DESIGN AND SYNTHESIS OF NOVEL CHEMICAL ENTITIES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

A THESIS SUBMITTED TO

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1. Introduction

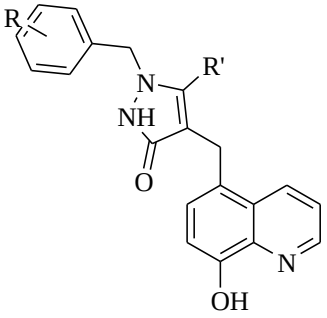
Alzheimer's disease (AD) is a progressive, age-related neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral disturbances. Its prevalence is increasing due to rising life expectancy[1]. As neurons degenerate, patients experience worsening symptoms, ultimately leading to death within 3 to 9 years post-diagnosis. Currently, no cure exists, and treatments focus on symptom management[2,3]. The U.S. FDA has approved four medications: three acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and an NMDA receptor antagonist (memantine). While these drugs provide some relief, they do not halt disease progression[4,5].

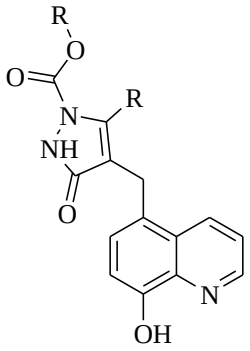
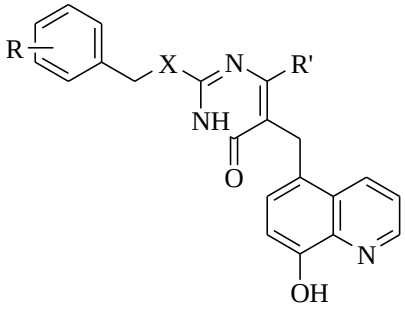
Recent AD research emphasizes the need for multi-target-directed ligands (MTDLs) to address multiple pathological factors within a single compound[6]. While cholinesterase inhibitors remain the primary treatment, they offer only temporary relief. Studies highlight oxidative stress and amyloid-beta (Aβ) aggregation as key contributors to AD pathology, but targeting these alone may be insufficient[7,8]. A promising approach is designing cholinesterase inhibitors with added antioxidant and MAO-B inhibitory properties, potentially providing neuroprotection and slowing disease progression. The study proposes novel molecules based on this strategy[9,10].

2. Aim and Objective

Despite its exceptional pharmacological profile, safety and tolerance, donepezil still has some serious issues. Donepezil could be effective for the treatment of early to intermediate stages of AD. It can be useful only to delay the progression of AD or manage the symptoms, it unfortunately failed to terminate or eliminate the cognitive impairments completely. It had been reported that

donepezil causes moderate improvements in the quality of life of patients suffering from AD. Moreover, there is no single report available claiming the long-term clinical efficacy and safety of donepezil. Due to its limited potential, use of donepezil becomes inadequate to treat severe and advanced stages of AD. This is the reason why so many efforts have continuously been made by the medicinal chemists involved in AD research to develop novel AChE inhibitors with long-term efficacy and safety. Based on the literature review we thought to design a new multifunctional ChEIs endowed with additional properties such as a MAO inhibition, anti-oxidant, matal chealation and amyloid inhibition. As per literature pyrazole moiety was reported as potent MAO-B inhibitor while pyrimidinone moiety was also reported with the AChE and BuChE inhibitory activity as well and quinoline was reported as a well-known anti-oxidant[11–14]. Additionally, The carbamate moiety act as an enzyme inhibitor by binding covalently to the active site of enzymes, resulting in the inhibition of enzyme activity while amine fragment forms strong cation-π interaction with amino acid residues in the active site of the enzymes. So, based on these findings, we thought it was logical to combining the quinoline with the pyrazolone and pyrimidinoone pharmacophore with benzyl amine moiety and carbamate moiety to develop novel pyrazolone based inhibitors of AD. (**Proposed derivatives (I-III**)

Derivative	Structure	Substitutions
8-Hydroxyquinoline fused pyrazolone derivatives **(I)**		R= H, C_6H_5 R'= H, 2,6-*di*F, 4-CF_3, 3,5-*di*F, 4-Cl, 2,6-*di*Cl, 2-CF_3, 2,5-*di*CH_3, 4-F, 3-Cl, 3,5-*di*CH_3, 4-CH_3

8-Hydroxyquinoline fused pyrazolone derivatives **(II)**		R= H, C_6H_5 R'= -Phenyl, Isobutyl, Ethyl, Methyl, n-Pentyl, Benzyl, 2,2,2-Trichloroethyl, 9(*H*) fluorenyl methyl
8-Hydroxyquinoline fused pyrimidinone derivatives **(III)**		R= H, C_6H_5 R'= H, 2,6-*di*F, 4-CF_3, 3,5-*di*F, 4-Cl, 2,6-*di*Cl, 2-CF_3, 2,5-*di*CH_3, 4-F, 3-Cl, 3,5-*di*CH_3, 4-CH_3 X = S, N

3. Research Methodology

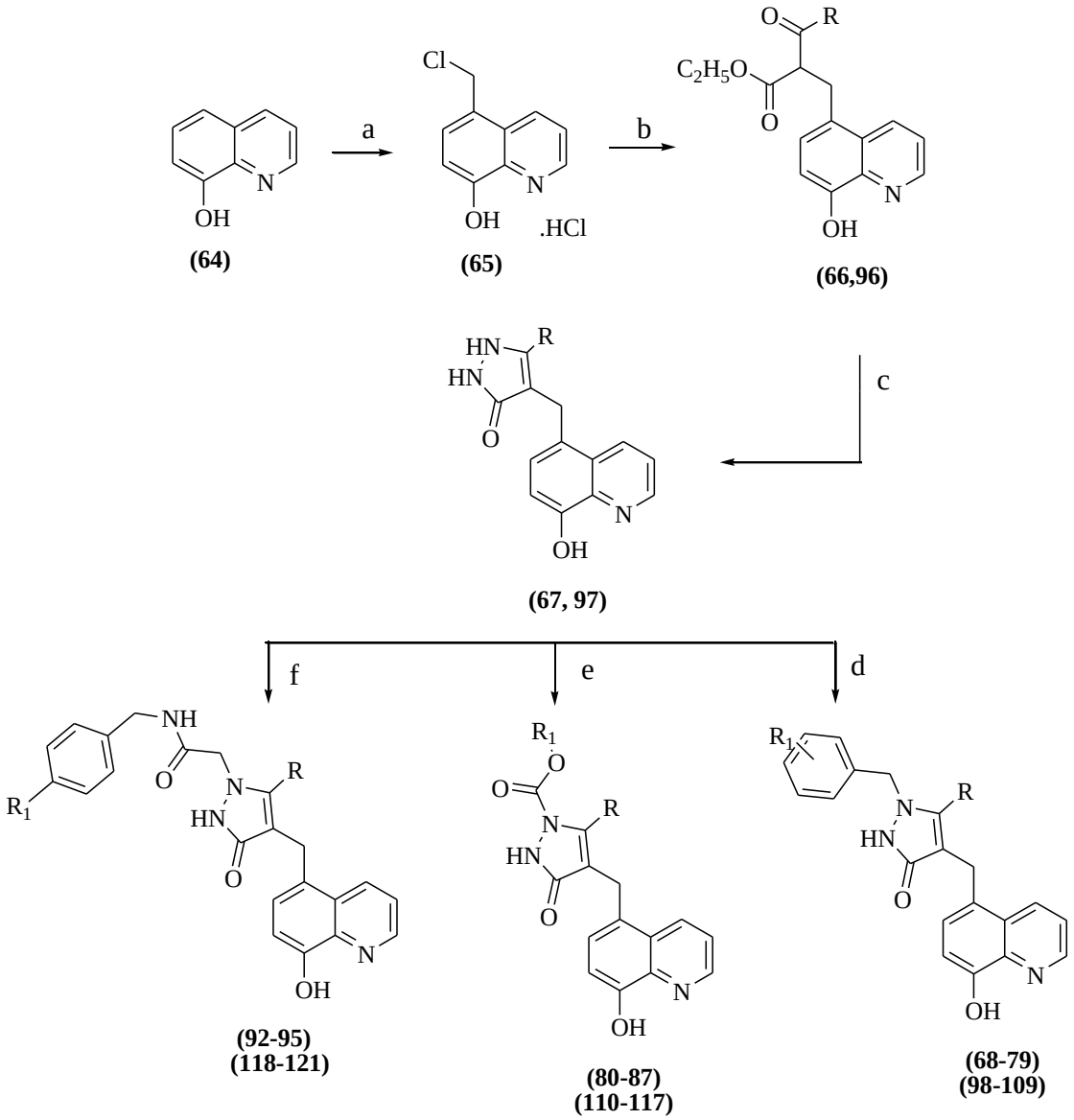
In order to synthesis the compounds of our interest two different schemes were followed (General scheme 1-2).

Under General scheme 1, 8-Hydroxyquinoline fused pyrazolone derivatives **(68-121)** were synthesized.

For the preparation of the proposed compounds, first 8-hydroxyquinoline **(64)** was treated with conc. HCl and formaldehyde in presence of HCl gas to obtain 5-(chloromethyl)-8-hydroxyquinoline hydrochloride[15] **(65)**. Further nucleophilic substitution reaction was carried out using aroyl acetoacetate and NaH to get compound **(66)** and **(96)**. Synthesis of *N*-substitited 1-benzyl-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-5-methylpyrazol-3-one derivatives **(68-79)** and **(98-109)** were carried out by reacting compound **(67)** and **(96)** with the respective benzyl bromide. Additionally, synthesis of *N*-substituted methyl 2,3-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-5-methyl-3-oxopyrazole-1-carboxylate derivatives **(80-87)** and **(110-117)** were carried out by reacting compound **(67)** and **(96)** with the various substituted chloroformates, while Compounds **(92-95)** and **(118-121)** synthesized by reacting compound **(67)** and **(96)** with

substituted benzylacetamide.

General Scheme-I



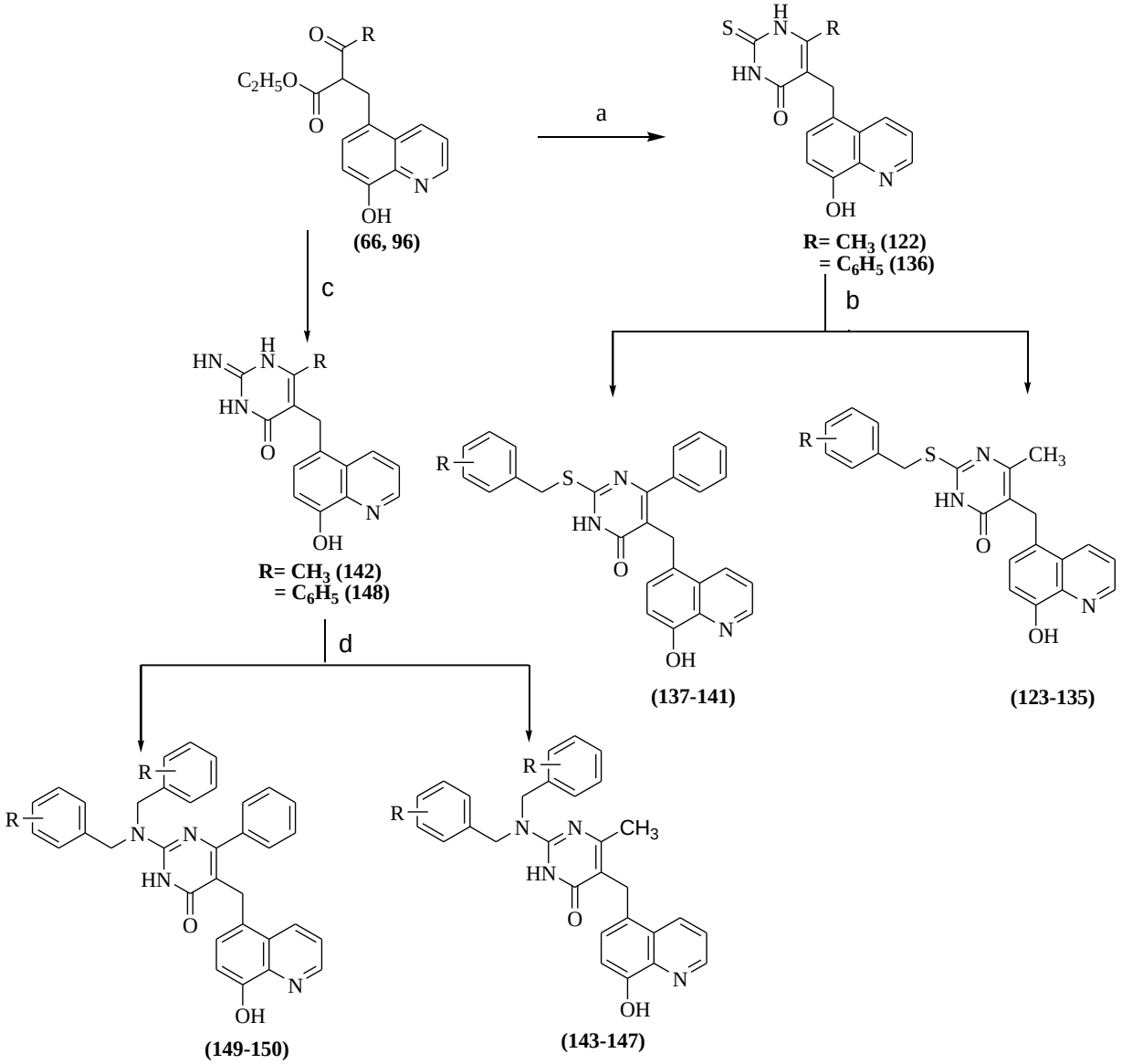
(64) **(65)** **(66,96)** **(67, 97)** **(92-95) (118-121)** **(80-87) (110-117)** **(68-79) (98-109)**

R= CH_3 (66, 67, 68-79, 80-87, 92-95);
C_6H_5 (96, 97, 98-109, 110-117, 118-121)

General synthetic scheme I for compounds reagents and conditions: (a) HCl, formaldyhide, HCl gas; (b) aroyl acetoacetate, DMF, NaH; (c) hydrazine hydrate, EtOH, reflux, 2hr; (d) substituted benzyl halide, DMF, K_2CO_3, RT; (e)substituted chloro formates, DMF, K_2CO_3, RT; (f) *N*-benzylacetamide, DMF, K_2CO_3, 80ºC.

Under the discussed scheme 2, 8-Hydroxyquinoline fused pyrimidinone derivatives **(122-150)** were synthesized.

General Scheme-II



General synthetic scheme II for compounds reagents and conditions; reagents and conditions: (a) DBU (diazabicyclo(5.4.0)undec-7-ene), thiourea, ethanol, reflux; (b) substituted benzyl halide, DMF, K_2CO_3, RT; (c) DBU(diazabicyclo(5.4.0)undec-7-ene), guanidine hydrochloride, ethanol, reflux; (d) substituted benzyl halide,DMF, K_2CO_3, RT.

Under the discussed scheme II, Compounds **(122 & 136)** and **(142 & 148)** were obtained by cyclization reaction of compound **(66)** and **(96)** with thiourea and Guanidine hydrochloride, respectively. Furthermore, this obtained compound reacted with the various substituted benzyl bromide to get the desired compounds **(123-150).**

4. Key Findings

All the proposed compounds from general schemes I & II were successfully synthesized and structurally confirmed through IR, ^{1}H NMR, HRMS, and mass spectrometry. All the synthesized derivatives were evaluated for their anti-Alzheimer activities, including AChE, BuChE inhibitory activity, MAO-B inhibition, metal chelation, anti-oxidant activity and cell toxicity study.

Among the tested compounds, several demonstrated noteworthy AChE inhibitory activity. Compounds **70 (50.7%)**, **72 (51.4%)**, **74 (53.3%)**, **83 (50.9%)**, and **86 (57.7%)** exhibited moderate inhibition. The most potent activity was observed for **102 (68.7%)** and **105 (67.2%)**, although both remained less active than physostigmine. Additionally, **107 (56.4%)** also showed moderate AChE inhibition. For BuChE inhibition, compound **75 (94.07%)** emerged as a highly potent inhibitor, with activity approaching that of physostigmine (97%).

Among the synthesized derivatives, several demonstrated significant MAO-B inhibitory activity. Compounds **93 (55.97%)** and **94 (56.81%)** exhibited moderate inhibition, although their efficacy was considerably lower than the reference inhibitor pargyline (99%). Notably, compound **107 (99.22%)** and compound **138 (100%)** matched the activity of pargyline, emerging as highly potent MAO-B inhibitors. Strong inhibition was also observed for compounds **118 (91.65%)**, **106 (90.94%)**, **102 (80.34%)**, and **101 (79.89%)**, while **98 (84.61%)**, **105 (69.58%)**, **99 (67.63%)**, **104 (67.45%)**, **121 (66.74%)**, and **149 (70.43%)** demonstrated moderate-to-strong activity.Based on these results, the four most effective compounds (**106, 107, 118, and 138**) were selected for IC_{50} determination. All exhibited nanomolar potency, with IC_{50} values of **15.11 ± 8.41 nM (118)**, **16.02 ± 4.48 nM (106)**, **19.14 ± 11.21 nM (138)**, and **26.23 ± 10.56 nM (107)**.

The four most active MAO-B inhibitors (**106, 107, 118, and 138**) were further evaluated for metal chelation, antioxidant potential, and cytotoxicity. At 10 μM, compound **118** demonstrated the highest metal chelation activity (21.39 ± 4.30%), though markedly lower than EDTA (93.12 ± 0.24%). In the DPPH assay, **118** also showed the best radical scavenging activity (5.19%), comparable to 8-hydroxyquinoline (5.97%). Cytotoxicity studies on STHdhQ7/Q7 cells revealed that all four compounds were non-toxic at lower concentrations (10–100 nM), whereas higher doses (≥1 μM) produced a moderate, dose-dependent reduction in cell viability, indicating relative

safety at low concentrations. Moreover, docking study revealed that all the synthesized compound showed strong binding interactions with the active sites of both AChE and MAO-B enzymes.

5. Conclusion

With an aim to design and develop novel therapeutics for Alzheimer's disease (AD), two series of derivatives based on **8-hydroxyquinoline–pyrazolone (66–121)** and **8-hydroxyquinoline–pyrimidinone (122–150)** scaffolds were synthesized and evaluated for their anti-Alzheimer's activity. These scaffolds, enriched with nitrogen-containing heterocycles, were designed to modulate basicity and potentially enhance blood–brain barrier (BBB) permeability. Biological screening revealed that several compounds exhibited dual enzyme inhibition, underscoring their potential for multi-target therapy.

Among the synthesized derivatives, compound **83** showed moderate dual inhibition of AChE (50.9%) and MAO-B (38.66%), while compound **72** demonstrated a similar profile with AChE (51.4%) and MAO-B (31.2%). Potent dual inhibition was observed for compound **102** (AChE: 68.7%; MAO-B: 80.34%) and compound **105** (AChE: 67.2%; MAO-B: 69.58%). Compound **107** combined strong MAO-B inhibition (99.22%) with moderate AChE inhibition (56.4%), whereas compound **149** exhibited activity against both AChE (48%) and MAO-B (70.43%). Notably, compound **100** inhibited AChE (42.7%) and BuChE (44.9%), while compound **75** showed pronounced BuChE inhibition (94.07 ± 0.19%). In contrast, compound **138** displayed exceptional selectivity toward MAO-B (100%).Based on these findings, the four most potent compounds (**106, 107, 118, and 138**) were selected for IC_{50} determination. Compound **118** emerged as the most active, with an IC_{50} value of 15.11 ± 8.41 nM, followed by **106 (16.02 ± 4.48 nM)**, **138 (19.14 ± 11.21 nM)**, and **107 (26.23 ± 10.56 nM)**. Additionally, compound **118** demonstrated metal-chelating activity (21.39 ± 4.30%) and antioxidant potential (5.19% radical scavenging) at 10 μM. Molecular docking further confirmed strong binding interactions of these lead molecules with the active sites of both AChE and MAO-B. Collectively, these results highlight the **8-hydroxyquinoline-pyrazolone and 8-hydroxyquinoline-pyrimidinone scaffolds** as promising leads for multi-target small-molecule therapeutics against Alzheimer's disease.

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6. Recommendation

The present findings suggest that derivatives of **8-hydroxyquinoline-pyrazolone** and **8-hydroxyquinoline-pyrimidinone** scaffolds possess considerable potential as multi-target agents for Alzheimer's disease. In particular, compounds **106, 107, 118, and 138** demonstrated strong MAO-B inhibition in the nanomolar range, with additional AChE inhibitory activity, highlighting their promise as lead molecules. Among them, **compound 118** exhibited the most favorable profile, combining potent MAO-B inhibition with moderate AChE inhibition, antioxidant activity, and metal-chelating properties. The observed non-cytotoxicity at low concentrations further strengthens their therapeutic relevance.

Finally, with all the work carried out can be concluded that the 8-hydroxyquinoline fused pyrazolone and 8-hydroxyquinoline fused pyrimidinone-based molecules are proved as a lead molecule and can be further investigated to develop as small molecules to treat Alzheimer's disease.

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