31):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 4-chloroaniline (0.37 g, 2.95 mM) was added to obtain compound (**31**) the desired product as white solid (0.94 g, 71.21 %), m.p. 70-73 ^{o}C, TLC (R_f): 0.56, IR: 3383, 2927, 1695, 1599, 1511, 1406, 1369, 1223, 1158, 1004 cm^{-1}.

4.3.1.17 ***tert-Butyl-4-(1-(4-fluorophenyl)-2-((4-hydroxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate*** **(32):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and 4-hydroxyaniline (0.32 g, 2.95 mM) was added to obtain compound (**32**) as brown solid (0.92 g, 73 %), m.p. 112-114 ^{o}C, TLC (R_f): 0.35 (20 % Ethyl acetate in hexane), IR: 3294, 2976, 1688, 1666, 1511, 1424, 1366, 12487, 1131, 1001 cm^{-1}.

4.3.1.18 ***tert-Butyl-4-(2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl)piperazine-1-carboxylate*** **(33):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4 fluorophenyl)acetic acid **(5)** (1.0 g, 2.95 mM), and cyclohexanamine (0.29 g, 2.95 mM) was added to obtain compound (**33**) as white solid (0.90 g, 72.58 %), m.p. 94-97 ^{o}C, TLC (R_f): 0.42 (20 % Ethyl acetate in hexane), IR: 3308, 2923, 2856, 1695, 1661, 1599, 1508, 1453, 1285, 1170, 1003 cm^{-1}.

4.3.1.19 ***tert-Butyl-4-(1-(4-methoxyphenyl)-2-oxo-2-(phenylamino)ethyl)piperazine-1-carboxylate*** **(34):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM) and aniline (0.26 g, 2.85 mM) was added to obtain compound (**34**) as brown solid (0.92 g, 76 %), m.p. 112-115 ^{o}C, TLC (R_f): 0.58 (20 % Ethyl acetate in hexane), IR: 3307, 2974, 1692, 1601, 1511, 1441, 1247, 1174, 754 cm^{-1}.

4.3.1.20 ***tert-Butyl-4-(1-(4-methoxyphenyl)-2-oxo-2-(o-tolylamino)ethyl)piperazine-1-carboxylate*** **(35):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 2-methylaniline (0.30 g, 2.85 mM) was added to obtain compound (**35**) as brown solid (0.86 g, 68.8 %), m.p. 107-110 ^{o}C, TLC (R_f): 0.60 (20 % Ethyl acetate in hexane), IR: 3356, 2975, 1692, 1607, 1511, 1454, 1247, 1172, 1002 cm^{-1}.

4.3.1.21 ***tert-Butyl-4-(1-(4-methoxyphenyl)-2-oxo-2-(p-tolylamino)ethyl)piperazine-1-carboxylate*** **(36):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 4-methylaniline (0.30 g, 2.85 mM) was added to obtain compound (**36**) as white solid (0.70 g, 70.4 %), m.p. 104-105 ^{o}C, TLC (R_f): 0.60 (20 % Ethyl acetate in hexane), IR: 3303, 2930, 2855, 1688, 1643, 1509, 1242, 1168, 1120 cm^{-1}.

4.3.1.22 ***tert-Butyl-4-(1-(4-methoxyphenyl)-2-((2-methoxyphenyl)amino)-2-oxoethyl)- piperazine-1-carboxylate*** **(37):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 2-methoxyaniline (0.35 g, 2.85mM) was added to obtain compound **(37**) as white solid (0.89 g, 68.46 %), m.p. 98-101 ^{o}C, TLC (R_f): 0.54 (20 % Ethyl acetate in hexane), IR: 3333, 2929, 2852, 1689, 1608, 1510, 1242, 1168, 1026 cm^{-1}.

4.3.1.23 ***tert-Butyl-4-(1-(4-methoxyphenyl)-2-((4-methoxyphenyl)amino)-2-oxoethyl)piperazine -1-carboxylate*** **(38):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 4-methoxyaniline (0.35 g, 2.85 mM) was added to

obtain compound (**38**) as white solid (0.95, 73 %), m.p. 95-97 ^{o}C, TLC (R_f): 0.54 (20 % Ethyl acetate in hexane), IR:3334, 2929, 2852, 1688, 1645, 1509, 1403, 1242, 1168, 1027 cm^{-1}.

4.3.1.24 ***tert*-Butyl-4-(2-((4-fluorophenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (39):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 4-fluoroaniline (0.31 g, 2.85 mM) was added to obtain compound (**39**) as white solid (0.92 g, 73 %), m.p. 94-95 ^{o}C, TLC (R_f): 0.56 (20 % Ethyl acetate in hexane), IR: 3305, 2974, 1690, 1611, 1511, 1458, 1248, 1172, 1033 cm^{-1}.

4.3.1.25 ***tert*-Butyl-4-(2-((4-chlorophenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (40):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 4-chloroaniline (0.36 g, 2.85 mM) was added to obtain compound (**40**), the desired product as brown solid (0.98 g, 74.8 %), m.p. 108-110 ^{o}C, TLC (R_f): 0.62 (20 % Ethyl acetate in hexane), IR: 3428, 2975, 1685, 1594, 1511, 1412, 1247, 1171,1008 cm^{-1}.

4.3.1.26 ***tert*-Butyl-4-(2-((4-hydroxyphenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)- piperazine-1-carboxylate (41):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and 4-hydroxyaniline (0.31 g, 2.85 mM) was added to obtain compound (**41**), the desired product as brown solid (0.93 g, 73.8 %), m.p. 120-122 ^{o}C, TLC (R_f): 0.40 (20 % Ethyl acetate in hexane), IR: 3333, 2929, 2852, 1690, 1645, 1510, 1403, 1242, 1168, 1027 cm^{-1}.

4.3.1.27 ***tert*-Butyl-4-(2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (42):** Prepared by **Method B** using 2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetic acid **(6)** (1.0 g, 2.85 mM), and cyclohexylamine (0.28 g, 2.85 mM) was added to obtain compound (**42**), the desired product as white solid (0.90 g, 73.1 %), m.p. 90-93 ^{o}C, TLC (R_f): 0.45 (20 % Ethyl acetate in hexane), IR: 3327, 2930, 2854, 1689, 1643, 1509, 1420, 1242, 1168, 1117 cm^{-1}.

4.4.1 *General Method for Boc-deprotection: (43-69)*: (Method C)

To a solution of the corresponding products (**16 - 42**) in DCM (7.5 mL), dioxane-HCl (7.5 mL) (dioxane saturated with hydrogen chloride gas) was added and stirred at 25 ^{o}C for 3 h. The reaction was monitored on TLC, after the completion of the reaction, solvent was removed under reduced pressure to obtain sticky Products (**43 - 69**) which were used as such for the next step.

4.4.1.1 ***N,2-Diphenyl-2-(piperazin-1-yl)acetamide* (43):** *tert*-Butyl-4-(2-oxo-1-phenyl-2-(phenylamino)ethyl)piperazine-1-carboxylate (**16**) (0.75 g, 1.76 mM) through **Method C** offered the product (**43**) (0.54 g, 96.42 %). TLC (R_f): 0.51 (70 % Ethyl acetate in hexane).

4.4.1.2 ***2-Phenyl-2-(piperazin-1-yl)-N-(o-tolyl)acetamide* (44):** *tert*-Butyl 4-(2-oxo-1-phenyl-2-(*o*-tolylamino)ethyl)piperazine-1-carboxylate (**17**) (0.75 g, 1.76 mM) through **Method C** offered the product (**44**) (0.53 g, 94.64 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.3 ***2-Phenyl-2-(piperazin-1-yl)-N-(p-tolyl)acetamide*** **(45):** *tert*-Butyl 4-(2-oxo-1-phenyl-2-(*p*-tolylamino)ethyl)piperazine-1-carboxylate (**18**) (0.75 g, 1.76 mM), through **Method C** offered the product (**45**) (0.52 g, 92.85 %). TLC (R_f): 0.46 (70 % Ethyl acetate in hexane).

4.4.1.4 ***N-(2-Methoxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide*** **(46):** *tert*-Butyl 4-(2-((2-methoxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**19**) (0.75 g, 1.76 mM), through **Method C** offered the product (**46**) (0.53 g, 94.64 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.5 ***N-(4-Methoxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide*** **(47):** *tert*-Butyl 4-(2-((4-methoxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**20**) (0.75 g, 1.76 mM), through **Method C** offered the product (**47**) (0.56 g, 98.24 %). TLC (R_f): 0.42 (70 % Ethyl acetate in hexane).

4.4.1.6 ***N-(4-Fluorophenyl)-2-phenyl-2-(piperazin-1-yl)acetamide*** **(48):** *tert*-Butyl 4-(2-((4-fluorophenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**21**) (0.75 g, 1.76 mM), through **Method C** offered the product (**48**) (0.54 g, 94.73 %). TLC (R_f): 0.43 (70 % Ethyl acetate in hexane).

4.4.1.7 ***N-(4-Chlorophenyl)-2-phenyl-2-(piperazin-1-yl)acetamide*** **(49):** *tert*-Butyl 4-(2-((4-chlorophenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**22**) (0.75 g, 1.76 mM), through **Method C** offered the product (**49**) (0.55 g, 98.21 %) which was further used for the final reaction. TLC (R_f): 0.47 (70 % Ethyl acetate in hexane).

4.4.1.8 ***N-(4-Hydroxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide*** **(50):** *tert*-butyl 4-(2-((4-hydroxyphenyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**23**) (0.75 g, 1.76 mM), through **Method C** offered the product (**50**) (0.54 g, 94.73 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.9 ***N-Cyclohexyl-2-phenyl-2-(piperazin-1-yl)acetamide*** **(51):** *tert*-butyl 4-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (**24**) (0.75 g, 1.76 mM), through **Method C** offered the product (**51**) (0.55 g, 98.21 %). TLC (R_f): 0.49 (70 % Ethyl acetate in hexane).

4.4.1.10 ***2-(4-Fluorophenyl)-N-phenyl-2-(piperazin-1-yl)acetamide*** **(52):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-oxo-2-(phenylamino)ethyl)piperazine-1-carboxylate (**25**) (0.75 g, 1.76 mM through **Method C** offered the product (**52**) (0.54 g, 94.73 %). TLC (R_f): 0.50 (70 % Ethyl acetate in hexane).

4.4.1.11 ***2-(4-Fluorophenyl)-2-(piperazin-1-yl)-N-(o-tolyl)acetamide*** **(53):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-oxo-2-(*o*-tolylamino)ethyl)piperazine-1-carboxylate (**26**) (0.75 g, 1.76 mM), through **Method C** offered the product (**53**) (0.53 g, 92.98 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.12 ***2-(4-Fluorophenyl)-2-(piperazin-1-yl)-N-(p-tolyl)acetamide*** **(54***): tert*-Butyl 4-(1-(4-fluorophenyl)-2-oxo-2-(*p*-tolylamino)ethyl)piperazine-1-carboxylate (**27**) (0.75 g, 1.76 mM), through **Method C** offered the product (**54**) (0.55 g, 96.49 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.13 ***2-(4-Fluorophenyl)-N-(2-methoxyphenyl)-2-(piperazin-1-yl)acetamide*** **(55):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-((2-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**28**) (0.75 g, 1.76 mM), through **Method C** offered the product (**55**) (0.56 g, 96.55 %). TLC (R_f): 0.44 (70 % Ethyl acetate in hexane).

4.4.1.14 ***2-(4-Fluorophenyl)-N-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide*** **(56):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-((4-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**29**) (0.75 g, 1.76 mM), through **Method C** offered the product (**56**) (0.55 g, 94.82 %). TLC (R_f): 0.44 (70 % Ethyl acetate in hexane).

4.4.1.15 ***N,2-Bis(4-fluorophenyl)-2-(piperazin-1-yl)acetamide*** **(57):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-((4-fluorophenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**30**) (0.75 g, 1.76 mM), through **Method C** offered the product (**57**) (0.56 g, 98.24 %). TLC (R_f): 0.45 (70 % Ethyl acetate in hexane).

4.4.1.16 ***N-(4-Chlorophenyl)-2-(4-fluorophenyl)-2-(piperazin-1-yl)acetamide*** **(58):** *tert*-Butyl 4-(2-((4-chlorophenyl)amino)-1-(4-fluorophenyl)-2-oxoethyl)piperazine-1-carboxylate (**31***)* (0.75 g, 1.76 mM), in DCM (7.5 mL), through **Method C** offered the product (**58**) (0.52 g, 89.65 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.17 ***2-(4-Fluorophenyl)-N-(4-hydroxyphenyl)-2-(piperazin-1-yl)acetamide*** **(59):** *tert*-Butyl 4-(1-(4-fluorophenyl)-2-((4-hydroxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**32**) (0.75 g, 1.76 mM), through **Method C** offered the product (**59**) (0.54 g, 94.73 %). TLC (R_f): 0.47 (70 % Ethyl acetate in hexane).

4.4.1.18 ***N-Cyclohexyl-2-(4-fluorophenyl)-2-(piperazin-1-yl)acetamide*** **(60):** *tert*-Butyl 4-(2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl)piperazine-1-carboxylate (**33**) (0.75 g, 1.76 mM), through **Method C** offered the product (**60)** (0.56 g, 98.24 %). TLC (R_f): 0.52 (70 % Ethyl acetate in hexane).

4.4.1.19 ***2-(4-Methoxyphenyl)-N-phenyl-2-(piperazin-1-yl)acetamide*** **(61):** *tert*-Butyl 4-(1-(4-methoxyphenyl)-2-oxo-2-(phenylamino)ethyl)piperazine-1-carboxylate (**34**) (0.75 g, 1.76 mM), through **Method C** offered the product (**61**) (0.52 g, 94.73%). TLC (R_f): 0.45 (70 % Ethyl acetate in hexane).

4.4.1.20 ***2-(4-Methoxyphenyl)-2-(piperazin-1-yl)-N-(o-tolyl)acetamide*** **(62):** *tert*-Butyl 4-(1-(4-methoxyphenyl)-2-oxo-2-(o-tolylamino)ethyl)piperazine-1-carboxylate (**35**) (0.75 g, 1.76 mM), in DCM (7.5 mL), through **Method C** offered the product (**62**) (0.53 g, 91.37 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.21 ***2-(4-Methoxyphenyl)-2-(piperazin-1-yl)-N-(p-tolyl)acetamide*** **(63):** *tert*-Butyl 4-(1-(4-methoxyphenyl)-2-oxo-2-(p-tolylamino)ethyl)piperazine-1-carboxylate (**36**) (0.75 g, 1.76 mM), in DCM (7.5 mL), through **Method C** offered the product (**63**) (0.56 g, 96.55 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.22 *N-(2-Methoxyphenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (64): *tert*-Butyl 4-(1-(4-methoxyphenyl)-2-((2-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**37**) (0.75 g, 1.76 mM), through **Method C** offered the product (**64**) (0.56 g, 96.55 %) which was further processed for the final reaction. TLC (R_f): 0.42 (70 % Ethyl acetate in hexane).

4.4.1.23 *N,2-Bis(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (65): *tert*-Butyl 4-(1-(4-methoxyphenyl)-2-((4-methoxyphenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**38**) (0.75 g, 1.76 mM), through **Method C** offered the product (**65**) (0.54 g, 93.10 %). TLC (R_f): 0.42 (70 % Ethyl acetate in hexane).

4.4.1.24 *N-(4-Fluorophenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (66): *tert*-Butyl 4-(2-((4-fluorophenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (**39**) (0.75 g, 1.76 mM), through **Method C** offered the product (**66**) (0.54 g, 96.42 %). TLC (R_f): 0.43 (70 % Ethyl acetate in hexane).

4.4.1.25 *N-(4-Chlorophenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (67): *tert*-Butyl 4-(2-((4-chlorophenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (**40**) (0.75 g, 1.76 mM), through **Method C** offered the product (**67**) (0.57 g, 96.61 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.26 *N-(4-Hydroxyphenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (68): *tert*-Butyl 4-(2-((4-hydroxyphenyl)amino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (**41**) (0.75 g, 1.76 mM), through **Method C** offered the product (**68**) (0.52 g, 89.85 %). TLC (R_f): 0.48 (70 % Ethyl acetate in hexane).

4.4.1.27 *N-Cyclohexyl-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide* (69): *tert*-Butyl 4-(2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl)piperazine-1-carboxylate (**42**) (0.75 g, 1.76 mM), through **Method C** offered the product (**69**) (0.56 g, 98.24 %) which was further processed for final reaction. TLC (R_f): 0.36 (70 % Ethyl acetate in hexane).

4.5.1 *5-Chloromethylquinolin-8-ol*(70):

A mixture of 8-hydroxyquinoline (10.0 g, 68 mM), concentrated hydrochloric acid (13 mL) and formalin (37 %formaldehyde and 12 % methanol, 12 mL, 399 mM)) was treated with hydrogen chloride gas and stirred for 3 h. The yellow solid obtained was collected on a filter paper, washed three times in acetone, and dried under vacuum to afford 5-chloromethyl-8-hydroxyquinoline (**70**) as a yellow solid hydrochloride salt, m.p. >260 ^{o}C, Reported >260 ^{o}C [42].

4.6.1 *General procedure for the synthesis of the target compounds* (71 – 79, 80 – 88 and 89 - 97): (Method D)

To a solution of the corresponding products (**43 - 69**) (1.0 equiv) in DMSO (7 mL), triethylamine (5.0 equiv) was added and the reaction mixture was stirred at 25 ^{o}C for 10 min followed by the addition of 5-

chloromethyl-8-hydroxyquinoline hydrochloride (**70**) (1.0 equiv) portion-wise. The reaction mixture was stirred at 100 ^{o}C for 16 h, and the progress of the reaction was monitored by TLC using (80 % ethyl acetate in hexane) After the consumption of the starting materials, the reaction mixture was poured into ice-cold water to obtain solid products (**71 - 97**) which were filtered, dried and further purified by column chromatography using #100-200 silica gel as stationary phase and ethyl acetate:hexane as mobile phase to afford the desired pure products (**71 - 97**).

4.6.1.1 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N,2-diphenylacetamide*** **(71):** Using *N*,2-diphenyl-2-(piperazin-1-yl)acetamide (**43**) (0.5 g, 1.69 mM) and **Method D** the desired compound (**71**) was obtained, m.p. 72-75 °C. TLC (Rf): 0.54 (80 % Ethyl acetate in hexane); IR: 3314, 2931, 2816, 1686, 1599, 1503, 1474, 1440, 1371, 1312, 1271, 1231, 1076, 827 cm^{-1}; ^{1}H NMR: δ 9.25 (s, 1H, N*H*), 8.79-8.78 (d, 1H, Ar*H*), 8.63-8.61 (d, 1H, Ar*H*), 7.61-7.59 (d, 2H, Ar*H*), 7.47-7.44 (dd, 1H, Ar*H*), 7.38-7.36 (d, 2H, Ar*H*), 7.33 (s, 5H, Ar*H*), 7.28 (s, 1H, Ar*H*), 7.15-7.11 (t, 1H, Ar*H*), 7.08-7.06 (d, 1H, Ar*H*), 3.98 (s, 1H, C*H*), 3.83 (s, 2H, CH_2), 2.63-2.50 (m, 8H, CH_2); $C_{28}H_{28}N_4O_2$ requires: C, 74.31; H, 6.24; N, 12.38; found C, 74.58; H, 6.41; N, 12.10; LC-MS (m/z): 453.2 (M+1); Purity 98.20 %.

4.6.1.2 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenyl-N-(o-tolyl)acetamide*** **(72):** Using 2-phenyl-2-(piperazin-1-yl)-N-(o-tolyl)acetamide (**44***)* (0.5 g, 1.61 mM) and **Method D** the desired compound (**72**) was obtained as a yellowish white solid (0.59 g, 78.66 %), which was further purified by column chromatography using #100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 110-112 °C. TLC (R_f): 0.52 (80 % Ethyl acetate in hexane); IR: 3338, 2923, 2812, 2766, 1669, 1596, 1505, 1475, 1229, 1136, 1007, 699 *cm^{-1}*; ^{1}H NMR; δ 9.71-9.61 (m, 2H, N*H,* O*H*), 8.85-8.79 (d, 1H, Ar*H*), 8.64-8.52 (d, 1H, Ar*H*), 7.57-6.98 (m, 11H, Ar*H*), 4.09 (s, 1H, Ar*H*), 3.78 (s, 2H, Ar*H*), 2.69- 2.56 (bs, 3H, CH_2), 2.41-2.35 (bs, 5H, CH_2), 2.18 (s, 3H, CH_3); $C_{29}H_{30}N_4O_2$ requires: C, 74.65; H, 6.48; N, 12.01; found C, 74.44; H, 6.56; N, 11.83; Mass (m/z): 467.4 (M+1).

4.6.1.3 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenyl-N-(p-tolyl)acetamide*** **(73):** Using 2-phenyl-2-(piperazin-1-yl)-*N*-(*p*-tolyl)acetamide (**45**) (0.5 g, 1.61 mM) and **Method D** the desired compound (**73**) was obtained as a greenish white solid (0.57 g, 76 %), which was further purified by column chromatography using #100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 165-168 °C. TLC (R_f): 0.53 (80 % Ethyl acetate in hexane); IR: 3329, 2817, 1658, 1595, 1472, 1363, 1230, 1133, 1004, 705 *cm^{-1}*; ^{1}H NMR: δ 9.16 (s, 1H, N*H*), 8.79-8.78 (d, 1H, Ar*H*), 8.63-8.61 (d, 1H, Ar*H*), 7.49-7.44 (m, 3H, Ar*H*), 7.34-7.32 (d, 6H, Ar*H*), 7.17-7.14 (d, 2H, Ar*H*), 7.08-7.06 (d, 1H, Ar*H*), 3.97 (s, 1H, C*H*), 3.84 (s, 2H, CH_2), 2.53 (d**,** 8H, CH_2), 2.34 (s, 3H, CH_3); $C_{29}H_{30}N_4O_2$ requires: C, 74.65; H, 6.48; N, 12.01; found C, 74.48; H, 6.65; N, 12.22; LC-MS (m/z): 467.5 (M+1); Purity 95.58 %.

4.6.1.4 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(2-methoxyphenyl)-2-phenyl- acetamide*** **(74):** Using *N*-(2-methoxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide (**46**) (0.5 g, 1.53 mM) and **Method D** the desired compound (**74**) was obtained as a light orange solid (0.57 g, 76 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate:

Hexane as mobile phase, m.p. 82-85 °C. TLC (R_f): 0.49 (80 % Ethyl acetate in hexane); IR: 3327, 2936, 2814, 1738, 1598, 1521, 1460, 1230, 1025, 787 *cm*$^{-1}$; ^{1}H NMR: δ 9.97 (s, 1H, N*H*), 9.72 (s, 1H, O*H*), 8.83-8.82 (d, 1H, Ar*H*), 8.60-8.58 (d, 2H, Ar*H*), 8.11- 8.09 (d, 1H, Ar*H*), 7.56-7.53 (dd, 1H, Ar*H*), 7.34 (d, 4H, Ar*H*), 7.28(d, 2H, Ar*H*), 7.08-7.07 (d, 2H, Ar*H*), 6.99-6.97 (d, 1H, Ar*H*), 6.92-6.84 (m, 1H, Ar*H*), 4.21 (s, 1H, C*H*), 3.94 (s, 3H, CH_2), 3.79-3.34 (m, 2H, OCH_3), 2.47-2.20 (m, 8H, CH_2); $C_{29}H_{30}N_4O_3$ requires: C, 72.18; H, 6.27; N, 11.61; found C, 71.81; H, 6.54; N, 11.43; Mass (m/z): 483.3 (M+1).

4.6.1.5 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(4-methoxyphenyl)-2-phenyl- acetamide*** **(75):** Using *N*-(4-methoxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide (**47**) (0.5 g, 1.53 mM) and **Method D** the desired compound (**75**) was obtained as a yellowish white solid (0.57 g, 77 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 175-178 °C. TLC (R_f): 0.49 (80 % Ethyl acetate in hexane); IR: 3332, 2955, 2808, 1664, 1520, 1473, 1248, 1135, 1030, 821 *cm*$^{-1}$; ^{1}H NMR; δ 9.92 (s, 1H, N*H*), 9.72 (s, 1H, O*H*), 8.85 (d, 1H, Ar*H*), 8.63-8.61 (d, 1H, Ar*H*), 7.59-7.57 (dd, 1H, Ar*H*), 7.51-7.46 (m, 4H, Ar*H*), 7.36-7.26 (m, 4H, Ar*H*), 6.99-6.97 (d, 1H, Ar*H*), 6.88-8.84 (d, 2H, Ar*H*), 3.94 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 3.70 (s, 3H, OCH_3), 2.35 (bs, 8H, CH_2); $C_{29}H_{30}N_4O_3$ requires: C, 72.18; H, 6.27; N, 11.61; found C, 71.88; H, 6.55; N, 11.31; Mass (m/z): 483.3 (M+1).

4.6.1.6 ***N-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenyl- acetamide*** **(76):** Using *N*-(4-fluorophenyl)-2-phenyl-2-(piperazin-1-yl)acetamide (**48**) (0.5 g, 1.59 mM) and **Method D** the desired compound (**76**) was obtained as a yellowish white solid (0.58 g, 77.33 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 89-90°C. TLC (R_f): 0.51 (80 % Ethyl acetate in hexane); IR: 3296, 2822, 1669, 1509, 1406, 1372, 1211, 1006, 835 *cm*$^{-1}$; ^{1}H NMR: δ 10.22- 10.11 (d, 1H, N*H*), 9.71 (bs, 1H, O*H*), 8.86-8.85 (d, 1H, Ar*H*), 8.64-8.62 (d, 1H, Ar*H*), 7.77- 7.59 (m, 2H, Ar*H*), 7.50-7.48 (m, 2H, Ar*H*), 7.37-7.36 (m, 4H, Ar*H*), 7.15-7.13 (d, 2H, Ar*H*), 7.01-6.99 (d, 1H, Ar*H*), 3.80 (s, 1H, C*H*), 3.41 (s, 2H, CH_2), 2.69 (s, 2H, CH_2), 2.43- 2.47 (d, 4H, CH_2); $C_{28}H_{27}FN_4O_2$ requires: C, 71.47; H, 5.78; N, 11.91; found C, 71.16; H, 5.96; N, 11.72; Mass (m/z): 471.3 (M+1).

4.6.1.7 ***N-(4-Chlorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenyl- acetamide*** **(77):** Using *N*-(4-chlorophenyl)-2-phenyl-2-(piperazin-1-yl)acetamide (**49**) (0.5 g, 1.51 mM) and **Method D** the desired compound (**77**) was obtained as a yellowish white solid (0.58 g, 77.33 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 89-90°C. TLC (R_f): 0.51 (80 % Ethyl acetate in hexane); IR: 3296, 2822, 1669, 1509, 1406, 1372, 1211, 1006, 835 *cm*$^{-1}$; ^{1}H NMR: δ 10.22- 10.11 (d, 1H, N*H*), 9.71 (bs, 1H, O*H*), 8.86-8.85 (d, 1H, Ar*H*), 8.64-8.62 (d, 1H, Ar*H*), 7.77- 7.59 (m, 2H, Ar*H*), 7.50-7.48 (m, 2H, Ar*H*), 7.37-7.36 (m, 4H, Ar*H*), 7.15-7.13 (d, 2H, Ar*H*), 7.01-6.99 (d, 1H, Ar*H*), 3.80 (s, 1H, C*H*), 3.41 (s, 2H, CH_2), 2.69 (s, 2H, CH_2), 2.43- 2.47 (d, 4H, CH_2); $C_{28}H_{27}ClN_4O_2$ requires: C, 69.06; H, 5.59; N, 11.50; found C, 68.78; H, 5.87; N, 11.34; Mass (m/z): 471.3 (M+1).

4.6.1.8 *N-(4-Hydroxyphenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenyl- acetamide* (78): Using *N*-(4-hydroxyphenyl)-2-phenyl-2-(piperazin-1-yl)acetamide (**50**) (0.5 g, 1.44 mM) and **Method D** the desired compound (**78**) was obtained as a brown solid (0.59 g, 79.72 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 155-158 °C. TLC (R_f): 0.52 (80 % Ethyl acetate in hexane); IR: 3331, 2928, 2813, 1677, 1592, 1474, 1398, 1270, 1134, 1005 cm^{-1}; ^{1}H NMR: δ 10.19 (s, 1H, N*H*), 9.71 (s, 1H, O*H*), 8.85- 8.84 (d, 1H, Ar*H*), 8.63-8.64 (d, 1H, Ar*H*), 7.65-7.63 (d, 2H, Ar*H*), 7.57-7.56 (d, 1H, Ar*H*), 7.49-7.47 (d, 2H, Ar*H*), 7.37-7.32 (m, 6H, Ar*H*), 7.0-6.99 (d, 1H, Ar*H*), 3.99 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.47-2.36 (bs, 8H, CH_2); $C_{28}H_{28}N_4O_3$ requires: C, 71.78; H, 6.02; N, 11.96; found C, 71.56; H, 6.35; N, 11.74; Mass (m/z): 487.3 (M^+). 488.3 (M+1), 489.2 (M+2).

4.6.1.9 *N-Cyclohexyl-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-phenylacetamide* (79): Using *N*-cyclohexyl-2-phenyl-2-(piperazin-1-yl)acetamide (**51**) (0.5 g, 1.44 mM) and **Method D** the desired compound (**79**) was obtained as a greenish-white solid (0.63 g, 82.89 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. >220 °C. TLC (R_f): 0.43 (80 % Ethyl acetate in hexane); IR: 3320, 2924, 2818, 1662, 1514, 1474, 1371, 1231, 1004 cm^{-1}; ^{1}H NMR: δ 9.79 (s, 1H, O*H*), 9.71 (s, 1H, O*H*), 9.20 (s, 1H, N*H*), 8.87-8.84 (d, 1H, Ar*H*), 8.63-8.57 (d, 1H, Ar*H*), 7.58-7.55 (m, 1H, Ar*H*), 7.45-7.46 (d, 2H, Ar*H*), 7.36-7.25 (m, 6H, Ar*H*), 6.99-6.97 (d, 1H, Ar*H*), 6.67-6.65 (d, 2H, Ar*H*), 3.91 (s, 1H), 3.76 (s, 2H, CH_2), 2.54 (s, 1H, C*H*), 2.50-2.33 (ds, 7H, CH_2); ^{13}C NMR δ: 169.01, 147.66, 138.80, 137.52, 133.72, 128.78, 128.51, 128.00, 127,80, 127.44, 121.32, 109.87, 74.12, 59.53, 52.55, 50.67, 47.15, 40.41, 32.32, 32.04, 25.11, 24.43. $C_{28}H_{34}N_4O_2$ requires: C, 73.33; H, 7.47; N, 12.22; found C, 73.05; H, 7.78; N, 12.04; Mass (m/z): 469.3 (M+1).

4.6.1.10 *2-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-phenyl- acetamide* (80): Using 2-(4-fluorophenyl)-*N*-phenyl-2-(piperazin-1-yl)acetamide (**52**) (0.5 g, 1.59 mM) and **Method D** the desired compound (**80**) was obtained as a greenish white solid (0.56 g, 74.66 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 74-77 °C, TLC (R_f): 0.48 (80 % Ethyl acetate in hexane), IR: 3305, 3056, 2931, 2815, 1685, 1507, 1439, 1353, 1246, 1176, 1133, 1030 cm^{-1}; ^{1}H NMR: δ 10.08 (s, 1H, N*H*), 9.72 (s, 1H, O*H*), 8.84 (d, 1H, Ar*H*), 8.62 (d, 1H, Ar*H*), 7.60-7.57 (m, 3H, Ar*H*), 7.55-7.53 (m, 2H, Ar*H*), 7.35-7.24 (m, 3H, Ar*H*), 7.18 (t, 2H, Ar*H*), 7.05 (t, 1H, Ar*H*), 6.98 (d, 1H, Ar*H*), 4.02 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.55-2.25 (m, 8H, CH_2); ^{13}C NMR δ: 168.95, 162.92, 160.50, 152.75, 147.69, 138.78, 133.66, 133.19, 130.55, 128.75, 127.79, 123.97, 123.58, 121.33, 119.53, 115.15, 114.94, 109.88, 73.69, 59.48, 52.48, 50.63. $C_{28}H_{27}FN_4O_2$ requires: C, 71.47; H, 5.78; N, 11.91; found C, 71.15; H, 5.95; N, 11.73; Mass (m/z): 471.3 (M+1).

4.6.1.11 *2-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(o-tolyl)- acetamide* (81): Using 2-(4-fluorophenyl)-2-(piperazin-1-yl)-*N*-(*o*-tolyl)acetamide (**53**) (0.5 g, 1.53 mM) and **Method D** the desired compound (**81**) was obtained as a greenish-white solid (0.57 g, 77 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 86-89 °C. TLC (Rf): 0.51 (80 % Ethyl acetate in hexane); IR: 3312, 2931, 1675, 1508,

1439, 1350, 1245, 1133, 1028 cm^{-1}; 1H NMR: δ 9.70 (s, 1H, O*H*), 9.61 (s, 1H, N*H*), 8.85-8.83 (dd, 1H, Ar*H*), 8.63-8.60 (d, 1H, Ar*H*), 7.58-7.55 (m, 1H, Ar*H*), 7.49-7.46 (m, 4H, Ar*H*), 7.34-7.32 (d, 1H, Ar*H*), 7.21-7.19 (m, 3H, Ar*H*), 7.17 (s, 1H, Ar*H*), 7.15-7.13 (d, 1H, Ar*H*), 7.09-7.05 (m, 2H, Ar*H*), 6.99-6.97 (d, 1H, CH_2), 4.12(s, 1H, C*H*), 3.81-3.74 (m, 3H, CH_3), 2.51-2.38 (bs, 4H, CH_2), 2.16 (s, 4H, CH_2); $C_{29}H_{29}FN_4O_2$ requires: C, 71.88; H, 6.03; N, 11.56; found C, 71.55; H, 6.46; N, 11.27; Mass (m/z): 485.4 (M+1).

4.6.1.12 ***2-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(p-tolyl)- acetamide*** **(82):** Using 2-(4-fluorophenyl)-2-(piperazin-1-yl)-*N*-(*p*-tolyl)acetamide (**54**) (0.5 g, 1.53 mM) and **Method D** the desired compound (**82**) was obtained as a yellowish white solid (0.58 g, 78.37 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 103-106 °C. TLC (R_f): 0.53 (80 % Ethyl acetate in hexane); IR: 3314, 2924, 2816, 1689, 1599, 1507, 1461, 1432, 1225 1115, 788, 749 cm^{-1}; ^{1}H NMR δ 9.96 (s, 1H, N*H*), 9.67 (s, 1H, O*H*), 8.85-8.84 (dd, 1H, Ar*H*), 8.64-8.61 (dd, 1H, Ar*H*), 7.58- 7.55 (m, 1H, Ar*H*), 7.52-7.48 (m, 2H, Ar*H*), 7.47-7.45 (d, 2H, Ar*H*), 7.33-7.32 (d, 1H, Ar*H*), 7.20-7.15 (t, 2H, Ar*H*), 7.10-7.08 (d, 2H, Ar*H*), 7.00-6.98 (d, 1H, Ar*H*), 4.00 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.54-2.35 (bs, 8H, CH_2), 2.24 (s, 3H, CH_3); $C_{29}H_{29}FN_4O_2$ requires; C, 71.88; H, 6.03; N, 11.56; found C, 71.76; H, 6.37; N, 11.23; Mass (m/z): 485.3 (M+1).

4.6.1.13 ***2-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(2-methoxyphenyl)acetamide*** **(83):** Using 2-(4-fluorophenyl)-*N*-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (**55**) (0.5 g, 1.45 mM) and **Method D** the desired compound (**83**) was obtained as a yellowish white solid (0.56 g, 76.71 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p.140-143 °C. TLC (R_f): 0.51 (80 % Ethyl acetate in hexane); IR: 3312, 2927, 2816, 1691, 1599, 1523, 1506, 1477, 1461, 1225, 1169, 1026, 789, 750 cm^{-1}; ^{1}H NMR: δ 9.97 (s, 1H, NH), 9.69 (s, 1H, OH), 8.84-8.83 (dd, 1H, Ar*H*), 8.61-8.59 (dd, 1H, Ar*H*), 8.10-8.08 (m, 1H, Ar*H*), 7.57-7.54 (dd, 1H, Ar*H*), 7.35-7.32 (t, 3H, Ar*H*), 7.21-7.16 (t, 2H, Ar*H*), 7.10-7.08 (m, 2H, Ar*H*), 7.00-6.98 (d, 1H, Ar*H*), 6.94-6.90 (m, 1H, Ar*H*), 4.30 (s, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.80 (s, 2H, CH_2), 2.68 (bs, 4H, CH_2), 2.37 (bs, 4H, CH_2); $C_{29}H_{29}FN_4O_3$ requires: C, 69.58; H, 5.84; F, 3.80; N, 11.19; found C, 69.36; H, 6.16; N, 10.91; LC-MS (m/z): 501.5 (M+1); Purity 97.11 %.

4.6.1.14 ***2-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(4-methoxyphenyl)acetamide*** **(84):** Using *N*,2-bis(4-fluorophenyl)-2-(piperazin-1-yl)acetamide (**56**) (0.5 g, 1.45 mM) and **Method D** the desired compound (84) was obtained as a yellowish white solid (0.54 g, 73.93 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 86-88 °C. TLC (R_f): 0.52 (80 % Ethyl acetate in hexane); IR: 3312, 2933, 2817, 1682, 1603, 1509, 1474, 1412, 1230, 1371, 1133, 1033, 1005, 828, 787cm^{-1}; ^{1}H NMR: δ 9.92 (s, 1H, NH), 9.67 (s, 1H, O*H*), 8.85-8.84 (d, 1H, Ar*H*), 8.64-8.61 (d, 1H, Ar*H*), 7.59-7.55 (dd, 1H, Ar*H*), 7.52-7.48 (m, 4H, Ar*H*), 7.34-7.32 (d, 1H, Ar*H*), 7.20-7.15 (t, 2H, Ar*H*), 7.0-6.98 (d, 1H, Ar*H*), 6.88-6.85 (m, 2H, Ar*H*), 3.97 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3), 2.69 (d, 4H, CH_2), 2.34 (s, 4H, CH_2); $C_{29}H_{29}FN_4O_3$ requires: C, 69.58; H, 5.84; N, 11.19; found C, 69.35; H, 5.96; N, 11.02; LC-MS (m/z): 501.4 (M+1); Purity 94.50 %.

4.6.1.15 ***N,2-bis(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)- acetamide*** **(85):** Using *N*-(4-chlorophenyl)-2-(4-fluorophenyl)-2-(piperazin-1-yl)acetamide (**57**) (0.5 g, 1.50 mM) and **Method D** the desired compound (**85**) was obtained as a greenish white solid (0.57 g, 79.16 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 85-88 °C. TLC (R_f): 0.48 (80 % Ethyl acetate in hexane); IR: 3391, 2923, 1693, 1507, 1474, 1271, 1227, 1005 *cm*$^{-1}$; ^{1}H NMR: δ 10.12 (s, 1H, N*H*), 9.68 (s, 1H, O*H*), 8.85-8.84 (d, 1H, Ar*H*), 8.63-8.61 (dd, 1H, Ar*H*), 7.63-7.59 (m, 2H, Ar*H*), 7.57 (m, 1H, Ar*H*), 7.53-7.49 (m, 2H, Ar*H*), 7.33-7.31 (d, 1H, Ar*H*), 7.20-7.16 (m, 2H, Ar*H*),7.15-7.10 (m, 2H, Ar*H*) 7.00-6.98 (d, 1H, Ar*H*), 4.00 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.47- 2.33 (bs, 8H, CH_2); $C_{28}H_{26}F2N_4O_2$ requires: C, 68.84; H, 5.36; F, 7.78; N, 11.47; found C, 68.68; H, 5.57; N, 11.15; LC-MS (m/z): 489.4 (M+1); Purity 98.68 %.

4.6.1.16 ***N-(4-Chlorophenyl)-2-(4-fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)- piperazin-1-yl)acetamide*** **(86)***:* Using *N*-(4-chlorophenyl)-2-(4-fluorophenyl)-2-(piperazin-1-yl)acetamide (**58**) (0.5 g, 1.45 mM) and **Method D** the desired compound (**73**) was obtained as a greenish white solid (0.57 g, 79.16 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 80-83 °C. TLC (R_f): 0.46 (80 % Ethyl acetate in hexane); IR: 3314, 2924, 2819, 1693, 1598, 1505, 1399, 1271, 1228, 1006, 828 *cm*$^{-1}$; 1H NMR: δ 10.21 (s, 1H, N*H*), 8.85-8.83 (d, 1H, Ar*H*), 8.64-8.61 (d, 1H, Ar*H*), 7.66-7.62 (m, 2H, Ar*H*), 7.59-7.56 (dd, 1H, Ar*H*), 7.53-7.49 (m, 2H, Ar*H*), 7.36-7.32 (m, 3H, Ar*H*), 7.21-7.16 (m, 2H, Ar*H*), 7.00-6.99 (d, 1H, Ar*H*), 4.02 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.71-2.34 (bm, 8H, CH_2); $C_{28}H_{26}ClFN_4O_2$ requires: C, 66.60; H, 5.19; N, 11.09; found C, 66.98; H, 5.49; N, 10.87; Mass (m/z): 505.3 (M+), 507.2 (M+2).

4.6.1.17 ***2-(4-Fluorophenyl)-N-(4-hydroxyphenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)- piperazin-1-yl)acetamide*** **(87):** Using 2-(4-fluorophenyl)-*N*-(4-hydroxyphenyl)-2-(piperazin-1-yl)acetamide (**59**) (0.5 g, 1.52 mM) and **Method D** the desired compound (**87**) was obtained as a obtain brown solid (0.56 g, 75.67 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 80-82 °C. TLC (R_f): 0.40 (80 % Ethyl acetate in hexane); IR: 3270, 2923, 2816, 1664, 1506, 1474, 1226, 1016, 832 *cm*$^{-1}$; ^{1}H NMR: δ 9.81 (s, 1H, N*H*), 9.68 (s, 1H, O*H*), 9.19 (s, 1H, O*H*), 8.85-8.84 (d, 1H, Ar*H*), 8.63-8.61 (d, 1H, Ar*H*), 7.59-7.55 (m, 1H, Ar*H*), 7.51-7.48 (m, 2H, Ar*H*), 7.36-7.31 (t, 3H, Ar*H*), 7.19-7.15 (t, 2H, Ar*H*), 6.99-6.98 (d, 1H, Ar*H*), 6.68-6.65 (d, 2H, Ar*H*), 3.95 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 2.68 (s, 4H, CH_2), 2.33 (s, 4H, CH_2); $C_{28}H_{27}FN_4O_3$ requires: C, 69.12; H, 5.59; N, 11.52; found C, 69.43; H, 5.87; N, 11.34; LCMS (m/z): 487.4 (M+1); Purity 98.85 %.

4.6.1.18 ***N-Cyclohexyl-2-(4-fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)acetamide*** **(88):** Using *N*-cyclohexyl-2-(4-fluorophenyl)-2-(piperazin-1-yl)acetamide (**60**) (0.5 g, 1.56 mM) and **Method D** the desired compound (**88**) was obtained as a yellowish white solid (0.61 g, 82.43 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 195-196 °C. TLC (R_f): 0.46 (80 % Ethyl acetate in hexane); IR: 3322, 2928, 2850, 1649, 1502, 1473, 1270, 1223, 1004, 829, 701 *cm*$^{-1}$; ^{1}H NMR: δ 9.71 (s, 1H, N*H*), 8.85-8,83 (dd, 1H, Ar*H*), 8.62-8.59 (d, 1H, Ar*H*), 7.86-7.85 (d, 1H, Ar*H*), 7.57-7.54 (q, 1H, Ar*H*), 7.41-7.38 (m, 2H, Ar*H*), 7.32-

7.30 (d, 1H, Ar*H*), 7.15-7.11(d, 2H, Ar*H*), 6.99-6.97 (d, 1H, Ar*H*), 3.77-3.75 (d, 3H, CH_2, C*H*), 3.49-3.47 (d, 1H, C*H*), 2.41 (bs, 4H, CH_2), 2.26 (bs, 3H, CH_2), 1.69-1.51 (m, 5H, CH_2), 1.22-1.09 (m, 5H, CH_2); $C_{28}H_{33}FN_4O_2$ requires: C, 70.56; H, 6.98; N, 11.76; found C, 70.38; H, 7.17; N, 11.48; Mass (m/z): 477.4 (M+1).

4.6.1.19 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)-N-phenyl- acetamide*** **(89):** Using 2-(4-methoxyphenyl)-*N*-phenyl-2-(piperazin-1-yl)acetamide (**61**) (0.5 g, 1.54 mM) and **Method D** the desired compound (**89**) was obtained as a white solid (0.56 g, 75.67 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 96-99 °C; TLC (R_f): 0.56 (80 % Ethyl acetate in hexane); IR: 3305, 3056, 2931, 2815, 1685, 1507, 1439, 1246, 1176, 1133, 1030 cm^{-1}; ^{1}H NMR: δ 9.97 (s, 1H, N*H*), 9.72 (s, 1H, O*H*), 8.84-8.83 (d, 1H, Ar*H*), 8.86-8.60 (d, 1H, Ar*H*), 7.59-7.55 (m, 3H, Ar*H*), 7.39-7.37 (d, 2H, Ar*H*), 7.32-7.25 (m, 3H, Ar*H*), 7.04-7.01 (t, 1H, Ar*H*), 6.98-6.96 (d, 1H, Ar*H*), 6.91-6.88 (d, 2H, Ar*H*), 3.90 (s, 1H, CH), 3.76 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.67 (s, 4H, CH_2), 2.33 (s, 4H, CH_2); $C_{29}H_{30}N_4O_3$ requires: C, 72.18; H, 6.27; N, 11.61; found C, 72.06; H, 6.49; N, 11.49; Mass (m/z): 483.3 (M+1).

4.6.1.20 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)-N-(o-tolyl) acetamide*** **(90):** Using 2-(4-methoxyphenyl)-2-(piperazin-1-yl)-*N*-(*o*-tolyl)acetamide (**62**) (0.5 g, 1.47 mM) and **Method D** the desired compound (**90**) was obtained as a greenish white solid (0.58 g, 79.45 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 86-88 °C. TLC (R_f): 0.62 (80 % Ethyl acetate in hexane); IR: 3332, 2922, 2833, 1689, 1608, 1582, 1505, 1229, 786 cm^{-1}; ^{1}H NMR: δ 9.70 (d, 1H, N*H*), 9.54 (d, 1H, O*H*), 8.90-8.78 (m, 2H, Ar*H*), 8.65-8.33 (m, 2H, Ar*H*), 7.62-7.48 (m, 2H, Ar*H*), 7.37-7.28 (m, 2H, Ar*H*), 7.25-7.04 (m, 2H, Ar*H*), 6.97 (d, 2H, Ar*H*), 6.87 (d, 1H, Ar*H*), 4.80-4.27 (m, 2H, CH_2), 3.97 (d, 1H, C*H*), 3.81-3.69 (m, 3H, OCH_3), 2.37 (bs, 4H, CH_2), 2.18 (bs, 3H, CH_3); ^{13}C NMR δ: 169.21, 158.81, 152.79, 151.90, 147.72, 138.83, 135.96, 133.70, 132.86, 130.23, 128.77, 127.77, 126.67, 126.08, 124.76, 123.28, 121.41, 113.59, 110.58, 109.88, 73.99, 59.53, 54.98, 52.67, 17.60. $C_{30}H_{32}N_4O_3$ requires: C, 72.56; H, 6.50; N, 11.28; found C, 72.75; H, 6.82; N, 11.06; Mass (m/z): 497.4 (M+1).

4.6.1.21 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)-N-(p-tolyl) acetamide*** **(91):** Using 2-(4-methoxyphenyl)-2-(piperazin-1-yl)-*N*-(*p*-tolyl)acetamide (**63**) (0.5 g, 1.47 mM) and **Method D** the desired compound (**91**) was obtained as a white solid (0.51 g, 69.86 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 103-105 °C. TLC (R_f): 0.48 (80 % Ethyl acetate in hexane); IR: 3327, 3037, 2921, 1686, 1580, 1506, 1473, 1418, 1370, 1274, 1224, 1192, 1251, 781 cm^{-1}; ^{1}H NMR: δ 9.69 (s, 2H, N*H,* O*H*), 8.87-8.88 (d, 2H, Ar*H*), 8.47-8.45 (d, 2H, Ar*H*), 7.66-7.37 (m, 3H, Ar*H*), 7.07-6.90 (m, 5H, Ar*H*), 4.68 (s, 2H, CH_2), 3.88-3.72 (m, 1H, C*H*), 3.36 (s, 3H, OCH_3), 2.57 (dd, 3H, CH_3), 2.34-2.14 (m, 4H, CH_2); $C_{30}H_{32}N_4O_3$ requires: C, 72.56; H, 6.50; N, 11.28; found C, 72.74; H, 6.73; N, 11.06; Mass (m/z): 497.4 (M+1).

4.6.1.22 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N-(2-methoxyphenyl)-2-(4-methoxyphenyl)acetamide*** **(92):** Using *N*-(2-methoxyphenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-

yl)acetamide (**64**) *(*0.5 g, 1.40 mM) and **Method D** the desired compound (**92**) was obtained as a yellowish white solid (0.58 g, 80.55 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 153-155 °C. TLC (R_f): 0.46 (80 % Ethyl acetate in hexane); IR: 3314, 2927, 2832, 1689, 1599, 1460, 1371, 1248, 1115, 1028 *cm*$^{-1}$; ^{1}H NMR: δ 9.95 (s, 1H, N*H*), 9.71 (s, 1H, O*H*), 8.85-8.83 (d, 1H, Ar*H*), 8.61-8.60 (d, 1H, Ar*H*), 8.11-8.09 (s, 1H, Ar*H*), 7.58-7.54 (m, 1H, Ar*H*), 7.36-7.34 (d, 1H, Ar*H*), 7.22-7.20 (d, 2H, Ar*H*), 7.11-7.07 (d, 2H, Ar*H*), 7.01-6.99 (d, 1H, Ar*H*), 6.94-6.90 (m, 3H, Ar*H*), 4.13 (s, 1H, C*H*), 3.95 (s, 3H, OCH_3), 3.84-3.79 (d, 2H, CH_2), 3.76 (s, 3H, OCH_3), 2.35 (s, 9H, CH_2); $C_{30}H_{32}N_4O_4$ requires: C, 70.29; H, 6.29; N, 10.93; found C, 70.08; H, 6.47; N, 10.76; Mass (m/z): 513.3 (M+1).

4.6.1.23 ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-N,2-bis(4-methoxyphenyl)- acetamide*** **(93)**: Using *N*,2-bis(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (**65**) (0.5 g, 1.40 mM) and **Method D** the desired compound (**93**) was obtained as a yellowish white solid (0.60 g, 83.33 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 145-147°C. TLC (R_f): 0.49 (80 % Ethyl acetate in hexane); IR: 3307, 2822, 1664, 1508, 1468, 1232, 1175, 1133, 1029 *cm*$^{-1}$; ^{1}H NMR: δ 9.84 (s, 1H, N*H*), 9.69 (s, 1H, O*H*), 8.85-8.83 (dd, 1H, Ar*H*), 8.63-8.60 (dd, 1H, Ar*H*), 7.58-7.55 (m, *J* = 4.5 Hz, 1H, Ar*H*), 7.50-7.47 (d, 2H, Ar*H*), 7.38-7.36 (d, 2H, Ar*H*), 7.33-7.31 (d,1H, Ar*H*), 6.99-6.97 (d, 1H, Ar*H*), 6.90-6.83 (m, 4H, Ar*H*), 3.86 (s, 1H, C*H*), 3.77 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 2.51-2.38 (s, 8H, CH_2); $C_{30}H_{32}N_4O_4$ requires: C, 70.29; H, 6.29; N, 10.93; found C, 69.95; H, 6.61; N, 10.76; Mass (m/z): 513.3 (M+1).

4.6.1.24 ***N-(4-Fluorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetamide*** **(94)***:* Using *N*-(4-fluorophenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (**66**) (0.5 g, 1.45 mM) and **Method D** the desired compound (**94**) was obtained as a yellowish white solid (0.57 g, 78 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 160-163°C. TLC (R_f): 0.51 (80 % Ethyl acetate in hexane); IR:3294, 3002, 2937, 2817, 1689, 1610, 1508, 1371, 1301, 1247, 1135, 1007, 832 *cm*$^{-1}$; ^{1}H NMR: δ 10.04 (s, 1H, N*H*), 9.71 (s, 1H, O*H*), 8.84-8.83 (d, 1H, Ar*H*), 8.62-8.60 (d, 1H, Ar*H*), 7.62-7.55 (m, 3H, Ar*H*), 7.42-7.30 (dd, 3H, Ar*H*), 7.13-7.09 (t, 2H, Ar*H*), 6.99-6.88 (dd, 3H, Ar*H*), 3.87 (s, 1H, C*H*), 3.76 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.45-2.33 (bs, 7H); $C_{29}H_{29}FN_4O_3$ requires: C, 69.58; H, 5.84; N, 11.19; found C, 69.34; H, 5.95; N, 11.06; Mass (m/z): 501.3 (M^+).

4.6.1.25 ***N-(4-Chlorophenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetamide*** **(95):** Using of *N*-(4-chlorophenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (**67**) (0.5 g, 1.38 mM) and **Method D** the desired compound (**95**) was obtained as a green solid (0.56 g, 77.77 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 135-138 °C. TLC (R_f): 0.52 (80 % Ethyl acetate in hexane); IR: 3316, 2817, 1692, 1583, 1504, 1473, 1372, 1232, 1177, 1005, 787 *cm*$^{-1}$; ^{1}H NMR: δ 10.12 (s, 1H, N*H*), 9.71 (s, 1H, O*H*), 8.88-8.84 (m, 1H, Ar*H*), 8.63-8.61 (m, 1H, Ar*H*), 7.65-7.63 (d, 2H, Ar*H*), 7.59-7.56 (dd, 1H, Ar*H*), 7.40-7.32 (m, 5H, Ar*H*), 6.99-6.98 (d, 1H, Ar*H*), 6.92-6.90 (d, 2H, Ar*H*), 3.91 (s, 1H,

C*H*), 3.78 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 2.46-2.35 (d, 8H, CH_2); $C_{29}H_{29}ClN_4O_3$ requires: C, 67.37; H, 5.65; N, 10.84; found C, 67.61; H, 5.98; N, 10.62; Mass (m/z): 517.3 (M^+), 519.1 (M+2).

4.6.1.26 *N-(4-Hydroxyphenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetamide* (96): Using *N*-(4-hydroxyphenyl)-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (**68**) (0.5 g, 1.46 mM) and **Method D** the desired compound (**96**) was obtained as an orange solid (0.54 g, 73.97 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate: Hexane as mobile phase, m.p. 170-172 °C. TLC (R_f): 0.38 (80 % Ethyl acetate in hexane); IR: 3317, 2948, 2820, 1659, 1607, 1510, 1476, 1371, 1233, 1180, 1032 cm^{-1}; ^{1}H NMR: δ 10.06 (bs, 1H, O*H*), 9.73 (s, 1H, N*H*), 9.20 (s, 1H, O*H*), 8.90-8.84 (dd, 1H, Ar*H*), 8.65-8.61 (t, 1H, Ar*H*), 8.01-7.85 (dd, 1H, Ar*H*), 7.71-7.66 (m, 2H, Ar*H*), 7.58-7.55 (m, 1H, Ar*H*), 7.45-7.41 (m, 1H, Ar*H*), 7.38-7.31 (m, 3H, Ar*H*), 7.10-6.97 (dd, 1H, Ar*H*), 6.90-6.88 (d, 1H, Ar*H*), 6.67-6.65 (d, 1H, Ar*H*), 3.85 (s, 1H, C*H*), 3.78 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 2.34 (bs, 8H, CH_2); $C_{29}H_{30}N_4O_4$ requires: C, 69.86; H, 6.07; N, 11.24; found C, 69.69; H, 6.25; N, 11.15; Mass (m/z): 499.3 (M+1).

4.6.1.27 *N-(4-Hydroxyphenyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)piperazin-1-yl)-2-(4-methoxyphenyl)acetamide* (97): Using *N*-cyclohexyl-2-(4-methoxyphenyl)-2-(piperazin-1-yl)acetamide (69) (0.5 g, 1.50 mM) and **Method D** the desired compound (**97**) was obtained as a white solid (0.63 g, 85.13 %), which was further purified by column chromatography using 100-200 silica gel as stationary phase and Ethyl acetate:Hexane as mobile phase, m.p. 197-199 °C. TLC (R_f): 0.52 (80 % Ethyl acetate in hexane); IR: 3325, 2934, 2852, 2817, 1644, 1509, 1475, 1376, 1246, 1180, 1135, 1006 cm^{-1}; ^{1}H NMR: δ 9.91 (s, 1H, N*H*), 8.84 (s, 1H, O*H*), 8.61-8.58 (d, 1H, Ar*H*), 7.78-7.76 (d, 1H, Ar*H*), 7.57-7.54 (m, 1H, Ar*H*), 7.31-7.25 (m, 3H, Ar*H*), 6.98-6.96 (d, 1H, Ar*H*), 6.86-6.84 (d, 2H, Ar*H*), 3.75 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.66 (s, 1H, C*H*), 3.47 (bs, 1H, C*H*), 2.44-2.40 (m, 8H, CH_2), 1.64-1.51 (m, 5H, CH_2), 1.23-1.09 (m, 5H, CH_2); $C_{29}H_{36}N_4O_3$ requires: C, 71.28; H, 7.43; N, 11.47; found C, 71.40; H, 7.75; N, 11.13; Mass (m/z): 489.4 (M+1).

4.7 Biological Activity

4.7.1 *Inhibition studies on AChE and BuChE*

The potential of the test compounds for cholinesterase inhibition was assessed using Ellman's essay. [39–41] The products that were purchased from Sigma-Aldrich included human AChE (product number C1682), equine serum BuChE (CAS 9001-08-5), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, product number T-D0944), acetylthiocholine iodide (ATCI, product number T-A0116), and butyrylthiocholine iodide (BTCI, product number T-B0775). Standard drugs were donepezil hydrochloride and tacrine hydrochloride hydrate. Every experiment was conducted at pH 8 in a 50 mM Tris-Hydrochloride buffer (Tris HCl, product number MB030). To ascertain the enzyme inhibitory activity, five distinct doses (0.001–100 µM) of every test chemical were employed. To summarize, 10 µL of the test or reference compounds were incubated in 50 µL of AChE (0.22 U/mL) or 50 µL of BuChE (0.06 U/mL)

4.7.2 *Antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity]*

a) Preparation of DPPH reagent: A solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1mM) was prepared in methanol.

b) Preparation of Sample/Standard

Based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), free radical scavenging activity of the synthesized compounds was determined by the method of Ali *et al* [43]. Different volumes (20 – 100µg/ml) of standard compound ascorbic acid and the synthesized compounds were taken from a stock solution in a set of test tubes, and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min.

C) Preparation of control

For control, DPPH (3 ml of 0.1mM solution) was taken and incubated for 30 min at room temperature in dark conditions. The absorbance of the control was taken against methanol (as blank) at 517 nm [44].

The percentage antioxidant activity of the sample/standard was calculated by using the formula:

% Inhibition = [(Ab of control- Ab of sample/Ab of control] x 100

The lower the absorbance, the higher the free radical scavenging activity. The curves were prepared and the IC_{50} values were calculated using linear regression analysis.

4.7.3 *Metal-chelating study*

The metal chelating ability of all the compounds was assessed using UV spectrophotometry [45]. The absorption spectra of the test compounds (25 µM) alone and in the presence of $CuSO_4$, $ZnCl_2$, $FeSO_4$, $FeCl_3$ and $AlCl_3$ (25 µM) in methanol for 30 min were recorded at room temperature in the UV-visible range.

4.7.4 *ADME Prediction*

Before a molecule is introduced into the market, its efficacy and safety are vital considerations. An examination of its ADMET (absorption, distribution, metabolism, excretion, and toxicity) profile can be one way to look at these features [46]. Using the online SwissADME server [47], the ADMET properties of the synthesized compounds were evaluated.

5. Conclusion

World's population is slowly inching towards a continuously growing pool of old-age people every year. Apart from other age-related ailments, Alzheimer's disease is posing a serious problem in the society. A worrying fact is poor understanding of the disease despite so much of advancements in the medical field, and absence of curative therapeutics. In our quest to develop some acceptable anti-Alzheimer's agents

we used molecular hybridization approach to combine some anti-Alzheimer's savvy molecular fragments, like piperazine, 8-hydroxyquinoline and acetamido groups into a singular molecular entity to design some potential anti-Alzheimer's agents. Modifications were made by attaching aromatic/alicyclic amines through acetamide linkers to the 5-(piperazin-1-ylmethyl)quinolin-8-ol scaffold, resulting in a novel series of anti-AD agents. The designed compounds displayed excellent affinity towards both the enzymes with docking scores in the range of -12.8 to -10.6 kcal/mol for AChE, and − 12.4 to -10.3 kcal/mol for BuChE which were higher than the scores obtained for the standard compound's donepezil (-10.8 kcal/mol) and Tacrine (-8.4 kcal/mol). Among them, compounds having a 4-chloroanilino moiety and a 4-methoxyphenyl group, exhibited the most promising inhibitory activities against AChE (with an IC_{50} value of 3.013 μM) and BuChE (with an IC_{50} value of 3.144 μM). Compound (**83**), with 2-methoxyaniline and 4-fluorobenzene substituents, offered the highest BuChE inhibition with an IC_{50} value of 1.888 μM. Additionally, compound **(79)** offered 93 times higher selectivity for BuChE over AChE. All the compounds displayed metal chelating ability with (Fe^{+2}, Fe^{+3}, Zn^{+2}, Cu^{+2}, and Al^{+3}), as well as moderate antioxidant activity. Molecular modelling studies indicated significant interactions between the most potent compounds (**83**, **95**) and the PAS and CAS sites of the enzymes. Furthermore, all the compounds offered acceptable *in silico* pharmacokinetic properties including twenty compounds showing BBB permeability. These results collectively suggested that compound (**95**) could be a leading candidate with high potential for further development as a novel anti-AD drug by inhibiting both AChE and BuChE. At the same time, compound **(79)** can be a potent and selective BuChE Inhibitor.

Declarations

Acknowledgements

We acknowledge Research and development cell, Parul University, Vadodara, Gujarat, India for providing funding as part of intramural research grant for the successful completion of the project.

Authors' contribution

M.R. Yadav conceptualized the whole study. A. A. Nagani, M. N. Shah and S. I. Patel carried out the synthesis and data collection, and H. A. Patel and M. N. Shah planned and executed computational studies. V. K. Parikh, A. D. Patel, and B. C. Bhimani assisted in data collection and data interpretation. K. V. Patel designed the biological studies and H. R. Parmar and S. P. Patel performed biological studies and data collection. A. A. Nagani, S. I. Patel, and M. N. Shah drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript. The authors declare that they do not have any conflict of interest. The authors declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere. We confirm that the

manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

References

1. https://alzheimersnewstoday.com/what-is-alzheimers-disease/
2. https://alzheimersnewstoday.com/alzheimers-disease-statistics/
3. Shah H, Patel A, Parikh V, Nagani A, Bhimani B, Shah U, Bambharoliya T (2020) The β-Secretase Enzyme BACE1: A Biochemical Enigma for Alzheimer's disease. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 19(3):184–194
4. Patel DV, Patel NR, Kanhed AM, Teli DM, Patel KB, Gandhi PM, Patel SP, Chaudhary BN, Shah DB, Prajapati NK, Patel KV (2020) Further studies on triazinoindoles as potential novel multitarget-directed anti-alzheimer's agents. ACS Chem Neurosci 11(21):3557–3574
5. Patel KB, Patel DV, Patel NR, Kanhed AM, Teli DM, Gandhi B, Shah BS, Chaudhary BN, Prajapati NK, Patel KV, Yadav MR (2022) Carbazole-based semicarbazones and hydrazones as multifunctional anti-Alzheimer agents. J Biomol Struct Dynamics 40(20):10278–10299
6. Madhav H, Abdel-Rahman SA, Hashmi MA, Rahman MA, Rehan M, Pal K, Nayeem SM, Gabr MT, Hoda N (2023) Multicomponent Petasis reaction for the identification of pyrazine based multi-target directed anti-Alzheimer's agents: In-silico design, synthesis, and characterization. Eur J Med Chem 254:115354
7. Zhou S, Huang G (2022) The biological activities of butyrylcholinesterase inhibitors. Biomed Pharmacother 146:112556
8. Jiang X, Zhang Z, Zuo J, Wu C, Zha L, Xu Y, Wang S, Shi J, Liu XH, Zhang J, Tang W (2021) Novel cannabidiol – carbamate hybrids as selective BuChE inhibitors: Docking-based fragment reassembly for the development of potential therapeutic agents against Alzheimer's disease. Eur J Med Chem 223:113735
9. Kanhed AM, Patel DV, Patel NR, Sinha A, Thakor PS, Patel KB, Prajapati NK, Patel KV, Yadav MR (2022) Indoloquinoxaline derivatives as promising multi-functional anti-Alzheimer agents. J Biomol Struct Dynamics 40(6):2498–2515
10. Umar T, Shalini S, Raza MK, Gusain S, Kumar J, Seth P, Tiwari M, Hoda N (2019) A multifunctional therapeutic approach: Synthesis, biological evaluation, crystal structure and molecular docking of diversified 1H-pyrazolo [3, 4-b] pyridine derivatives against Alzheimer's disease. Eur J Med Chem 175:2–19
11. Kumar J, Meena P, Singh A, Jameel E, Maqbool M, Mobashir M, Shandilya A, Tiwari M, Hoda N, Jayaram B (2016) Synthesis and screening of triazolopyrimidine scaffold as multi-functional agents for Alzheimer's disease therapies. Eur J Med Chem 119:260–277

12. Onaolapo OJ, Olofinnade AT, Ojo FO, Onaolapo AY (2022) Neuroinflammation and Oxidative Stress in Alzheimer's Disease; Can Nutraceuticals and Functional Foods Come to the Rescue? Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents). 21(2):75–89
13. Yang X, Cai P, Liu Q, Wu J, Yin Y, Wang X, Kong L (2018) Novel 8-hydroxyquinoline derivatives targeting β-amyloid aggregation, metal chelation and oxidative stress against Alzheimer's disease. Bioorg Med Chem 26(12):3191–3201
14. Liu F, Zhang Z, Zhang L, Meng RN, Gao J, Jin M, Li M, Wang XP (2022) Effect of metal ions on Alzheimer's disease. Brain Behav 12(3):e2527
15. Lane DJ, Ayton S, Bush AI (2018) Iron and Alzheimer's disease: an update on emerging mechanisms. J Alzheimers Dis 64(s1):S379–S395
16. Maher P (2018) Potentiation of glutathione loss and nerve cell death by the transition metals iron and copper: Implications for age-related neurodegenerative diseases. Free Radic Biol Med 115:92–104
17. van Duijn S, Bulk M, van Duinen SG, Nabuurs RJ, van Buchem MA, van der Weerd L, Natté R (2017) Cortical iron reflects severity of Alzheimer's disease. J Alzheimers Dis 60(4):1533–1545
18. Wang ZX, Tan L, Wang HF, Ma J, Liu J, Tan MS, Sun JH, Zhu XC, Jiang T, Yu JT (2015) Serum iron, zinc, and copper levels in patients with Alzheimer's disease: a replication study and meta-analyses. J Alzheimers Dis 47(3):565–581
19. Religa D, Strozyk D, Cherny RA, Volitakis I, Haroutunian V, Winblad B, Naslund J, Bush AI (2006) Elevated cortical zinc in Alzheimer disease. Neurology 67(1):69–75
20. Bucossi S, Polimanti R, Mariani S, Ventriglia M, Bonvicini C, Migliore S, Manfellotto D, Salustri C, Vernieri F, Rossini PM, Squitti R (2012) Association of K832R and R952K SNPs of Wilson's disease gene with Alzheimer's disease. J Alzheimers Dis 29(4):913–919
21. Squitti R, Ventriglia M, Simonelli I, Bonvicini C, Costa A, Perini G, Binetti G, Benussi L, Ghidoni R, Koch G, Borroni B (2021) Copper imbalance in Alzheimer's disease: Meta-analysis of serum, plasma, and brain specimens, and replication study evaluating ATP7B gene variants. Biomolecules 11(7):960
22. Squitti R, Polimanti R, Bucossi S, Ventriglia M, Mariani S, Manfellotto D, Vernieri F, Cassetta E, Ursini F, Rossini PM (2013) Linkage disequilibrium and haplotype analysis of the ATP7B gene in Alzheimer's disease. Rejuven Res 16(1):3–10
23. Squitti R, Ghidoni R, Siotto M, Ventriglia M, Benussi L, Paterlini A, Magri M, Binetti G, Cassetta E, Caprara D, Vernieri F (2014) Value of serum nonceruloplasmin copper for prediction of mild cognitive impairment conversion to Alzheimer disease. Ann Neurol 75(4):574–580
24. Tripathi PN, Srivastava P, Sharma P, Tripathi MK, Seth A, Tripathi A, Rai SN, Singh SP, Shrivastava SK (2019) Biphenyl-3-oxo-1, 2, 4-triazine linked piperazine derivatives as potential cholinesterase inhibitors with anti-oxidant property to improve the learning and memory. Bioorg Chem 85:82–96
25. Zhang J, Jiang CS (2018) Synthesis and evaluation of coumarin/piperazine hybrids as acetylcholinesterase inhibitors. Med Chem Res 27(6):1717–1727

26. Ostrowska K (2020) Coumarin-piperazine derivatives as biologically active compounds. Saudi Pharm J 28(2):220–232
27. Modh RP, Kumar SP, Jasrai YT, Chikhalia KH, Design (2013) Synthesis, Biological Evaluation, and Molecular Modeling of Coumarin–P iperazine Derivatives as Acetylcholinesterase Inhibitors. Arch Pharm 346(11):793–804
28. Piplani P, Danta CC (2015) Design and synthesis of newer potential 4-(N-acetylamino) phenol derived piperazine derivatives as potential cognition enhancers. Bioorg Chem 60:64–73
29. Makhaeva GF, Lushchekina SV, Kovaleva NV, Astakhova TY, Boltneva NP, Rudakova EV, Serebryakova OG, Proshin AN, Serkov IV, Trofimova TP, Tafeenko VA (2021) Amiridine-piperazine hybrids as cholinesterase inhibitors and potential multitarget agents for Alzheimer's disease treatment. Bioorg Chem 112:104974
30. Patel A, Patel S, Mehta M, Patel Y, Patel R, Shah D, Patel D, Shah U, Patel M, Patel S, Solanki N (2022) A review on synthetic investigation for quinoline-recent green approaches. Green Chem Lett Rev 15(2):337–372
31. Lad C, Panchal I, Patel A, Nagani A, Parikh V, Patel H, Bhimani B (2021) silico analysis, synthesis and biological evaluation of DHFR inhibitors. Folia medica 63(5):745–759
32. Li ZH, Yin LQ, Zhao DH, Jin LH, Sun YJ, Tan C SAR studies of quinoline and derivatives as potential treatments for Alzheimer’s disease. Arab J Chem 2022 Dec 9: 104502
33. McLachlan DC, Kruck TP, Kalow W, Andrews DF, Dalton AJ, Bell MY, Smith WL (1991) Intramuscular desferrioxamine in patients with Alzheimer's disease. Lancet 337(8753):1304–1308
34. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Aβ amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol 60(12):1685–1691
35. Wang L, Esteban G, Ojima M, Bautista-Aguilera OM, Inokuchi T, Moraleda I, Iriepa I, Samadi A, Youdim MB, Romero A, Soriano E (2014) Donepezil + propargylamine + 8-hydroxyquinoline hybrids as new multifunctional metal-chelators, ChE and MAO inhibitors for the potential treatment of Alzheimer's disease. Eur J Med Chem 80:543–561
36. Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, Harrison J, Masters CL, Targum S, Bush AI, Murdoch R, Wilson J (2008) Safety, efficacy, and biomarker findings of PBT2 in targeting Aβ as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. Lancet Neurol 7(9):779–786
37. Dileep KV, Ihara K, Mishima-Tsumagari C, Kukimoto-Niino M, Yonemochi M, Hanada K et al (2022) Crystal structure of human acetylcholinesterase in complex with tacrine: Implications for drug discovery. Int J Biol Macromolecule 210:172–181
38. Nachon F, Carletti E, Ronco C, Trovaslet M, Nicolet Y, Jean L, Renard PY (2013) Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl-and butyryl-cholinesterase. Biochem J 453(3):393–399

39. Sinha A, Tamboli RS, Seth B, Kanhed AM, Tiwari SK, Agarwal S, Nair S, Giridhar R, Chaturvedi RK, Yadav MR (2015) Neuroprotective role of novel triazine derivatives by activating Wnt/β catenin signaling pathway in rodent models of Alzheimer's disease. Mol Neurobiol. ; 52: 638 – 52.A.
40. Kanhed AM, Sinha A, Machhi J, Tripathi A, Parikh ZS, Pillai PP, Giridhar R, Yadav MR (2015) Discovery of isoalloxazine derivatives as a new class of potential anti-Alzheimer agents and their synthesis. Bioorg Chem 61:7–12
41. Shidore M, Machhi J, Shingala K, Murumkar P, Sharma MK, Agrawal N, Tripathi A, Parikh Z, Pillai P, Yadav MR (2016) Benzylpiperidine-linked diarylthiazoles as potential anti-Alzheimer's agents: synthesis and biological evaluation. J Med Chem 59(12):5823–5846
42. Rbaa M, Jabli S, Lakhrissi Y, Ouhssine M, Almalki F, Ben Hadda T et al (2019) Synthesis, antibacterial properties and bioinformatics computational analyses of novel 8-hydroxyquinoline derivatives. Heliyon 5(10):e02689
43. Ali MS, Amin MR, Kamal CM, Hossain MA (2013) In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the A. philippense L. leaves. Asian Pac J Trop Biomed 3(6):464–469
44. Al-Rimawi F, Rishmawi S, Ariqat SH, Khalid MF, Warad I, Salah Z (2016) Anticancer activity, antioxidant activity, and phenolic and flavonoids content of wild Tragopogon porrifolius plant extracts. Evidence-Based Complementary and Alternative Medicine. ; 2016
45. Savelieff MG, Lee S, Liu Y, Lim MH (2013) Untangling amyloid-β, tau, and metals in Alzheimer's disease. ACS Chem Biol 8(5):856–865
46. Kumar N, Goel N, Chand Yadav T, Pruthi V (2017) Quantum chemical, ADMET and molecular docking studies of ferulic acid amide derivatives with a novel anticancer drug target. Med Chem Res 26(8):1822–1834
47. http://www.swissadme.ch

Table

Table 4 is available in the Supplementary Files section.

Figures

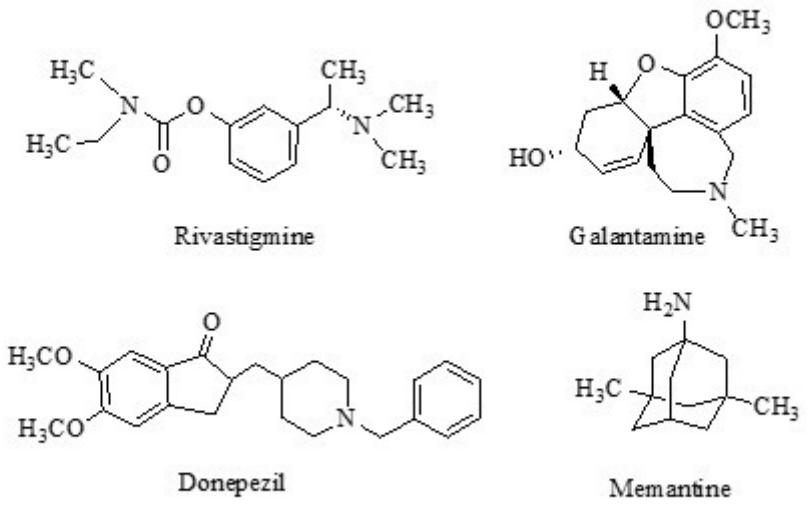

Figure 1

Chemical structures of the FDA-approved anti-Alzheimer's drugs.

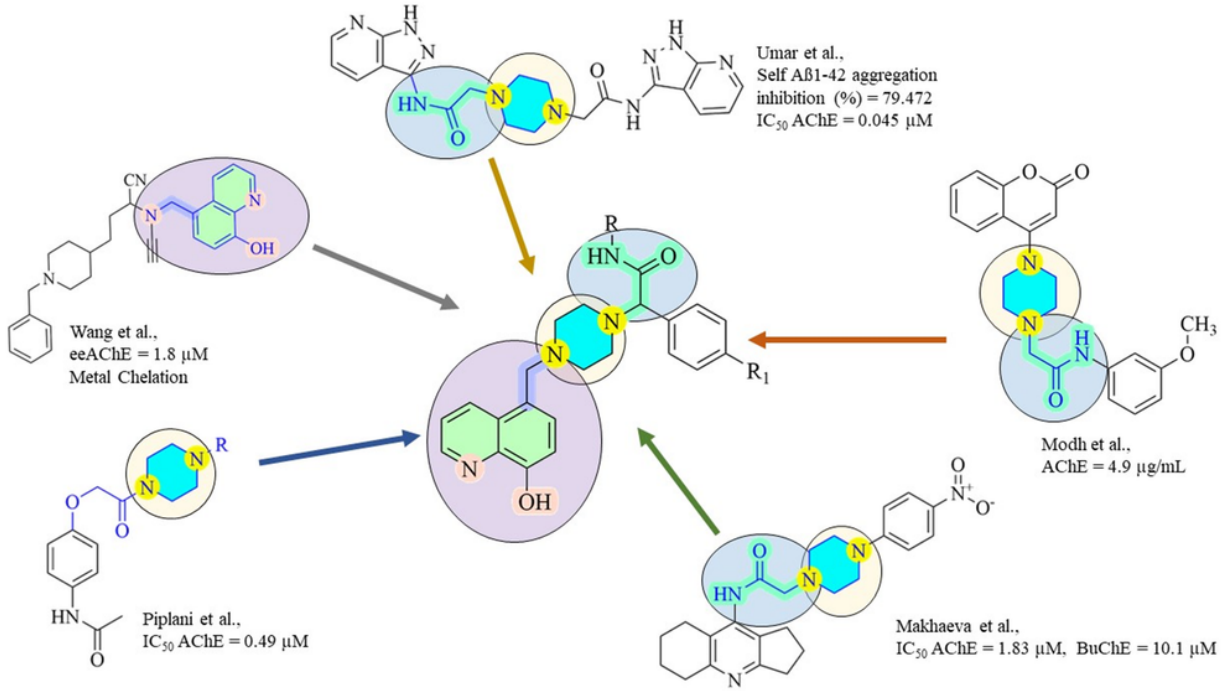

Figure 2

Designing strategy for the development of piperazine-quinoline-based MTDLs.

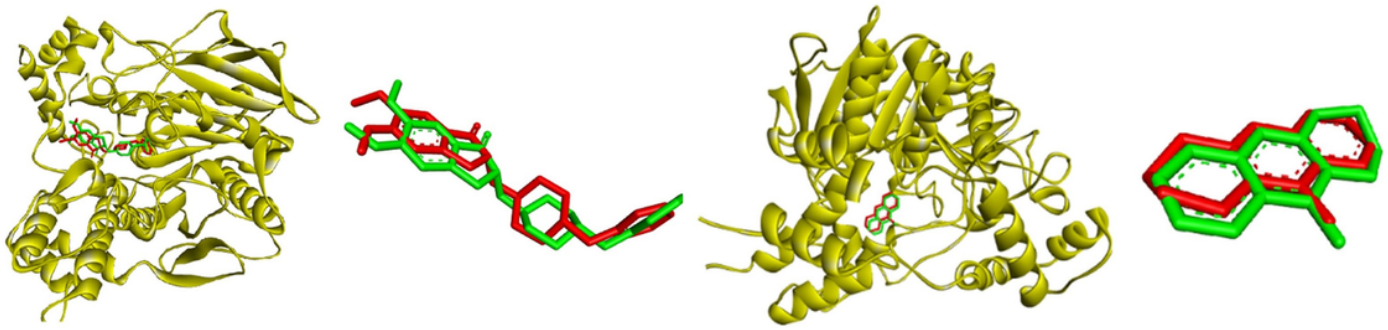

Figure 3

Validation results of the binding modes of donepezil and tacrine obtained using the AutoDock software. Donepezil, a potent AChE inhibitor (PDB: 7E3H) **(a);** and tacrine, a BuChE inhibitor (PDB: 4BDS) **(b)**. In green, the crystallographic pose; in red, the top-ranked docking solution.

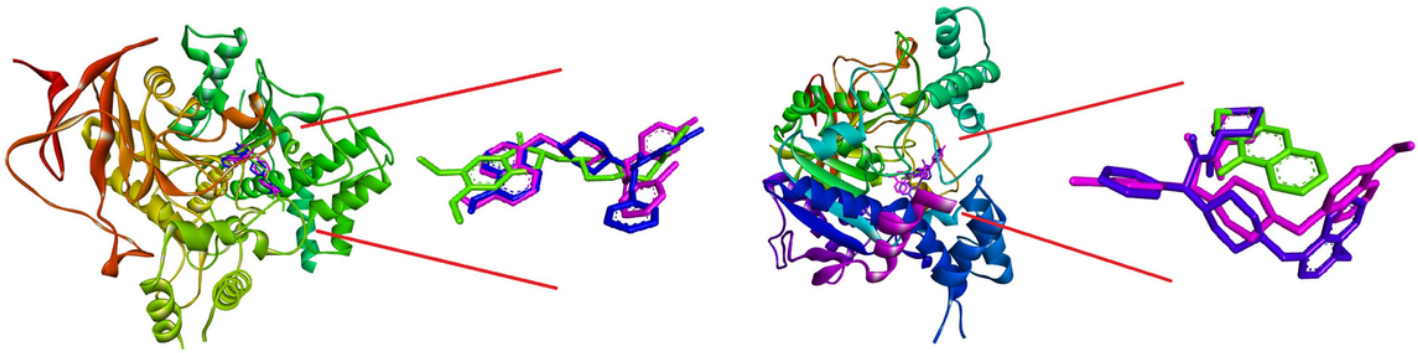

Figure 4

Superposed structures of reference drug donepezil and the designed compounds (**95, 96, 83** and **92**). (**a**) With AChE [PDB: 7E3H] (Donepezil: Green, **95**: Blue, **96**: Pink). (**b**) With BuChE [PDB: 4BDS] (Tacrine: Green, **83**: Blue, **92**: Pink).

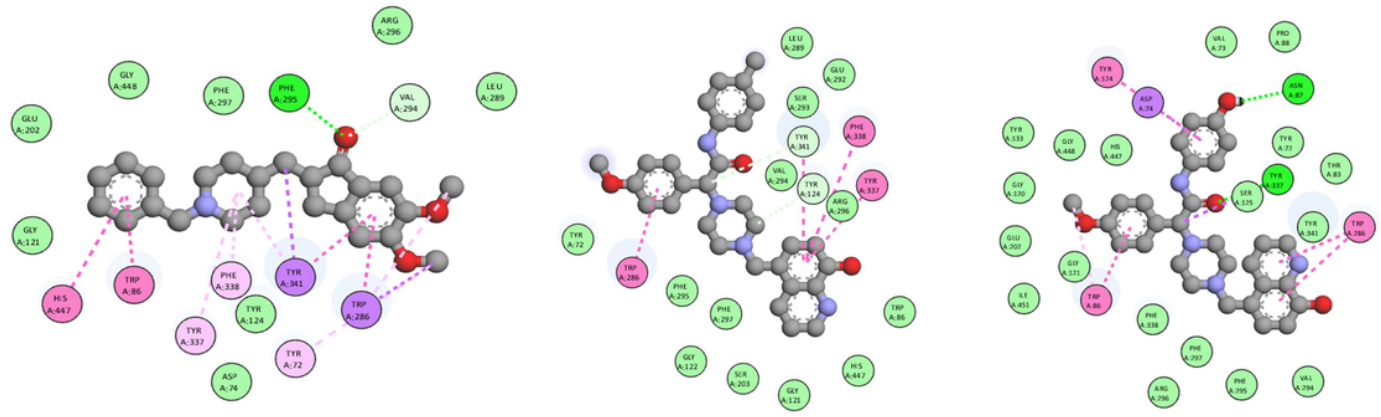

Figure 5

Docking conformations and AChE protein-ligand interactions of reference drug donepezil and the designed molecules. (a) Donepezil; **(b) compound (95); (c) compound (96).**

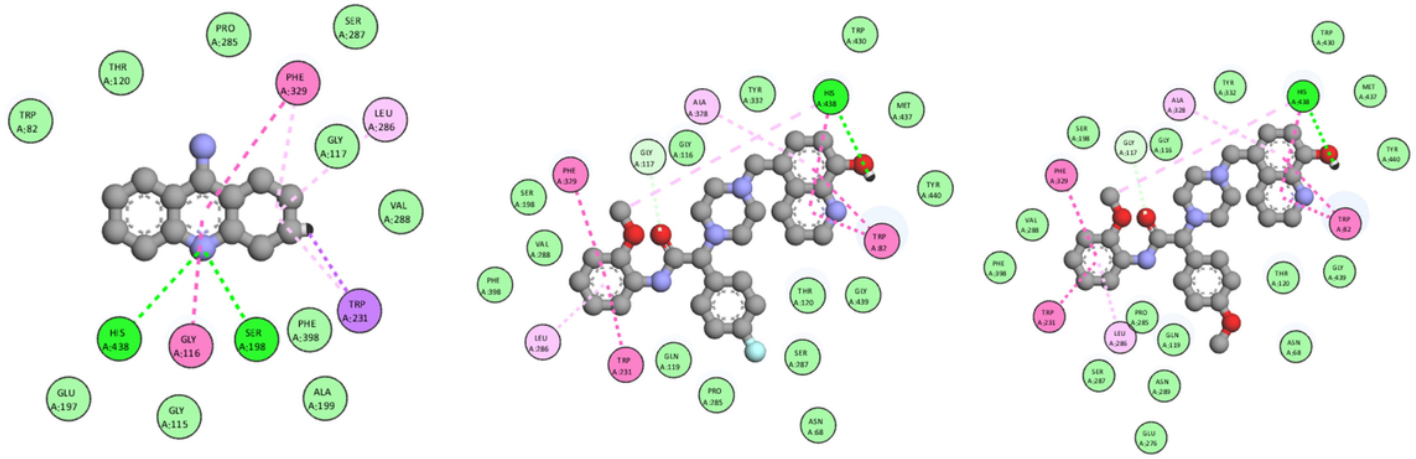

Figure 6

Docking conformations and BuChE protein-ligand interactions of compounds. (**a**) Tacrine; (**b**) compound (**83**); (**c**) compound (**92**).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SUPPORTINGINFORMATION.docx
- Table4.docx